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Corresponding author(s): RM Densham & JR Morris

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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 Authors & Referees 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	Microscopy images were collected using Leica Application Suite X, and FACS data using Summit 6.
Data analysis	Data Analysis was performed using Image J 1.50b (Densitometry, Foci Analysis, and Fibre Analysis), Summit 6.2 (FACS) and Excel 2016 (Colony Assays, Statistical Analysis). Graphs were prepared using GraphPad Prism7 and Figures assembled using Adobe Photoshop CS5.

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. 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 this current study are available from the corresponding authors on reasonable request

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

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No statistical method was used to predetermine sample size as this study did not include animal models or human participants. The number of repeats, cells or fibres scored per experiment was determined by using commonly accepted sample sizes by the field. In addition, control samples, with drug-vehicle, or cells transfected with Luciferase siRNA as a Non-Targeting control (NTC) were included as appropriate controls for each specific experiment.
Data exclusions	No data was excluded from the analysis
Replication	All attempts of replication were successful. All experiments were independently replicated on different days and, where possible, by different researchers.
Randomization	Randomisation was not required as no human participants or animal models were reported in this manuscript and experiments were performed on matched cell lines.
Blinding	Formal blinding was not done but, whenever possible, experiments were repeated by independent researchers who were not aware of previous findings. In all cases results were consistent between researchers.

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
☒	☐ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

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

Antibodies

Antibodies used

Antibody (clone), Host, Supplier, Cat. number, Lot number, Technique, Conc, RRID
β-actin, Rabbit, Abcam, Ab8227, GR3215935-1, WB, 1:3000, AB_2305186
BARD1, Rabbit, Abcam, ab226854, GR3197067-8, WB, 1:2000
BRCA1 (D-9), Mouse, Santa Cruz, Sc6954, C2519, IF, 1:500, AB_626761
BRCA1 (MS110), Mouse, MERCK Millipore, OP94, 3091924, WB, 1:500, AB_213438
pS114-BRCA1 (3C10G8), Mouse, Genscript, Custom design, , WB, 1:1000,
pS114-BRCA1, Rabbit, Genscript, Custom design, , WB/PLA, 1:1000
CDK1 (A17), Mouse, Abcam, Ab18, GR133813-4, WB, 1:2000, AB_2074906
CldU (BrdU), Rat, Abcam, Ab6326, GR3173537-9, Fibres/IF, 1:2000, AB_305426
Flag (M2), Mouse, SigmaAldrich, F1804, SLBT7654, WB, 1:1000, AB_262044
IdU (BrdU), Mouse, BD Biosciences, 347580, 8151735, Fibres, 1:500, AB_400326
γH2AX, Rabbit, Abcam, Ab2893, GR3242597-1, IF, 1:2000, AB_303388
PALB2, Rabbit, Bethyl, A301.246A, , WB, 1:2000, AB_890607,
PIN1, Mouse, R&D Systems, MAB2294, , WB, 1:1000, AB_2163944
RAD51 (H-92), Rabbit, Santa Cruz, SC8349, E0616, WB/IF, 1:1000, AB_2253533
RAD51 (Ab-1), Rabbit, Calbiochem, PC130, 3135376, PLA, 1:100, AB_2238184
Tubulin, Mouse, Santa Cruz, sc-5286, H0613, WB, 1:5000, AB_628411
Vinculin [EPR8185], Rabbit, Abcam, Ab129002, GR221671-50, WB, 1:2000, AB_11144129
BrdU-Biotin, Mouse, Jackson Immunoresearch, 200-002-211, 134961, PLA, 1:100, AB_2339006
BrdU-Biotin, Rabbit, Bethyl, A150-109A, , PLA ,1:100, AB_67327
Donkey α Mouse AlexaFluor 488, Donkey, Life technologies, A21202, 1975519, IF, 1:5000, AB_141607
Donkey α Rabbit AlexaFluor 488, Donkey, Life technologies, A21206, 1874771, IF, 1:5000, AB_2535792
Donkey α Mouse AlexaFluor 555, Donkey, Life technologies, A31570, 1774719, IF, 1:5000, AB_2536180
Donkey α Rabbit AlexaFluor 555, Donkey, Life technologies, A31572, 1945911, IF, 1:5000, AB_162543
Donkey α rat AlexaFluor 555, Donkey, Life technologies, A21434, 1987272, IF, 1:5000, AB_2535855
Rabbit α Mouse HRP, Rabbit, Dako, P0161, 20062080, WB, 1:10000, AB_2687969
Swine α Rabbit HRP, Swine, Dako, P0217, 20047666, WB, 1:10000, AB_2728719

Validation

The specificity of the phospho-specific pS114-BRCA1 rabbit polyclonal (Figure 1c) and mouse monoclonal (Extended data fig 4l) antibodies are confirmed by this study. In addition, peptide validation data obtained by Genscript during production are available on request. We refer all other antibody validation data to the corresponding commercial data sheet available from the manufacturers - see below for web links.

Antibody, catalogue number, manufacturer information
β-actin ab8227 https://www.abcam.com/beta-actin-antibody-ab8227.html
BARD1 ab226854 https://www.abcam.com/bard1-antibody-ab226854.html
BRCA1 (D-9) sc-6954 https://www.scbt.com/scbt/product/brca1-antibody-d-9
BRCA1 (MS110) OP94 https://www.merckmillipore.com/GB/en/product/Anti-BRCA1-Ab-1-Mouse-mAb-MS110,EMD_BIO-OP92?ReferrerURL=https%3A%2F%2Fwww.google.co.uk%2Furl%3Fsa%3Dt%26rct%3Dj%26q%3D%26esrc%3Ds%26source%3Dweb%26cd%3D1%26ved%3D2ahUKEwjK9JWl9pjiAhUFSBUIHVSPAkQQFjAAegQIAxAB%26url%3Dhttps%253A%252F%252Fwww.emdmillipore.com%252FUS%252Fen%252Fproduct%252FAnti-BRCA1-Ab-1-Mouse-mAb-MS110%252CEMD_BIO-OP92%26usg%3DAOvVaw1v4HDAI6d47HU40YiL2iWs&bd=1
CDK1 (A17) ab18 https://www.abcam.com/cdk1-antibody-a17-ab18.html
CldU (BrdU) ab6326 https://www.abcam.com/brdu-antibody-bu175-icr1-ab6326.html
Flag (M2) F1804 https://www.sigmaaldrich.com/catalog/product/sigma/f1804?lang=en&region=GB
IdU (BrdU) 347580 http://www.bdbiosciences.com/us/applications/research/apoptosis/purified-antibodies/purified-mouse-anti-brdu-b44/p/347580
γH2AX ab2893 https://www.abcam.com/gamma-h2ax-phospho-s139-antibody-chip-grade-ab2893.html
PALB2 A301.246A https://www.bethyl.com/product/A301-246A/PALB2+Antibody
PIN1 MAB2294 https://www.rndsystems.com/products/human-mouse-pin1-antibody-257417_mab2294
RAD51 (H-92) sc-8349 https://www.scbt.com/scbt/product/rad51-antibody-h-92?requestFrom=search
RAD51 (Ab-1) PC130 http://www.merckmillipore.com/GB/en/product/Anti-Rad51-Ab-1-Rabbit-pAb,EMD_BIO-PC130?bd=1
Tubulin sc-5286 https://www.scbt.com/scbt/product/alpha-tubulin-antibody-b-7?requestFrom=search
Vinculin [EPR8185] ab129002 https://www.abcam.com/vinculin-antibody-epr8185-ab129002.html
BrdU-Biotin 200-002-211 https://www.jacksonimmuno.com/catalog/products/200-002-211
BrdU-Biotin A150-109A https://www.bethyl.com/product/A150-109A/Biotin+Antibody
Donkey α Mouse AlexaFluor 488 A21202 https://www.thermofisher.com/antibody/product/Donkey-anti-Mouse-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21202
Donkey α Rabbit AlexaFluor 488 A21206 https://www.thermofisher.com/antibody/product/Donkey-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21206
Donkey α Mouse AlexaFluor 555 A31570 https://www.thermofisher.com/antibody/product/Donkey-anti-Mouse-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-31570
Donkey α Rabbit AlexaFluor 555 A31572 https://www.thermofisher.com/antibody/product/Donkey-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-31572
Donkey α rat AlexaFluor 555 A21434 https://www.thermofisher.com/antibody/product/Goat-anti-Rat-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21434
Rabbit α Mouse HRP P0161 https://www.agilent.com/en/product/immunohistochemistry/antibodies-controls/secondary-antibodies/rabbit-anti-mouse-immunoglobulins-hrp-(ig-fraction)-153249
Swine α Rabbit HRP P0217 https://www.agilent.com/store/productDetail.jsp?catalogId=P021702-2

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	FlpIn HeLa, FlpIn U20S and FlpIn HEK293 cells were from Morris lab stocks. All other cell lines were generated from these parental cell lines during the course of the study.
Authentication	None of these cell lines have been authenticated
Mycoplasma contamination	All cell lines tested negative for Mycoplasma contamination by Hoescht staining
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	U20S-DR3 reporter cells were grown in culture and transfected with ISce1 to induce a double-strand DNA break and either RFP or RFP-Flag-BARD1. ISce1 breaks which are repaired by Homologous Recombination in U20S-DR3 cells result in expression of a functional GFP. 72 hours after transfection, cells were trypsinised and fixed in 2% PFA before FACS analysis.
Instrument	CyAN ADP FACS Analyser
Software	Data collection and analysis was performed using Summit 6.2 software
Cell population abundance	U20S DR3 reporter cells were grown in culture, trypsinised, fixed, and 10,000 cells/sample were analysed. Each condition was prepared in triplicate.
Gating strategy	Cells were identified using Forward and Side scatter and data from 10,000 cells / sample was collected. Gating was used to measure the % GFP, %RFP and %RFP+GFP positive cells.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.