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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.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
☐	☒	An indication of 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 statistics 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
☐	☒	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 about availability of computer code

Data collection	scanR acquisition software 2.6.1.0, SoftWoRX 6.5.2, Cellquest Pro 6.0.
Data analysis	GraphPad Prism7, scanR analysis software 2.6.1.0, SoftWoRX 6.5.2, Flowjo 8.8.7, MaxQuant 1.6.0.16, Rosetta 5.0.37 macromolecular modeling package (www.rosettacommons.org)

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 mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD009281.

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 ☐ Ecological, evolutionary & environmental sciences

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

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. All experiments were included with multiple biological replicates based on previous experiences.
Data exclusions	No samples were excluded from analysis.
Replication	All results were tested and confirmed with at least two independent experiments
Randomization	No method of randomization was applied. Samples were organized into groups based on whether they were treated or not treated (IR/ chemical treatment, over-expression by tetracyclin inducible system or transient plasmid expression, knockdown by siRNA)
Blinding	No blinding assessment was performed as microscopy and FACS data were analyzed automatically. Exactly the same gate setting was used for all samples in high-content microscopy as well as flow cytometry analysis.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☐	☒ Unique biological materials
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☒	☐ Animals and other organisms
☒	☐ Human research participants

Methods

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

Unique biological materials

Policy information about availability of materials

Obtaining unique materials	All cells and plasmids established in this study are available on reasonable request.

Antibodies

Antibodies used	Target/Manufacturer/Catalogue number/Host/Dilution (Application) Immunofluorescence (IF), Western blot (WB) HA/Roche/1 867 423/Rat/1:200(IF) HA/Bio Legend/901509/Mouse/1:100(IF) HA/Covance/MMS-101P/Mouse/1:500(WB) GFP/Roche/11 814 460 001/Mouse/1:500(WB) SLF1/Novus Biologicals/NBP1-88358/Rabbit/1:500(WB) 53BP1/Santa Cruz Biotechnology/H-300/Rabbit/1:500(IF) BRCA1/Santa Cruz Biotechnology/sc-6954/Mouse/1:200(IF), 1:400(WB) BARD1/Bethyl/A300-263A/Rabbit/1:500(IF)(WB) BARD1/Abcam/ab64164/Rabbit/1:500(WB) H4K20me0/Abcam/ab227804/Rabbit/1:10,000(IF) H4K20me1/Abcam/ab9051/Rabbit/1:250(IF), 1:1000(WB) H4K20me2/diagenode/C15200205/Mouse/1:250(IF), 1:5,000(WB) Phospho-H2A.X (S139)/Cell Signaling Technology/2577/Rabbit/1:1000 (IF) OsTIR1/gift from Dr. Masato Kanemaki//Rabbit/1:1000 (WB) Beta-Actin/Sigma/A1978/Mouse/1:2000 (WB)

SET8/Millipore/06-1304/Rabbit/1:1000 (WB)
MCM2/BD Bioscience/610701/Mouse/1:150(IF)
BrdU/Eurobio/ABC117-7513/Rat/1:1000(IF)

Validation

H4K20me0(abcam ab227804; validated in Supplementary Fig. 2a), H4K20me1 (abcam ab9051; validated in Supplementary Fig. 2g), H4K20me2 (Diagenode C15200205; validated in Supplementary Data Fig. 2g). Other antibodies were validated by the manufactures.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

HeLa S3: Dr. Pat Nakatani
U-2-OS: Dr. Jiri Bartek
DR-GFP U2OS: Dr. Niels Mailand
DR-GFP BARD1-null mouse mammary carcinoma cells: Dr. Richard Baer
Flp-In T-Rex U-2-OS cells: Invitrogen
HCT116: Dr. Ian Tomlinson

Authentication

The Cell lines have been authenticated based on morphological criteria. BARD1-null mouse mammary carcinoma cell line was tested for BARD1 expression by western blot and confirmed the BARD1 deficient phenotypes can be rescued by exogenous BARD1 expression (22, and Fig. 4b and Supplementary Fig. 3d in this study). Flag-HA-BARD1 WT and ARD inducible cell lines (Flp-In T-Rex U-2-OS cells) and HCT116 Tet-OsTIR1 cells, and BARD1 AID/ AID cell line were authenticated by western blotting and/or immunofluorescence (in this study).

Mycoplasma contamination

All cell lines were tested negative for mycoplasma.

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

We used flow cytometry only for DR-GFP assay. Cells were trypsinized and suspended in PBS. Further details of the experimental procedures are provided in the Materials and Methods.

Instrument

FACSCalibur

Software

We analyzed the cells with CellQuest Pro (version 6.0) and analyzed the data with Flowjo 8.7.7.

Cell population abundance

N/A

Gating strategy

The FSC/SSC gates defined the single cell population and the percentage of GFP-positive cells in RFP positive population was calculated as HR repair efficiency. An RFP vector was used as a control for transfection efficiency. The gating strategy is provided in the Supplementary figure 3c.

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