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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 *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	Nikon NIS-Elements software (BR 4.500.0064-bit.lnk), InCyte Software (version 3.3), Gen5 v 2.01.14
Data analysis	Data analysis and plotting: GraphPad PRISM version 8; Microsoft Excel 2011; Image analysis: Fiji 2.0 d; CellProfiler 3.0 software. For ATAC-seq analysis: Trim galore (version 0.4.1), Picard (version 2.1.0), Macs (version 2.0.9), Bedtools (version 2.27.1) and Deseq2 package in R(version 3.6.1). For RNA-seq analysis: Lexogen Quantseq 2.3.1 FWD platform, STAR Aligner with modified ENCODE settings,HTSeq-count, DESeq2 (1.14.1). For synergy analysis: Combenefit

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

Sequencing data generated in this study has be deposited in the Gene Expression Omnibus with accession code # GSE149104 (for RNA-seq) and # GSE150955 (for ATAC-seq). Data from the CRISPR screen has been provided as mapped reads in Supplementary Table 1. Functionally conserved domains were identified using either NCBI Conserved Domain Search or Uniprot. Source data are provided with this paper. All other data supporting the findings of this study are available from the corresponding author upon 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

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 method was used to pre-determine sample size. Sample sizes are consistent with previously published studies on DNA repair. (Dilley et al, Nature,2016, Clairmount et al, Nat Cell Bio, 2020, Zimmermann, Nature 2018). Key validation experiments were performed using at least 2-3 independent replicates based on standard practices in the field. Every biological replicate was derived from independent sgRNA transductions and generation of a new stable line. For all cell viability assays, cells were plated in triplicates and treated separately. Values of the triplicates were averaged to derive data for one biological replicate.
Data exclusions	In the xenograft experiment, two individual flanks that failed to develop tumors were excluded from subsequent measurements. The GFP data was acquired using a 96-well plate, high-throughput Guava machine, where cell counts less than 500 tend to give fluctuations in the results. Therefore, for GFP competition experiments, reading were not taken when cell were less than 50% confluent and any value derived from less than 500 cells was omitted. We performed multiple biological independent transductions to confirm that all key results had at the minimum, values from three transductions for all time points.
Replication	All attempts at replication were successful. The number of replicates for each experiment is clearly presented in the corresponding figure legend. Most results were confirmed in multiple cell lines and using complementary assays.
Randomization	For xenograft studies animals were randomized. The two mice that developed a tumor only on one flank were included in the untreated group. Cell culture experiments are not subjected to randomization.
Blinding	The technician performing xenograft experiment was blinded to the experimental design. All IF analysis was blinded. For most other experiments, results were immediately revealed (eg. western) or data generation was automated (eg. GFP measurement or viability assay) and hence these experiments couldn't be subjected to blinding.

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	ALC1 (Santacruz Biotechnology, sc-81065, 1:200 dilution), XRCC1 (Abcam, ab1838, 1:200 dilution), PARG (Millipore,MABS61, 1:200 dilution), CHD4 (Proteintech, ab264417,1:1000), APE-1 (Santacruz Biotechnology, sc-17774, 1:200 dilution), NTHL1 (Santacruz Biotechnology, sc-2660C1a, 1:200 dilution), 53BP1 (Novus Biological NB100-94, 1:1000 dilution), PARP1 (Cell Signaling mAB#9532, 1:2000 dilution for western), PARP1 (1:500 for IF, Abcam, ab227244), PARP2 (1:500, Active Motif, 39743),Rev7 (Invitrogen PA5-49352, 1:1000 dilution), GAPDH (Cell Signaling 2118S, 1:2000 dilution),Histone H2A.X (Ser139) clone JBW301 antibody (Millipore, 05-636-1), RAD51 antibody (H-92, sc-8349, Santacruz), CldU antibody (Abcam,ab6326) and Anti-Histone H2A.X (phosphor S139) [EP854(2)Y] (Abcam, ab81299),Alexa Fluor 568 conjugated anti-mouse secondary (A-11031),Alexa Fluor 488 conjugated anti-rabbbit secondary (A-11034), Alexa Fluor 488 conjugated anti-mouse secondary (A-11029), Alexa Fluor 568 conjugated anti-rabbbit secondary (A-11036), anti-HA (Biolegend, 901514), rabbit anti-PCNA (Cell Signaling, 13110S),anti-IdU (BD-347580) ,rat anti-CldU (Serotec-OBT0030G),GFP (Cell Signaling mAb#2956),mouse Anti-FLAG (F1804, Sigma), anti-Histone H4 (Millipore, 05-858).
Validation	Antibodies validated by knockdown: ALC1 (Santacruz Biotechnology, sc-81065, 1:200 dilution), XRCC1 (Abcam, ab1838, 1:200

Validation	dilution), 53BP1 (Novus Biological NB100-94, 1:1000 dilution), PARP1 (Cell Signaling mAB#9532, 1:2000 dilution for western), PARP2 (1:500, Active Motif, 39743), Rev7 (Invitrogen PA5-49352, 1:1000 dilution), GFP (Cell Signaling mAb#2956). Antibodies validated by overexpression: mouse Anti-FLAG (F1804, Sigma), anti-HA (Biolegend, 901514). Antibodies validated by knockdown but data unpublished: NTHL1 (Santacruz Biotechnology, sc-2660C1a, 1:200 dilution), PARG (Millipore,MABS61, 1:200 dilution), rabbit anti-PCNA (Cell Signaling, 13110S). Validation for CHD4 (Proteintech, ab264417,1:1000): (https://www.ptglab.com/products/CHD4-Antibody-14173-1-AP.html). Validation for APE-1 (Santacruz Biotechnology, sc-17774, 1:200 dilution): Sica et al, 2019, Cell Reports and Wang et al, 2018, Cell death and disease PARP1 (1:500 for IF, Abcam, ab227244): Gatti et al, Cell Reports, 2020 GAPDH (Cell Signaling 2118S, 1:2000 dilution): This antibody is widely used as loading control: Wang et al, 2019,Nature ,Wu et al, 2019 Nature Med, Dilley et al 2016 Nature Histone H2A.X (Ser139) clone JBW301 antibody (Millipore, 05-636-1), RAD51 antibody (H-92, sc-8349, Santacruz), CIdU antibody (Abcam,ab6326), anti-IdU (BD-347580) , rat anti-CIdU (Serotec-OBT0030G), (Abcam, ab81299: These are gold-standard antibodies in DNA-repair field. Few examples using these antibodies: Zimmermann et al,2018, Nature, Dungrawala et al, Mol Cell, 2017, Mirman et al, Nature,2018, Quintet,2017, Methods Enymol., Phillips et al, Mol Cell, 2017. H2A.X (pSer139)) clone JBW301 antibody (Millipore, 05-636) antibody was also validated by comparing signals in control and cells treated with IR and MMS damage. No signal was detected in undamaged cells. CIdU antibody was also confirmed by comparing signals in control and cells treated with CIdU. No signal was detected in cells not treated with CIdU. Anti-Histone H2A.X (phosphorS139) [EP854(2)Y]: Validated as damaged marker by Abcam: https://www.abcam.com/gamma-h2ax-phospho-s139-antibody-ep8542y-ab81299.html?productWallTab=Abreviews#description_images_1 Anti-Histone H4 (Millipore, 05-858): Validated by Millipore https://www.emdmillipore.com/US/en/product/Anti-Histone-H4-Antibody-pan-clone-62-141-13-rabbit-monoclonal,MM_NF-05-858

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	293T, U-2 OS, CAPAN-1, UWB1.289 and UWB1.289+BRCA1(ATCC); DLD1 WT and DLD1 BRCA2-/- (Horizon discovery); SUM149PT(Asterand Bioscience); hTERT-RPE1 p53-/- Cas9 and hTERT-RPE1 p53-/-BRCA-/- Cas9 cells were gifted by D. Durocher (Univ.Toronto, Lunenfeld) (Zimmermann et al,Nature, 2018) , SUM149PT reversion mutants and parental control were a gift from Neil Johnson's lab (FoxChase), (Wang et al, Cancer Research, 2016), hTERT-RPE1 parental and XRCC1 KO from Keith Caldecott (Univ. of Sussex) (Hanzlikova et al,2017, NAR) and U-2 OS PARP1-/-, PARP2-/- and PARP1/2-/- cells from Nick Lakin lab (Univ. of Sussex) (Ronson et al,2018, Nature Comm).
Authentication	Purchase of UWB1.289 and DLD1 isogenic lines were made at the initiation of the project. CAPAN-1 and U-2 OS lines were validated by STR profiling at ATCC. SUM149PT was confirmed by immunoblotting and PARPi sensitivity. All other cell lines were procured from published studies as reported above and were not authenticated .
Mycoplasma contamination	All cell lines tested negative for mycoplasma.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in the study.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	5-week-old female NSG (NOD.Cg-Prkdcscidll2rgtm1Wjl/SzJ) mice from Jackson laboratory. Mice were kept at 72F with average humidity of 60% in 12h day light and 12h night cycle.
Wild animals	The study did not involve wild animals.
Field-collected samples	The study did not involve field-collected samples.
Ethics oversight	Xenograft studies were carried out under protocol number 803170 approved by the Institutional Animal Care & Use Committee at the University of Pennsylvania.

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