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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	Leica Zen 2012 software, Biorad CFX manager.
Data analysis	Graphpad Prism v7, FlowJo v10, TIDE, ProHits, SAINTexpress (v3.6.1), cytoscape, ImageJ, ImageQuant TL, MaGeCK, DrugZ, Python v3.5.1, Acapella, Image Studio Lite

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

Raw data of CRISPR Cas9 screens (Fig 1b and 2a) is included as supplementary tables. Raw data for IP-MS experiments has been deposited elsewhere (ftp://

massive.ucsd.edu/MSV000082207, Unique accession numbers are MSV000082207 and PXD009313). IP-MS data can also been viewed at the prohits website (prohits-web.lunenfeld.ca) under data set 29: Durocher lab.

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	All experiments represented in this manuscript are performed as biological replicates, with at least three independent replicates, as mentioned in each figure panel (except fig 3f, n=2, with a minimum of 20 cells each replicate). For the mouse transplantation studies, a power analysis was performed to calculate that a minimum of 8 mice per group were needed to achieve a power of 0.8 (two-sided test, alpha=0.05). Otherwise, no power analyses were performed a priori. For those experiments not having power analysis, biological replicates of n=3 or more were considered to have enough power to find biologically significant effects, with for IF studies, a minimum of 20 cells per sample for foci/ stripe intensity studies and a minimum of 100 cells for foci number analysis.
Data exclusions	Data from experiments was only excluded in the rare event when control samples didn't perform as expected. All graphs are represented as dot plots showing variation among individual biological replicates. The type of error bars are explained in the corresponding figure legends.
Replication	In addition to internal biological replicates within experiments, we show data in multiple mouse and human models in this manuscript, all confirming the role of the shieldin complex in NHEJ and limiting end resection. Since the data was acquired by multiple research groups, the data show a strong reproducibility.
Randomization	For the mouse transplantation study, animals were stratified into the diffferent treatment arms by randomly allocating them into the untreated or olaparib treated group. There was no additional randomization in the experiments described in this manuscript.
Blinding	For the mouse transplantation study, treatment of mice with tumours of the different genotypes was performed blind. No other experiments were collected or analyzed in a blinded manner.

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

Antibodies

Antibodies used

For a tabular overview: see Supplementary Table 7.
Primary antibodies
Rb anti RAD51 Santa Cruz, sc-8349 1:150
Rb anti RAD51 Bio Academia, #70-001 1:2,000
Rb anti REV7 Abcam, ab180579 1:1,000 1:500
Rb anti 53BP1 Santa Cruz, sc-22760 1:5,000
M anti 53BP1 Becton Dickinson, #612523 1:1,000-3,000
G anti RIF1 Santa Cruz, sc-55979 1:1,000 1:200
R anti RIF1 Bethyl A300-569A 1:7,500
M anti γH2AX Millipore, #05-636 1:5,000
Rb anti BRCA1 homemade 1:1,000
Rb anti pRPA32 (S4/S8) Bethyl, A300-245A 1:1,000
Rb anti KAP1 Bethyl, A300-274A 1:2,000

M anti Tubulin Calbiochem, CP06 1:2,000
M anti Tubulin Sigma, T6199 1:5,000
Rb anti Flag Cell Signalling Technologies, #2368S 1:2,000
M anti Flag-HRP Sigma, A8592 1:1,000
G anti GFP Homemade by Pelletier lab, Lunenfeld-Tanenbaum Research Institute 1:5,000
Rb anti GFP Abcam, ab290 1:1,000-5,000 1:2,000
M anti GFP Roche, #11814460001 1:2,000
M anti V5 Invitrogen, #46-0705 1:1,000
M anti HA Biolegend, #901502 1:1,000
G anti mouse IgA-PE Southern Biotech #1040-09 FACS: 1:100-150
M anti-AID Cell Signaling Technologies, #4975 1:1,000
Anti-β-actin Sigma 1:2,000

Secondary antibodies
HRP-tagged Rabbit anti Mouse DakoCytomation, P0260 1:5,000
HRP-tagged Goat anti Rabbit Jackson Laboratories, #111-035-144 1:5,000
HRP-tagged bovine-anti-goat Jackson Laboratories, #805-035-180 1:5,000
IRDye-conjgated Goat anti Mouse Li-cor, #926-32210 1;10,000
IRDye-conjgated Goat anti Rabbit Li-cor, #926-68071 1;10,000
AlexaFluor-488 Goat anti Mouse Invitrogen, A-11029 1:1,000
AlexaFluor-488 Goat anti Rabbit Invitrogen, A-11034 1:1,000
AlexaFluor-488 Donkey anti Goat Invitrogen, A-11055 1:1,000
AlexaFluor-555 Goat anti Mouse Invitrogen, A-21424 1:1,000
AlexaFluor-555 Goat anti Rabbit Invitrogen, A-21429 1:1,000
AlexaFluor-647 Goat anti Mouse Invitrogen, A-21236 1:1,000
AlexaFluor-647 Goat anti Rabbit Invitrogen, A-21245 1:1,000
Alexa fluor 568 F(ab')2 Fragment goat anti-rabbit Invitrogen, A-21069 1:1,000

Validation

All antibodies have been described before by the manufacturer, our groups or others for the purpose used in this study.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

All human cell lines original from ATCC or are derivatives of ATCC. More detailed information can be found in the Methods section. The mouse cell lines used in this study have been described before (Jaspers et al., 2013; Bouwman et al., 2013; Duarte et al., 2017; Nakamura et al., 1996

Authentication

We routinely authenticate our cell lines with STR analysis. Genetic knockouts were validated by genomic sequencing and using antibodies, when available.

Mycoplasma contamination

All cell lines were routinely tested negative for mycoplasma contamination.

Commonly misidentified lines
(See ICLAC register)

None of the used cell lines are listed in the ICLAC database.

Animals and other organisms

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

Laboratory animals

NMRI-nude female mice were purchased from Janvier Laboratories and used for transplantation studies at the age of 6-9 weeks. The derivation of KB1P4 tumor organoids was described previously (Duarte et al. Nat Methods, 2017).

Wild animals

No wild animals were used in this study.

Field-collected samples

No field-collected samples were used in this study.

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	For Traffic Light Reporter (TLR) assays, cells were collected from tissue culture plates by trypsinzation, diluted into a larger volume of PBS with 10% FBS, then sorted immediately. For class-switch recombination (CSR) assays, live cells were collected -/+ CSR induction and stained with PE-conjugated anti-IgA antibody prior to FACS.
Instrument	BD Biosciences Fortessa and BD Biosciences Fortessa X20-HTS.
Software	FlowJo version 10
Cell population abundance	No cell sorting was performed for this project.
Gating strategy	For all assays, live cells were first gated from the FSC/SSC plots, with events of very low or very high FSC and/or SSC being excluded. The live cell fraction constituted >80% of total events in TLR assays, and >50% of total events in CSR assays. For the TLR assay, the boundary between BFP-negative and BFP-positive was drawn from the plots of control samples where cells hadn't been transfected with BFP-IsceI. The BFP-positive population was then used to determine percent of GFP-positive cells. The boundary between GFP-negative and GFP-positive was drawn from the plots of the total live cells from control samples where cells hadn't been transfected with BFP-IsceI. For the CSR assay, the boundary between PE-negative and PE-positive cells were applied from the plots of simultaneously stained unstimulated control samples. For examples of how boundaries between "positive" and "negative" cells were drawn in the TLR assay, please see Extended Data Figure 3c,d. For examples of how boundaries between "positive" and "negative" cells were drawn in the CSR assay, please see Extended Data Figure 9a.

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