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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	Data collection using Microsoft Excel for Mac (Microsoft Inc, USA). Adobe Photoshop and Illustrator were used to process data for publication (Adobe Systems Inc, USA).
Data analysis	Above statistical parameters are indicated in the methods section. Graph display and statistical analysis was performed using Prism v7 for MacOs (GraphPad Software Inc., USA). ImageJ was used to quantify immunoblot bands (ImageJ 2.0.0). Flow cytometry analysis using Flowjo (BD Inc, USA). For RNA sequencing studies, prior to alignment, sequencing quality of the reads was enforced using Trim Galore! (v0.4.2). Reads were aligned to reference genomes using STAR (v2.5.2b). Counts were assigned to genome features using featureCounts(v1.5.2). Counts from multiple sequencing runs were merged/normalised using edgeR package. MaxQuant v1.5.2.8 was used for Mass Spectrometry data analysis. Raw OMX data was reconstructed and channel registered in SoftWoRx software version 6.5.2 (Applied Precision, a GE Healthcare company). FIJIv2.0.0 was used for OMX data analysis.

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 raw data files for the whole genome CRISPR cas9 screen in SUM149 cells is available on NIH Sequence Read Archive via the accession number PRJNA471892. Raw data files for the PDX RNA sequencing is available on NIH Sequence Read Archive via the accession number **PRJNA473981**. Raw data files for the mass spectrometry is available on PRIDE partner repository via PXD009830. FIJI custom source script is available on Fiji (https://github.com/gurdon-institute/OMX-Spatial-Analysis). Source data for figures can be found in Supplementary Table 5. All other data supporting the findings of this study are available from the corresponding authors on reasonable request.

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

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	Animal experiments were performed with the available BRCA1 PDX models generated. All experiments were conducted with cell lines with multiple available biological replicates and based on previous experience with specific experimental setup; no statistical method was used to determine sample size.
Data exclusions	No tumors/in-vitro cell line samples were excluded from analysis.
Replication	All experiments were reliably reproduced as stated in the text, and detailed methods provided to aid in their replication by others. Where further methods/data are sought corresponding authors will oblige reasonable requests.
Randomization	Mice were randomised into treatment arms.
Blinding	Group allocation and outcome assessment were performed in fully blinded manner.

Materials & experimental systems

Policy information about availability of materials

n/a	Involved in the study
☐	☒ Unique materials
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☐	☒ Research animals
☐	☒ Human research participants

Unique materials

Obtaining unique materials	All established cellular and plasmid reagents are available on reasonable request

Antibodies

Antibodies used	Antibody Species Source Application (Dilution) FAM35A Rabbit Abcam ab105521 WB (1:1000) C20orf196 (E-15) Rabbit Santa-Cruz sc-85394 WB (1:200) 53BP1 Rabbit Novus NB100-304 WB (1:5000) 53BP1 Mouse Millipore MAB3802 IF (1:250) RIF1 Rabbit Bethyl A300-569A WB (1:1000) REV7/MAD2L2 Mouse BD biosciences 612266 IF (1:100)/WB (1:1000) BRCA1 Rabbit Merck 07-434 WB (1:500) FANCD2 Mouse Santa-Cruz sc-20022 (FI17) IF(1:100)/WB (1:1000) HA Mouse Santa-Cruz sc7392 WB (1:500)

BLM Rabbit Bethyl A300-110A IF(1:100)
CTIP Mouse Hybridoma supernatant (Richard Baer) WB(1:50)
GFP Mouse Roche WB (1:1000)
Tubulin Rabbit Abcam ab52866 WB (1:1000)
GFP Rabbit Roche IF (1:1000)
PTIP Rabbit Abcam ab2614 WB (1:1000)
Abraxas Rabbit Bethyl A302-180A-M WB (1:1000)
Cyclin A Mouse BD Biosciences 611268 IF (1:100)
RAD51 Rabbit Santa-Cruz sc-8349 (H-92) IF (1:100)
γH2AX Mouse Millipore 05-636 IF (1:100)
RPA2 Mouse Abcam ab2175 IF (1:200)
γH2AX Rabbit Cell Signalling Technology 2577 IF (1:500)
XRCC4 Goat Santa Cruz sc-8285 (C-20) WB (1:1000)
SMC1 Rabbit Bethyl A300-055A WB (1:1000)
Total H2AX Rabbit Abcam ab11175 WB (1:5000)
GAPDH Mouse Abcam ab8245 WB (1:2500)
b-Actin Mouse Abcam ab8266 WB (1:5000)
GFP Rabbit Life Technology, A11122 WB (1:1000)
Phospho-RPA32 S4/S8 Rabbit Bethyl A300-245A WB (1:1000)
C20orf196 (E-15) Rabbit Santa-Cruz, sc-85394 WB (1:500)
GST Mouse Santa-Cruz, sc 138 WB (1:1000)
Alexa Fluor 488 anti mouse/rabbit Goat Molecular Probes A11029/A11034 IF (1:500-1:1000)
Alexa Fluor 594 anti mouse/rabbit Goat Molecular Probes A11005/A11037 IF (1:500-1:1000)
Alexa Fluor 647 anti mouse Goat Molecular Probes A21236 IF (1:500)
HRP anti mouse Rabbit Dako Ltd P0260 WB (1:10000)
HRP anti rabbit Goat Perbio Science 31462 WB (1:20000)
HRP anti goat Rabbit Dako Ltd P0449 WB (1:10000)
53BP1 Rabbit Bethyl, A300-272A WB (1:4000)
b-Catenin Mouse BD, 610154 WB (1:5000)
MAD2L2 Mouse Santa Cruz sc135977 IF (1:500)
V5 Mouse Invitrogen, R960-25 WB (1:1000)
Flag-M2 Mouse Sigma, F1804 IF (1:200)/WB (1:2000)

Validation

Each experiment had appropriate controls to validate the antibodies. Commercially available antibodies were validated by the supplier and by us using appropriate controls where needed; Supplementary Table 2. Please also refer to the manufacturers' websites for further details.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

U2OS(TREX), RPE1p53-(FRT), HEK293T, mESC: SP Jackson (Gurdon Institute, Cambridge, UK)
Lenti-X 293T: Clontech Laboratories Inc. (CA, USA)
SUM149PT: C. Lord (Institute of Cancer Research, London, UK)
U2OSLacSceIII: D. Durocher (University of Toronto, Canada)
CH12F3 cells: F. Alt (Harvard University, USA) and T. Honjo (Kyoto University, Japan)
MEFS(TRF2ts): J. Jacobs (NKI, Netherlands)

Authentication

All cells were originally obtained from the ATCC cell repository, and we have authenticated cell lines used in our study by STR profiling.

Mycoplasma contamination

All cells are routinely tested to be mycoplasma free.

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified cell lines were used.

Research animals

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

Animals/animal-derived materials

Animals/animal-derived materials PDTXs were generated by implantation of human tumour samples in female highly immunodeficient mice (NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ or NSGs) at around 4-5 months old. All experimental procedures were approved by the University of Cambridge Animal Welfare and Ethical Review Committee and by the Vall d'Hebron Hospital Clinical Investigation Ethical Committee and Animal Use Committee. Collection to mouse implantation ranges from 30-180 min. Surgically resected tissue samples were embedded in matrigel and then implanted subcutaneously into 2-4 female severe immune compromised NSG mice. Pleural effusion and ascites samples were centrifuged, washed with water twice to eliminate red blood cells, and cell pellets resuspended in 50% matrigel:FBS solution before subcutaneous injection into mice.

Human research participants

Policy information about studies involving human research participants

Population characteristics	Surgically resected primary breast cancer tissue, biopsies from brain, skin, liver, bone, axilla and lymph node metastasis, and pleural effusions or ascites samples were obtained from consenting patients (female, diagnosed with breast or ovarian malignancy). The research was done with the appropriate approval by the National Research Ethics Service, Cambridgeshire 2 REC (REC reference number: 08/H0308/178), and the Vall d'Hebron Hospital Clinical Investigation Ethical Committee (PR(AG)183/2012). Comprehensive clinical information on the patients and originating cancer sample implanted to generate PDTXs can be found in Bruna et al. Cell 2016 and Cruz et al. Annals of Oncology 2018.

Method-specific reporting

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ Magnetic resonance imaging

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	U2OS(TLR) cells (modified from Certo et al. 2011 Nat Methods, Jul 10;8(8):671-6) were treated with the indicated siRNAs and 8 h later cells were transfected with the HR-Donor and I-SceI expression plasmids. After ~72 h, cells were trypsinised and collected in 1% PBS/BSA.
Instrument	Samples were sorted using a BD LSRFortessa cell analyser (BD Biosciences).
Software	The data was collected and analysed using FlowJo(BD Inc, USA).
Cell population abundance	Cell sorting was not necessary to evaluate homologous recombination events, and was not performed for this assay.
Gating strategy	FSC/SSC gates define single cell population. For each condition, 10,000 live cells which were successfully expressing donor (BFP) and I-SCeI(IFP) were scored for GFP (HR) and mCherry (mutEJ). GFP and mCherry gates were defined using BFP/IFP negative cell population as a negative control. Each siRNA treatment was normalised to a negative control siRNA targeted to firefly luciferase.

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