nature portfolio

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Reporting Summary

Nature Portfolio 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 Portfolio 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

1) The acquisition of 3D super-resolution images with 0.125µm or 0.2µm z-slices was performed on LSM880 with Airyscan fast detection system confocal microscope with the oil immersion objective Plan-Apochromat 63×/1.4 oil DIC M27 and ZEN black software edition 2.3 (Zeiss) for acquisition, in sensitivity-versus-resolution (SR) mode with 8 bits, yielding an approximate range of 30 to 85 z-slices per nucleus.
2) Confocal 3D imaging and z-stack acquisition of slices at 0.125 µm, 0.3 µm, 0.25 µm or 0.5 µm step sizes was performed on a Leica TCS sp8 Lightning Confocal/STED microscope coupled to LasX (Leica) using 63x (1.4) oil-immersion objective, or an in-house Nikon Eclipse Ti2 C2+ confocal microscope coupled to NIS-Elements AR software (Nikon v4.10.00 and v4.50.00) at 100x (1.25) or 60x (0.85) at OFN25 oil objectives.
3) Live cell imaging was performed on a LSM880 microscope with Airyscan Fast detection system (Zeiss) coupled to ZEN black software edition 2.3 (Zeiss) and standard confocal imaging was performed using the Nikon A1R confocal microscope with a resonant scanner coupled to NIS-elements AR software (Nikon v4.10.00 and v4.50.00). Both microscopes were equipped with an incubation module at 37°C and 5% CO2. Airyscan
data files were acquired in sensitivity-versus-resolution (SR) mode at 8-bit, 0.125 µm or 0.2 µm z-slices and fitted zoom level with the oil immersion objective Plan-Apochromat 63x/1.4 oil DIC M27. Nikon A1R images were acquired with Plan-Apochromat, nano-crystal, 60x/1.4 NA, oil immersion lense, 0.125 µm or 0.2 µm z-slices.
4) Time-lapse live cell imaging was performed at 5 min intervals for 1 h with the oil immersion objective Plan-Apochromat 63x/1.4 oil DIC M27, and 0.125 µm z-slices, SR mode, with a LSM880 confocal microscope with Airyscan fast detection system (Zeiss) using ZEN black software edition 2.3 (Zeiss).
5) The acquisition of images of senescent cells was performed using the Leica DMIL microscope (Leica) with the Leica MC170HD camera and Las EZ software v3.4.
6) Metaphase chromosome spreads were imaged at 100X with a Leica DM4000B (Leica) fluorescent microscope.
7) Western blot membranes were developed with the ChemiDoc™ imaging system (Bio-Rad).

8) TCGA RNA-seq data were obtained from the Genomic Data Commons Data Portal (https://portal.gdc.cancer.gov) and corresponded to fragments per kilobase of transcript per million mapped reads upper quartile (FPKM-UQ).
9) Pediatric tumor data from the TARGET project (cancer types: Wilms tumor (WT), rhabdoid tumor (RT), and neuroblastoma (NBL)) and Metabric data were obtained preprocessed and normalized from the cBioPortal (http://www.cbioportal.org/public-portal/).
10) The data for chronic lymphocytic leukemia (CLLE) and malignant lymphoma (MALY) were obtained preprocessed and normalized from the ICGC Data Portal (https://dcc.icgc.org/).
11) The HR and NHEJ pathway gene signatures were obtained from a curation study (PMID: 29617664) and overlapping genes between signatures were excluded from the analysis (HR = 43 and NHEJ = 11 genes).
12) Representative whole slide images of breast cancer specimens were obtained as follows: the cBioPortal (http://www.cbioportal.org) was used to search the TCGA PanCancer Atlas for breast tumours containing mutations in BRCA1, BRCA2, or PIK3CA.
13) For the HR and NHEJ reporter assays, cells were run on the BD LSRfortessa cell analyzer and analyzed using FlowJo v10 software.
14) For the Comet Assay, images were acquired with the Nikon Nikon Eclipse Ti2 C2+ confocal microscope coupled to NIS-Elements AR software (Nikon v4.10.00 and v4.50.00) at 20x (NA 0.75).

Data analysis

1) Advanced image analysis was performed in BitPlane Imaris 9.7 or 10.0 and ImageJ/FIJI software v2.14.0. Airyscan data files were deconvoluted and processed in ZEN black version 2.3 (Zeiss) software. Maximum intensity projections were optimized using BitPlane Imaris 9.7 or 10.0 Image Proc function. Pixel intensity display settings were automatically or manually optimized using a thresholding method based on image quality and local contrast using BitPlane Imaris 9.7 or 10.0 baseline subtraction function followed by a Gaussian smoothing filter at a value of one.
2) To quantify the degree of tubular invagination of LaminB1 in each nucleus, DAPI-stained DNA and LMNB1 signal were reconstructed in 3D visualizing the invaginated signal and the boundary signal separately using the Surfaces MatLAB Xtension and masking function where every zplane in a single nucleus (30-85 z-slices) was contoured manually to ensure optimal separation of boundary and internal LMNB1 signal to detect LMNB1 nuclear tubules. For each 3D reconstructed surface, Surface Area was quantified using the BitPlane Imaris 9.7 detailed Statistics function. Alternatively, z-stacks of LMNB1-stained cells were segmented using the FIJI plugin LabKit and imported into BitPlane Imaris 10.0. The Labkit-defined LMNB1 surface area and DAPI surface area were quantified using the BipPlane Imaris 10.0 detailed statistics function. The tubular score or invagination ratio was quantified as total LMNB1 surface area divided by total DNA (DAPI) surface area.
3) The counting of 53BP1 foci was performed using the MatLAB Xtension of Bitplane Imaris 9.7 or Difference of Gaussians (DoG) and Analyze Particles functions on ImageJ/FIJI (v2.14.0).
4) To determine the association of 53BP1 foci with LMNB1 tubules, the foci were three-dimensionally reconstructed using the Spots MatLAB Xtension in BitPlane Imaris 9.7 and 10 with Different Spot Sizes option on to ensure capturing the actual size of each focus, followed by scoring the number of foci close to or far from the tubules using the Find Spots Close to Surface MatLAB Xtension. Threshold was set at smaller than the average diameter of voxels at 0.3µm which was determined based on the average diameter of the 53BP1 foci (0.70 ± 0.21µm,) and the average diameter of U2OS cells (13.0 ± 2.8 µm) that were selected randomly.
5) Cells were considered tubules-positive when two or more tubules extending deeper than the radius of the nucleus were detected using BitPlane Imaris 9.7 Section function. Alternatively, z-stacks of stained cells were analyzed using the Orthogonal Views function on ImageJ/FIJI (v2.14.0).
6) Using BitPlane Imaris 9.7 Section, cells were considered positive for nucleus-reshaping microtubules if the latter were detected at the mid-plane of the three-dimensional z-stack to ensure that only nuclear microtubules were selected.
7) The distance of single DSB foci to the nuclear edge, or incoming nuclear envelope tubules (marked via LMNB1) was measured in ImageJ/FIJI (v2.14.0) using the Straight Selection Tool by assessing the shortest distance from the DSB to the nuclear edge in 3D.
8) Nuclear envelope tubule width measurements were performed by analyzing 3D-confocal images of selected cell lines stained for LMNB1 in ImageJ/FIJI (v2.14.0) by detecting nuclear tubes in the XZ plane and measuring tubule diameters using the Straight Selection Tool.
9) The normalized gene signature scores of bioinformatic datasets were based on single sample Gene Set Expression Analysis (ssGSEA) computed using the Gene Set Variation Analysis (GSVA) software package.
10) 53BP1 foci clustering was analyzed via foci area size assessment measured in ImageJ/FIJI (v2.14.0)
11) Multivariate regression models including age at diagnosis and tumor stage as covariates, and log-rank tests computed in R software were used to assess gene expression associations with overall survival.
12) The whole slide images of human tumor sections were from TCGA (https://www.cancer.gov/tcga).
13) Statistical analysis was performed in GraphPad Prism Software v9 using Student's t-test, Mann-Whitney test, or one-way or two-way ANOVA with Tukey's, Dunnett's or Sidak's multiple comparisons test best suited for the specific experimental design and type of datasets analyzed (unless otherwise indicated).

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 Portfolio 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 description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

Data are in the Article, the Source Data files related to Figs. 1-6 and extended data Figs. 1-10, and Videos 1-5. All data and materials are available upon reasonable request. TCGA RNA-seq data were from the Genomic Data Commons Data Portal (https://portal.gdc.cancer.gov). Pediatric tumor data from the TARGET project (cancer types: Wilms tumor (WT), rhabdoid tumor (RT), and neuroblastoma (NBL)) and Metabric data were obtained preprocessed and normalized from the cBioPortal at http://www.cbioportal.org/public-portal/). The data for chronic lymphocytic leukemia (CLLE) and malignant lymphoma (MALY) were obtained preprocessed and normalized from the ICGC Data Portal (https://dcc.icgc.org/). Representative whole slide images of breast cancer specimens were obtained using the cBioPortal (http://www.cbioportal.org) to access the TCGA PanCancer Atlas and retrieve breast tumours with a mutation in the studied genes.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	N/A
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

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

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 methods were used to predetermine sample size. Experiments were conducted with cell lines with at least three biological replicates as specified in the manuscript, based on previous experience with specific experimental setups, and conforming to field standards. For growth assays in vitro and in vivo, sample size was based on previous experience with these assays (PMIDs: 33529165 and 37697435). For single cell microscopy experiments, cell counts used per experiment reflecting or excessing numbers routinely used in stringent quantitative cell biological experiments (PMID: 33707212). We consistently aimed to have as many cells imaged and analyzed as technically feasible, and our cell counts (as per the numerical source data files) across all experiments are most often significantly above common field standards for such assays, including for the 3D single-cell reconstruction where we quantified hundreds of cells as opposed to the typically much lower cell counts in similar time-consuming and tedious imaging reconstruction assays.
Data exclusions	Exclusion criteria were pre-determined based on internal controls and quality control indicators. For example, any experiment requiring transfection was assessed for successful transfection before inclusion in data analysis.
Replication	Observations were tested for their replication and generalizability by assessing 1) multiple cell lines to rule out cell type-specific effects, 2) where applicable multiple clones or chemical inhibitors to rule out clone-specific and reagent-specific effects, 3) three independent and successful biological replicates, and 4) suitable internal controls to establish experimental success and the validity and replication of findings between biological and technical replicates as indicated in the manuscript, and all control-confirmed experiments were successful.
Randomization	Randomization was not part of the experimental design. Since randomization was not part of the study, covariates were controlled by standardized cell culture conditions and techniques for all independent replicates and by the use of standardized protocols for all experiments. Data was performed on large sample sizes to rule out random error.
Blinding	Blinding was used for the quantification of images from microscopy and in vivo experiments. Due to time-limited and restricted access to common resources and to flexible work scheduling policies, blinding was not performed for in vitro experiments but this was addressed by multiple independent replicates by different experimenters at different times and through the careful interpretations of the results.

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
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

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

Antibodies

Antibodies used

1) KU70 rabbit polyclonal
Concentration: 1 mg/mL
Supplier: Abcam
Cat# ab83501
Application: IF, WB
Dilution/usage: 1:500
Lot# GR3422636-1,GR3220069-c

2) RAD51 rabbit monoclonal
Concentration: 1 mg/mL
Supplier: Abcam
Cat# ab133534
Application: IF
Dilution/usage: 1:500
Lot# EPR4030(3)

3) BRCA1 mouse monoclonal
Concentration: 2 mg/mL
Supplier: Millipore
Cat# OP92
Application: IF, WB
Dilution/usage: 1:500
Lot# 3698204

4) gamma-H2AX mouse polyclonal
Concentration: 1 mg/mL
Supplier: Abcam
Cat# ab11174
Application: IF, WB
Dilution/usage: 1:500
Lot# GR3382201-1

5) gamma-H2Ax rabbit monoclonal
Concentration: 1 mg/mL
Supplier: Abcam
Cat# 22551
Application: WB
Dilution/usage: 1:1000
Lot# GR3410391-2

6) SUN1 rabbit polyclonal
Concentration: 1 mg/mL
Supplier: Proteintech
Cat# 24568-I-AP
Application: IF, WB, IP
Dilution/usage: 1:500
Lot# 00020848

7) SUN2 mouse monoclonal
Concentration: 1 mg/mL
Supplier: Millipore
Cat# MABT880
Application: IF, WB
Dilution/usage: 1:500
Lot# 379213

8) SYNE1 rabbit polyclonal
Concentration: 0.6 mg/mL

Supplier: Sigma Prestige
Cat# HPA019113-25uL
Application: IF
Dilution/usage: 1:500
Lot# 000009216

9) NUP98 mouse monoclonal
Concentration: 1 mg/mL
Supplier: Invitrogen
Cat# MA5-14907
Application: IF, WB
Dilution/usage: 1:500
Lot# WF3318612A

10) NUP153 rabbit polyclonal
Concentration: 0.2 mg/mL
Supplier: Bethyl Lab
Cat# A301-788A
Application: IF, WB
Dilution/usage: 1:500
Lot# not provided

11) 53BP1 rabbit polyclonal
Concentration: 1 mg/mL
Supplier: Abcam
Cat# ab36823
Application: IF, WB
Dilution/usage: 1:500
Lot# GR279697-22, 1024518-1

12) 53BP1 rabbit polyclonal
Concentration: 1 mg/mL
Supplier: Bethyl Lab
Cat# A300-272A
Application: IF, WB
Dilution/usage: 1:500
Lot# 7

13) 53BP1, clone BP13, mouse monoclonal
Concentration: 1 mg/mL
Supplier: Millipore
Cat# MAB3802
Application: IF
Dilution/usage: 1:500
Lot# 3256018

14) LMNB1 rabbit polyclonal
Concentration: 1 mg/mL
Supplier: Abcam
Cat# ab16048
Application: IF, WB, IP
Dilution/usage: 1:500
Lot# GR3459550-1, GR3383070-1, GR3398320-1

15) LMNB1 mouse monoclonal
Concentration: 1 mg/mL
Supplier: Proteintech
Cat# 66095-1-Ig
Application: IF
Dilution/usage: 1:500
Lot# 10020247

16) LMNA/C mouse monoclonal
Concentration: 7ug/mL
Supplier: Cell Signaling
Cat# 4777
Application: IF
Dilution/usage: 1:500
Lot# 5

17) KIF5B rabbit monoclonal
Concentration: 1 mg/mL
Supplier: Abcam
Cat# ab167429
Application: IF, WB
Dilution/usage: 1:500

Lot# GR3276019-3

18) KIFC3 rabbit polyclonal
Concentration: 0.1 mg/mL
Supplier: Thermo Fisher
Cat# PA5-54359
Application: IF, WB
Dilution/usage: 1:500
Lot# XH3670797

19) β-Actin mouse monoclonal
Concentration: not determined
Supplier: Thermo Fisher
Cat# MA1-744
Application: IF, WB
Dilution/usage: 1:1000
Lot# XG348198

20) RNF8 mouse monoclonal
Concentration: N/A
Supplier: Santa Cruz
Cat# sc-271462
Application: IF, WB
Dilution/usage: 1:500
Lot# H3121

21 RAD50 rabbit polyclonal
Concentration: not provided
Supplier: Novus
Cat# NB100-1487
Application: IF, WB
Dilution/usage: 1:500
Lot# NB100

22) NBS1 rabbit polyclonal
Concentration: 2 mg/mL
Supplier: Bethyl Lab
Cat# A301-290A
Application: IF, WB
Dilution/usage: 1:500
Lot# 1

23) Phosphoserine rabbit polyclonal
Concentration: not provided
Supplier: Millipore
Cat# AB1603
Application: WB
Dilution/usage: 1:500
Lot# 2108486

24) Vinculin mouse monoclonal
Concentration: not provided
Supplier: Millipore
Cat# V9131-100uL
Application: WB
Dilution/usage: 1:1000
Lot# 079M4754V

25) alpha-Tubulin mouse monoclonal
Concentration: 1 mg/mL
Supplier: Abcam
Cat #: ab7291
Application: IF, WB
Dilution/usage: 1:500
Lot # GR3341361-10

26) acetyl-alpha-Tubulin rabbit monoclonal
Concentration: 317 ug/mL
Supplier: Cell Signaling Technology
Cat# 5335S
Application: IF, WB
Dilution/usage: 1:500
Lot # 6

27) DNA-PKcs phospho S2056 rabbit monoclonal
Concentration: 0.448 mg/mL

Supplier: Abcam
Cat# ab124918
Application: WB
Dilution/usage: 1:500
Lot # 1008802-1

28) phospho-CHK1 (Ser345) rabbit polyclonal
Concentration: 141 ug/mL
Cat# 2341S
Supplier: Cell Signaling Technology
Application: WB
Dilution/usage: 1:500
Lot # 6

29) Phospho-CHK2 (T68) rabbit polyclonal
Concentration: 0.2 mg/mL
Cat# AF1626
Supplier: R&D Systems
Application: WB
Dilution/usage: 1:500
Lot # JBC0522051

30) XRCC4 mouse monoclonal
Concentration: 0.2 mg/mL
Supplier: Santa Cruz
Cat# sc-271087
Application: WB, ChIP
Dilution/usage: 1:100 (WB), 5 ug/IP (ChIP)
Lot# GR3341361-10

31) Alexa-488 anti-mouse polyclonal
Concentration: 2 mg/mL
Supplier: Invitrogen
Cat# A11001
Application: IF
Dilution/usage: 1:1000
Lot# 2318440

32) Alexa-488 anti-rabbit polyclonal
Concentration: 2 mg/mL
Supplier: Invitrogen
Cat# A11008
Application: IF
Dilution/usage: 1:1000
Lot# 514957

33) Alexa-568 anti-mouse polyclonal
Concentration: 2 mg/mL
Supplier: Invitrogen
Cat# A11004
Application: IF
Dilution/usage: 1:1000
Lot# 2447869

34) Alexa-568 anti-rabbit polyclonal
Concentration: 2 mg/mL
Supplier: Invitrogen
Cat# A11011
Application: IF
Dilution/usage: 1:1000
Lot# 2088068

35) Alexa-647 anti-mouse polyclonal
Concentration: 2 mg/mL
Supplier: Invitrogen
Cat# A21235
Application: IF
Dilution/usage: 1:1000
Lot# 1987304

36) Rabbit IgG polyclonal
Concentration: 1 mg/mL
Supplier: Abcam
Cat# ab171870
Application: IP
Dilution/usage: 5 ug per sample

Lot# GR3409731-1, GR3353004-3

37) HRP-Mouse IgG
Concentration: not provided
Supplier: Amersham
Cat# NA931-1ML
Application: WB
Dilution/usage: 1:4000
Lot# 17479274, 17573047

38) HRP-Rabbit IgG
Concentration: not provided
Supplier: Amersham
Cat# NA934-1ML
Application: WB
Dilution/usage: 1:4000
Lot# 17457635, 17599760

39) Ubiquitin mouse monoclonal
Supplier: Santa Cruz
Concentration: 200 ug/ml
Cat# sc-8017
Application: WB
Dilution/usage: 1:1000
Lot# not available

40) Phalloidin-iFluor reagent 488
Concentration: N/A
Supplier: Abcam
Cat# 76753
Application: IF
Dilution/usage: 1:1000
Lot# Not provided

Validation

Commercially available antibodies were validated for specificity by the manufacturer using knockdown or knockout of conjugate transcript/gene. Validation statements for the antibodies used in this study can be found at the following links:
Ku70: https://www.abcam.com/products/primary-antibodies/ku70-antibody-ab83501.html
Rad51: https://www.abcam.com/products/primary-antibodies/rad51-antibody-epr40303-ab133534.html
BRCA1: https://www.sigmaaldrich.com/CA/en/product/mm/op92
yH2Ax: https://www.abcam.com/products/primaryantibodies/gamma-h2ax-phospho-s139-antibody-ab11174.html; https://www.abcam.com/products/primary-antibodies/gamma-h2ax-phospho-s139-antibody-3f2-ab22551.html
SUN1: https://www.ptglab.com/products/UNC84A-Antibody-24568-1-AP.htm
SUN2: https://www.sigmaaldrich.com/CA/en/product/mm/mabt880
SYNE1: https://www.sigmaaldrich.com/CA/en/product/sigma/hpa019113
NUP98: https://www.thermofisher.com/antibody/product/NUP98-Antibody-clone-J-750-3-Monoclonal/MA5-14907
NUP153: https://www.fortislife.com/products/primary-antibodies/rabbit-anti-nup153-antibody/BETHYLA301-788
53BP1: https://www.abcam.com/products/primary-antibodies/53bp1-antibody-ab36823.html; https://www.fortislife.com/products/primary-antibodies/rabbit-anti-53bp1-antibody/BETHYL-A300-272; https://www.sigmaaldrich.com/CA/en/product/mm/mab3802
LMNB1: https://www.abcam.com/products/primary-antibodies/lamin-b1-antibody-nuclear-envelope-marker-ab16048.html; https://www.ptglab.com/products/LMNB1-Antibody-66095-1-Ig.htm
LMNA/C: https://www.cellsignal.com/products/primary-antibodies/lamin-a-c-4c11-mouse-mab/4777
KIF5B: https://www.abcam.com/products/primary-antibodies/kif5bkif5c-antibodyepr10276b-ab167429.html
KIFC3: https://www.thermofisher.com/antibody/product/KIFC3-Antibody-Polyclonal/PA5-54359
Actin: https://www.thermofisher.com/antibody/product/Actin-Antibody-clone-mAbGEa-Monoclonal/MA1-744
RNF8: https://www.scbt.com/p/rnf8-antibody-b-2
Rad50: https://www.novusbio.com/products/rad50-antibody_nb100-1487
Nbs1: https://www.fortislife.com/products/primary-antibodies/rabbit-anti-nbs1-antibody/BETHYL-A301-290
Phosphoserine: https://www.emdmillipore.com/CA/en/product/Anti-Phosphoserine-Antibody,MM_NF-AB1603
Vinculin: https://www.sigmaaldrich.com/CA/en/product/sigma/v9131
Alpha-tubulin: https://www.abcam.com/products/primary-antibodies/alpha-tubulin-antibody-dm1a-loading-control-ab7291.html
Acetylated tubulin: https://www.cellsignal.com/products/primary-antibodies/acetyl-a-tubulin-lys40-d20g3-xp-174-rabbitmab/5335
DNAPK p2056: https://www.abcam.com/products/primary-antibodies/dna-pkcs-phospho-s2056-antibody-epr5670-ab124918.html
Phospho-Chk1: https://www.cellsignal.com/products/primary-antibodies/phospho-chk1-ser345-antibody/2341
Phospho-Chk2: https://www.rndsystems.com/products/human-phospho-chk2-t68-antibody_af1626
XRCC4: https://www.scbt.com/p/xrcc4-antibody-c-4; Ubiquitin: https://www.scbt.com/p/ubiquitin-antibody-p4d1

We further validated the specificity of antibodies via small RNA-knockdown of KU70, NBS1, RAD50, RNF8, SUN1, SUN2, NUP153, NUP98, KIF5B, KIFC3, BRCA1; or via ATAT1 knockdown for Ac-alphaTub; or via pharmacological inhibition of ATR for phospho-CHK1, inhibition of DNAPKcs for phospho-pDNAPKcs, or inhibition of ATM, DSB induction, or enoxacin treatment for phospho-CHK2.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	U2OS, IMR-90, HEK293T, HeLa, MDA-MB-231, MDA-MB-436, and MCF10A cells were purchased from ATCC. The U2OS-derived 2-6-5 cell line was obtained from Roger A. Greenberg (U. of Pennsylvania). The EJ5 and DR-GFP U2OS cell lines were obtained from Jeremy Stark (City of Hope).
Authentication	Purchased cell lines were commercially authenticated by ATTC. Cells obtained from Roger A. Greenberg and Jeremy Stark were previously authenticated (PMID: 20550933, PMID: 25629353). For all cell lines, cultures were not maintained for more than 6 months.
Mycoplasma contamination	The cell lines used tested negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals	For xenografts, 10 female 12-14-week-old NOD-Scid (NSG, JAX strain # 005557) mice were injected with equal parts MDA-MB-436 human breast cancer cells (1.5X10^6 cells) and Matrigel into inguinal mammary fat pads. Mice were monitored for mammary tumor onset by palpation and tumours were measured with calipers starting at 13 days after cell injection. Tumors were then measured every 2-3 days up to 55 days. Mice were given acidified water supplemented with 1 mg/mL doxycycline (Sigma, cat# D9891) and 5% sucrose (BioShop, cat# SUC507).
Wild animals	Wild animals were not part of the experimental design.
Reporting on sex	Experimental findings apply to female animals. Female animals were included in the experimental design considering the nature of the studied tumor model (breast cancer is significantly more frequently occurring in female individuals).
Field-collected samples	The study does not include field-collected samples.
Ethics oversight	All mice in this study were housed in a pathogen-free animal facility at the Princess Margaret Cancer Centre (PMCC) and all procedures were performed in compliance the PMCC Animal Care Committee guidelines and the University of Toronto Animal Care Committee. Mice were housed on ventilated racks supplied with autoclaved micro isolator cages. The housing facility was maintained at a temperature between 22 and 23°C and 40% and 60% humidity with a 12-hour dark and 12-hour light cycle.

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