

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 (<i>n</i>) 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 <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☒	☐ 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
☒	☐ 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 <i>d</i> , Pearson's <i>r</i>), 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	All DNA fiber data, immunofluorescence, and SIRC assay data were collected using an inverted spinning disk confocal (Andor Dragonfly) using Fusion (2.3.0.54). All flow cytometry data were collected using the ThermoFisher Attune NxT instrument or using the BD Accuri C6 Plus. Cells for live cell imaging were sorted on the BD FACSaria II instrument. Some immunoblots were imaged using the Li-Cor Odyssey Imager. Live cell imaging was acquired using a Keyence BZ-X810 microscope with BZ-X800 Analyzer software. Luciferase assay data were collected using a DLReady TD-20/20 Luminometer. Proteomics data were collected using an Easy nLC 1200 coupled to a QExactive HF (Thermo Scientific)
Data analysis	CRISPR screen data were analyzed using Völundr v2.1.0 and the DrugZ pipeline (PMID: 31439014) ImageJ (FIJI 2.14.0) was used for colony formation assay, live cell imaging, immunoblot, DNA fiber, and SIRC assay analyses. FlowJo v10.8 and the BD Accuri C6 workspace v1.0.34.1 was used to analyze flow cytometry data. MaxQuant software suite (version 1.6.12.0) and Perseus software (version 1.6.14.0) was used to analyze proteomics data. R (version 4.1.0) and R package ComplexHeatmap (https://doi.org/10.1093/bioinformatics/btw313) was used to analyze gene expression data from TCGA. GraphPad Prism v9.4.1 and v10.1.1 was used for statistical 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 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 Availability

Requests for additional information and resources may be directed to Cyrus Vaziri (cyrus_vaziri@med.unc.edu). Reagents generated in this study are available upon request. The mass spectrometry proteomics data generated in this study have been deposited in the ProteomeXchange Consortium via the PRIDE partner repository under accession code PXD056698 [<https://www.ebi.ac.uk/pride/archive/projects/PXD056698>]. The CRISPR-Cas9 genetic screen data have been deposited at Zenodo under accession code 13930006 [<https://zenodo.org/records/13930006>]. The DepMap gene effect scores were downloaded from Figshare using accession code 27993248 [<https://doi.org/10.25452/figshare.plus.27993248.v1>]. All other data analyzed in this paper are available in the published article, Supplementary Information, or Source Data. Source Data are provided with this paper.

Code Availability

This paper does not report original code.

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](https://www.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 sizes. Sample sizes were chosen based on standard practices of the field and using published papers with similar methods (see recent Nature Communications papers PMID: 31337767, PMID: 37591859, PMID: 34772932, PMID: 37805499, PMID: 36075897, PMID: 39043663, PMID: 34645815, PMID: 36085347). All sample sizes and number of replicates are indicated in figures and/or figure legends or methods. Where applicable, all individual data points are reported.

Data exclusions

Only EdU positive cells were scored in SIRC assays for PLA foci formation. No other data were excluded.

Replication

All replication details are noted in the figure legends or methods for each experiment, with the number of technical replicates and the number of biological replicates indicated. Experiments were successfully replicated at least twice.

Randomization

For DNA fiber assays, immunofluorescence, live cell imaging, and SIRC assays the windows of imaging were randomly selected. For live cell imaging, cells that were tracked were randomly selected. During cell culture, cells were allocated randomly to plates to test different conditions.

Blinding

Blind analysis of the data was not performed and is not a standard practice in our field (see recent Nature Communications papers PMID: 31337767, PMID: 37591859, PMID: 34772932, PMID: 38521768, PMID: 38789420, PMID: 35365626, PMID: 37612308).

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

Primary antibodies used: TRIM11 (ab111694), pIRF3 S386 (ab76493), MUS81 (ab14387), and MRE11 (ab214) from Abcam; RNF25 (A303-844A), pRPA S33 (A300-246A), pRPA S4/S8 (A300-245A), HLTf (A300-230A), Pol η (A301-231A), Pol κ (A301-975A), Pol ι (A301-304A), RAD18 (A301-340A), and HA (A190-138A) from Bethyl Laboratories; Vinculin (V4505), SHLD3 (HPA068764), and γH2AX (05-636) from Sigma Millipore; RPA 34 (NA19L) from Calbiochem; pATM S1981 (sc-47739), β-actin (sc-47778), GAPDH (sc-32233), TNKS1/2 (sc-365897), PARP1 (sc-8007), UBE2D2 (sc-100617), REV1 (sc-393022), FANCD2 (sc-28194), Histone H2B (sc-10808), WEE1 (sc-9037), CDK2 pT14/Y15 (sc-28435-R), CDK2 (sc-6248), and PCNA (sc-56) from Santa Cruz Biotech; CHK1 (CST 2360), CHK1 pS317 (CST 2344), CDC2 pTyr15 (CST 9111), PIAS1 (CST 3550), Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (CST 9106), pSTING Ser366 (CST 19781), STING (CST 13647), pSTAT1 Tyr701 (CST 9167) and STAT1 (CST 9175) from Cell Signaling Technology; REV7 (12683-1-AP), EXO1 (16253-1-AP) from Proteintech; CtIP (61141) from Active Motif; rat anti-BrdU Abcam ab6326; mouse anti-BrdU, BD Biosciences 347580.

Secondary antibodies used: anti-rabbit (Bethyl A120-101P), anti-mouse (Bethyl A90-116P), anti-goat (Santa Cruz Biotech sc-2020). Invitrogen A32816, A21434, A11029.

Most primary antibodies for western blot were used at a dilution of 1:1000, except HA, Vinculin, γH2AX, β-actin, GAPDH, PCNA were used at 1:3000, and UBE2D2 and REV1 used at 1:500. Secondary antibodies for western blot were used at 1:5000 dilution.

Primary antibodies were used for IF at 1:100, and secondary antibodies were used for IF at 1:300.

Primary antibodies were used for DNA fiber assays at 1:500 (Abcam ab6326) and 1:600 (BD Biosciences 347580.). Secondary antibodies were used for DNA fiber assays at 1:500 (Invitrogen A21434) and 1:600 (Invitrogen A11029).

Validation

In our study, all protein bands are observed at the correct size as indicated by the manufacturer. Additional validation by the manufacturer and additional citations for these antibodies can be found on the manufacturer's website.

TRIM11: validated in this study with TRIM11 knockout cells, <https://www.abcam.com/en-us/products/primary-antibodies/trim11-antibody-ab111694>

pIRF3: <https://www.abcam.com/en-us/products/primary-antibodies/irf3-phospho-s386-antibody-epr2346-ab76493>

MUS81: validated in this study with siRNA targeting MUS81, <https://www.abcam.com/en-us/products/primary-antibodies/mus81-antibody-mta30-2g10-3-ab14387>

MRE11: validated in this study with siRNA targeting MRE11, <https://www.abcam.com/en-us/products/primary-antibodies/mre11-antibody-12d7-bsa-and-azide-free-ab214>

RNF25: validated in this study with RNF25 knockout cells and siRNA targeting RNF25, <https://www.fortislife.com/products/primary-antibodies/rabbit-anti-rnf25-antibody/BETHYL-A303-844>

pRPA S33: <https://www.fortislife.com/products/primary-antibodies/rabbit-anti-phospho-rpa32-s33-antibody/BETHYL-A300-246>

pRPA S4/S8: <https://www.fortislife.com/products/primary-antibodies/rabbit-anti-phospho-rpa32-s4-s8-antibody/BETHYL-A300-245>

HLTF: validated in this study with siRNA targeting HLTf, <https://www.fortislife.com/products/primary-antibodies/rabbit-anti-smarca3-antibody/BETHYL-A300-230>

Pol η: <https://www.fortislife.com/products/primary-antibodies/rabbit-anti-polh-antibody/BETHYL-A301-231>

Pol K: <https://www.fortislife.com/products/primary-antibodies/rabbit-anti-pol-kappa-antibody/BETHYL-A301-975>

Pol ι: <https://www.fortislife.com/products/primary-antibodies/rabbit-anti-pol-iota-antibody/BETHYL-A301-304>

RAD18: <https://www.fortislife.com/products/primary-antibodies/rabbit-anti-rad18-antibody/BETHYL-A301-340>

HA: validated in this study with expression of HA-tagged proteins, <https://www.fortislife.com/products/primary-antibodies/goat-anti-ha-tag-antibody/BETHYL-A190-138>

Vinculin: <https://www.sigmaaldrich.com/US/en/product/sigma/v4505>

SHLD3: validated in this study with siRNA targeting SHLD3, <https://www.sigmaaldrich.com/US/en/product/sigma/hpa068764>

γH2AX: <https://www.sigmaaldrich.com/US/en/product/mm/05636>

RPA 34: <https://www.citeab.com/antibodies/227763-na19l-anti-replication-protein-a-ab-3-mouse-mab-rp>

pATM S1981: <https://www.scbt.com/p/p-atm-antibody-10h11-e12>

β-actin: <https://www.scbt.com/p/beta-actin-antibody-c4>

GAPDH: <https://www.scbt.com/p/gapdh-antibody-6c5>

TNKS1/2: <https://www.scbt.com/p/tankyrase-1-2-antibody-e-10>

PARP1: validated in this study using siRNA targeting PARP1, <https://www.scbt.com/p/parp-1-antibody-f-2>
 UBE2D2: <https://www.scbt.com/p/ube2d2-antibody-y-20>
 REV1: validated in this study with siRNA targeting REV1, <https://www.scbt.com/p/rev1-antibody-a-11>
 FANCD2: <https://www.scbt.com/p/fancd2-antibody-h-300>
 Histone H2B: <https://datasheets.scbt.com/sc-10808.pdf>
 WEE1: <https://www.scbt.com/p/wee-1-antibody-h-300>
 CDK2 pT14/Y15: <https://datasheets.scbt.com/sc-28435.pdf>
 CDK2 (sc-6248): <https://www.scbt.com/p/cdk2-antibody-d-12>
 PCNA: <https://www.scbt.com/p/pcna-antibody-pc10>
 CHK1: <https://www.cellsignal.com/products/primary-antibodies/chk1-2g1d5-mouse-mab/2360>
 CHK1 pS317: <https://www.cellsignal.com/products/primary-antibodies/phospho-chk1-ser317-antibody/2344>
 CDC2 pTyr15: <https://www.cellsignal.com/products/primary-antibodies/phospho-cdc2-tyr15-antibody/9111>
 PIAS1: validated in this study with PIAS1 knockout cells, <https://www.cellsignal.com/products/primary-antibodies/pias1-d33a7-xp-rabbit-mab/3550>
 Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204): <https://www.cellsignal.com/products/primary-antibodies/phospho-p44-42-mapk-erk1-2-thr202-tyr204-e10-mouse-mab/9106>
 pSTING Ser366: <https://www.cellsignal.com/products/primary-antibodies/phospho-sting-ser366-d7c3s-rabbit-mab/19781>
 STING: <https://www.cellsignal.com/products/primary-antibodies/sting-d2p2f-rabbit-mab/13647>
 pSTAT1 Tyr701: <https://www.cellsignal.com/products/primary-antibodies/phospho-stat1-tyr701-58d6-rabbit-mab/9167>
 STAT1: validated in this study with siRNA targeting STAT1, <https://www.cellsignal.com/products/primary-antibodies/stat1-42h3-rabbit-mab/9175>
 REV7: validated in this study using siRNA targeting REV7, <https://www.ptglab.com/products/MAD2L2-Antibody-12683-1-AP.htm>
 EXO1: validated in this study with siRNA targeting EXO1, <https://www.ptglab.com/products/EXO1-Antibody-16253-1-AP.htm>
 CtIP: validated in this study with siRNA targeting CtIP, <https://www.activemotif.com/catalog/details/61141>
 BrdU (rat): <https://www.abcam.com/en-us/products/primary-antibodies/brdu-antibody-bu1-75-icr1-proliferation-marker-ab6326>
 BrdU (mouse): <https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/clinical-discovery-research/single-color-antibodies-ruo-gmp/purified-mouse-anti-brdu.347580>
 anti-rabbit (Bethyl A120-101P): <https://www.fortislife.com/products/secondary-antibodies/goat-anti-rabbit-igg-heavy-and-light-chain-antibody/BETHYL-A120-101?selected=A120-101D5>
 anti-mouse (Bethyl A90-116P): <https://www.fortislife.com/products/secondary-antibodies/goat-anti-mouse-igg-heavy-and-light-chain-antibody/BETHYL-A90-116?selected=A90-116P>
 anti-goat (Santa Cruz Biotech sc-2020): <https://www.scbt.com/p/donkey-anti-goat-igg-hrp>
 Invitrogen A32816: <https://www.thermofisher.com/antibody/product/Donkey-anti-Goat-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A32816>
 Invitrogen A21434: <https://www.thermofisher.com/antibody/product/Goat-anti-Rat-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21434>
 Invitrogen A11029: <https://www.thermofisher.com/antibody/product/Goat-anti-Mouse-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11029>

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)	Pa02C and Pa03C cells were from Dr. Channing Der (UNC Chapel Hill). H1299 (CRL-5803) and hTERT-HPNE (CRL-4023) cells were purchased from the American Type Culture Collection (ATCC).
Authentication	Cell lines were verified by immunoblot and/or genomic sequencing for the desired protein/gene expression or deletion.
Mycoplasma contamination	All cell lines used were tested for mycoplasma and confirmed to be negative.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in this study.

Plants

Seed stocks	<i>Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.</i>
Novel plant genotypes	<i>Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.</i>
Authentication	<i>Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosaicism, off-target gene editing) were examined.</i>

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 cell cycle distribution assay: Pa03C sgRNF25 and sgPIAS1 polyclonal cell lines, along with Pa03C sgNT control cells, were treated with DMSO or WEE1i for 24 h. Cells were collected by trypsinization, fixed in 35% ethanol overnight at 4°C. The lysates were then washed twice with DPBS and stained with 1mg/ml propidium iodide (Invitrogen #P3566) with 8ug/ml RNaseA (Sigma Aldrich R4642) to stain DNA. Unstained samples were used to distinguish cell populations.

For ssDNA assay: sub-confluent cells were cultured for 24-36 h in medium containing 10 µM BrdU to label genomic DNA, in duplicate. Following BrdU labeling, media was changed to fresh BrdU-free medium and cells were incubated overnight for 12 h. WEE1i was diluted in fresh media and incubated with cells for another 24 h. Then cells were harvested by trypsinization, resuspended in 65% PBS with 35% ethanol, and fixed overnight at 4°C. Fixed cells were washed with PBS and incubated with 4:10 FITC conjugated anti-BrdU antibody (BD Biosciences 556028) for 1 h at RT. The samples were then incubated 1 hr-overnight in PBS containing 10 µg/ml of propidium iodide (Invitrogen P3566) and 8 µg/ml of RNaseA (Sigma Aldrich R4642) to stain total DNA. For FACS controls, unstained sample and FITC-BrdU sample with denaturation by HCl (followed by neutralization with Borax and PBS wash) were prepared

For cell sorting: Pa03C sgRNF25 and sgPIAS1 polyclonal cell lines and Pa03C sgNT control cells stably expressing H2B-GFP (Addgene #69550) were generated with polybrene assisted transduction and infected cells were selected by flow sorting with the BD FACSAria II.

Instrument

Thermo Fisher Attune NxT, BD FACSAria II, BD Accuri C6 Plus

Software

ThermoFisher Attune NxT software, BD FACSAria II software, and FlowJo (v10.8), BD Accuri C6 workspace (v1.0.34.1)

Cell population abundance

All analyzed samples that were fixed and stained were from pure populations and processed at the same time.

Gating strategy

Cells were first gated using FSC/SSC to exclude debris. Then cells were gated on PI-area/PI-height to identify singlets. Unstained cells and single stain cells were used as controls to define gating boundaries.

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