Sister chromatid exchanges induced by perturbed replication can form independently of BRCA1, BRCA2 and RAD51

Anne Margriet Heijink1,11,13, Colin Stok1,12,13, David Porubsky2,3, Eleni Maria Manolika4, Jurrian K. de Kanter5,6, Yannick P. Kok1, Marieke Everts1, H. Rudolf de Boer1, Anastasia Audrey, Femke J. Bakker5, Elles Wierenga1, Marcel Tijsman7, Victor Guryev2, Diana C. J. Spierings2, Puck Knipscheer2,8, Ruben van Boxtel5,6, Arnab Ray Chaudhuri2, Peter M. Lansdorp2,9,10 & Marcel A. T. M. van Vugt1

Sister chromatid exchanges (SCEs) are products of joint DNA molecule resolution, and are considered to form through homologous recombination (HR). Indeed, SCE induction upon irradiation requires the canonical HR factors BRCA1, BRCA2 and RAD51. In contrast, replication-blocking agents, including PARP inhibitors, induce SCEs independently of BRCA1, BRCA2 and RAD51. PARP inhibitor-induced SCEs are enriched at difficult-to-replicate genomic regions, including common fragile sites (CFSs). PARP inhibitor-induced replication lesions are transmitted into mitosis, suggesting that SCEs can originate from mitotic processing of under-replicated DNA. Proteomics analysis reveals mitotic recruitment of DNA polymerase theta (POLQ) to synthetic DNA ends. POLQ inactivation results in reduced SCE numbers and severe chromosome fragmentation upon PARP inhibition in HR-deficient cells. Accordingly, analysis of CFSs in cancer genomes reveals frequent allelic deletions, flanked by signatures of POLQ-mediated repair. Combined, we show PARP inhibition generates under-replicated DNA, which is processed into SCEs during mitosis, independently of canonical HR factors.

Double-stranded DNA breaks (DSBs) are toxic DNA lesions that can lead to cell death or genomic alterations if left unrepaired. Cells have evolved multiple DNA repair mechanisms to deal with DNA breaks12. In G1 phase of the cell cycle, DNA breaks are predominantly repaired through non-homologous end-joining (NHEJ), which involves ligation of DNA ends independently of sequence homology and frequently results in the introduction of small indels across the break site1. In contrast, when DNA has been replicated during S-phase, the sister chromatids can be used as templates for error-free repair through homologous recombination (HR)3. Cyclin-dependent kinase (CDK)-mediated activation of CtIP promotes the resection of the broken DNA ends by BRCA1/BARD1 and the MRE11/RAD50/NBS1 (MRN) complex, generating single-stranded DNA (ssDNA) overhangs. The resected ends are poor substrates for NHEJ, marking a point of no return for initiation of HR. Subsequently, BRCA2 promotes loading of RAD51 recombinase onto ssDNA stretches35. RAD51 monomers are assembled into nucleoprotein filaments, which ultimately perform the homology search and invasion of the repair template35,40. Upon finding homology with the sister chromatid, DNA synthesis takes place via synthesis-dependent strand annealing (SDSA) or through the formation of a joint DNA molecule known as a Holliday junction (HJ). In order to allow faithful chromosome segregation, HJs need to be removed before the onset of mitosis8. HJs can be either ‘dissolved’ by the BLM/RMI1/RMI2/TopIIIa (BTR) complex or ‘resolved’ by the SLX1/SLX4/MUS81/EME1 complex.
complex or the GEN1 nuclease\(^8\). Upon completion of DNA synthesis by
SDSA and after BTR-mediated dissolution of a HJ, the two ends of the
DNA break are rejoined to the original sister chromatid, giving rise to a
so-called ‘non-crossover’ event. Alternatively, when the DNA ends of
opposing sister chromatids are rejoined, this results in a ‘crossover’
event or sister chromatid exchange (SCE)\(^{20–22}\). Thus, whereas HJ
dissolution exclusively gives rise to non-crossover events\(^1\), HJ resolution
can give rise to either non-crossover events or crossover end products.

Cells that lack functional BRCA1 or BRCA2, as for instance observed in
hereditary breast or ovarian cancers, are defective in HR
and display high levels of genomic instability\(^{23–26}\). Due to their DNA
repair defect, HR-deficient cancer cells display enhanced sensitivity to
DNA damaging agents, including DNA cross-linking agents such as
cisplatin\(^22\). Particularly, HR-deficient cells are sensitive to inhibition
of PARP1, an enzyme involved in DNA single-strand break repair\(^{23,24}\).
The synthetic lethal interaction between BRCA deficiency and PARP1
inhibitors was initially explained by accumulation of single-strand DNA
breaks due to PARP inhibition, which are converted into DSBs that are
toxic in the absence of HR repair. However, PARP inhibitors also trap
PARP molecules onto DNA\(^23\). PARP trapping induces stalling and col-
lapse of replication forks\(^24,25\), which are resolved, at least in part, by the
HR machinery\(^26\). As a consequence, PARP inactivation leads to elevated
HR-de
iciency\(^29\). Using differential
chromatid staining of
Brca2
-cells and
Brca2IBAC
-cells35 were
observed a loss of
centchromatin complexes or the GEN1 nuclease9. Upon completion of DNA synthesis by
SDSA and after BTR-mediated dissolution of a HJ, the two ends of the
DNA break are rejoined to the original sister chromatid, giving rise to a
so-called ‘non-crossover’ event. Alternatively, when the DNA ends of
opposing sister chromatids are rejoined, this results in a ‘crossover’
event or sister chromatid exchange (SCE)\(^{20–22}\). Thus, whereas HJ
dissolution exclusively gives rise to non-crossover events\(^1\), HJ resolution
can give rise to either non-crossover events or crossover end products.

Cells that lack functional BRCA1 or BRCA2, as for instance observed in
hereditary breast or ovarian cancers, are defective in HR
and display high levels of genomic instability\(^{23–26}\). Due to their DNA
repair defect, HR-deficient cancer cells display enhanced sensitivity to
dNA damaging agents, including DNA cross-linking agents such as
cisplatin\(^22\). Particularly, HR-deficient cells are sensitive to inhibition
of PARP1, an enzyme involved in DNA single-strand break repair\(^{23,24}\).The synthetic lethal interaction between BRCA deficiency and PARP1
inhibitors was initially explained by accumulation of single-strand DNA
breaks due to PARP inhibition, which are converted into DSBs that are
toxic in the absence of HR repair. However, PARP inhibitors also trap
PARP molecules onto DNA\(^23\). PARP trapping induces stalling and col-
lapse of replication forks\(^24,25\), which are resolved, at least in part, by the
HR machinery\(^26\). As a consequence, PARP inactivation leads to elevated
HR-de
iciency\(^29\). Using differential
cromatin complexes or the GEN1 nuclease9. Upon completion of DNA synthesis by
SDSA and after BTR-mediated dissolution of a HJ, the two ends of the
DNA break are rejoined to the original sister chromatid, giving rise to a
so-called ‘non-crossover’ event. Alternatively, when the DNA ends of
opposing sister chromatids are rejoined, this results in a ‘crossover’
event or sister chromatid exchange (SCE)\(^{20–22}\). Thus, whereas HJ
dissolution exclusively gives rise to non-crossover events\(^1\), HJ resolution
can give rise to either non-crossover events or crossover end products.

Cells that lack functional BRCA1 or BRCA2, as for instance observed in
hereditary breast or ovarian cancers, are defective in HR
and display high levels of genomic instability\(^{23–26}\). Due to their DNA
repair defect, HR-deficient cancer cells display enhanced sensitivity to
dNA damaging agents, including DNA cross-linking agents such as
cisplatin\(^22\). Particularly, HR-deficient cells are sensitive to inhibition
of PARP1, an enzyme involved in DNA single-strand break repair\(^{23,24}\).
The synthetic lethal interaction between BRCA deficiency and PARP1
inhibitors was initially explained by accumulation of single-strand DNA
breaks due to PARP inhibition, which are converted into DSBs that are
toxic in the absence of HR repair. However, PARP inhibitors also trap
PARP molecules onto DNA\(^23\). PARP trapping induces stalling and col-
lapse of replication forks\(^24,25\), which are resolved, at least in part, by the
HR machinery\(^26\). As a consequence, PARP inactivation leads to elevated
HR-de
iciency\(^29\). Using differential
cromatin complexes or the GEN1 nuclease9. Upon completion of DNA synthesis by
SDSA and after BTR-mediated dissolution of a HJ, the two ends of the
DNA break are rejoined to the original sister chromatid, giving rise to a
so-called ‘non-crossover’ event. Alternatively, when the DNA ends of
opposing sister chromatids are rejoined, this results in a ‘crossover’
event or sister chromatid exchange (SCE)\(^{20–22}\). Thus, whereas HJ
dissolution exclusively gives rise to non-crossover events\(^1\), HJ resolution
can give rise to either non-crossover events or crossover end products.

Cells that lack functional BRCA1 or BRCA2, as for instance observed in
hereditary breast or ovarian cancers, are defective in HR
and display high levels of genomic instability\(^{23–26}\). Due to their DNA
repair defect, HR-deficient cancer cells display enhanced sensitivity to
dNA damaging agents, including DNA cross-linking agents such as
cisplatin\(^22\). Particularly, HR-deficient cells are sensitive to inhibition
of PARP1, an enzyme involved in DNA single-strand break repair\(^{23,24}\).
The synthetic lethal interaction between BRCA deficiency and PARP1
inhibitors was initially explained by accumulation of single-strand DNA
breaks due to PARP inhibition, which are converted into DSBs that are
toxic in the absence of HR repair. However, PARP inhibitors also trap
PARP molecules onto DNA\(^23\). PARP trapping induces stalling and col-
lapse of replication forks\(^24,25\), which are resolved, at least in part, by the
HR machinery\(^26\). As a consequence, PARP inactivation leads to elevated
HR-de
iciency\(^29\). Using differential
cromatin complexes or the GEN1 nuclease9. Upon completion of DNA synthesis by
SDSA and after BTR-mediated dissolution of a HJ, the two ends of the
DNA break are rejoined to the original sister chromatid, giving rise to a
so-called ‘non-crossover’ event. Alternatively, when the DNA ends of
opposing sister chromatids are rejoined, this results in a ‘crossover’
event or sister chromatid exchange (SCE)\(^{20–22}\). Thus, whereas HJ
dissolution exclusively gives rise to non-crossover events\(^1\), HJ resolution
can give rise to either non-crossover events or crossover end products.

Cells that lack functional BRCA1 or BRCA2, as for instance observed in
hereditary breast or ovarian cancers, are defective in HR
and display high levels of genomic instability\(^{23–26}\). Due to their DNA
repair defect, HR-deficient cancer cells display enhanced sensitivity to
dNA damaging agents, including DNA cross-linking agents such as
cisplatin\(^22\). Particularly, HR-deficient cells are sensitive to inhibition
of PARP1, an enzyme involved in DNA single-strand break repair\(^{23,24}\).
The synthetic lethal interaction between BRCA deficiency and PARP1
inhibitors was initially explained by accumulation of single-strand DNA
breaks due to PARP inhibition, which are converted into DSBs that are
toxic in the absence of HR repair. However, PARP inhibitors also trap
PARP molecules onto DNA\(^23\). PARP trapping induces stalling and col-
lapse of replication forks\(^24,25\), which are resolved, at least in part, by the
HR machinery\(^26\). As a consequence, PARP inactivation leads to elevated
HR-de
iciency\(^29\). Using differential
cromatin complexes or the GEN1 nuclease9. Upon completion of DNA synthesis by
SDSA and after BTR-mediated dissolution of a HJ, the two ends of the
DNA break are rejoined to the original sister chromatid, giving rise to a
so-called ‘non-crossover’ event. Alternatively, when the DNA ends of
opposing sister chromatids are rejoined, this results in a ‘crossover’
event or sister chromatid exchange (SCE)\(^{20–22}\). Thus, whereas HJ
dissolution exclusively gives rise to non-crossover events\(^1\), HJ resolution
can give rise to either non-crossover events or crossover end products.
chemotherapeutics that target DNA replication was tested, including the DNA crosslinking agents mitomycin C (MMC) and cisplatin, and the topoisomerase inhibitors camptothecin (CPT) and etoposide. Although these agents induced SCEs to various extents, very similar amounts of SCEs were observed in control and BRCA2-depleted cells (Fig. 3B). Only for etoposide, a small but significant decrease in SCEs was observed in BRCA2-depleted cells (Fig. 3B). Overall, our data suggests that the capacity to block DNA replication forks is a key determinant in the formation of HR-independent SCEs.

To investigate whether PARP inhibitor-induced SCEs are associated with specific genomic features, we performed Strand-seq in olaparib-treated KBM-7 cells, expressing control or BRCA2-targeting
shRNAs. The near haploid karyotype of KBM-7 cells allows for robust mapping of SCEs. In line with our previous observations, PARP inhibition induced SCEs in KBM-7 cells independently of BRCA2 (Supplementary Fig. 4A). KBM-7 cells displayed deletions, amplifications and copy number variations in BRCA2-depleted cells, which were further increased upon PARP inhibition, underscoring a functional HR defect in these cells (Supplementary Fig. 4B–D). SCE locations were mapped using HapSCElocatoR, as described previously (Supplementary Fig. 4E)49. Genomic locations of SCEs were mapped against the locations of previously described human CFSs49. A significant enrichment of olaparib-induced SCEs was observed within CFS regions in BRCA2-deficient cells (Fig. 3C, D and Supplementary Data 1), in line with CFSs being regarded as difficult-to-replicate loci. For example, in BRCA2-deficient cells treated with olaparib, a substantial number of SCEs were detected within the FRAP1 and FRAP4 CFSs (Fig. 3C). Subsequently, SCEs were mapped against centromeres and telomeres to test enrichment at other difficult-to-replicate regions (Supplementary Fig. 4F, G). Whereas no SCEs were observed at telomeric regions, olaparib also significantly induced centromeric SCEs in both BRCA2-deficient and control cells (Supplementary Fig. 4F, G). Moreover, BRCA2-depleted cells showed a significant depletion of SCEs within gene bodies (Supplementary Fig. 4H). Finally, no significant enrichments were observed at putative G4 structures (Supplementary Fig. 4I). The observation that SCEs in BRCA2-deficient cells are enriched at CFSs and centromeric regions is in accordance with our hypothesis that these SCEs are associated with DNA replication fork stalling.

To further investigate the relation between DNA replication and SCE formation, RPE-1 cells were treated with olaparib for different time periods, treating cells either during or after S-phase (Fig. 3E, F). Edu incorporation was used to assess the time required for RPE-1 cells to progress from S-phase to mitosis (Fig. 3E). After 8 h of Edu incorporation, only 4.9% of all mitoses (phospho-histone H3-positive cells) had incorporated Edu, suggesting that the majority of mitotic cells did not progress through S-phase at the time of collection (Fig. 3E). Accordingly, 8 h olaparib treatment induced only a minor number of SCEs (Fig. 3F). In contrast, 12 or 24 h Edu treatment resulted in a considerable population of Edu-positive mitotic cells (Fig. 3E), which coincided with significantly larger numbers of SCEs being induced (Fig. 3F). Overall, these data suggest that olaparib needs to be present during S-phase in order to induce SCEs in the following mitosis. Combined, these findings illustrate that PARP inhibition in HR-deficient cells leads to extensive replication perturbation, and that HR-independent SCEs are observed in a range of conditions, with perturbed replication as a shared mechanism-of-action.

Processing of olaparib-induced DNA lesions during mitosis

We previously reported that olaparib-induced replication lesions are transmitted into mitosis50,51. To further test whether olaparib treatment induces mitotic DNA lesions in BRCA2-deficient RPE-1 TP53−/− cells, we measured the DNA damage markers yH2AX and FANC2D in mitotic cells (Fig. 4A). Both endogenous and olaparib-induced yH2AX and FANC2D foci were enriched in BRCA2-depleted cells (Fig. 4A). Since mitotic FANC2D foci reflect the presence of unresolved under-replicated DNA, we next assessed if olaparib treatment leads to increased mitotic DNA synthesis (MiDAS). We observed an increase in mitotic Edu foci in response to olaparib treatment in BRCA2-depleted cells (Fig. 4B), suggesting that DNA replication in these cells is incomplete at the moment of mitotic entry. Although MiDAS was most prominently induced in BRCA2-depleted cells, we also observed a mild increase in MiDAS foci in control cells treated with olaparib (Fig. 4B). To further investigate the link between mitosis and SCE formation, RPE-1 cells were treated with an inhibitor of the ATR checkpoint kinase to force cells into mitosis with under-replicated DNA (Fig. 4C). Cells treated with ATR inhibitor showed elevated numbers of SCEs, independently of BRCA2 (Fig. 4D). Similarly, inhibition of the cell cycle checkpoint kinase Wee1 also resulted in premature mitotic entry (Fig. 4E), along with elevated numbers of BRCA2-independent SCEs (Fig. 4F). Combined, these observations suggest that mitotic processing of under-replicated DNA may be the source for HR-independent SCEs.

Recently, it was hypothesized that SCEs could also originate from mitotic processing of stalled replication forks. This would involve cleavage of both leading or both lagging strands, introducing DSBs at under-replicated DNA (Fig. 4C). Cells treated with ATR inhibitor showed increased numbers of SCEs, independently of BRCA2 (Fig. 4D). Moreover, we observed severe fragmentation of mitotic chromosomes, consistent with an inability to process mitotic breaks resulting from under-replicated DNA (Fig. 5D). Chromosome fragmentation was also observed in BRCA2-deficient cells treated with the recently described POLQ inhibitor novobiocin52 (Supplementary Fig. 5C, D). Moreover, POLQ inhibition resulted in a minor extension of mitotic duration of BRCA2-deficient cells, suggesting a function for POLQ in the repair of DNA lesions during mitosis (Fig. 5E).

Means and standard deviations are plotted. E, F DT40 RAD51−/− cells harboring a dox- repressed hRad51 transgene were treated with doxycycline for indicated time periods, and treated with olaparib (E) or irradiation (F). SCEs in macrochromosomes were quantified by microscopy analysis of at least 29 metaphase spreads per condition from one biologically independent experiment. Exact n values are indicated in the figure. Means and standard deviations are plotted. G, H RPE-1 TP53−/− cells were incubated with olaparib (G) or IR (H) in the absence or presence of the RAD51 inhibitor BO2. SCEs were quantified by microscopy analysis of at least 28 metaphase spreads per condition from one biologically independent experiment. Exact n values are indicated in the figure. Means and standard deviation are plotted. Statistics in panels B, C, E, F, G, H were performed using unpaired two-tailed t-tests (ms: non-significant). Gray bars indicate HR-proficient conditions, green bars indicate HR-defective conditions. Source data are provided with this paper.
Fig. 3 | HR-independent SCEs are associated with defective replication. A RPE-1-TP53-/- shBRCA2 cells were pre-treated with doxycycline (dox) and transfected with siPARP or control siRNAs. Lysates were immunoblotted for PARP1 and Actin. Data are representative for two independent experiments. B RPE-1-TP53-/- shBRCA2 cells were pre-treated with doxycycline (dox) and subsequently treated with indicated agents for 48 h or transfected with PARP1 siRNAs 48 h before harvest. SCEs were quantified by microscopy analysis of at least 21 metaphase spreads per condition from one biologically independent experiment. Exact n values are indicated in the graph. Averages and standard deviations are indicated. C, D KBM-7 cells harboring doxycycline-inducible control or BRCA2 shRNAs were pre-treated with doxycycline and subsequently treated with olaparib where indicated. SCEs were mapped using StrandSeq of n = 64 (shLUC/DMSO), n = 31 (shLUC/OLA), n = 50 (shBRCA2/DMSO), and n = 52 (shBRCA2/OLA) libraries per condition from one biologically independent experiment. Observed SCEs were mapped to CFSs. Statistical analysis was performed using one-sided permutation with 10,000 iterations (D). P values indicate deviation of the observed number of SCEs compared to the mean of all permutations. SCE mapping to the common fragile sites FRA1F and FRA4F are presented as illustrative examples (C). E, F Doxycycline pre-treated RPE-1 TP53-/- shBRCA2 cells were treated with ethynyl deoxyuridine (EdU) for the indicated time periods, and subsequently analyzed for mitotic cells by flow cytometry of phospho-histone H3 (Ser10). Percentages of mitotic cells that were EdU-positive are indicated (E). Doxycycline pre-treated RPE-1 TP53-/- shBRCA2 cells were treated with olaparib for the indicated time points, and SCEs were quantified by microscopy analysis of 30/31/29/30 metaphase spreads per condition from one biologically independent experiment (F). Means and standard deviations are plotted. Statistics in panels B and F were performed using unpaired two-tailed t-tests (ns: non-significant), and gray bars indicate HR-proficient conditions, green bars indicate HR-defective conditions. Source data are provided with this paper.
**Fig. 4 | SCEs originate from mitotic processing of under-replicated DNA.** A RPE1 TP53-/- shBRCA2 were pre-treated with doxycycline (dox), synchronized using RO-3306 for 4 h, and subsequently treated with olaparib where indicated. γH2AX and FANCD2 foci in mitotic cells were quantified by immunofluorescence microscopy. Means and standard deviation of pooled data from three independent experiments are shown, with n = 30 mitoses per experiment. B RPE1 TP53-/- shBRCA2 were treated as for panel A. 24 h after olaparib treatment, cells were incubated for 25 min with EdU. Mitotic EdU foci were quantified in n = 30 mitoses per experiment. Means and standard deviation of pooled data from three independent experiments are shown. C, D RPE1 TP53-/- shBRCA2 cells were treated with doxycycline (dox) for 48 h, with olaparib for 24 h, with or without the ATR inhibitor VE-821 (ATRi) for 3 h. Cells were treated with colcemid for 3 h before harvesting, fixed and stained for the mitotic marker phospho-Histone-H3 (C). In parallel, SCEs were quantified by microscopy analysis of at least 18 metaphase spreads per condition. Exact n values are indicated in the figure (D). Exact n values are provided in the figure. E, F RPE1 TP53-/- shBRCA2 cells were treated with doxycycline (dox) for 48 h, with olaparib for 24 h, with or without the Wee1 inhibitor AZD-1775 (Wee1i) for 3 h. Cells were treated with colcemid for 3 h before harvesting, fixed and stained for the mitotic marker phospho-Histone-H3 (E). In parallel, SCEs were quantified by microscopy analysis of at least 18 metaphase spreads per condition. Exact n values are indicated in the figure (F). Statistics in panels A, B, D, and F were performed using two-sided Mann-Whitney tests (ns: non-significant). Gray bars indicate HR-proficient conditions, green bars indicate HR-defective conditions. Source data are provided with this paper.
Our data fit a model in which transmission of under-replicated DNA into mitosis results in SCE formation that can arise in a HR-independent fashion (Fig. 5F). We hypothesize that upon removal of the replisome at the onset of mitosis, DNA breaks are induced, flanking the under-replicated DNA, consistent with previously reported data51. Subsequently, the ssDNA gaps flanking the under-replicated region are filled, and ligation of the two broken sister chromatids is promoted by POLQ (Fig. 5F). As a consequence of mitotic processing of under-replicated DNA according to this model, an HR-independent SCE is formed, which is predicted to be accompanied by allelic deletions with a size reflecting the extent of under-replication (Fig. 5F). To test this model, we analyzed whole-genome sequencing (WGS) data of a cohort.
of 507 breast cancers. Since SCEs occur stochastically, bulk sequencing data cannot directly assess scars at spontaneous SCEs. Instead, we assessed CFSSs, as these loci are enriched for SCEs (Fig. 3C, D). We separately analyzed HR-proficient and HR-deficient breast cancers, as assessed by the CHORD algorithm (Fig. 6A), or by BRCA1/2 mutation status (Supplementary Fig. 6A). We frequently observed allelic deletions that are positioned in CFSSs, which was more frequently observed in HR-deficient cancers when compared to HR-proficient cancers (19% vs 15%, \( p = 7.55 \times 10^{-11} \), Fig. 6B), and more frequently observed in BRCA1/2 mutant cancers (Supplementary Fig. 6B). Deletions frequently spanned mega-base regions (Supplementary Fig. 6C), in line with the previously reported length of under-replicated areas. As HR-deficient tumors have previously been characterized by deletions flanked by ≥2 bp microhomology, we next tested whether allelic deletions at CFSSs displayed microhomology at the break sites (Fig. 6C, D and Supplementary Fig. 6D, E), which reflects usage of polymerase theta-mediated end joining. Indeed, a large fraction of deletions at CFSSs showed 2 bp microhomology, with more CFSS deletions in HR-deficient tumors harboring breakpoints with microhomology when compared to HR-proficient tumors (56% vs 47%, \( p = 8.6 \times 10^{-5} \)). Similar results were obtained when comparing BRCA1/2 mutant with BRCA1/2 wildtype cancers (\( p = 5.4 \times 10^{-4} \); Fig. 6C and Supplementary Fig. 6D).

Combined, our data suggest that HR-independent SCEs can originate from mitotic processing of under-replicated DNA, and suggests the involvement of the alternative end-joining polymerase POLQ, leading to allelic loss of under-replicated loci.

**Discussion**

We here show that agents that perturb DNA replication, including PARP inhibitors, induce sister chromatid exchanges in the absence of canonical HR factors BRCA1, BRCA2, and RAD51. Conversely, these HR components are required for induction of SCEs upon irradiation. Our findings challenge the current dogma that SCEs solely arise as a result of homologous recombination. Interestingly, models in which formation of SCEs upon replication forklift stalling occur independently of HR have been proposed in the past, although these have lost support in favor of the HR-dependent models over the years. The observation that SCEs form independently of RAD51 is of particular interest. SCEs are considered to involve the formation of joint molecules, which typically require strand invasion by RAD51. Previously observed replication stress-associated SCEs in BRCA2 mutant cells were explained by BRCA2-independent RAD51 recruitment. Yet, spontaneous and replication-induced SCEs have been reported frequently in cells lacking canonical HR factors, underscoring the notion that SCEs can arise independently of HR, and suggesting that PARP-inhibitor induced SCEs and spontaneous SCEs may share common mechanisms. Surprisingly, loss of the RAD51 paralogs RAD54, RAD53C, RAD53D, XRC2, and XRCC3 has previously been reported to reduce MMC-induced and spontaneous SCEs, although these effects were limited and were attributed to defective RAD51 functioning.

Our data point towards incompletely replicated DNA as the source of DNA lesions that cause HR-independent SCEs. We and others find that PARP inhibition perturbs DNA replication in HR-defective cells, and that this leads to elevated levels of mitotic FANCD2 foci, a previously established marker of under-replicated DNA. Moreover, we observed moderate MiDAS activation in BRCA2-proficient cells, and further elevated levels of MiDAS in BRCA2-depleted cells upon PARP inhibition. Increased MiDAS was observed upon PARP inhibition in both BRCA2-depleted cells and control-depleted cells, although it occurred more frequently in BRCA2-depleted cells. MiDAS is commonly observed in situations of perturbed DNA replication, and may reflect an attempt of cells to finalize stalled DNA replication at the G2/M transition. Importantly, incomplete DNA replication results in sister chromatids that are connected as ‘joint DNA molecules’ (Fig. 5F).
and would explain how SCEs could arise independently of strand invasion via BRCA1, BRCA2, and RAD51.

It is currently unclear if the ssDNA gaps that we observed at replication forks (Fig. 2B) play a role in the formation of HR-independent SCEs. The enrichment of both olaparib-induced SCEs and ssDNA gaps in BRCA2-deficient cells, warrants further research into potential shared mechanisms between SCE and ssDNA gap formation at stalled forks. If SCE formation at CFSs is directly linked to stalled replication forks, this would suggest that SCEs at CFSs may arise through different mechanisms than SCEs elsewhere in the genome.

ssDNA gaps in BRCA1/2-deficient cell have previously been attributed to PRIMPOL activity7. Analysis of PRIMPOL-deficient cells may shed light on the role of ssDNA gap formation in HR-independent SCEs.

Of note, differential BrdU staining protocols for SCE detection require scoring of mitotic chromosome spreads. A limitation of this method is that cells with extensive DNA damage will likely activate a G2 cell cycle checkpoint and fail to enter mitosis. By selectively analyzing mitotic cells, we may be looking at an underestimate of the amount of DNA lesions in these cells. Yet, the cells that do enter mitosis clearly demonstrate that SCEs can arise independently of canonical HR components.

In good agreement with our data, coordinated cleavage of stalled replication forks in mitosis was recently hypothesized to yield SCEs, at the cost of local deletions12,13. This process requires that the stalled replication forks flanking the under-replicated DNA are cleaved either at both leading strands, or at both lagging strands (Fig. 5E). Coordinated cleavage of replication forks could be initiated by TRAIL/p97-dependent unloading of the CMG helicase36, leaving stretches of vulnerable ssDNA at the leading strands. Although the responsible nuclease in processing under-replicated DNA upon PARP inhibition remains elusive, MUS81 has been shown to be active during mitosis12,13,14, to localize to under-replicated DNA in mitosis36, and to act on stalled replication forks in mitosis72,73. However, our data showed that depletion of MUS81 alone was not sufficient to reduce SCEs, possibly due to redundancy with other endonucleases that are active during mitosis74,75.

Cleavage and end-joining of cleaved under-replicated DNA regions during mitosis would yield SCEs as well as alelic deletions of under-replicated genomic regions. We report a potential role for POLQ in this process. Recently, POLQ was shown to act on DNA lesions during mitosis82, although a role of POLQ in processing of mitotic DNA lesions into SCEs was not previously demonstrated to our knowledge.

Of note, we do not find a full loss of HR-independent SCEs in POLQ-deficient cells. We therefore cannot rule out that other parallel pathways can mediate HR-independent SCEs. In contrast to our findings, Poly loss in mouse embryonic fibroblasts (MEFs) resulted in increased numbers of MMC-induced SCEs, although these SCEs may be a product of canonical HR in these cells37.

The allelic deletions that are predicted in our model to arise during processing of under-replicated DNA during mitosis have been reported previously at common-fragile sites72. Moreover, the observed submicroscopic deletions that span CFS regions were shown to be flanked by microhomology regions, suggesting the involvement of POLQ-mediated end joining, consistent with the mutational signatures observed in BRCA1/2-deficient tumours13,14. Moreover, a role for POLQ in the mitotic processing of stalled replication forks in BRCA1/2-deficient cells fits well with the previously described synthetic lethality between POLQ and BRCA212,14, and between POLQ and other regulators of DSB repair82. Also, a model in which POLQ-mediated processing of under-replicated DNA functions in parallel to a pathway involving HR explains the observed synthetic lethal effects of POLQ inhibition in BRCA1/2-deficient cells, particularly in the context of PARP-inhibitor treatment83.

Interestingly, our observation that mitotic SCEs arise independently of RAD52 implicates that we are not looking at a direct end-product of break-induced replication (BIR)83,84 or MiDAS70,85, which are both dependent on RAD52. Interestingly, MiDAS involves nuclelease activity, and this pathway could therefore potentially compete with POLQ-dependent mitotic SCE formation. Indeed, RAD52 inactivation resulted in higher SCE numbers, suggesting that RAD52 inhibits SCE formation during mitosis. These findings are in good agreement with a recently described interaction between RAD52 and POLQ, in which RAD52 blocks POLQ activity during mitosis86. How MiDAS-mediated joint molecules can be formed during mitosis, when multiple nucleases are active to resolve such structures remains unclear. Interestingly, MiDAS was recently suggested to reflect completion of DNA replication at the end of G2 phase, rather than during mitosis82, suggesting that MiDAS and mitotic SCE formation could be consecutive processes.

Although the synthetic lethal interaction between HR loss and PARP inhibition has been validated in various models and has been successfully exploited in the clinic, the fate of PARP inhibitor-induced DNA lesions in HR-deficient cells remains unclear. Although we find that PARP inhibitor-induced DNA lesions are processed into SCEs in HR-deficient cells, we predict that this goes along with accumulation of large deletions and translocations due to mitotic POLQ activity, which may underlie loss of viability observed in these cells. In this context, a requirement for mitotic replisome unloading by TRAIL/p97, fits well with observed sensitization of HR-deficient cells for PARP inhibition upon inactivation of TRAIL87, and the observation that progression through mitosis promotes PARP inhibitor-mediated cell death88. Our observation that PARP inhibitor-induced DNA lesions in HR-deficient cells are transmitted into mitosis also aligns well with the recent identification of the DNA tethering factor CIP2A being essential in HR-deficient cells89, and was identified in our proteomics analysis of mitotic factors that bind DNA ends. Further research is warranted to investigate whether tethering of DNA ends is required to ligate DNA ends upon cleavage of stalled replication forks during mitosis.

Methods

Cell lines

HTERT-immortalized human retina epithelial RPE-1 cells and HEK293T cells were obtained from ATCC, and were maintained in Dulbecco’s Minimum Essential Media (DMEM, Thermofisher), supplemented with 10% fetal calf serum (FCS, Lonza), 50 units/mL penicillin and 50 µg/mL streptomycin (P/S, Gibco) at 37 °C, 5% CO2 and 5% O2. KB2P3.4 and KB2P3.4R3 cell lines were a kind gift from Jos Jonker (The Netherlands Cancer Institute, Amsterdam, The Netherlands) and were maintained in Iscove’s Modified Dulbecco’s Media (IMDM, Thermofisher) supplemented with 10% FCS and P/S. DT-40 cells were a kind gift from Shunichi Takeda (Kyoto University, Japan) and were grown in Roswell Park Memorial Institute (RPMI)-1640 media supplemented with 10% FCS, 1% chicken serum (Sigma) and P/S (Gibco) at 39.5 °C, 20% O2 and 5% CO2.

KB2P3.4 and KB2P3.4R3 cell lines were cultured in DMEM/F-12 medium, supplemented with 10% FCS, 50 units/mL penicillin, 50 µg/mL streptomycin, 5 µg/mL insulin (Sigma), 5 ng/mL epidermal growth factor (Life Technologies) and 5 ng/mL chola toxin (Gentaur), at 37 °C and hypoxic conditions (1% O2, 5% CO2).

Knockdown and knockout cell line models

To generate RPE-1 and KBM-7 cell lines expressing doxycycline-inducible short-hairpin RNAs (shRNAs), DNA oligos were cloned into Tet-plKO-puro (Addgene plasmid #21915) vector. Tet-plKO-puro was
a kind gift from Dmitri Wiederschain, shRNAs directed against luciferase (shLUC, 5′-AAGAGCTTTTGGAGGCC-3′), BRCA2 (#1: 5′-GAAGATCTGAGGTTTA-3′ and #2: 5′-AACAATTACTGAGCAAAC-AC-3′), BRCA1 (#1: 5′-CCCCACTTTCATTGCTATAGTATG-3′ and #2: 5′-GAGTATGCAAACCTTATATG-3′). RAD51 (#1: 5′-CTGCTGAGATCATCATA-AGTATT-3′ and #2: 5′-GGTAAGATCTGAGGCTATTG-3′), MUS81 (#1: 5′-AGTTGTCATCTGACCAT-3′ and #2: 5′-CTATGTCACCATTCTA-3′), SLX4 (#1: 5′-ATTCTGCTCATTAGGTTT-3′ and #2: 5′-ACTGGACACTCATTAGGCT-3′), and ERCC1 (#1: 5′-CCAGAACCTTATTCCGATC-3′ and #2: 5′-CAGAGAGATCTGGCCTTAT-3′) were cloned into the Tet-pLOKO plasmid. Lentiviral particles were produced as described previously. In brief, HEK293T packaging cells were transfected with 4 μg of plKO plasmid in combination with the packaging plasmids lenti-VSVG and lenti-DVPR using a standard calcium phosphate protocol. Virus-containing supernatant was harvested at 48 and 72 h after transfection and filtered through a 0.45 μm syringe filter. Supernatants were used to infect target cells in medium with a final concentration of 4 μg/mL polybrene (Sigma-Aldrich). RPE-1 cells harboring a TP53 mutation were generated by introducing a single-guide RNA (sgRNA) targeting exon 4 of the TP53 gene as described previously. To generate RAD52 and POLQ knock-out cells, sgRNAs targeting exon 4 of RAD52 (AGAAT-CAACCTGTTTCTGAGGAGCC-3′ and #1: 5′-CTTAATGGTCACCACTT-3′) and POLQ (GCCGCCGCCGCCCTCAGCA) were cloned into the PX435 vector, which was a gift from Feng Zhang (Addgene plasmid # 48138). POLQ sgRNAs were a kind gift from Marcel Tijsterman (Leiden University Medical Centre, Leiden, the Netherlands). Plasmids were introduced in RPE-1 cells using Fugene HD transfection reagent and cells were selected based on GFP-expression or using 7 μg/mL puromycin (Sigma Aldrich) for 5 days.

**siRNA transfection**

Cells were transfected with 40 nM siRNAs (Ambion Stealth RNAi, Thermofisher) targeting exon 4 of the TP53 gene. siRNA transfection was increased to 64 h. Inhibitors were added for 48 h, simultaneously with BrdU treatment at the following concentrations: 1.0 μM olaparib, 5 nM campthothecin (Sigma), 250 nM etoposide (Accord), 5 nM cisplatin (Accord), and 0.5 μM novobiocin (POLQi; Sigma–Aldrich), 16 μM veliparip (Axon Medchem), 7 nM talazoparip (Axon Medchem), 50 nM mitomycin C (Sigma), 5 μM cisplatin (Accord), 5 μM camptothecin (Sigma), 250 nM etoposide (Sigma), 20 μM BO2 (Axon Medchem), and 50 μM novobioin (POLQi: Sigma–Aldrich), VE-821 (ATRI; Axon Medchem) or AZD-1775 (WeelI; Axon Medchem) were added simultaneously with colcemid for 3 h at a concentration of 1.0 μM. Alternatively, cells were treated with 2 Gy γ-irradiation 8–10 h prior to fixation using an IBL 637 Cesium137 γ-ray source. Cells were collected in 10 μg/mL colcemid (Roche) for 3–6 h, fixed in 3:1 methanol:acetic acid solution and infiltrated in a hypotonic 0.075 M KCl solution. Metaphase spreads were made by dripping the cell suspensions onto microscope glasses from a height of ~30 cm. Slides were stained with 10 μg/mL bis-Benzimide H 33258 (Sigma) for 30 min, exposed to 245 nM UV light for 30 min, incubated in 2x SSC buffer (Sigma) at 60 °C for 1 h, and stained in 5% Giemsa (Sigma) for 15 min. DT40 cells were treated with BrdU for 48 h, doxycycline for 24 h and 0.5 μM olaparip for 24 h. Alternatively, DT40 cells were treated with BrdU and doxycycline as stated above, irradiated with 4 Gy and fixed at 8 h later. For DT40 cells, only macrochromosomes were included for analysis. KB2P3.4R3 cells were treated with BrdU for 32 h and KB2P3.4 for 40 h. Both KB2P3.4 cells lines were treated with 1μM olaparip for 48 h.

**Immunofluorescence microscopy**

RPE-1 cells were seeded on glass coverslips in 6-well plates and treated with doxycycline (1 μg/mL) and olaparip (0.5 μM). Cells were then treated for 4 h with the CDK inhibitor RO-3306 (5 μM). Upon washout of RO-3306, cells were incubated with EdU (20 μM) for 25 min. Cells were fixed using 2% formaldehyde in 0.1% Triton X-100 PBS for 10 minutes and subsequently permeabilized for 10 min in PBS with 0.5% Triton X-100. Staining was performed using primary antibodies against FANCD2 (Novusbio, Centennial, CO, USA; NB100-182, 1:200) and γH2AX Millipore, 05-636, 1:200). Cells were then incubated with corresponding Alexa-488 or Alexa-647-conjugated secondary antibodies and counterstained with DAPI (Sigma). For analysis of DNA damage response components, prophase and prometaphase cells were identified based on condensed chromatin conformation, and included for analysis. Images were acquired on a Leica DM6000B microscope using a ×63 immersion objective (PL- APO, numerical aperture: 1.30) with Las af software (Leica, Wetzlar, Germany).

For RAD51 analysis, RPE1 cells were left untreated or were irradiated using a Cesium137 source (CIS International/IBL 637 irradiator, dose rate: 0.0083 Gy per second). After 3 h, cells were washed in phosphate-buffered saline (PBS) and then fixed in 2% paraformaldehyde with 0.1% Triton-X100 in PBS for 30 min at room temperature. Cells were permeabilized in 0.5% Triton X-100 in PBS for 10 min. Subsequently, cells were extensively washed and incubated with PBS containing 0.65% Tween 20 and 4% bovine serum albumin (fraction V) (PBS-Tween-BSA) for 1 h to block nonspecific binding. Cells were incubated overnight at 4 °C with primary antibodies targeting RAD51 (GeneEx, GTX70230, 1:400). Cells were extensively washed and incubated for 1 h with Alexa-conjugated secondary antibodies (1:400) and counterstained with 4,6-diamidino-2-phenylindole (DAPI). Slides were mounted with ProLong Antifade Mountant (Thermofisher). Images were acquired on a Leica DM-6000RXA fluorescence microscope, equipped with Leica Application Suite software.

**Cell viability assays**

RPE-1 cells were plated in 96-wells plates at a concentration of 800 cells per well. After 24 hours, cells were treated with indicated concentrations of olaparip, veliparip, or talazoparip (all from Axon Medchem) for 3 days. Methyl-thiazol tetrazolium (MTT, Sigma) was added to cells at a concentration of 5 mg/mL for 4 hours, after which culture medium was removed and formazan crystals were dissolved in DMSO. Absorbance values were determined using a Bio-Rad benchmark III Biorad microtiter spectrophotometer at a wavelength of 520 nm.

**Western blot analysis**

Cells were lysed in Mammalian Protein Extraction Reagent (MEP, Thermo Scientific), supplemented with protease inhibitor and phosphatase inhibitor (Thermo Scientific). Protein concentrations were measured using a Bradford assay. Proteins were separated by SDS-PAGE gel electrophoresis, transferred to Polyvinylidene fluoride (PVDF, immobilon) membranes and blocked in 5% skimmed milk (Sigma) in TBS-buffered saline (TBS) containing 0.05% Tween-20 (Sigma). Immunodetection was performed with antibodies directed against BRCA2 (Calbiochem, OP95, 1:1000), BRCA1 (Cell Signaling, 9010, 1:1000), RAD51 (GeneTex, gtx70230, 1:1000), PARP1 (Cell Signaling, 2173, 1:1000), H2AX (Millipore, 05-636, 1:200), RAD52 (Santa Cruz, sc-635431, 1:250), SLX4 (BTBD12; Novus Biologicals, NBPI-28680, 1:1000), MUS81 (Abcam, ab14387, 1:1000), ERCC1 (Cell Signaling, 8427S, 1:1000), and beta-Actin (MP Biomedicals, 69100, 1:10000). Horseradish peroxidase (HRP)-conjugated secondary antibodies (DAKO) were used for visualization using chemiluminescence (Lumi-Light, Roche Diagnostics) on a Bio-Rad Bioluminescence device, equipped with Quantity One/ChemiDoc XRS software (Bio-Rad).
**Strand-seq library preparation and sequencing**

Strand-seq libraries were prepared as previously described, with a few modifications. Prior to sorting single cells, KB2P3.4 and KB2P4.4R3 were treated with 1 µM olarip and KBM-7 with 0.15 µM olarip for 48 h. To incorporate BrdU during one cell cycle, BrdU (Invitrogen) was added to exponentially growing cell cultures at 40 µM final concentration. Timing of BrdU pulse was 16 h for KB2P3.4 and KBM-7, and 20 h for KB2P4.4 cells. After BrdU pulse, cells were resuspended in nuclei isolation buffer (100 mM Tris-HCl pH 7.4, 150 mM NaCl, 1 mM CaCl2, 0.5 mM MgCl2, 0.1% NP-40, and 2% bovine serum albumin) supplemented with 10 µg/mL Hoechst 33258 (Life Technologies) and propidium iodide (Sigma Aldrich). Single nuclei were sorted into 5 µL Pro-Freeze-CDM NAO freeze medium (Lonza) supplemented with 7.5% dimethyl sulfoxide, in 96-well skirted PCR plates (4Titude), and purified. DNA quality and concentrations were assessed on the Qubit 2.0 Fluorometer (Life Technologies) and using the High Sensitivity dsDNA kit (Agilent) on the Agilent 2100 Bio Analyzer. Single-end 50 bp sequencing reads from the Strand-seq libraries were generated using the HiSeq 2500 or the NextSeq 500 sequencing platform (Illumina).

**Detection and mapping of breakpoints**

Indexed bam files were aligned to mouse (GRCm38) or human genomes (GRCh38) using Bowtie2. Different R-based packages were used for the detection and mapping of breakpoints: Aneufinder2 was used for libraries with arbitrary copy number profiles (KB2P3.4 and KB2P4.4R3), while HapSCElocatoR was used for libraries derived from the haploid cell line KBM-7. Aneufinder2 was used to locate and classify any type of breakpoint, not only template strand switches, using standard settings. In short, copy numbers for both the Watson (negative) and Crick (positive) strand were called and breakpoints were defined as changes in copy number state. These breakpoints are then refined with read-resolution to make full use of the sequencing data. As Aneufinder2 also detects stable chromosomal rearrangements, clonal aberrations were defined as events that occurred at the exact same locations in ≥25% of the libraries from one cell line. HapSCElocatoR is implemented in the R package fastsega, and uses circular binary segmentation to localize SCEs in haploid Strand-seq libraries as a change in read directionality from Crick to Watson or vice versa. Only non-duplicate reads with a mapping quality greater than or equal to 10 were analyzed. We considered only strand state changes with at least three directional reads on both sides of the putative SCE site as an SCE event. Single directional reads embedded within an extended region with the opposite directionality were considered as errors and their directionality was flipped. Computationally localized SCE or somatic copy number alteration (SCNA) events were further manually verified by visual inspection of chromosome ideograms (obtained from Aneufinder2 or BAFF; see Figs. IA and 5A respectively).

**Genomic analysis of SCE localization**

A custom Perl script was used for the permutation model (https://github.com/Vityay/GenomePermute). For each of 1000 permutations, a random number n was generated and all SCEs were shifted downstream by n bases on the same chromosome. To prevent small-scale local shifts, n was confined to be a random number between 2 and 50 Mb. When the resulting coordinates exceeded chromosome size and the size of chromosome was subtracted, so that the SCE is mapped to beginning part of the chromosome, as if the chromosome was circular. All annotated assembly gaps were excluded before our analysis, to prevent permuted SCE mapping to one of the gap regions. The number of SCEs overlapping with a feature of interest in each permutation was then determined, as well as the original SCE regions. All values were normalized to the median permuted value, in order to determine relative SCE enrichments over expected, randomized distributions and to allow for comparison of the different cell lines. Significance was determined based on the amounts of permutations that showed the same or exceeding overlap (enrichment) or the same or receding (depletion) overlap with a given genomic feature compared to overlap between the original SCEs and the same feature. Any experimental overlap that lies outside of the 95% confidence interval found in the permutations has a p-value below 0.05 and was deemed significant. Experimental overlaps lying outside of the permuted range were given a p-value below 0.001, as there was a < 0.1% (1/1000) chance of such an overlap occurring by chance.

Enrichment analyses for G4 motifs were performed using a 10 kb region size cutoff. Putative G4 motifs were predicted using custom Perl script by matching genome sequence against following patterns: G3 + N x G3 + N x G3 + N x G3, where x could be the ranges of 1-3, 1-7, or 1-12 bp. Enrichment analysis for coding genes, CFSs, centromeres, and telomeres were performed using a 100 Kb size cutoff. Genome and gene annotations were obtained from Ensembl release 88 (GRCh38 assembly, https://www.ensembl.org). Gene bodies were defined as regions between transcription start sites and transcription end sites.

**Flow cytometry**

RPE-1 cells were treated with 20 µM EdU for 0, 8, 12 or 24 h, subsequently fixed in ice-cold ethanol (70%) for at least 16 h, and stained with primary antibody against phospho-histone-H3-Ser10 (Cell Signaling; 9701L, 1:100) and Alexa-488-conjugated secondary antibodies (1:200). EdU Click-it reaction was performed with Alexa-647 azide according to the manufacturer's instructions (InvitrogenTM). DNA was stained using propidium iodide following RNase treatment. At least 10,000 events per sample were analyzed on an LSR-II flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA). Data were analyzed using flowjo software (Becton Dickinson).

**Xenopus laevis egg extracts and biotin-oligonucleotide pull-downs**

Cytostatic factor (mitotic) and low speed supernatant (LSS) extracts were prepared according to Murray and Blow respectively. Biotin-oligonucleotide pull-down MS was performed as previously described. In short, a biotinylated-oligo (5`-ACCAGTGCCTTGCTAGGACATCTTTGCCCACCTGCAGGTTCACCC-3`) was annealed to its reverse complement at a concentration of 10 µM in 50 mM Tris pH 8.0 buffer. The oligo-duplicates were diluted to 100 nM, after which 10 µl oligo was coupled to 60 µl streptavidin-coupled magnetic beads (Dynabeads MyOne Streptavidin C1, Invitrogen) by incubation for 60 mins in wash buffer 1 (50 mM Tris pH 7.5, 150 mM NaCl, 1 mM EDTA pH 8.0, 0.02% Tween-20). Excess oligo-duplicates were removed by three washes in IP buffer (ELB-sucrose buffer: 10 mM HEPES-KOH pH 7.7, 50 mM KCl, 2.5 mM MgCl2, 250 mM sucrose; 0.25 mg/mL BSA; 0.02% Tween-20), after which the oligo-beads mixture was suspended in 40 µl IP buffer. Mitotic and interphase extracts were thawed on ice from -80 °C and supplemented with 20X...
energy mix (20 mM ATP, 150 mM Creatine Phosphate, 20 mM MgCl2, 2.5 mM EGTA). For biotin-oligonucleotide pulldown 8 μl mitotic or interphase extract was incubated with 4 μl of oligo-beads mixture for 10 mins. Beads-extract mixture was washed two times with 400 μl of IP Buffer, two times with IP-buffer minus BSA, and lastly one time with ELB-sucrose buffer. After the final wash, beads were taken up in 50 μl denaturing buffer (8 M Urea, 100 mM Tris pH8.0) and snap frozen.

Mass spectrometry of oligonucleotide-bound proteins was performed by on-bead digestion as previously described for plasmid pull-down. Microscopy of extracted tryptic peptides was performed using an Ultimate 3000 HPLC system (Thermo Fisher Scientific) coupled online to a Q-Exactive Plus mass spectrometer with a NanoFlex source (Thermo Fisher Scientific), equipped with a stainless-steel emitter. Tryptic digests were loaded onto a 5 mm x 300 μm internal diameter (i.d.) trapping micro column packed with PepMAP100, 5 μm particles (Dionex) in 0.1% formic acid at the flow rate of 20 μl/min. After loading and washing for 3 min, trapped peptides were back-flush eluted onto a 50 cm x 75 μm i.d. nanocolumn, packed with Acclaim C18 PepMAP100 C2 2 μm particles (Dionex). Eluents used were 100% H2O/acetonitrile (volume/volume (V/V)) with 0.1% formic acid (Eluent A) and 0:100 H2O/acetonitrile (v/v) with 0.1% formic acid (Eluent B). The following mobile phase gradient was delivered at the flow rate of 250 nl/min: 1–50% of solvent B in 90 min; 50–80% B in 1 min; 80% B during 9 min, and back to 1% B in 1 min and held at 1% A for 19 min which results in a total run time of 120 min. MS data were acquired using a data-dependent acquisition (DDA) top-10 method, dynamically choosing the most abundant not-yet-sequenced precursor ions from the survey scans (300–1630 Th) with a dynamic exclusion of 20 s. Survey scans were acquired at a resolution of 70,000 at mass-to-charge (m/z) 200 with a maximum inject time of 50 ms or AGC 3E6. DDA was performed via higher energy collisional dissociation fragmentation with a target value of 1x10E5 ions determined with predictive automatic gain control in centroid mode. Isolation of precursors was performed with a window of 1.6 m/z. Resolution for HCD spectra was set to 17,500 at m/z 200 with a maximum ion injection time of 50 ms. Normalized collision energy was set to 28. The S-lens RF level was set at 60 and the capillary temperature was set at 250 °C. Precursor ions with single, unassigned, or six and higher charge states were excluded from fragmentation selection. Statistics analysis was conducted in Perseus using two-sided unpaired Students’ T tests with equal variance. A false discovery rate (FDR) of 0.01 was used to indicate significant hits.

Live cell microscopy

RPE-1 TPS3 shBRCA2 cells were seeded in 8-well cover glass chambers (Lab-Tek-II, Nunc) at 50% confluency. 48 hours prior to plating, cells were treated with doxycycline (0.1 μg/mL). 16 hours prior to imaging, olaparib (0.5 μM) was added where indicated. Novobiocin (POLQ) was added at the start of imaging at a final concentration of 50 μM. DIC images were obtained every 7 minutes over a period of 10 hours using a Nikon Eclipse Ti-E inverted microscope, equipped with a Hamamatsu C11440-22CU digital camera, and 12 V/100 W halogen lamp. In the Z-plane, 5 images were acquired at 1-micron interval. Image analysis was performed using NIS-Elements software.

Electron microscopy analysis of DNA intermediates

Electron microscopy (EM) analysis was performed according to the standard protocol9,10, with modifications. For DNA extraction, cells were lysed in lysis buffer and digested at 50 °C in the presence of Proteinase-K for 2 h. The DNA was purified using chloroform/isooamyl alcohol and precipitated in isopropanol and given 70% ethanol wash and resuspended in elution buffer. Isolated genomic DNA was digested with PvuII HF restriction enzyme for 4 to 5 h. DNA was washed with TE buffer and concentrated using Amicon size-exclusion column. The benzylidemethylalkylammonium chloride (BAC) method was used to spread the DNA on the water surface and then loaded on carbon-coated nickel grids and finally DNA was coated with platinum using high-vacuum evaporator MED 010 (Bal Tec). Microscopy was performed with a transmission electron microscope FEI Talos, with 4 K by 4 K cmos camera. Images were processed and analyzed using the MAPS software (FEI) and Imagej software.

Analysis of whole-genome sequence data

Deletion calls were downloaded from the ICGC Data Portal (https://dcc.icgc.org/releases/release_28/Projects/BRCA-EU). HRD status, as previously assessed by the CHORD algorithm, was acquired from Nguyen et al17. BRCA1/2 mutation status was obtained from Davies et al18. The coordinates of common fragile site were acquired from Georgakilas et al19. FRA1C, FRA5D and FRA13C were excluded as they were fully enveloped within another CFS. Furthermore, only autosomal deletions and CFS were considered. A deletion was only considered as being in a CFS if the entire deletion was positioned within a single CFS. Microhomology was determined by calculating the length of consecutive overlapping nucleotides from the 3’ and 5’ deletion breakpoints. Microhomology of larger equal or to 2 bp was used for analysis.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

The mass spectrometry proteomics data generated in this study have been deposited in the ProteomeXchange Consortium via the PRIDE partner repository with identifier PXD028670. The Strand-seq data have been deposited in the European Nucleotide Archive (ENA) at EMBL-EBI under accession number PRJEB47697. Source data are provided with this paper. Uncropped Western Blots for Fig. 2a are not available. Source data are provided with this paper.

Code availability

HapSCElocatoR can be found at https://github.com/daewoooo/HapSCElocatoR.

References

1. Jackson, S. P. & Bartek, J. The DNA damage response in human biology and disease. Nature 461, 1071–1078 (2009).
2. Ciccia, A. & Elledge, S. J. The DNA damage response: making it safe to play with knives. Mol. Cell https://doi.org/10.1016/j.molcel.2010.09.019 (2010).
3. Lieber, M. R. The mechanism of double-strand DNA break repair by the nonhomologous DNA end-joining pathway. Annu. Rev. Biochem. 79, 181–211 (2010).
4. Wyman, C., Ristic, D. & Kanaar, R. Homologous recombination-mediated double-strand break repair. DNA Repair 3, 827–833 (2004).
5. Sharar, S. K. et al. Embryonic lethality and radiation hypersensitivity mediated by Rad51 in mice lacking Brca2. Nature 386, 804–810 (1997).
6. Yang, H. et al. BRCA2 function in DNA binding and recombination from a BRCA2-DSS1-ssDNA structure. Science 297, 1837–1848 (2002).
7. Sigurdsson, S., Van Komen, S., Petukhova, G. & Sung, P. Homologous DNA pairing by human recombination factors Rad51 and Rad54. J. Biol. Chem. 277, 42790–42794 (2002).
8. Petukhova, G., Stratton, S. & Sung, P. Catalysis of homologous DNA pairing by yeast Rad51 and Rad54 proteins. Nature 393, 91–94 (1998).
9. West, S. C. et al. Resolution of recombination intermediates: mechanisms and regulation. Cold Spring Harb. Symp. Quant. Biol. 80, 103–9 (2015).

10. Svendsen, J. M. et al. Mammalian BTB12/SLX4 assembles a holli-
day junction resolvase and is required for DNA repair. Cell 138, 63–77 (2009).

11. Wyatt, H. D. M., Sarbajna, S., Matos, J. & West, S. C. Coordinated
actions of SLX1-SLX4 and MUS81-EME1 for holli-
day junction resolution in human cells. Mol. Cell 52, 234–247 (2013).

12. Garner, E., Kim, Y., Lach, F. P., Kottemann, M. C. & Smor
gorzewska, A. Human GEN1 and the SLX4-associated nucleases MUS81 and
SLX1 are essential for the resolution of replication-induced holli-
day junctions. Cell Rep. 5, 207–215 (2013).

13. Fekairi, S. et al. Human SLX4 is a holli-
day junction resolvase sub-
unit that binds multiple DNA repair/recombination endonu-
clases. Mol. Cell 52, 221–233 (2013).

14. Castor, D. et al. Cooperative control of holli-
day junction resolution and DNA Repair by the SLX1 and MUS81-EME1 nuclease.
Mol. Cell 52, 111 (2013).

15. Wu, L. & Hickson, I. O. The Bloom
helicase suppresses crossing over during homologous
recombination. Nature https://doi.org/10.1038/nature02253 (2003).

16. Davies, H. et al. HRDetect is a predictor of BRCA1 and BRCA2
deficiency based on mutational signatures. Nat. Med. 23, 517–525 (2017).

17. Jonkers, J. et al. Synergistic tumor suppressor activity of BRCA2
and p53 in a conditional mouse model for breast cancer. Nat.
Genet. 29, 418–425 (2001).

18. Liu, X. et al. Somatic loss of BRCA1 and p53 in mice induces
mammary tumors with features of human BRCA1-mutated basa-
like breast cancer. Proc. Natl Acad. Sci. USA 104, 12111–12116 (2007).

19. Byrski, T. et al. Pathologic complete response rates in young
women with BRCA1-positive breast cancers after neoadjuvant
chemotherapy. J. Clin. Oncol. 28, 375–379 (2010).

20. Silver, D. P. et al. Efficacy of neoadjuvant cisplatin in triple-
negative breast cancer. J. Clin. Oncol. 28, 1145–1153 (2010).

21. Farmer, H. et al. Targeting the DNA repair defect in BRCA mutant
cells as a therapeutic strategy. Nature 434, 917–921 (2005).

22. Bryant, H. E. et al. Specific killing of BRCA2-deficient tumours
with inhibitors of poly(ADP-ribose) polymerase. Nature 434, 913–917 (2005).

23. Murray, J. et al. Trapping of PARP1 and PARP2 by Clinical PARP
Inhibitors. Cancer Res. 72, S588–S99 (2012).

24. Zellweger, R. et al. Rad51-mediated replication fork reversal is a
global response to genotoxic treatments in human cells. J. Cell
Biol. 208, 563–579 (2015).

25. Schoonen, P. M. et al. Progression through mitosis promotes PARP
inhibitor-induced cytotoxicity in homologous recombination-
deficient cancer cells. Nat. Commun. https://doi.org/10.1038/s41467-022-34519-8 (2021).

26. Arnaudeau, C., Lundin, C. & Helleday, T. DNA double-strand
breaks associated with replication forks are predominantly
repaired by homologous recombination involving an exchange
mechanism in mammalian cells. J. Mol. Biol. 307, 1235–1245 (2001).

27. Menissier De Murcia, J. et al. Requirement of poly(ADP-ribose)
polymerase in recovery from DNA damage in mice and in cells.
Proc. Natl Acad. Sci. USA 94, 7303–7307 (1997).

28. Wang, Z. Q. et al. PARP is important for genomic stability but
dispensable in apoptosis. Genes Dev. 11, 2347–2358 (1997).

29. Ito, S., Murphy, C. G., Doubrovina, E., Jasim, M. & Moynahan, M. E.
PARP inhibitors in clinical use induce genomic instability in normal
human cells. PLoS ONE 11, e0159341 (2016).

30. Wilson, D. M. & Thompson, L. H. Molecular mechanisms of sister-
chromatid exchange. Mutat. Res. 616, 11–23 (2007).

31. Sonoda, E. et al. Sister chromatid exchanges are mediated by
homologous recombination in vertebrate cells. Mol. Cell. Biol. 19, 5166–5169 (1999).

32. Sanders, A. D., Falconer, E., Hills, M., Spierings, D. C. J. & Landsdorp,
P. M. Single-cell template strand sequencing by Strand-seq
enables the characterization of individual homologs. Nat. Protoc.
12, 1151–1176 (2017).

33. Falconer, E. et al. DNA template strand sequencing of single-cells
maps genomic rearrangements at high resolution. Nat. Methods 9, 1107–1112 (2012).

34. van Wietmarschen, N. et al. BLM helicase suppresses recombi-
nation at G-quadruplex motifs in transcribed genes. Nat.
Commun. 9, 271 (2018).

35. Evers, B. et al. A high-throughput pharmaceutical screen identifies
compounds with specific toxicity against BRCA2-deficient tumors.
Clin. Cancer Res. 16, 99–108 (2010).

36. Oikawa, A., Tohda, H., Kanai, M., Miwa, M. & Sugimura, T. Inhibitors
of poly(adenosine diphosphate ribose) polymerase induce sister
chromatid exchanges. Biochem. Biophys. Res. Commun. 97, 1311–1316 (1980).

37. Heijink, A. M. et al. BRCA2 deficiency instigates cGAS-mediated
inflammatory signaling and confers sensitivity to tumor necrosis
factor-alpha-mediated cytotoxicity. Nat. Commun. 10, 1 (2019).

38. Panzarino, N. J. et al. Replication gaps underlie BRCA deficiency
and therapy resistance. Cancer Res. 81, 1388–1397 (2021).

39. Cong, K. et al. Replication gaps are a key determinant of PARP
inhibitor synthetic lethality with BRCA deficiency. Mol. Cell 61, 3227 (2021).

40. Paes Dias, M. et al. Loss of nuclear DNA ligation III reverts PARP
inhibitor resistance in BRCA1/S3BP2 double-deficient cells by
exposing ssDNA gaps. Mol. Cell 81, 4692–4708.e9 (2021).

41. Tirman, S., Cybula, E., Quinet, A., Meroni, A. & Vindigni, A. PRIM-
POL ready, set, reprime! Crit. Rev. Biochem. Mol. Biol. https://doi.org/10.1080/09499698.2020.1841089 (2020).

42. Conrad, S., Künzel, J. & Löbrich, M. Sister chromatid exchanges
occur in G2-irradiated cells. Cell Cycle 10, 222–228 (2011).

43. Sonoda, E. et al. Rad51-deficient vertebrate cells accumulate
chromosomal breaks prior to cell death. EMBO J. 17, 598–608 (1998).

44. Huang, F. et al. Identification of specific inhibitors of human RAD51
recombinase using high-throughput screening. ACS Chem. Biol. 6, 628–35 (2011).

45. El-Khamisy, S. F., Masutani, M., Suzuki, H. & Caldecott, K. W. A
requirement for PARP-1 for the assembly or stability of XRC1
nuclear foci at sites of oxidative DNA damage. Nucleic Acids Res.
31, 5526–33 (2003).

46. Maya-Mendoza, A. et al. High speed of fork progression induces DNA
replication stress and genomic instability. Nature 559, 279–284 (2018).

47. Bryant, H. E. et al. PARP is activated at stalled forks to mediate
Mre11-dependent replication restart and recombination. EMBO J. 28, 2601–2615 (2009).

48. Clauedin, C. et al. Genome-wide mapping of sister chromatid
exchange events in single yeast cells using strand-seq. Elife 6, e30560 (2017).

49. Georgakilas, A. G. et al. Are common fragile sites merely structural
domains or highly organized ‘functional’ units susceptible to
oncogenic stress? Cell. Mol. Life Sci. 71, 4519–4544 (2014).

50. Schoonen, P. M. et al. Premature mitotic entry induced by ATR
inhibition potentiates olaparib inhibition-mediated genomic
instability, inflammatory signaling, and cytotoxicity in BRCA2-
deficient cancer cells. Mol. Oncol. 13, 2422–2440 (2019).
51. Deng, L. et al. Mitotic CDK promotes replisome disassembly, fork breakage, and complex DNA rearrangements. Mol. Cell 73, 915–929.e6 (2019).

52. Orthein, A. et al. Mitosis inhibits DNA double-strand break repair to guard against telomere fusions. Science 344, 189–93 (2014).

53. Benada, J., Burdová, K., Lidák, T., von Morgen, P. & Macurek, L. Polo-like kinase 1 inhibits DNA damage response during mitosis. Cell Cycle 14, 219–31 (2015).

54. Leimbacher, P.-A. et al. MDC1 Interacts with TOPBP1 to Maintain Chromosomal Stability during Mitosis. Mol. Cell 74, 571–583 (2019).

55. Adam, S. et al. CIP2A is a prime synthetic-lethal target for BRCA-deficient cells. Cell Synth 35, 1–19 (2021).

56. Zhou, J. et al. A synthetic lethal strategy identifies MDC1 as a pivotal target for BRCA-deficient cells. Cell Synth 35, 1–19 (2021).

57. Nguyen, L. J. W. M. Martens, Van Hoeck, A. & Cuppen, E. Pan-cancer landscape of homologous recombination deficiency. Nat. Commun. 11, 5584 (2020).

58. Macheret, M. et al. High-resolution mapping of mitotic DNA synthesis regions and common fragile sites in the human genome through direct sequencing. Cell Res. https://doi.org/10.1038/s41422-020-0388-x (2020).

59. Nik-Zainal, S. et al. Landscape of somatic mutations in 560 breast cancer whole-genome sequences. Nature 534, 47–54 (2016).

60. Schimmel, J., Kool, H., Schendel, R. & Tijsterman, M. Mutational signatures of non-homologous and polymerase theta-mediated end-joining in embryonic stem cells. EMBO J. 36, 3634–3649 (2017).

61. Smiraldo, P. G., Gruver, A. M., Osborn, J. C. & Pittman, D. L. Extensive chromosomal instability in Rad51d-deficient mouse cells. Cancer Res. 65, 2098–2096 (2005).

62. Polato, F. et al. CtIP-mediated resection is essential for viability and can operate independently of BRCA1. J. Exp. Med. 211, 1027–36 (2014).

63. Dronkert, M. L. G. et al. Mouse RAD54 affects DNA double-strand break repair and sister chromatid exchange. Mol. Cell. Biol. 20, 3147–3156 (2000).

64. Ishii, Y. & Bender, M. A. Effects of inhibitors of DNA synthesis on spontaneous and ultraviolet light-induced sister-chromatid exchanges in Chinese hamster cells. Mutat. Res. 79, 19–32 (1980).

65. Ray Chaudhuri, A. et al. Replication fork stability confers chemo-resistance in BRCA-deficient cells. Nature 535, 382–7 (2016).

66. Lambert, S. & Lopez, B. S. Role of RAD51 in sister-chromatid exchanges in mammalian cells. Oncogene 20, 6627–6631 (2001).

67. Tutt, A. et al. Mutation in Brca2 stimulates error-prone homology-directed repair of DNA double-strand breaks occurring between repeated sequences. EMBO J. 20, 4704–4716 (2001).

68. Takata, M. et al. Chromosome instability and defective recombinational repair in knockout mutants of the five Rad51 paralogs. Mol. Cell. Biol. 21, 2858–2866 (2001).

69. Slade, D. PARP and PARP inhibitors in cancer treatment. Genes Dev. 34, 360–394 (2020).

70. Bhowmick, R., Minocherhomji, S. & Hickson, I. D. RAD52 facilitates mitotic DNA synthesis following replication stress. Mol. Cell 64, 1117–1126 (2016).

71. Quinet, A. et al. PRIMPOL-mediated adaptive response suppresses replication fork reversal in BRCA-deficient cells. Mol. Cell 77, 461–474.e9 (2020).

72. Wu, R. A., Pettman, D. S. & Walter, J. C. The ubiquitin ligase TRAP1: double-edged sword at the replisome. Trends Cell Biol. 31, 75–85 (2021).

73. Wild, P. et al. Network rewiring of homologous recombination enzymes during mitotic proliferation and meiosis. Mol. Cell 75, 859–874.e4 (2019).

74. Calzetta, N. L., Besteiro, M. A. G. & Gottifredi, V. Mus81–Eme1–dependent aberrant processing of DNA replication intermediates in mitosis impairs genome integrity. Sci. Adv. 6, eabc8257 (2020).

75. Garcia-Luis, J. & Machín, F. Mus81-Mms4 and Yen1 resolve a novel anaphase bridge formed by noncanonical Holliday junctions. Nat. Commun. 5, 5652 (2014).

76. Di Marco, S. et al. REQS helicase cooperates with MUS81 endonuclease in processing stalled replication forks at common fragile sites during mitosis. Mol. Cell 66, 658–671 (2017).

77. Fugger, K. et al. Targeting the nucleotide salvage factor DNPH1 sensitizes BRCA-deficient cells to PARP inhibitors. Science 372, 156–165 (2021).

78. Zimmermann, M., Lottersberger, F., Buonomo, S. B., Sfeir, A. & De Lange, T. S3BP1 regulates DSBr repair using Rif1 to control S′-end resection. Science 339, 700–704 (2013).

79. Chan, Y. W. & West, S. C. Spatial control of the GEN1 Holliday junction resolvase ensures genome stability. Nat. Commun. 5, 4844 (2014).

80. Llorens-Agost, M. et al. POLβ-mediated end joining is restricted by RAD52 and BRCA2 until the onset of mitosis. Nat. Cell. Biol. https://doi.org/10.1038/s41556-021-00764-0 (2021).

81. Feng, W. et al. Genetic determinants of cellular addiction to DNA polymerase theta. Nat. Commun. 10, 4286 (2019).

82. Durkin, S. G. & Glover, T. W. Chromosome fragile sites. Annu. Rev. Genet. https://doi.org/10.1146/annurev.genet.41.042007.165900 (2007).

83. Glover, T. W., Wilson, T. E. & Artl, M. F. Fragile sites in cancer: more than meets the eye. Nat. Rev. Cancer 17, 489–501 (2017).

84. Stok, C., Kok, Y. P., Van Den Tempel, N. & Van Vught, M. A. T. M. Shaping the BRCAnes mutational landscape by alternative double-strand break repair, replication stress and mitotic aberrations. Nucleic Acids Res. 49, 4239–4257 (2021).

85. Mateos-Gomez, P. A. et al. Mammalian polymerase theta promotes alternative-NHEJ and suppresses recombination. Nature 518, 254–257 (2015).

86. Cecchaldi, R. et al. Homologous-recombination-deficient tumours are dependent on Polβ-mediated repair. Nature 518, 258–262 (2015).

87. Mengwasser, K. E. et al. Genetic screens reveal FEN1 and APEX2 as BRCA2 synthetic lethal targets. Mol. Cell 73, 885–899 (2019).

88. Zatreanu, D. et al. Polθ-mediated repair. Nature 579, 47–50 (2017).

89. Sotiriou, S. K. et al. Mammalian RAD52 functions in break-induced replication repair of collapsed DNA replication forks. Mol. Cell 64, 1127–1134 (2016).

90. Kramara, J., Osia, B. & Malkova, A. Break-induced replication: the where, the why, and the how. Trends Genet. 34, 518–531 (2018).

91. Minocherhomji, S. et al. Replication stress activates DNA repair synthesis in mitosis. Nature 528, 286–90 (2015).

92. Mocanu, C. et al. DNA replication is highly resilient and persistent under the challenge of mild replication stress. Cell Rep. 39, 110701 (2022).

93. Helleday, T. The underlying mechanism for the PARP and BRCA synthetic lethality: Clearing up the misunderstandings. Mol. Oncol. 5, 387–393 (2011).

94. Fugger, K. et al. Targeting the nucleotide salvage factor DNPH1 sensitizes BRCA-deficient cells to PARP inhibitors. Science 372, 156–165 (2021).

95. Evers, B. et al. Selective inhibition of BRCA2-deficient mammary tumor cell growth by AZD2281 and cisplatin. Clin. Cancer Res. 14, 3916–3925 (2008).

96. Heijink, A. M. et al. A haploid genetic screen identifies the G1/S regulatory machinery as a determinant of Wee1 inhibitor sensitivity. Proc. Natl Acad. Sci. USA 112, 15160–5 (2015).
van Vugt, M. A. T. M. et al. A mitotic phosphorylation feedback network connects Cdk1, PIlk1, 53BP1, and Chk2 to inactivate the G2/M DNA damage checkpoint. PLoS Biol. 8, e1000287 (2010).

Kok, Y. P. et al. Overexpression of Cyclin E1 or Cdc25A leads to replication stress, mitotic aberrancies, and increased sensitivity to replication checkpoint inhibitors. Oncogenesis 9, 88 (2020).

Perry, P. & Evans, H. J. Cytological detection of mutagenic carcinogen exposure by sister chromatid exchange. Nature 258, 121–5 (1975).

Bakker, B. et al. Single-cell sequencing reveals karyotype heterogeneity in murine and human malignancies. Genome Biol. 17, 115 (2016).

Klaibauer, G. et al. MOPS: mixture of Poissons for discovering copy number variations in next-generation sequencing data with a low false discovery rate. Nucleic Acids Res. 40, e69 (2012).

Murray, A. W. Cell cycle extracts. Methods Cell Biol. 36, 581–605 (1999).

Blow, J. J. Preventing re-replication of DNA in a single cell cycle: evidence for a replication licensing factor. J. Cell Biol. 122, 993–1002 (1993).

Budzowska, M., Graham, T. G. W., Sobeck, A., Waga, S. & Walter, J. C. Regulation of the Rve1-pol ε complex during bypass of a DNA interstrand cross-link. EMBO J. 34, 1971–85 (2015).

Larsen, N. B. et al. Replication-coupled DNA-protein crosslink repair by SPRTN and the proteasome in Xenopus egg extracts. Mol. Cell 73, 574–588.e7 (2019).

Neelsen, K. J., Chaudhuri, A. R., Follonier, C., Herrador, R. & Lopes, M. Visualization and interpretation of eukaryotic DNA replication intermediates in vivo by electron microscopy. Methods Mol. Biol. 1094, 177–208 (2014).

Acknowledgements

This work was supported by grants from the Netherlands Organization for Scientific Research (NWO-VIDI 917.13334 to M.A.T.M.v.V. and Gravitation program ‘CancerGenomiCs’ to P.K.), the European Research Council (ERC-Consolidator grant #681572 ‘TENSION’ to M.A.T.M.v.V.), ERIBA-UMCG funding to A.M.H., P.L. and M.A.T.M.v.V. We thank Jos Jonkers, Thijn Brummelkamp and Shunichi Takeda for sharing reagents. ERIBA-UMCG funding to A.M.H., P.L. and M.A.T.M.v.V. We thank Jos Jonkers, Thijn Brummelkamp and Shunichi Takeda for sharing reagents. We thank members of the Medical Oncology Department and ERIBA for feedback on the manuscript.

Author contributions

A.M.H., P.L., and M.A.T.M.v.V. conceived the project. A.M.H., C.S., Y.P.K., M.E., R.H.d.B., A.A., F.J.B., and E.W. conducted cell biological experiments. A.A., R.H.d.B., and P.K. coordinated and performed mass spec analyses using Xenopus egg extracts. M.T. provided reagents. D.C.J.S. coordinated sequencing analyses. D.P. and V.G. performed bioinformatics analysis. J.K.d.K., M.T., and R.v.B. analyzed WGS tumor data. E.M.M. and A.R.C. conducted and analyzed ssDNA and EM analyses. A.M.H., C.S., and M.A.T.M.v.V. wrote the manuscript. All authors provided feedback on the manuscript.

Competing interests

M.A.T.M.v.V. has acted on the scientific advisory board of RepareTx, which is unrelated to this work. The other authors declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41467-022-34519-8.

Correspondence and requests for materials should be addressed to Peter M. Lansdorp or Marcel A. T. M. van Vugt.

Peer review information Nature Communications thanks the anonymous reviewers for their contribution to the peer review of this work. Peer reviewer reports are available.

Reprints and permissions information is available at http://www.nature.com/reprints

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2022

3Department of Medical Oncology, University Medical Center Groningen, University of Groningen, the Netherlands, 9713GZ Groningen, the Netherlands.
2European Institute for the Biology of Ageing, University Medical Center Groningen, University of Groningen, 9713GZ Groningen, the Netherlands.
3Department of Genome Sciences, University of Washington School of Medicine, Seattle, WA 98195, USA. 4Department of Molecular Genetics, Erasmus MC Cancer Institute, Erasmus University Medical Center, 3000 CA Rotterdam, the Netherlands. 5Princess Máxima Center for Pediatric Oncology, 3584 CS Utrecht, the Netherlands. 6Oncode Institute, 3521 AL Utrecht, the Netherlands. 7Department of Human Genetics, Leiden University Medical Center, 2333 ZC Leiden, the Netherlands. 8Hubrecht Institute-KNAW and University Medical Center Utrecht, Utrecht, the Netherlands. 9Terry Fox Laboratory, BC Cancer Agency, Vancouver, BC V5Z 1L3, Canada. 10Department of Medical Genetics, University of British Columbia, Vancouver, BC V6T 1Z4, Canada. 11Present address: Department of Genetics, University Medical Center Groningen, University of Groningen, the Netherlands, 9713GZ Groningen, the Netherlands.
12Department of Medical Genetics, University of British Columbia, Vancouver, BC V6T 1Z4, Canada. 13These authors contributed equally: Anne Margriet Heijink, Colin Stok. E-mail: plansdor@bccrc.ca; m.vugt@umcg.nl.