PARP3 is a promoter of chromosomal rearrangements and limits G4 DNA

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Chromosomal rearrangements are essential events in the pathogenesis of both malignant and nonmalignant disorders, yet the factors affecting their formation are incompletely understood. Here we develop a zinc-finger nuclease translocation reporter and screen for factors that modulate rearrangements in human cells. We identify UBC9 and RAD50 as suppressors and 53BP1, DDB1 and poly(ADP)ribose polymerase 3 (PARP3) as promoters of chromosomal rearrangements across human cell types. We focus on PARP3 as it is dispensable for murine viability and has druggable catalytic activity. We find that PARP3 regulates G quadruplex (G4) DNA in response to DNA damage, which suppresses repair by nonhomologous end-joining and homologous recombination. Chemical stabilization of G4 DNA in PARP3−/− cells leads to widespread DNA double-strand breaks and synthetic lethality. We propose a model in which PARP3 suppresses G4 DNA and facilitates DNA repair by multiple pathways.
Chromosomal rearrangements, including translocations, are essential events in the pathogenesis of malignant and nonmalignant disorders\(^1\)\(^-\)\(^3\). Both germline defects in DNA repair and certain clastogens increase the risk for oncogenic rearrangements that drive tumour progression and therapeutic resistance\(^4\)\(^,\)\(^5\).

Rearrangements are believed to occur when two DNA double-strand breaks (DSBs) fuse inappropriately. Because DSBs pose a serious threat to genomic stability, multiple mechanisms exist to repair them. Nonhomologous end-joining (NHEJ) pathways repair DSBs throughout the cell cycle by direct re-ligation of the broken ends; nucleotides may be lost or added at the broken ends before ligation, resulting in sequence modifications. NHEJ pathways include classical NHEJ (c-NHEJ), which is essential for ionizing radiation (IR) resistance and V(D)J recombination, as well as one or more pathways of alternative NHEJ. In contrast to NHEJ, homologous recombination (HR) repairs DSBs during S and G2 phases by copying a portion of the sister chromatid to bridge the lesion.

Because they largely result from the fusion of nonhomologous sequences, chromosomal rearrangements are thought to primarily occur through NHEJ. Indeed, a recent study demonstrated that loss of XRCC4-LIG4, the essential ligase complex involved in c-NHEJ, reduces translocation formation between endonuclease-induced DSBs in human cells\(^6\).

Chromosomal rearrangements between endonuclease-induced DSBs occur in Saccharomyces cerevisiae at frequencies of up to 50% (ref. 7). These high rates have allowed for agnostic strategies to identify factors that modulate rearrangements\(^8\)\(^-\)\(^10\). In contrast, rearrangements in mammalian cells between endonuclease-induced DSBs are much less frequent, which has precluded an effort to screen for modulators. As a result, only a small number of c-NHEJ and alternative NHEJ factors are known to affect rearrangements\(^6\)\(^,\)\(^11\)\(^-\)\(^15\). We developed a tractable strategy for inducing and quantifying targeted translocations across a range of human cell types. Using this approach, we perform a medium-throughput loss-of-function screen to identify factors that promote or suppress chromosomal rearrangements in human cells.

**Results**

A screen for modulators of chromosomal rearrangements. Previous approaches to induce targeted translocations in human cells have utilized PCR to quantify frequency\(^6\), which precluded the use of pooled loss- or gain-of-function screens. To facilitate screening for genetic factors that modulate chromosomal rearrangements, we established a flow cytometry-based zinc-finger nuclease translocation reporter (ZITR) assay by randomly integrating either a GFP or CD4 transgene downstream of sequence from the AAVS1 locus (Fig. 1a; Supplementary Fig. 1a). Cleavage of the integrated AAVS1 sequence by zinc-finger nucleases (ZFNs)\(^16\) and cleavage at the endogenous AAVS1 locus on chr.19 followed by rearrangement between the heterologous DSBs resulted in transgene expression in 0.5–1.5% of cells (Fig. 1b; Supplementary Fig. 1b,c).

We performed a short hairpin RNA (shRNA) screen targeting 169 DNA repair-associated genes with 5 shRNA/gene plus controls (Fig. 1b–d; Supplementary Data 1). A549 lung adenocarcinoma cells carrying a GFP- or CD4 reporter were transduced with individual shRNAs at high multiplicity of infection and then pooled. After 7 days, ZFNs were delivered by adenovirus and transgene-positive cells were flow-sorted 48 h later. In total, we performed 7 replicates (3 GFP-reporter and 4 CD4-reporter) with 2.5 × 10\(^7\) shRNA-expressing cells per replicate. We quantified the representation of individual shRNAs within transgene-positive and transgene-negative populations by massively parallel sequencing and compared the abundance of each shRNA in these two populations.

Of the 169 genes, 5 merited further consideration, based on ≥ 2 shRNAs with ≥ 2 fold change in the same direction compared to controls, without causing significant toxicity (Fig. 1e; Supplementary Fig. 2a; Supplementary Data 2). It is likely that additional factors assayed in the screen are capable of affecting translocation frequency but did not meet our threshold for further consideration because of either incomplete gene suppression (for example, KU70; Supplementary Fig. 2b) or excessive toxicity from the shRNA (for example, MRE11 or RFC1; Supplementary Fig. 2c).

To validate the five hits in a separate lineage, we performed the ZITR assay in HeLa cervical adenocarcinoma cells and confirmed that all five hits affected rearrangement frequency as expected. Specifically, RAD50 and UBC9 suppressed rearrangements, while 53BP1, DDB1 and poly(ADP)ribose polymerase 3 (PARP3) promoted them (Fig. 2a–e). In validation, all 5 (100%) genes met the criteria of ≥ 3 shRNAs resulting in the predicted phenotype (Fig. 2) without causing significant changes in cell cycle distribution (Supplementary Fig. 3). We observed striking correlations between the extent of knockdown and fold effect on rearrangement frequency, with R\(^2\) > 0.5 for 3 of the 5 factors (Fig. 2). Thus, multiple factors from diverse protein families have dose-dependent effects on chromosomal rearrangements.

Among the five factors, rad50 (refs 8–10) and ubc9 (ref. 17) were previously identified as suppressors of translocations in yeast. 53BP1 is known to promote long-range end-joining events, including class switch recombination\(^18\). However, DDB1 and PARP3 have not been previously implicated as promoters of chromosomal rearrangements.

**PARP3 promotes rearrangements across lineages and reporters.** We elected to focus on PARP3, as it has druggable catalytic activity\(^19\) and is dispensable for murine and cellular viability\(^20\)\(^,\)\(^21\).

The PARP family of 17 proteins contains 3 enzymes (PARP1, PARP2 and PARP3) that have been implicated in DNA repair. PARP3 is known to cooperate in the recruitment of DNA repair factors, including mediators of c-NHEJ, and PARP3 loss delays the repair of DSBs\(^22\)\(^,\)\(^23\). A recent study catalogued PARylation targets of PARP1, PARP2 and PARP3; PARP3 had the highest number of total targets and only ~10% of these were shared with the other PARP enzymes\(^24\), indicating unique but poorly elucidated cellular roles for PARP3.

Three different PARP3 shRNAs reduced chromosomal rearrangement frequency between 60 and 70% in HeLa cells (Fig. 3a,b). We considered multiple ways in which this observation could be the result of a confounding effect from PARP3 knockdown. Importantly, PARP3 knockdown did not affect either: (1) the extent of cleavage at the targeted locus 18 h after ZFN transduction, (2) cell cycle distribution, (3) the number of basal DSBs (that is, without ZFN transduction), or (4) the ability of IR to induce cell cycle checkpoint activation (Fig. 3c; Supplementary Fig. 4a–c). PARP3 re-expression after depletion with an untranslated region-directed shRNA rescued the shRNA effect on rearrangements (Fig. 3d,e). Notably, overexpression of PARP3 did not further increase rearrangement frequency above the repair of cells transduced with control shRNA (Fig. 3d,e).

To test the effects from complete PARP3 loss, we generated PARP3\(^{-/-}\) A549 cells (Supplementary Fig. 4d) carrying the AAVS1 ZITR. As expected, re-expression of wild-type PARP3 in these cells increased the frequency of ZFN-induced rearrangements (Fig. 3f,g).

Next, we devised a CRISPR translocation reporter (CRITR) assay in unmodified 293T human embryonic kidney cells by targeting CAS9-mediated DSBs downstream of the constitutively
expressed CD71 promoter on chr.3 and upstream of the non-expressed CD4-coding sequence on chr.12. Translocation between these DSBs results in aberrant CD4 expression on der(3) (Fig. 3h,i). PARP3/C0/cells had significantly reduced translocation frequency compared to wild-type cells (Fig. 3k,l). As with the ZFNs, loss of PARP3 did not affect the extent of cleavage by CAS9 at the target site (Fig. 3m).

PARP3 knockdown had a similar effect on translocations to PARP3 deletion (Supplementary Fig. 4e,f). We also generated 293T cells lacking either 53BP1 or LIG4. These cells had a similar decrease in translocation frequency using the CRITR as PARP3−/− cells (Fig. 3k–o); the reduced frequency of translocations was in LIG4−/− cells was previously shown using a PCR-based assay6.

PARP3 is known to localize to laser-induced DNA damage20. To confirm that PARP3 localizes to a single, targeted DSB, we used CAS9-based transgenesis to knock-in a haemagglutinin (HA) tag at the endogenous PARP3 locus. Chromatin immunoprecipitation (ChIP) using an anti-HA antibody confirmed that PARP3 accumulates at sequences flanking a CAS9-mediated DSB (Fig. 3p; Supplementary Fig. 4g).

**Figure 1 | Identification of promoters and suppressors of chromosomal rearrangements in human cells.** (a) Flow cytometry-based assay for chromosomal rearrangements. Scissors, AAVS1 zinc-finger nucleases (ZFNs). Dotted lines, targeted cutting at AAVS1 recognition sequence upstream of either GFP or CD4. (b) Design of shRNA rearrangement screen. A549 cells harbouring a randomly integrated GFP or CD4 reporter were transduced clonally with 1 of 966 shRNA, pooled and 2.5 × 10^7 shRNA-expressing cells were infected with AAVS1 ZFN adenovirus, and flow-sorted by transgene expression. (c) Representation of different pathways in the shRNA library of 169 genes. (d) Representation of different protein functions in the shRNA library of 169 genes. (e) Gene hits identified in the shRNA screen. Average of log_2 reads in transgene-positive population/average log_2 reads in transgene-negative population for GFP replicates (x axis) and CD4 replicates (y axis).
PARP3 loss sensitizes cells to pyridostatin. To further explore the mechanism of PARP3 in mediating DSB repair, we next determined the sensitivity of PARP3−/− A549 cells to genotoxins with distinct effects on DNA. PARP3−/− A549 cells were not significantly sensitive to IR (Fig. 4a), as previously shown25 and were only slightly sensitive to etoposide and bleomycin at higher concentrations (Fig. 4b,c), as previously shown21.

In contrast, PARP3−/− cells were profoundly sensitive to pyridostatin (Fig. 4d) and PhenDC3 (Supplementary Fig. 4h), small molecules that stabilize G quadruplex (G4) DNA. Estimates of the occurrence of G4 DNA in the human genome range from ~375,000 from computational predictions29–31 to >700,000 experimentally observed G4 DNA structures32. G4 DNA can obstruct replication and repair33–36. In fact, pyridostatin leads to cell cycle arrest by inducing replication- and transcription-dependent DNA damage that preferentially, but not exclusively, occurs within gene bodies enriched for G-quadruplex forming sequences37. Although pyridostatin is an imperfect tool for studying G4 DNA, two recent studies have reported clear enrichments for G4 motifs at sites of pyridostatin-induced damage37 and pyridostatin binding37. Therefore, we used pyridostatin as a tool to preferentially target regions that are enriched for G4 DNA. Furthermore, the observation that PARP3−/− cells are also sensitive to PhenDC3, a structurally distinct G4 DNA ligand, lends support to the hypothesis that...
PARP3−/− cells are preferentially susceptible to stabilization of G4 DNA.

At a concentration of 2.5 μM, pyridostatin reduced colony formation of wild-type A549 cells by < 1 log10 but completely eliminated colony formation of PARP3−/− cells (Fig. 4d). The pyridostatin sensitivity of PARP3−/− cells was complemented by re-expression of PARP3 (Supplementary Fig. 4i,j). Knockdown of PARP3 with short interfering RNA (siRNA) resulted in

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**Graphs and Figure Legends**

- **a**: Rearrangements in HeLa

- **b**: PARP3 qRT-PCR

- **c**: Cleavage

- **d**: Rearrangements

- **e**: PARP3 qRT-PCR

- **f**: A549 PARP3−/−

- **g**: PARP3 and β-actin

- **h**: Chr3, Chr12, der(3)

- **i**: CD4-PE

- **j**: GFP

- **k**: 293T qRT-PCR

- **l**: qRT-PCR

- **m**: Cleavage

- **n**: LIG4

- **o**: S3BP1

- **p**: HA-PARP3 occupancy

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**Tables and Data**

- **Distance in bp from breaksite**: The table shows distances in base pairs from the breaksite with various levels of significance.

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**Supplementary Material**

- Supplementary Fig. 4i,j: Additional experiments showing the complementation of pyridostatin sensitivity by PARP3 re-expression.

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**References**

- [1] Nature Communications 8:15110, DOI: 10.1038/ncomms15110

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**Notes**

- The paper discusses the role of PARP3 in the stabilization of G4 DNA structures and how this is affected by pyridostatin and PARP3 knockdown.

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pyridostatin sensitivity that was similar to the PARP3−/− cells (Fig. 4e; Supplementary Fig. 4k). In contrast, siRNA knockdown of either PARP1 or PARP2 did not increase sensitivity to pyridostatin (Fig. 4e).

A recent study demonstrated that PARP1 can influence transcription38, raising the possibility that the DNA repair abnormalities we observed are related to alterations in transcription from loss of PARP3. To agnostically assess transcriptional effects, we performed RNA-sequencing (RNA-seq) of wild-type and PARP3−/− cells in the presence or absence of pyridostatin. We found that only 61 genes were significantly differentially expressed between wild-type and PARP3−/− cells, which identified only 64 differentially expressed genes (Supplementary Table 4; Supplementary Fig. 7). These genes were highly overlapping with those identified in the absence of pyridostatin, suggesting relatively minor differences in the transcriptional response to pyridostatin across genotypes. Again, neither gene ontology enrichment analysis nor KEGG pathway analysis of differentially expressed genes following pyridostatin treatment revealed any pathways that might explain the observed phenotype (Supplementary Figs 6b and 8). Thus, and in contrast with PARP1, PARP3 does not appear to serve a major regulatory role in transcription, at least within our experimental system.

To test whether the effect on pyridostatin resistance depends on PARP3 catalytic activity, we treated wild-type cells with ME0328, a PARP3-specific inhibitor that inhibits PARP3 with sevenfold greater potency compared with PARP1 (ref. 19). ME0328 (3 μM) did not elicit any cytotoxicity (Supplementary Fig. 9a), but the same concentration sensitized cells to pyridostatin (Fig. 4f). Treatment with the PARP inhibitor KU0058948 at 500 nM concentration (half-maximal inhibitory concentration for PARP1 3.4 nM; PARP2 ~6 nM, PARP3 85 nM)24,25 conferred the same sensitization to pyridostatin as ME0328 (Fig. 4f). Taken together, these results suggest a unique function for PARP3 in the regulation of G4 DNA.

The BLM helicase is known to bind and unwind G4 DNA in vitro46–47. In addition, BLM facilitates replication through G4 DNA in telomeric sequences in vivo48,49. However, the role of BLM in promoting DSB repair specifically within regions that harbour abundant G4 DNA remains unclear. On the basis of the in vitro data, one possibility is that BLM facilitates the repair of DSBs within G4-rich regions by unwinding secondary DNA structures. We examined the recruitment of BLM to a CRISPR-CAS9-targeted DSB in the CD4 locus by ChiP. We selected the CD4 locus because two different in silico algorithms (QGRS and Quadbase2) predicted that the CD4 locus contains abundant guanine-rich repeats predicted to form G4 DNA (Fig. 4g)49–51. Furthermore, a genome-wide sequencing-based study reported a high concentration of G4 DNA at this locus (Fig. 4h)52.

After induction of a DSB at the CD4 locus, BLM recruitment was significantly increased in PARP3−/− cells by ChiP relative to wild-type cells (Fig. 4i). One possible explanation for this finding is that BLM is recruited to unwind G4 DNA that is more abundant in PARP3−/− cells. We validated the ChiP signal given by the BLM antibody by immunoprecipitation (IP) followed by western blot (Supplementary Fig. 9b) and by using an siRNA specificity control (Supplementary Fig. 9c). Consistent with the finding at a single DSB, overall chromatin-bound BLM was increased in PARP3−/− cells in the absence of exogenous damage (Supplementary Fig. 9i). If recruitment of BLM in PARP3−/− cells is dependent on the presence of G4 DNA, it would follow that PARP3 status would not influence the recruitment of BLM to a CRISPR-CAS9-targeted DSB at a locus with minimal G4 DNA. In contrast to the CD4 locus, the oestrogen receptor 1 (ESR1) locus is predicted and observed to contain relatively little G4 DNA (Fig. 4i). As predicted, we found that there was no significant difference between the recruitment of BLM to a DSB in ESR1 between wild-type and PARP3−/− cells (Fig. 4j).

A recent study described h2, an engineered single-chain antibody specific for G4 DNA52. To validate h2 at sites of enriched G4 DNA in our experimental system, we tested loci previously characterized for G4 DNA content: (1) telomeric DNA features the most abundant G4 DNA in the cell, (2) the MYC promoter is known to contain abundant G4 DNA53,54 (Supplementary Fig. 9b), as mentioned above, (3) the ESR1 enhancer locus55 (Fig. 4i) and (4) two loci on chromosomes 8 and 22 that are predicted and observed to contain very little G4 DNA (Supplementary Fig. 9i). Following IP with h2 using pyridostatin-treated 293T cells, we observed no enrichment at the ESR1, chr8 and chr22 loci, but significant enrichments at the MYC promoter and at telomeres in the absence of DNA damage (Fig. 4k). We therefore measured the G4 DNA content at the CD4 locus following induction of a targeted DSB following treatment with pyridostatin. G4 DNA was enriched to a greater extent in PARP3−/− cells compared to wild-type cells at sites flanking the DSB (Fig. 4l). In contrast, at the ESR1 locus, we did not observe any enrichment of G4 DNA in PARP3−/− cells following a targeted DSB (Supplementary Fig. 10a). These data are consistent with a role for PARP3 in negatively regulating G4 DNA induced by a DSB.

PARP3−/− cells are susceptible to pyridostatin-induced DSBs. On the basis of the increased sensitivity of PARP3−/− cells to pyridostatin, we hypothesized that ligand-stabilized G4 structures would result in more extensive DSBs. To test this, we performed immunofluorescence (IF) to quantify γH2AX and 53BP1 foci following pyridostatin treatment. PARP3−/− cells exhibited significantly more γH2AX (Fig. 5a) and 53BP1 foci (Supplemen-

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**Figure 3 | PARP3 promotes chromosomal rearrangements in several human cell types.** (a–c) Normalized frequency of rearrangements (a) compared to control (CT), PARP3 transcript levels (b) and cleavage by the AAV51 ZFns measured by quantitative PCR across the targeted site (c) in HeLa cells transduced with shRNA targeting PARP3, P3, PARP3, (d,e) Normalized frequency of rearrangements (d) and qRT-PCR (e) in HeLa ZITR cells transduced with lentivirus expressing control (CT) shRNA or shRNA targeting PARP3 3′-untranslated region (P3) and expressing control (CT) cDNA or PARP3 cDNA. (fg) Normalized frequency of rearrangements (f) and immunoblots (g) in PARP3−/−/C0 A549 cells with adenovirus-mediated re-expression of wild-type (WT) PARP3. (h,j) Schematic of CRITR assay for translocations between CD71 and CD4 loci (h), flow cytometry (I) and metaphase fluorescence in situ hybridization (j) of CD4+ 293T cells 48 h after transfection of CA59 and gRNAs. Green probe, RP11-436M6, CD71. Red probe, RP11-277E18, CD4. Scale bar, 10 μm. (k-o) Normalized frequency of rearrangements using CRITR assay in wild-type (WT), 53BP1−/−, PARP3−/−, and LIG4−/− 293T cells (k) with qRT-PCR of PARP3 (l), immunoblots of indicated proteins (m,n) and intact locus after expression of CA59 and gRNA measured by quantitative PCR across the targeted site (o). HA-PARP3 occupancy at CD4 18 h after transfection expression of CA59 and gRNA by ChiP, represented as ratio of CA59 with CD4-directed gRNA to CA59 with empty vector (EV) (p), bp, base pairs. Data are presented as mean ± s.e. of n = 3. P values were calculated using unpaired Student’s t-test. *P < 0.05, **P < 0.01, ***P < 0.001.
Figure 4 | PARP3−/− cells are sensitive to the stabilization of G4 DNA (a–d) Colony survival assays using wild-type and PARP3−/− A549 cells with the indicated doses of ionizing radiation (a), etoposide (b), bleomycin (c) and pyridostatin (d). (e) Colony survival assay in wild-type A549 cells with siRNA for control (siCont), PARP1, PARP2 or PARP3 with corresponding immunoblots. (f) Colony survival assay in wild-type A549 cells treated with vehicle, 3.0 μM ME0328 or 500 nM KU58948 and pyridostatin. (g) Schematic of sequences predicted by QGRS (purple) and Quadbase2 (blue) and experimentally observed to form G4 DNA by Chambers et al.32 (green) in a 2,000 bp window surrounding the CD4 CRISPR-CAS9 gRNA. Scissors, CRISPR-CAS9 cut site. (h) BLM occupancy at the CD4 locus measured by ChIP 18 h after transient expression of CAS9 alone or with gRNA targeting CD4. (i) Schematic of sequences G4 DNA sequences depicted as in g in a 2,000 bp window surrounding ESR1 CRISPR-CAS9 gRNA. Scissors, CRISPR-CAS9 cut site. (j) BLM occupancy at the ESR1 locus measured by ChIP 18 h after transient expression of CAS9 alone or with gRNA targeting CD4. (k) G4 DNA content measured by immunoprecipitation (IP) with hf2 antibody following 24 h treatment with vehicle or pyridostatin (PDS) at the telomere (TEL), MYC promoter (MYC), ESR1 enhancer, chr8 or chr22 loci. (l) Quantification of G4 DNA content in the indicated genotypes at the CD4 locus measured by IP with hf2 antibody 24 h after pyridostatin (PDS) treatment and 18 h after transfection with CRISPR-CAS9 with or without gRNA for CD4, bp, base pairs; PDS, pyridostatin. Data are mean ± s.e. of n = 3. P values calculated using unpaired Student’s t-test. *P < 0.05, **P < 0.01, ***P < 0.001. NS, not significant.
PARP3–/– cells are more susceptible to pyridostatin-induced DSBs. (a) Quantification of immunofluorescence (IF) for γH2AX at the indicated time points following treatment with 5 μM pyridostatin (PDS) in wild-type and PARP3–/– A549 cells. (b,c) Quantification of IF for γH2AX (b) and G4 DNA using the H6 antibody (c) at the indicated time points, and immunoblots (d) in PARP3–/– A549 cells infected with adenovirus-expressing PARP3 (ad PARP3) or β-galactosidase (ad β-gal). (e) Cell cycle dynamics for wild-type (WT) or PARP3–/– A549 cells mock treated or treated with 5 μM pyridostatin. Data are presented as mean ± s.e. of n = 3. P values calculated using unpaired Student’s t-test. A two-way analysis of variance (alpha set at 0.05) gave the following P values for cell cycle phases: G1, P = 0.5955, S, P = 0.0152, and G2, P = 0.0111. *P < 0.05, **P < 0.01, ***P < 0.001.

PARP3 promotes CtIP and RPA deposition at DNA DSBs. We hypothesized that the increased prevalence of G4 structures in PARP3-deficient cells would suppress binding by the endonuclease CtIP, which converts DSB ends into single-stranded DNA tails that are intermediates for HR. In fact, the efficiency of HR at a single DSB was reduced in PARP3-depleted cells containing a HR reporter (Supplementary Fig. 10f–h), consistent with a previous report.37. Cutting by CAS9 at the CD4 locus was similar in wild-type and PARP3–/– cells (Fig. 6a), but PARP3–/– cells had less CtIP accumulation flanking the DSB (Fig. 6b). The ChIP signal given by the CtIP antibody was validated by IP (Supplementary Fig. 9d) and siRNA specificity control (Supplementary Fig. 9e). Processing by CtIP facilitates deposition of the single-stranded DNA-binding protein RPA. PARP3–/– cells also had reduced RPA deposition around the targeted DSB (Fig. 6c) but significantly increased γH2AX signal (Fig. 6d). The latter finding is consistent with a report that deletion of PARP3 leads to the persistence of γH2AX foci. In contrast, we did not observe any significant PARP3-dependent differences in CtIP or RPA deposition or γH2AX signal at a targeted DSB in ESRI (Supplementary Fig. 14a–c), where little or no G4 DNA is expected to form.

To further support these findings, we examined RPA occupancy following targeted DSBs at six additional loci: three in G4-rich regions and three in G4-poor regions (Supplementary Fig. 15). We observed that RPA deposition was significantly reduced in PARP3–/– cells near DSBs in G4-rich regions (Supplementary Fig. 15a–c,g–i) but not affected by PARP3 deficiency in G4-poor regions (Supplementary Fig. 15d–e,j–l). To confirm these findings using an orthogonal method, we performed IF microscopy for RPA at 2 h after 10 Gy IR. Compared to wild-type cells, fewer PARP3–/– cells had both γH2AX and RPA foci (Fig. 6e,f). We observed the same reduction in PARP3–/– cells with double-positive foci after synchronizing the cells in S-phase by serum starvation (Fig. 6g; Supplementary Fig. 16a). Thus, the reduction in RPA foci was not attributable to alterations in cell cycle. Together, these data suggest that PARP3 deficiency inhibits CtIP and RPA deposition at DSBs in G4-rich regions, perhaps leading to a delay in repair as evidenced by persistent γH2AX signal.

PARP3 and BLM cooperate to promote NHEJ. Previous studies have demonstrated that PARP3 loss reduces the efficiency of NHEJ21, which delays the repair of IR-induced DNA damage.23. We confirmed the effect of PARP3 deletion on NHEJ between targeted DSBs (Supplementary Fig. 16b–d).55. Because G4 DNA can be a substrate for BLM in vitro and at telomeres,46,48 we reasoned that loss of BLM in PARP3-deficient cells might further suppress the resolution of G4 DNA structures, which could reduce both HR and NHEJ, leading to the persistence of DSBs. We used siRNA to deplete BLM in wild-type and PARP3–/– cells, and measured the kinetics of IR-induced 53BP1 and...
H2AX foci. Deficiency of either PARP3 or BLM decreased the formation of 53BP1 foci between 1 and 6 h after 10 Gy IR (Fig. 7a,c). Knockdown of BLM in PARP3−/− cells further suppressed 53BP1 focus formation after IR (Fig. 7a,c). Knockdown of BLM in PARP3−/− cells also resulted in significantly more γH2AX foci as early as 1 h following 10 Gy compared to cells lacking BLM or PARP3 alone (Fig. 7b,c). In wild-type cells and in cells lacking either BLM or PARP3, 40% or more of γH2AX and 53BP1 were co-localized, compared to <10% of foci in PARP3−/− cells with BLM knockdown (Supplementary Fig. 16e). Together, these data support a model (Fig. 8), in which BLM and PARP3 cooperate to regulate G4 DNA structures and promote DSB repair.

**Discussion**

We performed a medium-throughput screen for genetic factors that modulate the formation of chromosomal rearrangements. We identified and validated two genes that suppress (UBC9 and RAD50) and three genes that promote (53BP1, DDB1 and PARP3) chromosomal rearrangements. The results of our screen are useful both for significantly expanding the catalogue of factors involved in rearrangements in human cells and for demonstrating the feasibility of genetic screening for factors involved in rare chromosomal events. On the basis of our stringent definition of 'hits', all five factors validated in an alternate cell lineage, but the stringent definition likely resulted in multiple false-negatives. Importantly, we intentionally utilized shRNA-based knockdown
rather than CRISPR-based knockout for the screen, as this allowed for the identification of factors required for viability (for example, DDB1) and the assessment of dosage effects.

We examined PARP3 in detail and observed that, as previously reported, PARP3-deficient cells had reduced efficiencies of both HR and NHEJ but relatively little or no sensitivity to traditional DSB-inducing agents\(^21,23\) (Fig. 4a–c; Supplementary Figs 10g,h and 16c,d). This creates a conundrum, as cells must presumably either repair DSBs, arrest or undergo apoptosis. A key insight came from the finding that PARP3-deficient cells are highly sensitive to pyridostatin and PhenDC3, small molecules that preferentially but not exclusively stabilize and damage regions of G4 DNA. This raised the possibility that PARP3-deficient cells are susceptible to G4 DNA-mediated damage. In fact, PARP3\(^{-/-}\) cells had increased G4 DNA compared to wild-type cells at sites flanking DSBs within G4-rich regions. Taken together, these findings suggest that PARP3\(^{-/-}\) cells accumulate more G4 DNA than wild-type cells. Of note, the EJ5 (ref. 55) and DR-GFP\(^56\) reporters used to measure NHEJ and HDR, respectively, contain high concentrations of sequence predicted to form G4 DNA (Supplementary Fig. 16f,g).

We propose a model in which the existence of G4 DNA delays but does not prevent the repair of DSBs in the absence of PARP3 (Fig. 8). There are several aspects of the model that require experimental clarification: (1) As discussed above, the specificity of G4 DNA ligands is imperfect\(^37\). Therefore, it will be important to conclusively define the mechanism of sensitization to pyridostatin and PhenDC3 in the absence of PARP3. The RNA-seq experiments described in Supplementary Figs 5–8 represent an important first step but further experiments are needed. (2) The role of BLM helicase at DNA DSBs within G4-rich regions remains unclear. We have shown that BLM accumulates to a greater extent in the absence of PARP3 at a DNA DSB targeted to a region of abundant G4 DNA.

Figure 7 | PARP3 and BLM cooperate to repair DNA DSBs. (a–c) Quantification of IF for 53BP1 (a) and \(\gamma\)H2AX (b) at the indicated time points in wild-type (WT) or PARP3\(^{-/-}\) A549 cells transfected with siRNA targeting control (siCont) or BLM (siBLM). Data are represented as mean ± s.e. of three biological replicates. \(P\) values calculated using unpaired Student’s \(t\)-test. *\(P < 0.05\), **\(P < 0.01\), ***\(P < 0.001\). (c) Immunoblot after transfection with siRNA targeting control (Ct) or BLM.

Figure 8 | Model of PARP3 regulation of G4 DNA at DNA DSBs. PARP3 negatively regulates G4 DNA after DNA DSBs to facilitate repair of the damage (left). In the absence of PARP3, G4 DNA accumulates at sites of damage and delays repair of DSBs by preventing deposition of repair factors.
potential. Given that BLM has been shown to unwind G4 DNA in vitro, one possibility is that BLM unwinds G4 DNA in the context of DSBs in vivo as well. However, several other functions for BLM have been reported during repair of DSBs including unwinding Holliday junctions, promoting DNA DSB resection by recruiting EXO1 and DNA2 (ref. 58), and blocking recombination by disrupting RAD51 nucleoprotein filaments. Further experiments are required to distinguish between these mechanistic possibilities. (3) Our data showing PARP3-dependent differences in accumulation of G4 DNA and BLM helicase at DNA DSBs is based on profiling of two targeted loci: one with high G4 DNA potential (CD4) and one lacking the potential to form G4 DNA structures (ESR1). Altogether, our data showing PARP3-dependent differences in RPA accumulation assessed eight loci with varying G4 DNA potential; however, this remains a relatively small set of loci. Therefore, to strengthen our model and overcome potential artefacts resulting from comparison of a small number of loci, data from a larger set of loci with varying G4 DNA content is needed. Techniques that will allow profiling of the PARP3 dependency of G4 DNA within a genome-wide manner were recently reported. The combination of these techniques with a larger set of targeted DSBs will provide important future data to refine or even refute our model. (4) Further experiments are needed to determine the extent to which PARP3 plays a role in G4 DNA resolution in the absence of damage, at sites of damage other than DSBs (for example, interstrand crosslinks), and in species other than humans.

Recent studies have begun to shed light on the role of chromatin structure in G4 DNA formation. For example, ATRX, a member of the SWI–SNF nucleosome remodelling complex, binds specifically to G4 DNA. A recent study identified PARP3-specific targets of mono(ADP)riboseylation, including several histones and chromatin-bound factors; among the latter was ATRX. One possible hypothesis generated by our data is that PARP3 modifies chromatin structure to establish an environment that limits G4 DNA. As previously reported, PARP3-deficient cells were not sensitized to IR but were partially sensitized to bleomycin. Bleomycin has been shown to preferentially induce DSBs near DNA secondary structures and in particular in telomeric regions. Therefore, bleomycin could be selectively targeting DNA DSBs to regions with abundant G4 DNA, and thereby confer a slight sensitivity in PARP3-deficient cells. Several additional questions remain unanswered by our findings. First, are the suppression of NHEJ and binding of CIP and RPA by PARP3 loss mechanistically linked to the lower frequency of chromosomal rearrangements in cells lacking PARP3? Although significant, the reduced binding of RPA at the targeted break in the CD4 locus in PARP3 cells was modest and thus bears further exploration in other systems. How does PARP3 catalytic and/or scaffold function mediate the suppression of G4 structures on chromatin in the presence of DNA damage? Finally, does PARP3 inhibition have a therapeutic role, either alone or in combination with G4-stabilizing therapeutics? In fact, G4 DNA structures are enriched in proto-oncogenes such as MYC, suggesting the potential for simultaneously targeting of DNA damage and modulating oncogenic expression. Small molecules that bind G4 structures have shown promise in early studies. Certain malignancies, including those of the stomach and liver, have abundant G4 DNA relative to the surrounding stromal tissue and may therefore be ideal targets for G4 DNA ligands. Selective PARP3 inhibitors could represent a tractable strategy to combine with G4 DNA ligands to exacerbate G4 DNA formation, DSB induction and tumour cell death.

Methods

Cell culture and reagents. A549, HeLa, 293T and U2OS cells were cultured in DMEM (Gibco) supplemented with 10% fetal bovine serum (FBS), and 50 μg ml⁻¹ penicillin and 50 μg ml⁻¹ streptomycin (Gibco). None of the cell lines used in this study are listed in the database of commonly misidentified cell lines maintained by the International Cell Line Authentication Committee. The A549 cell line was a gift from the Bradner Lab at Dana-Farber Cancer Institute and was verified by short tandem repeat (STR) profiling. The HeLa and U2OS cells originated from the Jasin Lab at Memorial Sloan Kettering Cancer Center. The 293T cells were gifts from the Ali Lab at Boston Children’s Hospital. Cells were tested for mycoplasma contamination by the IDEXX Laboratories or using the Lonza Mycoplasma kit. Puromycin (Gibco) selection in A549 and 293T cells was at 1.0 μg ml⁻¹. Hypoxia (B (Invitrogen) selection in A549 cells was at concentration (200 μg ml⁻¹). Cell synchronization of A549 cells was achieved by growing cells to confluence, reducing FBS to 0.1% for 72 h and releasing cells into DMEM with 20% FBS. A549 cells were treated at the indicated concentrations of pyridostatin (Sigma Aldrich), PhenDC3 (Polymedecines), ME0328 (Selleck), KU0058948 (Axon MedChem) or Bleomycin (Santa Cruz Biotechnology) when indicated. All cells were cultured under normal oxygen conditions (21% O₂, 5% CO₂, 37°C).

Preparation of adenovirus. Complementary DNAs (cDNAs) encoding AAVS1 ZFNs 1 and 2 separated by a ribosomal insertion site, AAVS1 ZFN 1 alone, or PARP3 were subcloned into pAd/CMV (Invitrogen) using Gateway cloning (Invitrogen). The resulting shuttle vectors were transfected into 293A cells (Invitrogen) to generate recombinant adenovirus. Adenovirus was concentrated by centrifugation in a caesium chloride gradient. A549 and HeLa cells were routinely showing PARP3-dependent differences in accumulation of G4 DNA and between these mechanistic possibilities. (3) Our data showing PARP3-dependent differences in RPA accumulation assessed eight loci with varying G4 DNA potential; however, this remains a relatively small set of loci. Therefore, to strengthen our model and overcome potential artefacts resulting from comparison of a small number of loci, data from a larger set of loci with varying G4 DNA content is needed. Techniques that will allow profiling of the PARP3 dependency of G4 DNA within a genome-wide manner were recently reported. The combination of these techniques with a larger set of targeted DSBs will provide important future data to refine or even refute our model. (4) Further experiments are needed to determine the extent to which PARP3 plays a role in G4 DNA resolution in the absence of damage, at sites of damage other than DSBs (for example, interstrand crosslinks), and in species other than humans.

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incubator overnight. Twenty-four hours after transduction, cells were cultured in 1.0 μg ml⁻¹ puromycin for 48 h.

Flow cytometry. To avoid cleavage of the CD4 transgene in the A549 rearrangement reporter cells, Cell Stripper (MediaTech Inc.) was used to dissociate the cells. For FACS analysis of the A549 rearrangement CD4 reporter cell line, CD4-PET (VIT4, Miltenyi Biotec) was used at a dilution of 1:500 to stain for 20 min at room temperature.

Cytogenetics. Interphase and metaphase fluorescent in situ hybridization (FISH) was performed with either a break-apart probe at the AAVS1 locus (RP11-1535P5 and RP11-46I13) for interphase FISH or probes for CD4 (RP11-277E18) and CD71 (RP11-436M6) for metaphase FISH. All cytogenetics for this study were performed at the CytoGenomics Core at Brigham and Women’s Hospital.

CRITR assay. Guide RNAs targeting CD4 (5’-CAGAGGTTGCTTTACCTCAG-3’) and CD71 (5’-CTAGATTGATGCAGTCAT-3’) were cloned into pX330 (Addgene 42230), a plasmid that expresses Cas9 and the chimeric guide RNA. Using Lipofectamine 2000, 20 pmol of siRNA.

Real-time qRT-PCR. Total RNA was extracted from cells using the RNeasy Mini Kit (Qiagen). Quantitative PCR with reverse transcription (qRT–PCR) was performed on a CFX96 Real Time System (Bio-Rad) machine using iTaq Universal SYBR Green (Bio-Rad). qRT–PCR conditions were 50 °C for 10 min; 95 °C for 10 min; 39 cycles of 95 °C for 10 s and 60 °C for 20 s. qRT–PCR primers can be found in Supplementary Table 5.

Western blotting. Cells were washed with ice-cold PBS and lysed on ice for 30 min with RIPA lysis buffer supplemented with protease inhibitors (Complete Mini, Roche). Protein concentration was determined using the Bradford Protein Assay kit (Bio-Rad) and a calibration standard curve created from bovine serum albumin standards. The samples were prepared for loading by adding 4 × Laemmli sample buffer (Life Technologies) and heating the samples at 90 °C for 10 min. Total proteins were separated by SDS–polyacrylamide gel electrophoresis on 4–12% Bis-Tris gradient gels (Life Technologies). Proteins in the gel were electrophoretically transferred to the nitrocellulose membrane (Bio-Rad) and then the membrane was blocked in 5% milk with Tris-Buffered Saline Triton X-100 buffer (100 mM Tris, pH 7.4, 500 mM NaCl, 0.1% Triton X-100; TBST). Membranes were incubated with primary antibodies in 5% milk in TBST overnight at 4 °C. Horseradish peroxidase-conjugated secondary antibody incubation was performed for 2 h at room temperature in 5% milk in TBST and signals were visualized by enhanced chemiluminescence (ECL) (Perkin Elmer). Whole portions of western blots are included in Supplementary Fig. 17.

Immunofluorescence. At the indicated times after irradiation, cells were pre-extracted with 25 mM HEPES, pH 7.4, 50 mM NaCl, 1 mM EDTA, 3 mM MgCl₂, 300 mM sucrose and 0.5% Triton X-100 for 5 min on ice. Cells were fixed with 4% paraformaldehyde for 12 min at room temperature, washed twice with PBS with 1% FBS for blocking and co-stained for 16 h at 4 °C with γH2AX S139 (20E3, Cell Signaling) and RPA32 (A300-244A, Bethyl Labs) at dilutions of 1:500, mouse anti-DNA-G quadruplex (G4), (1H6, EMD Millipore; dilution of 1:500, rabbit anti-BLM (A300-110, Bethyl Labs; dilution of 1:1000), and rabbit anti-H2AX (D-001810-10). Nucleofection of A549 and 293T cells was performed according to the manufacturer’s instructions (Amaxa, Lonza) using 2 × 10⁶ cells and 2.0 μg of a plasmid constitutively expressing puromycin. Cells were grown 24 h without selection, 48 h with puromycin selection, and subsequently cloned by serial dilution (293T and A549 cells) or allowed to proliferate for three days and colonies were isolated and analysed by PCR for the presence of the deletion. RNA guides can be found in Supplementary Table 5.

Construction of knockout cells. To produce homoygous deletions in 293T and A549 cells using transient expression of Cas9/CRISPR, 2 μg of cells were nucleofected (Amaxa, Lonza) with 2.0 μg each of pX330 (Addgene plasmid 42230), a plasmid that expresses Cas9 and the chimeric guide RNA. Using Lipofectamine 2000, 1 μg of siRNA and cDNA transfection or nucleofection isolated and analysed by PCR for the presence of the deletion. RNA guides can be found in Supplementary Table 5.

Propidium iodide cell cycle assay. Cells were irradiated and pulsed with 10 μM BrdU (Sigma) for 1 h at the indicated time points and fixed in 35% ethanol in DMEM. Cells were resuspended and incubated in 2 N HCl for 20 min, washed in PBS containing 1% FBS and stained for 2 h in the dark at room temperature with Alexa Fluor 594 goat anti-rabbit (eBioscience) and Alexa Fluor 488 goat anti-mouse (eBioscience) at dilutions of 1:1,000. Slides were then stained with propidium iodide with 4,6-diamidino-2-phenylindole (DAPI; Vectashield). Images were acquired at 488 nm with an Andor iXon 897 EMCCD camera. Acquisition parameters, shutters and filter positions were controlled using the Andor iQ software. Confocal microscopy images for this study were acquired in the Confocal and Light Microscopy Core facility at the Dana-Farber Cancer Institute. Additional images were acquired using a Zeiss Axiolmager Z1 microscope equipped with an Axioscan MR Rev.3 colour digital camera and Plan APO × 63/1.4 oil M27 lens (magnification × 63). Acquisition software and image processing used the Zeiss AxioVision software package (Zeiss Imaging). Images were analysed with the US National Institutes of Health Image J Fiji program (http://rsbweb.nih.gov/ij/). Specifically, Image J was used to quantify in fluorescence microscopy experiments. Images from the vehicle-treated condition were used to set an appropriate noise tolerance for each antibody stain. The same noise threshold was used for all images in a given experiment (noise tolerances were as follows: 10 for γH2AX (JWB301), 10 for G4 DNA (H16) and 5 for 53BP1 (Bethyl)). The ‘Find Maxima’ tool was used to count foci for the indicated number of nuclei. Just another co-localization plugin (JacoJ) was used to calculate degree of co-localization between foci.

Colonies survival assay. Cells were seeded at a density of 200 cells per 10 cm tissue culture plate and 24 h later treated with the indicated dose of genotoxin. Cells were treated with bleomycin for 1 h and pyridostatin for 48 h before washing twice with PBS, adding fresh media and returning to the incubator for 10 days to develop colonies. Colonies were fixed with methanol and stained with 0.5% crystal violet for counting. Colony survival assays are presented as representative experiments from n = 3 biological replicates.

Propidium iodide knockdown

Chromatin immunoprecipitation. ChIP assays were performed using the SimpleChIP Chromatin IP kit (Cell Signaling Technology). Cells were crosslinked with formaldehyde (5 min) and quenched with glycine. Cell pellets were solubilized in CHIP buffer (Cell Signaling Technology) and sonicated (Fisher BioRuptor U200). Part of the supernatant was digested with proteinase K (65 °C for 2 h), the DNA isolated by spin columns, and input DNA quantified by real-time PCR. Equivalent amounts of chromatin were incubated with primary antibody (overnight at 4 °C for RPA, BLM, and HA, 2 h at 4 °C for γH2AX) followed by protein-G magnetic beads (Cell Signaling Technology). Immune complexes were washed in low- and high-salt CHIP buffers (Cell Signaling Technology), eluted and incubated in NaCl (65 °C for 2 h), and then digested with proteinase K, PCR-quantified DNA was identified by qPCR using the SsoAdvanced plus real-time PCR system (Applied Biosystems). ChIP-graded antibodies included anti-HA magnetic beads (HA-7, Pierce), mouse anti-RPA3A (NA19L, Calbiochem), mouse anti-γH2AX (JWB301, Millipore), rabbit anti-ChIP (AS00-487, Bethyl Labs), rabbit anti-BLM (A300-110, Bethyl Labs) and normal mouse IgG (Santa Cruz). The anti-RPA3A (NA19L, Calbiochem) and mouse anti-γH2AX (JWB301, Millipore) reagents have been used extensively for ChIP74,75. Primer sequences are listed in Supplementary Table 5.
Preparation of hf2 antibody. pSANG10-3F-BG4 (Addgene 55756) was transformed into BL21 (DE3) competent Escherichia coli, (Invitrogen) grown in LB broth and induced for 6 h with 10 mM isopropyl-β-D-thiogalactoside (Life Technologies). Bacterial pellets were snap-frozen, lysed and sonicated (Fisher BioRuptor U200) before centrifuging at 12 K rpm. Lysates were incubated at 4 °C with the end-over-end rotation overnight with Flag-agarose beads (Sigma Aldrich). Beads were washed in lysis buffer (50 mM Tris, pH 7.5, 150 mM NaCl, 0.05% NP-40) and used immediately for IP to G4 DNA.

Preparation of DNA and IP of G4 DNA with hf2 antibody. Wild-type or PARP3−/− 293T cells were treated with 5 μM pyridostatin for 24 h and transfected with the px330 plasmid expressing CAS9 with or without a gRNA targeting the CDN locus 16 h before isolation of DNA. Isolation of genomic DNA and pulldown of G4 DNA were performed as described with slight modifications. Genomic DNA was extracted from 293T cells using the QIAamp DNA Mini kit (Qiagen) according to the manufacturer’s protocol. The purified DNA was eluted from columns in 10 mM Tris, pH 7.4. Genomic DNA was sheared by sonication (Fisher BioRuptor U200) before pull-down experiment. An amount of 60 μg of DNA was used per condition and incubated overnight with Flag beads bound to hf2. Following 4 °C incubation overnight with the end-over-end rotation, beads were washed six times with 10 mM Tris, pH 7.4, 100 mM KCl and 0.1% Tween followed by a wash with 10 mM Tris pH 7.4, 100 mM KCl. DNA was eluted in 50 μl of 1% SDS, 0.1 M NaHCO3, at 30 °C for 1 h and then purified with Qiagen PCR purification columns. Purified DNA was quantified by qPCR using the Step One Plus real time PCR system (Applied Biosystems). Primer sequences are listed in Supplementary Table 7.

Statistics. Statistical tests used were unpaired, two-sided Student’s t-test to test for differences between the means of two independent sets of normally distributed data. P < 0.05 as the significance level was used. No marked differences in variance within individual experiments were observed across conditions. Error bars show s.e.m. On the basis of the observations from previous shRNA screens, we used per condition and incubated overnight with Flag beads bound to hf2. According to the manufacturer’s protocol. The purified DNA was eluted from columns in 10 mM Tris, pH 7.4. Genomic DNA was sheared by sonication (Fisher BioRuptor U200) before pull-down experiment. An amount of 60 μg of DNA was used per condition and incubated overnight with Flag beads bound to hf2. Following 4 °C incubation overnight with the end-over-end rotation, beads were washed six times with 10 mM Tris, pH 7.4, 100 mM KCl and 0.1% Tween followed by a wash with 10 mM Tris pH 7.4, 100 mM KCl. DNA was eluted in 50 μl of 1% SDS, 0.1 M NaHCO3, at 30 °C for 1 h and then purified with Qiagen PCR purification columns. Purified DNA was quantified by qPCR using the Step One Plus real time PCR system (Applied Biosystems). Primer sequences are listed in Supplementary Table 7.

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Data availability. DNA primer sequences and CRISPR-Cas9 gRNA sequences are all found in Supplementary Tables 5–7. Any other relevant sequences are available upon request from the authors. RNA-seq data are publicly available in the NCBI GEO repository under the accession number GSE94588.

References
1. Kong, F. et al. dbCRID: a database of chromosomal rearrangements in human diseases. Nucleic Acids Res. 39, D895–D900 (2011).
2. Piomboni, P., Stendardi, A. & Gambera, L. Chromosomal aberrations and clonal diversity and progression of acute myeloid leukemia. Proc. Natl Acad. Sci. USA 97, 228–233 (2000).
3. Beck, C. et al. PARP3 affects the relative contribution of homologous recombination and nonhomologous end-joining pathways. Nucleic Acids Res. 42, 5616–5632 (2014).
4. Fenton, A. L., Shirodkar, P., Macrae, C. J., Meng, L. & Koch, C. A. PARP-3 and ATM-dependent phosphorylation of APLF facilitates DNA double-strand break repair. Nucleic Acids Res. 41, 4080–4092 (2013).
5. Rullen, S. L. et al. PARP-3 and APLF function together to accelerate nonhomologous end-joining. Mol. Cell. 41, 33–45 (2011).
6. Gibson, B. A. et al. Chemical genetic discovery of PARP targets reveals a role for PARP-1 in transcription elongation. Science 353, 45–50 (2016).
7. Boehler, C. & Dantzer, F. PARP-3, a DNA-dependent PARP with emerging roles in DNA strand break repair and mitotic progression. Cell Cycle 10, 1023–1024 (2011).
8. Rodriguez, R. et al. A novel small molecule that alters shelterin integrity and triggers a DNA-damage response at telomeres. J. Am. Chem. Soc. 130, 15758–15759 (2008).
9. Halder, R., Riou, J. F., Teulade-Fichou, M. P., Frickey, T. & Hartig, J. S. Bisiquinolinium compounds induce quadruplex-specific transcriptome changes in HeLa S3 cell lines. BMC Res. Notes 5, 138 (2012).
10. Maizels, N. & Gray, L. T. The G4 genome. PLoS Genet. 9, e1003468 (2013).
11. Huppert, J. L. & Balasubramanian, S. Prevalence of quadruplexes in the human genome. Nucleic Acids Res. 33, 2908–2916 (2005).
12. Todd, A. K., Johnston, M. & Neidle, S. Highly prevalent putative quadruplex sequence motifs in human DNA. Nucleic Acids Res. 33, 2901–2907 (2005).
13. Bedrat, A., Lacroix, L. & Merjny, J. L. Re-evaluation of G-quadruplex propensity with G4Hunter. Nucleic Acids Res. 44, 1746–1759 (2016).
14. Chambers, V. S. et al. High-throughput sequencing of DNA G-quadruplex structures in the human genome. Nat. Biotechnol. 33, 877–881 (2015).
15. Papadopoulou, C., Guillaud, G., Schiavone, D. & Sale, J. E. Nucleotide pool depletion induces G-quadruplex-dependent perturbation of gene expression. Cell Rep. 13, 2491–2503 (2015).
16. Zimmer, J. et al. Targeting BRCA1 and BRCA2 deficiencies with G-quadruplex-interacting compounds. Mol. Cell 61, 449–460 (2016).
17. Bohdanecky, M. et al. Stability and function of G-quadruplex structures. Nat. Rev. Genet. 13, 770–780 (2012).
18. Tarsounas, M. & Tijsterman, M. Genomes and G-quadruplexes: for better or for worse. J. Mol. Biol. 425, 4782–4789 (2013).
19. Rodriguez, R. et al. Small-molecule-induced DNA damage identifies alternative DNA structures in human genes. Nat. Chem. Biol. 8, 301–310 (2012).
20. Ashburner, M. et al. Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. Nat. Genet. 25, 25–29 (2000).
21. Gene Ontology Consortium. Gene Ontology Consortium: going forward. Nucleic Acids Res. 43, D1049–D1056 (2015).
22. Kaneshia, M., Sato, Y., Kawashima, M., Furumichi, M. & Tanabe, M. KEGG as a reference resource for gene and protein annotation. Nucleic Acids Res. 44, D457–D462 (2016).
23. Kaneshia, M. & Goto, S. KEGG: kyoto encyclopedia of genes and genomes. Nucleic Acids Res. 28, 27–30 (2000).
24. Loseva, O. et al. PARP-3 is a mono-ADP-ribosylase that activates PARP-1 in the absence of DNA. J. Biol. Chem. 285, 8054–8060 (2010).
25. Hay, T. et al. Efficient deletion of normal Brca2-deficient intestinal epithelium by poly(ADP-ribose) polymerase inhibition models potential prophylactic therapy. Cancer Res. 65, 1043–1048 (2005).
26. Wu, W. Q., Hou, X. M., Li, M., Dou, S. X. & Xi, X. G. BLM unfolds G-quadruplexes in different structural environments through different mechanisms. Nucleic Acids Res. 43, 4614–4626 (2015).
45. Chatterjee, S. et al. Mechanistic insight into the interaction of BLM helicase with intra-strand G-quadruplex structures. Nat. Commun. 5, 5556 (2014).

46. Sun, H., Ka, A.-C., Hickson, I. D. & Maizels, N. G4 DNA unwinding by BLM and Sgs1p: substrate specificity and substrate-specific inhibition. Nucleic Acids Res. 30, 3954–3961 (2002).

47. Huber, M. D., Lee, D. C. & Maizels, N. G4 DNA unwinding by BLM and Sgs1p: facilitation of telomere replication during leading strand synthesis of telomeres. J. Cell Biol. 210, 191–208 (2015).

48. Drosopoulos, W. C., Kosiyatrakul, S. T. & Schildkraut, C. L. BLM helicase causes strand displacement of telomeres. J. Biol. Chem. 273, 27587–27592 (1998).

49. Huber, M. D., Lee, D. C. & Maizels, N. G4 DNA unwinding by BLM and Sgs1p: substrate specificity and substrate-specific inhibition. Nucleic Acids Res. 30, 3954–3961 (2002).

50. Dhapola, P. & Chowdhury, S. QuadBase2: web server for multiplexed guanine quadruplex DNA as a potential target for induced synthetic lethality in cancer cells. J. Am. Chem. Soc. 135, 9640–9643 (2013).

51. Riffel, G., Tannahill, D., Adey, J., Howat, W. J. & Balasubramanian, S. Elevated levels of G-quadruplex formation in human stomach and liver cancer tissues. PLoS ONE 9, e102711 (2014).

52. Capes-Davis, A. et al. Check your cultures! A list of cross-contaminated or misidentified cell lines. Int. J. Cancer 127, 1–8 (2010).

53. Schindelin, J. et al. Fiji: an open-source platform for biological-image analysis. Nat. Methods 9, 676–682 (2012).

54. Bolte, S. & Cordelieres, F. P. A guided tour into subcellular colocalization analysis in light microscopy. J. Microsc. 224, 213–232 (2006).

55. Nakanishi, K., Cavallo, F., Brunet, E. & Jasien, M. Homologous recombination assay for interstrand cross-link repair. Methods Mol. Biol. 745, 283–291 (2011).

56. Yamane, A. et al. RPA accumulation during class switch recombination represents 5’-3’ DNA-end resection during the S-G2/M phase of the cell cycle. Cell Rep. 3, 138–147 (2013).

57. Xu, Y. et al. The p400 ATPase regulates nucleosome stability and chromatin ubiquitination during DNA repair. J. Cell Biol. 191, 31–43 (2010).

58. Luo, B. et al. Highly parallel identification of essential genes in cancer cells. Proc. Natl Acad. Sci. USA 105, 20380–20385 (2008).

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Author contributions
T.A.D., A.C.S., J.D., D.E.R., W.C.H. and D.M.W. conceived and designed the experiments; T.A.D., J.V.L., J.P.C., S.G., T.T., S.K. and F.I. performed the experiments, T.A.D., A.C.S., J.D., D.E.R., W.C.H. and D.M.W. wrote the paper; and J.D. and B.D.P. contributed to preparing the manuscript.

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Corrigendum: PARP3 is a promoter of chromosomal rearrangements and limits G4 DNA

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In this Article, the antibody 'BG4' is consistently referred to incorrectly as 'hf2'. These errors appear in the Results and Methods. Additionally, the Article incorrectly cites reference 52 in the sentences ‘A recent study described hf2, an engineered single-chain antibody specific for G4 DNA52 and ‘pSANG10-3F-BG4 (Addgene 55756)52 was transformed into….’ The correct citation is given below.

Biffi, G., Tannahill, D., McCafferty, J. & Balasubramanian, S. Quantitative visualization of DNA G-quadruplex structures in human cells. Nat. Chem. 5, 182–186 (2013).

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