ALC1 links chromatin accessibility to PARP inhibitor response in homologous recombination-deficient cells

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The response to poly(ADP-ribose) polymerase inhibitors (PARPi) is dictated by homologous recombination (HR) DNA repair and the abundance of lesions that trap PARP enzymes. It remains unclear, however, if the established role of PARP in promoting chromatin accessibility impacts viability in these settings. Using a CRISPR-based screen, we identified the PAR-binding chromatin remodeler ALC1/CHD1L as a key determinant of PARPi toxicity in HR-deficient cells. ALC1 loss reduced viability of breast cancer gene (BRCA)-mutant cells and enhanced sensitivity to PARPi by up to 250-fold, while overcoming several resistance mechanisms. ALC1 deficiency reduced chromatin accessibility concomitant with a decrease in the association of base damage repair factors. This resulted in an accumulation of replication-associated DNA damage, increased PARP trapping and a reliance on HR. These findings establish PAR-dependent chromatin remodelling as a mechanistically distinct aspect of PARPi responses and therapeutic target in HR-deficient cancers.

Results

Loss of ALC1 confers PARPi hypersensitivity in BRCA-mutant cells. We performed a CRISPR-Cas9 genetic screen in BRCA-mutant cells to identify loss-of-function mutations in chromatin regulators that generate PARPi hypersensitivity. The single-guide RNAs (sgRNAs) targeted functional domains, an approach that imparts higher editing efficiency16. A sgRNA library targeting 197 functional domains of 179 chromatin regulators was transduced into Streptococcus pyogenes (SpCas9) expressing BRCA-mutant cells. These included the BRCA1 exon 11 mutant ovarian and breast cancer cell lines UWB1.289 and SUM149PT, respectively, and CAPAN-1, a pancreatic cancer line that harbours the 6174delT BRCA2 mutation. The screen was performed at 10 nM olaparib, which approximates the lethal dose 20 (dose that kills 20% of cells, LD20) for these BRCA-mutant lines in a two-week clonogenic assay (Fig. 1a and Supplementary Table 1).

ALC1 loss conferred olaparib hypersensitivity across all cell lines and ranked as the top hit in both SUM149PT and CAPAN-1 cells (Fig. 1b and Extended Data Fig. 1a). Olaparib hypersensitivity was validated in a growth-based competition assay that monitors changes in the percent of GFP co-expressed from the sgRNA vector (Fig. 1c and Extended Data Fig. 1b,c). Loss of ALC1 also conferred a growth defect in all lines tested, albeit to a lesser degree in UWB1.289 cells, suggesting a synthetic sick relationship between ALC1 loss and BRCA deficiency.

We next employed paired isogenic cell lines to examine if ALC1 loss selectively impacts PARPi sensitivity of BRCA-knockout (KO) cells in comparison to their wild-type (WT) counterparts. We utilized the dual Staphylococcus aureus SaCas9 and SpCas9 approach to achieve efficient simultaneous knockdown of two proteins17.
Loss of ALC1 selectively impaired proliferation in both BRCA1 KO hTERT-retinal pigmented epithelial cells (RPE1) and BRCA2 KO colon cancer DLD1 cells, revealing a synthetic sick relationship with BRCA loss. Additionally, ALC1 depletion sensitized BRCA-mutant cells to low doses of olaparib (1 nM), while having minimal effects on their WT counterparts (Fig. 1d). Given that the half-maximal inhibitory concentration (IC50) of olaparib for purified PARP1 is 5 nM (ref. 23), ALC1 loss appears to sensitize HR-deficient cells to PARPi concentrations that do not inhibit the majority of cellular PARP activity. Moreover, clonogenic survival and cellular ATP content-based viability assays revealed up to ~250-fold decreases in IC50 for PARPi upon ALC1 loss in several BRCA1- and BRCA2-mutant cell lines (Extended Data Figs. 2 and 3a).

To examine the impact of ALC1 in a BRCA-mutant tumour model, SUM149PT Cas9 cells were transduced with either sgNegative (sgNeg) or sgALC1 and the heterogeneous pool of cells was

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**Fig. 1** Loss of ALC1 reduces proliferation and confers olaparib hypersensitivity in BRCA-mutant cells. a, Schematic of the CRISPR screen to identify regulators of olaparib (ola) sensitivity. b, Protein domains ranked on the basis of CRISPR score (CS) for ola sensitivity in BRCA1-mutant SUM149PT cells (left) and BRCA2-mutant CAPAN-1 cells (right). c, Schematic of the green fluorescent protein (GFP) competition experiments. For a given cell line, Tfinal indicates the day when maximum GFP expression is achieved for a sgRNA targeting an essential gene.

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subcutaneously implanted in NOD-scid gamma (NSG) mice on day 5 after transduction, when maximum sgRNA expression occurs. The gap between sgRNA expression and protein depletion with CRISPR editing allowed the tumours to reach ~100 mm³, when olaparib treatment was initiated (Fig. 1e). Tumour growth in sgALC1 xenografts was significantly slower compared to the olaparib-treated sgNeg group, consistent with the synthetic sick interaction between ALC1 loss and BRCA mutation. The volume of PARPi-treated sgNeg tumours increased by ~7.8-fold, compared to a ~2-fold increase in sgALC1 counterparts by 38 days post olaparib administration (Fig. 1f). Kaplan–Meier analyses indicated that the median overall survival was significantly longer for the untreated sgALC1 group compared to the olaparib administered sgNeg counterparts, and further extended in the PARPi-treated sgALC1 cohort (Fig. 1g). An immunoblot of late-stage sgALC1 tumours revealed residual ALC1 protein, potentially resulting from cells that escaped genetic editing. By contrast, ALC1 was not detected 15 days post-transduction in sgALC1 cells that were used to inoculate the mice (Extended Data Fig. 3b). These findings suggest that selective pressure exists to maintain ALC1 expression in BRCA-mutant tumours.

PARPi sensitivity in ALC1-deficient cells is not epistatic with other DNA repair pathways. We systematically examined epistasis between ALC1 and a selected group of DNA repair pathways for PARPi response. An arrayed sgRNA library was designed that targeted genes in the HR, non-homologous end joining (NHEJ), single-strand break repair (SSBR), nucleotide excision repair (NER) and microhomology-mediated end joining (MMEJ) pathways. The proliferation of BRCA1-proficient UWB1.289 ALC1 KO cells was monitored following the loss of targeted genes in the absence or presence of 50 nM olaparib (Fig. 2a). Targeting ATR, BLM, SLX4, Mus81 and Fen1 selectively sensitized ALC1 KO cells to 50 nM olaparib (Fig. 2b and Extended Data Fig. 3c). ALC1 deficiency was also synthetically sick with XRCC1 loss and further increased PARPi sensitivity, suggesting that ALC1 loss may generate lesions that increase reliance on SSBR in addition to HR. Reciprocal experiments with ALC1 depletion in both sgXRCC1 DEL1 cells and XRCC1 KO hTERT-RPE1 cells also enhanced PARPi sensitivity. Notably, loss of ALC1 alone conferred a modest increase in talazoparib sensitivity of BRCA-proficient DEL1 cancer cells, while having no effects in hTERT-RPE1 counterparts (Extended Data Fig. 4a–d). ALC1 inactivation did not affect PARPi response following the loss of c-NHEJ or NER proteins (Fig. 2b and Extended Data Fig. 3c). Collectively, these findings reveal that ALC1 loss is not epistatic to either of the primary determinants of PARPi response, HR or SSBR.

ALC1 deficiency restores PARPi sensitivity in cells with engineered resistance. The profound PARPi response in ALC1-deficient BRCA-mutant cells led us to examine if this relationship could be leveraged to overcome therapeutic resistance. Loss of 53BP1 or Rev7 permits partial restoration of HR in BRCA1-mutant cells coincident with both reduced radial chromosome formation and PARPi resistance[26,28]. In agreement, PARPi sensitivity and Rad51 foci formation in 53BP1 KO UWB1.289 cells were intermediate between the parental and BRCA1-addback counterparts (Extended Data Fig. 4e,f). ALC1 loss in either 53BP1- or Rev7-deficient BRCA1-mutant cells restored PARPi sensitivity, albeit at levels comparable to the parental control (Fig. 2c,d). ALC1-deficient UWB1.289 cells formed increased radial chromosomes upon PARPi treatment that were eliminated by concomitant 53BP1 depletion (Extended Data Fig. 4g,h). Increased chromosome breaks and Rad51 foci were still observed in PARPi-treated ALC1-deficient 53BP1 KO BRCA1-mutant cells, potentially accounting for the continued drug sensitivity.

We extended this analysis to reversion mutations that produced functional BRCA1 protein. ALC1 loss failed to increase olaparib sensitivity in SUM149PT cells that have become resistant due to restoration of the BRCA1 reading frame[25]. By contrast, some degree of sensitivity was observed with the more potent PARPi, talazoparib (Fig. 2e,f).

Reduced PARPi expression renders PARPi resistance, suggesting that PARPi trapping is the major contributor to PARPi cytotoxicity[19,25]. Remarkably, cells depleted of both PARPi and ALC1 remained PARPi hypersensitive compared to the parental control (Fig. 3a–c). PARPi also traps PARP2[20,21], potentially accounting for the continued sensitivities in ALC1- and PARPi-depleted cells. Using a quantitative immunofluorescence assay, we observed increased PARPi and PARP2 trapping in ALC1-deficient cells compared to the parental control (Extended Data Fig. 5). Combined loss of PARPi and PARP2 rendered ALC1-deficient BRCA1-mutant cells fully resistant to PARPi, whereas PARPi loss alone caused resistance when ALC1 was present (Fig. 3a–c). We propose that ALC1 loss increases the abundance of genomic lesions that trap both PARPi and PARP2.

Loss of poly(ADP-ribose) glycohydrolase (PARG) results in PAR accumulation concomitant with less PARP trapping and resistance[22]. ALC1 depletion restored olaparib sensitivity at levels comparable to parental controls in cells treated with either an inhibitor or sgRNA targeting PARG (Fig. 3d,e). These observations (Figs. 2 and 3) demonstrate that ALC1 loss restores PARPi efficacy across a broad range of resistance mechanisms.

ALC1 deficiency increases genomic instability and reliance on BRCA-dependent HR. We next characterized the genomic lesions arising from ALC1 loss in BRCA-mutant cells. Depletion of ALC1 in DLD1 BRCA2-deficient cells increased breaks and radial chromosomes, which were exacerbated by olaparib treatment (Fig. 4a,b). Loss of ALC1 increased Rad51 foci formation upon olaparib treatment in BRCA-proficient U-2 OS and UWB1.289 cells, suggesting the genesis of lesions that require HR (Fig. 4c and Extended Data Fig. 6a). ALC1 loss did not enhance sensitivity to cisplatin in BRCA-proficient or deficient settings, emphasizing that it does not contribute to homology-directed repair as is observed for factors that affect PARPi sensitivity through HR or MMEJ (Fig. 4d).

ALC1 depletion in BRCA-mutant cells increased γH2AX foci specifically in S-phase, consistent with the generation of replication-coupled double-strand breaks (DSBs) (Fig. 4e and Extended Data Fig. 6b). We next quantified single-strand DNA (ssDNA) using non-denaturing CldU immunofluorescence. ALC1 loss resulted in a significant increase in non-γH2AX-positive CldU foci, an outcome that was more pronounced in BRCA-mutant settings and was further elevated upon olaparib treatment (Fig. 4f).

ALC1 deficiency confers sensitivity to agents that induce single-strand breaks (SSBs)[14,18]. We could recapitulate the reported methyl methanesulfonate (MMS) sensitivity across several ALC1-depleted lines, with a notable exception in hTERT-RPE1 cells. BRCA loss exacerbated MMS sensitivity in ALC1-depleted cells, consistent with channelling of unrepaired damage into HR[10,17]. By contrast, ALC1 depletion did not confer sensitivity to camptothecin (CPT; Fig. 5a and Extended Data Fig. 6c). Repair of MMS damage necessitates base excision and nick generation, but CPT traps the TOP1 enzyme on already nicked DNA[16]. Although repair of MMS-induced lesions would require nucleosome sliding to provide accessibility to damaged bases, this may not be required for CPT-induced lesions (Fig. 5b). In agreement, ALC1 has been shown to function in the pre-incision step of NER by recognizing damaged nucleotides in a PARPi-dependent manner[11]. Moreover, ALC1 loss enhanced MMS but not CPT sensitivity in XRCC1-deleted cells (Fig. 5c and Extended Data Fig. 6d).

The presence of persistent base lesions can lead to the generation of replication-coupled single-strand (ss)-gaps[12]. Indeed, loss of ALC1 increased the S1 nuclease sensitivity of nascent replication
Fig. 2 | ALC1 loss causes PARPi hypersensitivity in HR-deficient cells. a, Schematic of the dual Cas9 CRISPR system for the GFP competition experiments. b, GFP competition experiment in the UWBI.289 + BRCA1 addback line to examine cell proliferation and ola sensitivity following the combined loss of ALC1 and the indicated DNA repair protein. Data are presented as mean ± s.e.m., normalized to Tmut. After every two population doublings, cells were passaged (P) and percent GFP was recorded (n = 4 independent transductions, except for sgXRC1 and sgERCC4, where n = 6 independent transductions were made). c, Immunoblot showing ALC1, 53BP1 and Rev7 depletion in UWB1.289 cells. Consistent results were obtained across two independent blots. d, Sensitivities of the indicated UWBI.289 cell lines to ola using the CellTiter-Glo assay; n = 3 biologically independent experiments. Data are presented as mean ± s.e.m. e, Immunoblot showing BRCA1 and ALC1 levels in the indicated SUM149PT cells. Consistent results were obtained across two independent blots. f, Sensitivities of the indicated SUM149PT cell lines to ola (left) and talazoparib (tal; right) using the CellTiter-Glo assay. cf1 and #7 indicate two different clones with restored BRCA1 reading frames. Data are presented as means from two biologically independent experiments.

ALC1 localizes to chromatin in a PARP1- and PARP2-dependent manner. ALC1 recruitment to damaged chromatin requires PARylation. We reasoned that owing to its nanomolar binding affinity for PAR chains, ALC1 localization to damaged chromatin would not be diminished by low-dose PARPi treatments. Treatment of cells with PARPi revealed accumulation of ALC1 specifically on S-phase chromatin. This suggests that ALC1 primarily associates with replicating DNA, consistent with PAR levels being highest in S-phase. ALC1 chromatin association was evident in both replicating and non-S-phase cells following MMS treatment and did not require PARPi to elicit detection (Fig. 6a,b). ALC1 chromatin localization was

tracts and this was exacerbated by PARPi treatment (Fig. 5d and Extended Data Fig. 6e). Together, these results demonstrate that ALC1 prevents the accumulation of toxic genomic lesions that feed into SSB and HR repair pathways.
PAR-binding proteins (Extended Data Fig. 7)44. In accordance with the dose of olaparib required to nullify chromatin recruitment of other PARP1 or PARGi mutants, ALC1 localization was eliminated at 20 nM olaparib. Consistent results were obtained across two independent blots. Colonies with more than 50 cells were included in the quantification. Data are presented as mean ± s.e.m. from n = 3 biologically independent experiments.

**Fig. 3** | **ALC1 loss mitigates PARPi resistance in BRCA-mutant cells that are deficient in PARPi or PARG.** **a.** Immunoblot showing ALC1, PARP1 and PARP2 depletion in UWB1.289 cells. Consistent results were obtained across two independent blots. **b–c.** Sensitivities of the indicated UWB1.289 cell lines to ola (b) and tal (c) using the CellTiter-Glo assay. Data are presented mean ± s.e.m. from n = 3 biologically independent experiments. **d.** Representative images (top) and quantification (bottom) of the clonogenic survival assay of UWB1.289 cells treated with the indicated doses of ola and PARG inhibitor (PARGi). Data are presented as mean ± s.e.m. from n = 3 biologically independent experiments. **e.** Sensitivities of the indicated DLD1 BRCA2−/− cell lines to ola using the CellTiter-Glo assay. sgPARG#1 and sgPARG#2 indicate two sgRNAs targeting PARG. Data are presented as mean ± s.e.m. from n = 3 biologically independent experiments.

**Fig. 4** | **ALC1 responses to PARPi and MMS require its chromatin remodelling and PAR binding activities.** We investigated the biochemical properties underlying ALC1 function in PARPi responses. The K77R mutation in the Walker A motif of the ALC1 ATPase domain abrogates its nucleosome sliding activity45. Overexpression of the K77R ALC1 mutant led to a pronounced loss of viability of BRCA-mutant cells, suggesting a dominant negative phenotype. Depletion of the endogenous ALC1 protein in the surviving population of K77R-expressing cells pre-incubated with 100 nM olaparib or 1 µM talazoparib. Conversely, ALC1 localization was eliminated at 20 µM PARPi, consistent with the dose of olaparib required to nullify chromatin recruitment of other PAR-binding proteins (Extended Data Fig. 7)44. In accordance with PAR-dependent recruitment, ALC1 chromatin association was abolished in cells lacking both PARP1 and PARP2, and ALC1 loss did not increase MMS sensitivity in these lines (Fig. 6c–g).
Fig. 4 | Loss of ALC1 increases genomic instability. a, b, Representative images (a) of the chromosomal aberrations (indicated by red arrowheads) and quantification (b) of breaks and radials per metaphase upon ALC1 depletion in DLD1 BRCA2−/− cells. Data are presented as mean ± s.e.m. from n = 3 biologically independent experiments; P value, unpaired t-test. For each experiment, at least 50 spreads were analysed per sample. c, Quantification of γH2AX-RAD51 co-localization in indicated UWB1.289 + BRCA1 addback and U-2 OS cells. Median values are indicated. P values determined by Mann–Whitney test were derived from n ≥ 67 cells examined over two biologically independent experiments. d, Sensitivities of the indicated cell lines to cisplatin and ola as determined by CellTiter-Glo assay. Data are presented as mean ± s.e.m. from n = 3 biologically independent experiments; P value, unpaired t-test. For each experiment, at least 50 spreads were analysed per sample. e, Sensitivities of the indicated cell lines to cisplatin and ola as determined by CellTiter-Glo assay. Data are presented as mean ± s.e.m. from n = 3 biologically independent experiments; P value, unpaired t-test. For each experiment, at least 50 spreads were analysed per sample. f, Schematic and quantification of non-γH2AX-positive CldU foci in the indicated cell lines to specifically detect ssDNA that was not generated by end-resection at double-strand breaks (DSBs). Median values are indicated. P values determined by Mann–Whitney test were derived from n ≥ 105 cells sampled over two biologically independent experiments. Cells were incubated with the indicated concentrations of ola for either 2 h (f) or 24 h (b,c,e).
cells resulted in PARPi hypersensitivity comparable to ALC1-deficient cells (Fig. 7a,b and Extended Data Fig. 8a,b).

Like other chromatin remodelers, interaction of ALC1 with the basic histone H4 tail is essential for its nucleosome sliding activity. Based on the cryo-electron microscopy (cryo-EM) structure of the ATPase domain of an ISWI remodeller (PDB: 6PWF), we complementation with the single mutants D723A, G749F, L751D interactions with ADP-ribose in the macrodomain. Although AF1521 G41 and V43 residues, which form hydrogen-bonding possibility.

ALC1 and PARP activity cooperate to promote chromatin accessibility. ALC1 affects chromatin decondensation at laser-induced damage sites. To examine if ALC1 loss altered genome-wide chromatin accessibility, we performed assay for transposable-accessible chromatin (ATAC)-seq analysis in BRCA2-mutant DLD1 cells. Chromatin accessibility was assessed in ALC1-deficient cells treated with 5µM olaparib for 4h (Fig. 7c). A longer treatment was avoided to negate chromatin changes due to differences in the cell cycle. Pairwise comparisons of accessible sites using DESeq2 analysis did not identify large locus-specific changes (log2 fold change) of ≥0.5 or ≤−0.5, false discovery rate (FDR)<0.05, suggesting that ALC1 does not play a major role in regulating gene expression (Supplementary Table 2). In agreement, RNA-seq analysis in ALC1-depleted delp53 BRCA1−/− hiTERT-RPE1 and BRCA2−/− DLD1 cells revealed no consistent gene expression changes (Supplementary Table 3).

We next examined if ALC1 loss had an effect on overall chromatin accessibility. Metagene analysis revealed a significant 1.6-fold overall reduction in chromatin accessibility upon ALC1 depletion alone. A 4-h olaparib treatment resulted in 1.3- and 1.84-fold changes in overall accessibility in sgNeg and sgALC1 cells, respectively, compared to the control (paired t-test P<1×10−4) (Fig. 7d). We reasoned that for a short-duration treatment, a more potent PARPi may manifest in larger accessibility changes. We performed ATAC-seq analysis in UWB1.289 cells, which are less sensitive to ALC1 loss in the absence of PARPi treatment and thus avoid confounding issues of toxicity from genetic interactions. ALC1 loss alone revealed no significant reduction in overall chromatin accessibility in UWB1.289 cells. By contrast, ALC1 depletion had a profound impact on chromatin accessibility when combined with the
Fig. 6 | ALC1 function in the DNA damage response requires PARP1 and PARP2. a,b, Representative images (a) and quantification (b) of HA-ALC1 localization to chromatin with the indicated treatments. Scale bar, 10 µm. The median values were normalized to non-S-phase untreated control. Data are presented as mean ± s.e.m. from n = 3 biologically independent experiments. P values are from an unpaired Student’s t-test. For each experiment, at least 50 cells were analysed per sample.

c, Immunoblot showing stable expression of HA-ALC1 in the indicated cell lines. Samples were analysed once across two independent experiments. Values are from an unpaired Student’s t-test. For each experiment, at least 50 cells were analysed per sample.

d, Immunoblot showing depletion of ALC1 in the indicated cell lines. Consistent results were obtained across biologically independent experiments.

Fig. 7 | ALC1 loss reduces sensitivities to several DNA damaging agents. a, Sensitivities of the indicated U-2 OS lines to MMS in the CellTiter-Glo assay. Data are presented as mean ± s.e.m. from n = 5 biologically independent experiments. b, Representative images and quantification of the indicated ALC1KO cell lines after 4 h of treatment with the indicated concentrations of MMS. Data are presented as mean ± s.e.m. from n = 5 biologically independent experiments. P values are from an unpaired Student’s t-test. For each experiment, at least 50 cells were analysed per sample.

c, Immunoblot showing stable expression of cDNA: HA-ALC1 in the indicated cell lines. Samples were analysed once across biologically independent experiments.

PARP-dependent chromatin relaxation has been implicated in the recruitment of repair proteins to the sites of laser damage39,49. Strikingly, loss of ALC1 alone reduced the association of several proteins dedicated to processing base damage in DLD1 BRCA2-mutant cells (Extended Data Fig. 9a,b). This included the DNA glycosylase NTHL1, the apurinic/apyrimidinic endonuclease APE1, and...
Fig. 7 | ALC1 PAR-recognition and chromatin-remodelling activities are essential for responses to PARPi and MMS. a, Domain organization and mutants of ALC1 used in this study. The helicase ATP-binding (light blue), helicase C-terminal (dark blue) and macro (grey) domains are indicated. Red bars show the position of the residues that were mutated. b, Sensitivities of SUM149PT cells expressing various ALC1 mutants to ola and MMS using the CellTiter-Glo assay (EV, empty vector; WT, wild type). Data are presented as means from three biologically independent experiments. c, Schematic of the experiment used to examine the effects of ALC1 loss and PARPi treatment on chromatin accessibility. d, ATAC-seq analysis in DLD1 BRCA2−/− cells to assess global accessibility of chromatin. Cells were treated with ola for 4 h. Data are from three biologically independent replicates for each condition. e, ATAC-seq analysis in UWB1.289 cells to assess the global accessibility of chromatin. Cells were treated with tal for 4 h. Data are from three biologically independent replicates of untreated sgNeg and sgALC1+ tal and two biologically independent replicates of sgNeg + tal and untreated sgALC1 cells. For plotting of both the ATAC-seq graphs (d,e), the 2,000 base pairs (bp) flanking the centre of the accessible sites (that is, from −1 to 1 on the x axis) were divided into equally sized 20-bp regions. The differential accessibility was calculated at each of these 20-bp regions.

XRCC1. These reductions were more pronounced on combining ALC1 loss with olaparib, suggesting that ALC1 and PARP act together to ensure chromatin accessibility to certain repair factors. Similar changes in chromatin association of repair factors after PARPi treatment were also observed in UWB1.289 cells (Extended Data Fig. 9c). XRCC1 chromatin localization was also significantly lower in PARPi-treated ALC1-deficient UWB1.289 cells compared to its WT counterpart, even in the presence of MMS that induce PARylation (Extended Data Fig. 9d–g).

To determine if this coordination affects response to other genotoxic insults, we combined PARPi with ALC1 loss and examined responses to ionizing radiation (IR). ALC1 loss by itself did not increase sensitivity to IR in either BRCA1-mutant hTERT-RPE1 or UWB1.289 cells. By contrast, treatment with nanomolar concentrations of PARPi showed synergy with ALC1 loss to achieve cell killing at low doses of IR. Only modest or no synergy with IR was observed at these PARPi concentrations in ALC1-proficient cells (Fig. 8a–c and Extended Data Fig. 10). These results support a role for ALC1 in promoting diverse PARP-dependent damage responses.

Discussion

PAR-dependent changes in chromatin accessibility were reported nearly four decades ago, yet its relationship to the selective toxicity of PARPi in HR-deficient cells has remained unexplored. Our study identifies PARP-dependent chromatin remodelling by ALC1 as a vulnerability in HR-deficient cancers. ALC1 deficiency reduced the proliferation of BRCA-mutant cells and enhanced the efficacy of PARPi in a distinct manner from perturbations in canonical
SSBR or HR pathways. ALC1 modulates chromatin accessibility to enable processing of damaged bases. We propose that PARPi treatment in ALC1-deficient cells results in an accumulation of persistent lesions that trap PARP1 and PARP2, necessitating a transition to BRCA-dependent HR repair during S-phase (Fig. 8d). The requirement for both PARP1 and PARP2 loss for resistance to PARPi or MMS provides evidence that PARP2 becomes important upon ALC1 loss. This differentiates PARPi response determinants or PARPi by translesion polymerases or repaired by replication restart or template switching with the sister chromatid. The role of HR proteins in replication restart and template switching may account for the lower frequency of gaps in BRCA-proficient settings upon ALC1 loss (Fig. 4f). ALC1 has been reported as a component of active replication forks, raising possibilities for its role in replication-associated repair.

A recent genome-wide CRISPR screen reported that loss of enzymes involved in processing base damage confers PARPi sensitivity. Together with our findings, these observations suggest that increased accumulation of base damage and resultant replication-coupled gaps may provide an avenue for enhancing...
PARPi cytotoxicity. ALC1 loss has also been reported to produce PARPi sensitivity in genome-wide screens in BRCA-proficient cell lines passage over several weeks at olaparib concentrations of 0.5–2 µM (50- to 200-fold higher doses than used in our studies) or 5 µM veliparib, albeit investigation of the underlying mechanisms was not described \(^\text{59-61}\). These findings, together with our data from isogenic cell lines (Extended Data Fig. 2), indicate that a large therapeu tic window can be obtained by targeting ALC1 in HR-deficient tumours.

The ATPase and macrodomain of ALC1 were essential for mediating PARPi response, presenting two moieties for small-molecule inhibitor development. The relative dispensability of ALC1 in cellular settings with intact DNA repair presents potential advantages over combinatorial approaches that include cytotoxic agents with PARPi. Targeting PAR-dependent chromatin accessibility may offer possibilities that extend beyond inactivation of a singular repair pathway.

**Online content**

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41556-020-00624-3.

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Methods

Cell culture. UW1.289 and UW1.289 + BRCA1 cells were purchased from ATCC and maintained in 1:1 Mammary Epithelial Cell Growth Basal Medium (MEBM) (Lonza) RPMI 1640 with Glutamax (Thermo Fisher) and supplemented with 10% fetal bovine serum (FBS, Atlanta Biologicals) and 1X penicillin-streptomycin (100 U/ml-1, Gibco). DLD1 WT and DLD1 BRCA2−/− cells were purchased from Horizon Discovery and cultured in RPMI 1640 with Glutamax media and supplemented with 10% FBS and 1X P/S. 293T and U-2 OS cells were purchased from ATCC and were maintained in DMEM medium (Thermo Fisher) with 10% bovine cell serum (BCS, GE Healthcare) and 1X P/S. CytoH2, hTERT-RPE1 p53−/−, Cas9§ and hTERT-RPE1 p53−/−/Cas9§ cells were grown in DMEM with 10% FBS and 1X P/S. SUM149PT cells were cultured in Ham’s F-12 (Thermo Fisher) medium supplemented with 5% FBS,1X P/S, hydrocortisone (1 mg/ml−1), streptomycin (100 µg/ml−1), and 1X P/S. 

Chemicals used in this study. The chemicals used include olaparib (LC Laboratories, cat. no. 0-9201), talazoparib (Selleck Chemicals, cat. no. S7048), and DMSO. The chemicals used include olaparib (LC Laboratories, cat. no. 0-9201), talazoparib (Selleck Chemicals, cat. no. S7048), camptothecin (Cayman Chemicals, cat. no. 11694), H2O2 (Sigma, cat. no. H1009), cisplatin (Tocris, cat. no. 2255), MMS (Sigma, cat. no. 129925) and PARPi (PD1, cat. no. 00017273; Fisher, cat. no. 5952). Most solid compounds were reconstituted in dimethylsulfoxide (DMSO) at 1000x the required concentration, such that the final concentration of DMSO was 0.1%. To all untreated controls, a final concentration of 0.1% DMSO was added. Cisplatin was freshly dissolved in water to yield a stock concentration of 5 mM.

Vector construction and sgRNA cloning. Single knockouts were generated either using a two-vector Streptococcus pyogenes (Sp) Cas9 system (LentiV, SpCas9-9_puro (Addgene, 108100) and LR2G.1 backbone (Addgene, 108098)) or an all-in-one (expressing both SpCas9 and sgRNA) single vector Staphylococcus aureus (Sa) Cas9 system. The SaCas9 all-in-one vector, henceforth referred to as SaLCg, was derived by cloning the SaCas9 coding sequence (dCAS-VP64-Blast, Addgene, 61425) and its associated sgRNA expression cassette into a lentiviral vector. For double knockout experiments, ALCl was knocked out using the single vector SaCas9 system, and the two-vector SpCas9 system was used for the depletion of the second protein of interest. Plasmids generated in this study will be available through Addgene: (1) SaLCg (U6-sgRNA-EFS-SaCas9-P2A-GFP) (Addgene,164563), (2) SaLCgP (U6-sgRNA-EFS-SaCas9-P2A-Puro) (Addgene,164562) and (3) SalgCn (U6-sgRNA-EFS-SaCas9-P2A-Neo). sgRNAs were designed to target the functional domain of protein and were cloned by annealing the two complementary DNA oligos into a BsmBI-digested vector using T4 DNA ligase. To improve U6 promoter transcription efficiency, an additional 5′ G nucleotide was added to all sgRNA oligos that did not already start with a 5′ G. A list of sgRNAs used in the study is provided in Supplementary Table 4.

Lentivirus generation and transduction. Lentivirus production and stable cell representation of sgRNAs during the screen, the number of sgRNA-positive cells to solvent-exposed cells. The data were fitted in GraphPad Prism using the above. To ensure a single copy sgRNA transduction per cell, the MOI was set to 0.3–0.4. At Tstart a fraction of cells were collected to prepare the reference representation and the rest were propagated either in the presence of DMSO or 10 mM olaparib, which approximately corresponds to the LD1s (in a two-week clonogenic assay) of the cells used for screening. Cells were passaged for 14 population doublings and were collected at the final time point. To maintain the representation of sgRNAs during the screen, the number of sgRNA-positive cells was kept at least 1.000 times the sgRNA number in the library. The CRISPR library was generated as described previously6. A protein domain CRISPR score was calculated by averaging the log(fold change) of all CRISPR RNA targeting a given protein domain. Log(fold change) = final CRISPR RNA abundance−1/(initial CRISPR RNA abundance). The log(fold change) values for each protein domain are provided in Supplementary Table 1.

Clonogenic assay. For clonogenic experiments with SUM149PT, DLD1, UW1.289 and UW1.289 + BRCA1, 500 cells were plated in technical triplicates and analysed after 10–14 days. For hTERT-RPE1, 250 cells were plated in a 10-cm dish. hTERT-RPE1 p53−/− and p53−/−/Cas9§ cells were grown at 3% oxygen for 8 and 11 days, respectively. For cellular complementation analysis with hTERT-RPE1 and hTERT-RPE1 p53−/−/Cas9§, a protein domain CRISPR score was generated as described previously. A protein domain CRISPR score was calculated by averaging the log(fold change) of all CRISPR RNA targeting a given protein domain. Log(fold change) = final CRISPR RNA abundance−1/(initial CRISPR RNA abundance). The log(fold change) values for each protein domain are provided in Supplementary Table 1.

CellTitre-Glo assay. One thousand cells in a volume of 100 µl were plated into each well of a 96-well clear-bottomed black plate (Corning, Neta Scientific, USA) on day 0. On the next day, 2x drug dilutions were prepared and 100 μl of the drug dilution was added to cells in technical triplicates. Although other drugs were retained, MMS-treated cells were released into fresh medium after 24 h. Viability was measured using a CellTitre-Glo luminescent cell viability assay (Fisher, G7572) either after five days of drug addition (for RPE-1 and U-2 OS) or after seven days (for DLD1, UW1.289, UW1.289 + BRCA1 and SUM149PT) using the Gen5 software (v2.01.14) on a Biotek Synergy microplate reader. When plotting the survival curves, the luminescence of the drug-treated population was normalized to solvent-exposed cells. The data were fitted in GraphPad Prism using the following equation: y = min +(max − min)/(1 + 10(logEC50−x)).

Xenograft experiment. Xenograft studies were carried out under protocol no. 803170 approved by the Institutional Animal Care & Use Committee at the University of Pennsylvania. Five-week-old female NSG (NOD, Cg:PrkdcscidIl2rgtm1Wjl/Svl) mice procured from Jackson Mice were kept at 72°F with an average humidity of 60% and under a 12-hour day and 12-hour night.
cycle. Laboratory tumour implantation was performed once these mice were seven weeks old. SUM149PT-Cas9 cells were engineered to stably express either sg

was dissolved in DMSO to a concentration of 50 mg ml

A 1:10 dilution of DMSO in 15% hydroxypropyl

was used to vehicle control. Tumour dimensions were measured twice a week using a digital caliper and the volume was calculated using the formula: length × width × height / 2. Mice were euthanized once the tumour size reached >10.5 mm in any direction. The harvested tumours were snap-frozen in liquid nitrogen for western studies.

**Metaphase spreads.** Metaphase spreads were prepared as described previously14. Slides were stained with Giemsa for 3–6 min, washed, and mounted using Permount.

**Immunofluorescence.** PARP1 and PARP2 trapping was performed as described previously14,15. Images from two biologically independent experiments were captured at the same time using a Zeiss Axio wide-field (20x/0.8) microscope. Immunofluorescence was independently analysed using CellProfiler 3.0 software. A specialized pipeline was implemented to identify and measure PARP1, PARP2 and DAPI signal intensity (CellProfiler Module: IdentifyPrimaryObjects/Measure ObjectIntensity). Illumination correction was applied to each image prior to intensity measurements to account for non-uniformities in illumination. In addition, the PARP2 signal was masked with DAPI-stained nuclei (CellProfiler Module: MaskImagetoObject) to ensure PARP2 signal intensity was measured. From this analysis, an output measure of IntegratedIntensity per object / per field-of-view was obtained for each experimental condition.

To examine yH2AX foci during S-phase, cells were grown on Poly-K coated cover slips and treated with 10 mM EdU for 20 min before collection. Cells were pre-extracted on ice for 5 min using Triton X-100 (0.5%) in CSK buffer (10 mM PIPES pH 6.8, 100 mM NaCl, 300 mM sucrose, 3 mM MgCl2 and 1 mM EGTA) and fixed using PFA (4%) in PBS for 15 min at room temperature (RT). Following 3–4 washes with PBS, cells were permeabilized using Triton X-100 (0.5%) in PBS (0.5% hydrogen peroxide for 5 min) to reveal yH2AX signal. yH2AX signal intensity was measured. From this analysis, an output measure of IntegratedIntensity per object / per field-of-view was obtained for each experimental condition.

**Chromatin fractionation.** Chromatin fractionation was performed using the Thermo Scientific Subcellular Protein Fractionation Kit for cultured cells (Fisher, cat. no. 78840), following the manufacturer’s instructions. Modifications in the protocol included scraping cells in cold PBS supplemented with PARP1 used for the respective experiment and MNase digestion was performed at 37°C for 15 min. An extra wash step with the nuclear extraction buffer was introduced before MNase digestion.

**Immunoprecipitation.** Exponentially growing HEK293T cells were transfected in a 10-cm dish with 3x FLAG-tagged ALC1 constructs using a 1:1 ratio of DNA:PEI. At 48 h after transfection, cells were scraped in cold PBS and the cell pellet was collected after spinning down at 500g for 5 min at 4°C. Cell pellets were suspended in lysis buffer (1 ml, 50 mM Tris, pH 8.0, 150 mM NaCl, 1% Triton X-100, 1 mM PMSF and 1 mM EDTA) supplemented with 100 μg ml

For quantifying RAD51-yH2AX co-localization, cells grown on Poly-K cover slips were pre-extracted on ice for 3 min using Triton X-100 (0.5%) in CSK buffer and fixed using PFA (4%) in PBS for 15 min at RT. Following washes with PBS, cells were incubated with a 1:2,000 dilution of anti-phospho-Histone H2A.X (Ser139) chain JBW031 antibody (Millipore, cat. no. 05–856–1) for 1 h at RT. Cells were washed with PBS and then incubated with the Alexa Fluor

For quantifying ssDNA, cells grown on Poly-K cover slips were labelled with CldU (10 μM, Sigma, cat. no. C6891) for 48h. After pre-extraction on ice for 5 min using Triton X-100 (0.5%) in CSK buffer, cells were fixed in PBS containing PFA (4%) for 15 min at RT, washed with PBS and blocked using the blocking buffer for 1 h at RT. Cells were incubated with a 1:100 dilution of CldU antibody (Abcam, ab36326) and 1:2,000 dilution of anti-Histone H2A.X (phosphor S139) [EP8542] Y (Abcam, ab81299) for 1 h at 37°C. This was followed by washes with PBS and incubation with Alexa Fluor 488 conjugated anti-mouse (1:200 dilution) and Alexa Fluor 594 conjugated anti-rabbit secondary antibodies (1:1,000 dilution) for 1 h at RT. Cover slips were mounted and imaged as described above. CldU antibody that did not co-localize with yH2AX were manually counted, keeping the values for image brightness and contrast constant throughout the analysis.

**Assay for ATAC-seq.** ATAC-seq was performed as previously described16. Reads from ATAC-seq experiments were trimmed with Trim Galore (version 0.4.1) with the parameters -q 15 –phred33 –gzip –stringency 5 –e 0.1–10. Trimmed reads were aligned to the Ensembl GRCh37.75 primary assembly including chromosome 1–22, chrX, chrY, chrM and contigs using BWA (version 0.7.13) with the parameters bowtie aln -q 3 -1 32 -k 2 -t 12 and paired-end reads were group with bwa sample -P o 10000000 . Reads mapped to contigs, the ENCODE blacklist and marked as duplicates by Picard (version 2.1.0) were discarded, and the remaining reads were used in downstream analyses. Peaks in each sample were identified using MACS (version 2.0.8) with the parameters -p 1E-5 –nomodel –nomatch –format = BAM -q bs –bw = 3000–keep-dup = F . A union of all peaks in untreated wild type, PARP1-treated wild type, untreated ALC1 KO and PARP1-treated ALC1 KO samples were generated using bedtools (version 2.27.1) ‘merge’ function.

To determine the change in accessibility, aligned reads of each ATAC-seq sample were quantified on the peak of peaks and normalized to fragments per kilobase of genomic DNA (FPKM). The log (fold change) of accessibility was calculated as log$_2$(averaged FPKM) on replicates of other conditions versus untreated wild type. The significance of change was determined using unnormalized quantification as the input to the ‘DESeq’ function from the DESeq2 package in R (version 3.6.1) with parameters test = Wald, betaPrior = F, fitType = parametric. P-values were adjusted for multiple hypothesis testing using FDR. The average ATAC-seq signal ± 1 kb round the centre of the union of peaks was calculated using the ‘metagene’ R package and the metagene plot was generated using the ggplot2 R package. A one-tailed paired t-test of the average profiles was performed for other conditions versus untreated wild type using the ‘t.test’ function in R with parameters paired = TRUE and alternative = less.

**Antibodies used for immunoblotting.** The following antibodies were used: ALC1 (Santa Cruz Biotechnology, sc-81065, 1:200 dilution), XRCC1 (Abcam, ab1838, 1:200 dilution), PARG (Millipore, MAB561, 1:200 dilution), CHD4 (Proteintech, ab51613, 1:500 dilution), APE1 (Santa Cruz Biotechnology, sc-17774, 1:200 dilution), NTHL1 (Santa Cruz Biotechnology, sc-26061c1, 1:200 dilution), 53BP1 (Novus Biological, NB100-941, 1:1,000 dilution), PARP1 (Cell Signaling, mAb9532, 1:2,000 dilution), PARP2 antibody (Active Motif, 39743, 1:1,000 dilution), HA (BioLegend, 901541, 1:1,000 dilution), GFP (Cell Signaling, mAb92956, 1:1,000 dilution), Rev7 (Inviogene PAS-49352, 1:1,000 dilution), GAPDH (Cell Signaling, 2118S, 1:2,000 dilution), mouse anti-FLAG (F1804, Sigma) and anti-Histone H4 (Millipore, 05-858).

**ATAC-seq analysis.** ATAC-seq was performed as previously described16. Reads from ATAC-seq experiments were trimmed with Trim Galore (version 0.4.1) with the parameters -q 15 –phred33 –gzip –stringency 5 –e 0.1–10. Trimmed reads were aligned to the Ensembl GRCh37.75 primary assembly including chromosome 1–22, chrX, chrY, chrM and contigs using BWA (version 0.7.13) with the parameters bowtie aln -q 3 -1 32 -k 2 -t 12 and paired-end reads were group with bwa sample -P o 10000000 . Reads mapped to contigs, the ENCODE blacklist and marked as duplicates by Picard (version 2.1.0) were discarded, and the remaining reads were used in downstream analyses. Peaks in each sample were identified using MACS (version 2.0.8) with the parameters -p 1E-5 –nomodel –nomatch –format = BAM -q bs –bw = 3000–keep-dup = F . A union of all peaks in untreated wild type, PARP1-treated wild type, untreated ALC1 KO and PARP1-treated ALC1 KO samples were generated using bedtools (version 2.27.1) ‘merge’ function. To determine the change in accessibility, aligned reads of each ATAC-seq sample were quantified on the peak of peaks and normalized to fragments per kilobase of genomic DNA (FPKM). The log (fold change) of accessibility was calculated as log$_2$(averaged FPKM) on replicates of other conditions versus untreated wild type. The significance of change was determined using unnormalized quantification as the input to the ‘DESeq’ function from the DESeq2 package in R (version 3.6.1) with parameters test = Wald, betaPrior = F, fitType = parametric. P-values were adjusted for multiple hypothesis testing using FDR. The average ATAC-seq signal ± 1 kb round the centre of the union of peaks was calculated using the ‘metagene’ R package and the metagene plot was generated using the ggplot2 R package. A one-tailed paired t-test of the average profiles was performed for other conditions versus untreated wild type using the ‘t.test’ function in R with parameters paired = TRUE and alternative = less.
RNA-seq. BRCA1-mutant hTERT-RPE1 cells and BRCA2-mutant DLD1 cells were infected with lentivirus expressing sgRNAs targeting ALC1 and selected using the appropriate selection marker. One to five million cells were collected at the fourth population doubling time after their respective T_{0}. The RNA-seq library was prepared and analysed as described before. Software used for analysis included the Lexogen Quantsave 2.3.1 FWD platform, STAR Aligner with modified ENCODE settings, HTSeq-count and DESeq2 (1.14.1).

Statistics and reproducibility. All statistical analyses were performed using Microsoft Excel 2011 and GraphPad PRISM 8 software. Significance was calculated either by the two-tailed Student's t-test or Mann–Whitney test, unless otherwise specified. All experiments were biologically repeated and confirmed.

**Reporting Summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this Article.

**Data availability.** Sequencing data generated in this study have been deposited in the Gene Expression Omnibus with accession codes GSE149104 (for RNA-seq) and GSE150955 (for ATAC-seq). Data from the CRISPR screen have been provided as mapped reads in Supplementary Table 1. Functionally conserved domains were identified using either the NCBI Conserved Domain Search or UniProt. All other data supporting the findings of this study are available from the corresponding author upon reasonable request. Source data are provided with this paper.

**Code availability.** All the analyses were based on standard algorithms described in the Methods and referenced accordingly. There are no custom algorithms to make available.

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**Author contributions.** P.V., J.S. and R.A.G. designed the study. P.V. did most of the experiments, with assistance from P.V.D., M.D., Y.S., Y.L. and S.P. Z.C. and E.A. performed the CRISPR screen in SUM149PT and CAPAN-1 cells and the RNA-seq experiments. Y.Z. carried out the ATAC-seq experiment under the guidance of R.B.F. W.L. performed the mouse work. L.P. imaged and analysed PARP1 and PARP2 trapping under the guidance of R.H.M. P.V. and R.A.G. wrote the manuscript with contributions from J.S.

**Competing interests.** R.A.G. is a founder and scientific advisory board member of RADD Pharmaceuticals. The other authors declare no competing interests.

**Additional information.**

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Extended Data Fig. 1 | ALC1 loss renders olaparib hypersensitivity and proliferation defects in various BRCA-mutant lines. 

a, Protein domains ranked on the basis of the CRISPR score (CS) for ola sensitivity in BRCA1-mutant UWB1.289 cells. b, Immunoblot showing depletion of ALC1 using a sgRNA vector with GFP selection marker. GFP-positive cells were sorted and analyzed. The blot is a representative image of two biologically independent experiments. c, GFP competition assay to examine the effects of ALC1 depletion on ola sensitivity in CAPAN-1, SUM149PT and UWB1.289 cells. Ola sensitivity in CAPAN-1 and SUM149PT was confirmed using six and seven independent guides respectively and data for each guide are from one experiment performed at three different ola concentrations. Ola sensitivity in UWB1.289 was confirmed using seven independent guides and data for each guide is mean of two biologically independent experiments in the absence and presence of 50 nM ola concentration. Source data are provided.
Extended Data Fig. 2 | ALC1 loss enhances the therapeutic window of olaparib sensitivity in BRCA-mutant cells. a, Sensitivities of the indicated cell lines to ola using CellTiter-Glo. Data are mean of 2 (hTERT-RPE1) or mean ± s.e.m. of 3 (UWB1.289, SUM149PT and DLD1) biologically independent experiments.

b, Representative images (left) and quantification (right) of the clonogenic survival of ALC1-depleted DLD1 WT and BRCA2-mutant cells grown in the presence of increasing concentrations of ola. Data are mean ± s.e.m. from 3 biologically independent experiments. Source data are provided.
Extended Data Fig. 3 | Extended analysis of PARPi sensitivity upon ALC1 loss. a, Sensitivities of the indicated DLD1 BRCA2−/− cells to vel (veliparib), ola and tal in CellTiter-Glo assay. Data are mean ± s.e.m. from n = 3 biologically independent experiments. b, Immunoblot showing ALC1 levels in cells used for xenograft studies (first four left lanes) and in tumors that reached >10.5 mm in any dimension, which is when the mice were euthanized. Data from two biologically independent tumors per condition are shown. c, GFP competition experiment in UWB1.289+BRCA1 addback line to examine the effects of the combined loss of ALC1 and the indicated DNA repair proteins on cell proliferation and ola sensitivity. Data are normalized to T_initial and indicate mean ± s.e.m. After every two population doublings, cells were passaged (P) and GFP percent was recorded (n=4 independent transductions except for sgFEN1, where n = 6 independent transductions were performed). Source data are provided.
Extended Data Fig. 4 | See next page for caption.
Extended Data Fig. 4 | Genomic lesions in PARPi treated ALC1-deficient cells are repaired by SSBR and NHEJ. a-b, Immunoblot showing levels of ALC1 and XRCC1 in indicated DLD1 (a) and hTERT-RPE1 (b) cells. The western samples were analyzed once to check the efficiency of the sgRNAs for protein depletion before drug sensitivity assays. c, Sensitivities of the indicated DLD1 cells lines to ola and tal using the CellTiter-Glo assay. Data are mean ± s.e.m. from n = 3 biologically independent experiments. d, Sensitivities of the indicated hTERT-RPE1 cells lines to tal using the CellTiter-Glo assay. Data are mean ± s.e.m. from n=2 biologically independent experiments. e, Sensitivities of the indicated UWB1.289 cell lines to ola using the CellTiter-Glo assay. Data are mean ± s.e.m. from n = 3 biologically independent experiments. f, Quantification of γH2AX-Rad51 foci in indicated cell lines. Cells were fixed 16 hrs. after treatment with 10 Gy ionizing radiation (IR). Median is indicated. p-value determined by Mann–Whitney was derived from n≥54 cells examined over two biologically independent experiments. g, Quantification of γH2AX-Rad51 foci in indicated cell lines. Cells were treated with 5 µM ola for 24 hrs. before fixation. Median is indicated. p-value determined by Mann–Whitney was derived from n≥114 cells examined over three biologically independent experiments. h, Representative images and quantification of radials (indicated by red arrowheads) and breaks (indicated by yellow arrowheads) in the indicated UWB1.289 cell lines, post treatment with 1 µM ola for 24 hrs. For each experiment, at least 50 spreads were analyzed per sample. Data are mean from two biologically independent experiments. Source data are provided.
Extended Data Fig. 5 | ALC1 deficiency results in increased trapping of PARP1 and PARP2 by PARPi upon DNA damage. a-b Representative images (left) and quantification (right) of PARP1 (a) and PARP2 (b) trapping in UWB1.289 cells. Indicated treatments were performed for 4 hours. Median is indicated. p-value determined by Mann–Whitney was derived from n≥107 cells sampled over two biologically independent experiments.
Extended Data Fig. 6 | ALC1 loss confers MMS sensitivity and results in replication-coupled gaps. 

a, Representative images of Rad51–γH2AX foci in U-2 OS (left) and UWB1.289 (+BRCA1 addback) (right) cell lines. Scale bar: 2 microns. Images represent n≥67 cells examined over two biologically independent experiments. b, Representative images of γH2AX signal in EdU negative (left) and positive (right) hTERT-RPE1 BRCA1−/− cells. Scale bar: 2 microns. Images represent n≥99 cells examined over two biologically independent experiments. c, Sensitivities of the indicated cell lines to MMS and CPT using the CellTiter-Glo assay. Data are mean ± s.e.m. from n=3 biologically independent experiments. d, Sensitivities of the indicated hTERT-RPE1 cells lines to MMS and CPT using the CellTiter-Glo assay. Data are mean ± s.e.m. from n=3 biologically independent experiments. e, Representative images of fibers from the S1 nuclease experiment. Scale bar: 2 microns. Images represent n≥75 fibers examined over two biologically independent experiments. Source data are provided.
Extended Data Fig. 7 | ALC1 is recruited to the damaged chromatin under conditions of reduced PARylation. a, Schematic of the experiment. b-c, Representative images (b) and quantification (c) of HA-ALC1 localization to chromatin upon indicated treatments in U-2 OS cells. d, Schematic of the experiment. e-f, Representative images (e) and quantification (f) of HA-ALC1 localization to chromatin upon indicated treatments in SUM149PT cells. Scale bar, 10 microns. The median value was normalized to untreated control. Data are mean ± s.e.m. from n = three biologically independent experiments, p-value, unpaired Student’s t-test. For each experiment, at least 50 cells were analyzed per sample. Source data are provided.
Extended Data Fig. 8 | See next page for caption.
Extended Data Fig. 8 | ATPase activity, H4 interaction and macrodomains of ALC1 are essential for protecting BRCA-mutant cells from ola hypersensitivity. a, Representative images (left) and quantification (right) of the clonogenic survival assay using hTERT-RPE1 BRCA1-/- cells expressing sgALC1 and the indicated ALC1 mutants treated with ola (1 nM). Data are mean from two biologically independent experiments. b, Representative images (left) and quantification (right) of the clonogenic survival assay (left) using SUM149PT cells expressing sgALC1 and ALC1 K77R mutant treated with ola (0.5 nM). Data are mean ± s.e.m from n = three independent experiments. Number of colonies in the ola treated condition were normalized to its respective untreated counterpart. c, Sequence alignment of various chromatin remodelers using Clustal Omega. Histone H4 interacting residues as predicted by PDB:6PWF are highlighted and marked by a blue star. d, Immunoblots showing interactions of FLAG ALC1 WT and FLAG ALC1 D377A+D381A with histone H4. Experiment was repeated twice with similar outcomes. e, Representative images of the clonogenic survival assay (left) and quantification (right) of SUM149PT cells expressing sgALC1 and indicated ALC1 macrodomain mutants treated with ola (1 nM). Data are mean ± s.e.m. from n = three independent experiments. Number of colonies in the ola treated condition were normalized to its respective untreated counterpart. Source data are provided.
Extended Data Fig. 9 | ALC1 co-operates with PARP activity to permit association of repair proteins with chromatin. a, DLD1 BRCA2-- cells were fractionated and the chromatin-bound proteins were immunoblotted. Cells were treated with 5 µM ola for 4 hrs. Data for DLD1 BRCA2-- cells are from the same sample, from two different western blots and the histone levels for each blot are shown by the ponceau staining. The data for XRCC1 is representative of 5 biologically independent experiments and the data for NTHL1 and APE1 is representative of 3 biologically independent experiments. b, Immunoblot of whole cell lysates of DLD1 BRCA2-- cells showing total proteins levels upon ALC1 depletion and PARPi treatment. Cells were treated with indicated PARPi for 4 hrs. Data is representative of two biologically independent experiments. c, UWB1.289 cells were fractionated and the chromatin-bound proteins were immunoblotted. Cells were treated with 1 µM tal for 4 hrs. Data for XRCC1 is representative of 4 biologically independent experiments and the data for APE1 is representative of 3 biologically independent experiments. d, Immunoblot showing expression levels of HA-XRCC1. The blot was performed once to access the expression level of the tagged protein. e, Schematic of the IF experiment. f-g, Representative images (f) and quantification (g) of HA-XRCC1 localization to chromatin upon indicated treatments. Scale bar, 50 microns. Data are mean ± s.e.m. from n = three biologically independent experiments, p-value, unpaired Student’s t-test. For each cell line, the median value upon MMS treatment was normalized to its respective untreated control. Source data are provided.
Extended Data Fig. 10 | ALC1 loss synergistically enhances IR sensitivity at low olaparib doses. a-b Representative images of two independent clonogenic survival assay to monitor the effect of combining low doses of ola and IR upon ALC1 depletion in UWB1.289 (a) and hTERT-RPE1 BRCA1−/− cells (b). c-d, Quantification of clonogenic survival (c) and heat map of bliss scores (d) obtained from BRCA1−/− hTERT-RPE1 cells treated with the indicated doses of ola and IR. Data are mean from two biologically independent experiments. Bliss score >0, synergistic; Bliss score <0, antagonistic; Bliss score = 0, additive. Number of colonies in IR-treated conditions were normalized to their respective un-irradiated counterparts. Colonies with more than 50 cells were included in the analysis. Source data are provided.
Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- n/a
- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
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- The statistical test(s) used AND whether they are one- or two-sided
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- A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted
  - Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

Software and code

Policy information about availability of computer code

Data collection: Nikon NIS-Elements software (BR 4.500.0064-bit.Ink), InCyte Software (version 3.3), Gen5 v 2.0.1.14

Data analysis: Data analysis and plotting: GraphPad PRISM version 8, Microsoft Excel 2011; Image analysis: Fiji 2.0 d, CellProfiler 3.0 software. For ATAC-seq analysis: Trim galore (version 0.4.1), Picard (version 2.1.0), Macs (version 2.0.9), Bedtools (version 2.27.1) and Deseq2 package in R version 3.6.1. For RNA-seq analysis: Lexogen Quantseq 2.3.1 FWD platform, STAR Aligner with modified ENCODE settings, HTSeq-count, DESeq2 [1.14.1]. For synergy analysis: Combenefit

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Sequencing data generated in this study has been deposited in the Gene Expression Omnibus with accession code # GSE149104 (for RNA-seq) and # GSE150955 (for ATAC-seq). Data from the CRI/SPA screen has been provided as mapped reads in Supplementary Table 1. Functionally conserved domains were identified using either NCBI Conserved Domain Search or Uniprot. Source data are provided with this paper. All other data supporting the findings of this study are available from the corresponding author upon reasonable request.
Field-specific reporting

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- Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size
No statistical method was used to pre-determine sample size. Sample sizes are consistent with previously published studies on DNA repair. (Dilley et al, Nature, 2016; Clairmount et al, Nat Cell Bio, 2020; Zimmermann, Nature 2018). Key validation experiments were performed using at least 2-3 independent replicates based on standard practices in the field. Every biological replicate was derived from independent sgRNA transductions and generation of a new stable line. For all cell viability assays, cells were plated in triplicates and treated separately. Values of the triplicates were averaged to derive data for one biological replicate.

Data exclusions
In the xenograft experiment, two individual flanks that failed to develop tumors were excluded from subsequent measurements. The GFP data was acquired using a 96-well plate, high-throughput Guava machine, where cell counts less than 500 trend to give fluctuations in the results. Therefore, for GFP competition experiments, reading were not taken when cell were less than 50% confluent and any value derived from less than 500 cells was omitted. We performed multiple biological independent transductions to confirm that all key results had at the minimum, values from three transductions for all time points.

Replication
All attempts at replication were successful. The number of replicates for each experiment is clearly presented in the corresponding figure legend. Most results were confirmed in multiple cell lines and using complementary assays.

Randomization
For xenograft studies animals were randomized. The two mice that developed a tumor only on one flank were included in the untreated group. Cell culture experiments are not subjected to randomization.

Blinding
The technician performing xenograft experiment was blinded to the experimental design. All IF analysis was blinded. For most other experiments, results were immediately revealed (e.g. western) or data generation was automated (e.g. GFP measurement or viability assay) and hence these experiments couldn’t be subjected to blinding.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
| ☐   | Antibodies            |
| ☑   | Eukaryotic cell lines |
| ☐   | Palaeontology and archaeology |
| ☑   | Animals and other organisms |
| ☐   | Human research participants |
| ☑   | Clinical data |
| ☑   | Dual use research of concern |

Methods

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

Antibodies

**Antibodies used**

- ALC1 (Santa Cruz Biotechnology, sc-81065, 1:200 dilution), XRCC1 (Abcam, ab1838, 1:200 dilution), PARG (Millipore, MA5651, 1:200 dilution), CHD4 (Proteintech, ab264417, 1:1000), APE1 (Santa Cruz Biotechnology, sc-17774, 1:200 dilution), NTHL1 (Santa Cruz Biotechnology, sc-2660C1a, 1:200 dilution), S3BP1 (Novus Biological N8100-94, 1:1000 dilution), PARP1 (Cell Signaling mAb#9532, 1:2000 dilution for western), PARP2 (1:500 for IF, Abcam, ab227244), PARP2 (1:500, Active Motif, 39743), Rev7 (Invitrogen P10-59352, 1:1000 dilution), GAPDH (Cell Signaling 21185, 1:2000 dilution), Histone H2AX [Ser139] clone BW301 antibody (Millipore, 05-636-1), RAAS1 antibody (H-92, sc-8349, Santa Cruz), CidU antibody (Abcam, ab6326) and Anti-Histone H2A.X [phosphor S139] [EP5842] antibody (Abcam, ab12199), Alexa Fluor 568 conjugated anti-mouse secondary [A11031], Alexa Fluor 488 conjugated anti-rabbit secondary [A11034], Alexa Fluor 488 conjugated anti-mouse secondary [A11029], Alexa Fluor 568 conjugated anti-rabbit secondary (A-11036), anti-HA [Biologend, 5011541], rabbit anti-PCNA (Cell Signaling, 13110S), anti-IdU (BD-347580), rat anti-CidU (Serotec-OBI0035G), GFP (Cell Signaling mAb#2950), mouse Anti-FLAG (F1804, Sigma), anti-Histone H4 (Millipore, 05-858).

**Validation**

Antibodies validated by knockdown: ALC1 (Santa Cruz Biotechnology, sc-81065, 1:200 dilution), XRCC1 (Abcam, ab1838, 1:200 dilution).
Validation

dilution]; S3BP1 [Novus Biological NB100-94, 1:1000 dilution], PARP1 [Cell Signaling mAB#9532, 1:2000 dilution for western], PARP2 [1:1500, Active Motif, 39743], Rev7 [Invitrogen PAS-49352, 1:1000 dilution], GFP [Cell Signaling mAB#2956].

Antibodies validated by overexpression: mouse Anti-FLAG [FL804, Sigma], anti-HA [Biologend, 901514].

Antibodies validated by knockdown but data unpublished: NTHL1 [Santacruz Biotechnology, sc-266001A, 1:200 dilution], PARG [Millipore,MAB561, 1:200 dilution], rabbit anti-PCNA [Cell Signaling, 13110S].

Validation for ChD4 [Proteintech, ab264417, 1:1000]: (https://www.proteintech.com/products/ChD4-Antibody-14173-1-AP.html).

Validation for APT-1 [Santacruz Biotechnology, sc-17774, 1:200 dilution]: Sica et al, 2019, Cell Reports and Wang et al, 2018, Cell death and disease

PARP1 [1:500 for IF, Abcam, ab227244]; Gatti et al, Cell Reports, 2020

GAPDH [Cell Signaling 21185, 1:2000 dilution]: This antibody is widely used as loading control: Wang et al, 2019; Nature, Wu et al, 2019 Nature Med. Dilley et al 2016 Nature

Histone H2AX (Ser139) clone JBW301 antibody [Millipore, 05-636-1], RAD51 antibody (H-92, sc-8349, Santacruz), CldU antibody [Abcam, ab6216], anti-IκB (BD-345750), rat anti-CdK6 (Ser860), Abcam, ab81299: These are gold-standard antibodies in DNA repair field. Few examples using these antibodies: Zimmermann et al, 2018, Nature, Dungrawala et al, Mol Cell, 2017, Mirman et al, Nature, 2018, Quintet, 2017, Methods Enyimol., Phillips et al, Mol Cell, 2017. H2AX (pSer139 ) clone JWB301 antibody [Millipore, 05-636] antibody was also validated by comparing signals in control and cells treated with IR and MMS damage. No signal was detected in undamaged cells. CldU antibody was also confirmed by comparing signals in control and cells treated with CldU. No signal was detected in cells not treated with CldU.

Anti-Histone H2AX (phosphoS139) [EPB542Y]: Validated as damaged marker by Abcam: https://www.abcam.com/gamma-h2ax-phospho-s139-antibody-epb542y-ab81299.html#productWallTabAbreviews#description_images_1

Anti-Histone H4 [Millipore, 05-858]: Validated by Millipore https://www.emdmillipore.com/US/en/product/anti-histone-h4-antibody-par-clone-62-141-13-rabbit-monoclonal-MM-05-858

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

293T, U-2 OS, CAPAN-1, UW81.289 and UW81.289+BRCAl [ATCC]; DLD1 WT and DLD1 BRCA2-/- (Horizon discovery); SUM149PT (Asterand Bioscience); hTERT-RPE1 p53-/-; Cas9 and hTERT-RPE1 p53-/-/BRCA-/-; Cas9 cells were gifted by D. Durocher (Univ. Toronto, Lunenfeld) [Zimmermann et al, Nature, 2018]. SUM149PT reversion mutations and parental control were a gift from Neil Johnson’s lab [FoxChase, (Wang et al, Cancer Research, 2016)], hTERT-RPE1 parental and XRCC1 KO from Keith Caldecott (Univ. of Sussex) [Hanslikova et al, 2017, NAR] and U-2 OS PARP1/-; PARP2/- and PARP1/-; cells from Nick Lakin lab (Univ. of Sussex) [Ronson et al, 2018, Nature Comm.].

Authentication

Purchased of UW81.289 and DLD1 isogenic lines were made at the initiation of the project. CAPAN-1 and U-2 OS lines were validated by STR profiling at ATCC. SUM149PT was confirmed by immunoblotting and PARP sensitivity. All other cell lines were procured from published studies as reported above and were not authenticated.

Mycoplasma contamination

All cell lines tested negative for mycoplasma.

Commonly misidentified lines

No commonly misidentified cell lines were used in the study.

Animals and other organisms

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

Laboratory animals

5-week-old female NSG (NOD.Cg-PrkdcscidIl2rgtm1Wjl/SzJ) mice from Jackson laboratory. Mice were kept at 72F with average humidity of 60% in 12h day light and 12h night cycle.

Wild animals

The study did not involve wild animals.

Field-collected samples

The study did not involve field collected samples.

Ethics oversight

Xenograft studies were carried out under protocol number 03176 approved by the Institutional Animal Care & Use Committee at the University of Pennsylvania.

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