CRISPR screens identify genomic ribonucleotides as a source of PARP-trapping lesions

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The observation that BRCA1- and BRCA2-deficient cells are sensitive to inhibitors of poly(ADP–ribose) polymerase (PARP) has spurred the development of cancer therapies that use these inhibitors to target deficiencies in homologous recombination1. The cytotoxicity of PARP inhibitors depends on PARP trapping, the formation of non-covalent protein–DNA adducts composed of inhibited PARP1 bound to DNA lesions of unclear origins1–4. To address the nature of such lesions and the cellular consequences of PARP trapping, we undertook three CRISPR (clustered regularly interspersed palindromic repeats) screens to identify genes and pathways that mediate cellular resistance to olaparib, a clinically approved PARP inhibitor5. Here we present a high-confidence set of 73 genes, which when mutated cause increased sensitivity to PARP inhibitors. In addition to an expected enrichment for genes related to homologous recombination, we discovered that mutations in all three genes encoding ribonuclease H2 sensitized cells to PARP inhibition. We establish that the underlying cause of the PARP-inhibitor hypersensitivity of cells deficient in ribonuclease H2 is impaired ribonucleotide excision repair5. Embedded ribonucleotides, which are abundant in the genome of cells deficient in ribonucleotide excision repair, are substrates for cleavage by topoisomerase 1, resulting in PARP-trapping lesions that impede DNA replication and endanger genome integrity. We conclude that genomic ribonucleotides are a hitherto unappreciated source of PARP-trapping DNA lesions, and that the frequent deletion of RNASEH2B in metastatic prostate cancer and chronic lymphocytic leukaemia could provide an opportunity to exploit these findings therapeutically.

We carried out dropout CRISPR screens with olaparib in three human cell lines of diverse origins, representing both neoplastic and non-transformed cell types (Fig. 1a, Extended Data Fig. 1a, b). The cell lines selected were HeLa, which is derived from a human papilloma virus-induced cervical adenocarcinoma; RPE1-hTERT, a telomerase-immortalized retinal pigment epithelium cell line; and SUM149PT, originating from a triple-negative breast cancer with a hemizygous BRCA1 mutation6. SUM149PT cells express a partially defective BRCA1 protein (BRCA1-Δ11q)7 and thus provided a sensitized background to search for enhancers of PARP-inhibition cytotoxicity in cells that have compromised homologous recombination. The screens were performed in technical triplicates, and a normalized deletion score for each gene was computed using Drug2. To identify high-confidence hits, we used a stringent false discovery rate (FDR) threshold of 1% in one cell line. To this initial list, we added genes that were found at an FDR threshold of less than 10% in at least two cell lines. This analysis identified 64, 61 and 116 genes, the inactivation of which caused sensitization to olaparib in the HeLa, RPE1-hTERT and SUM149PT cell lines, respectively, giving a total of 155 different genes (Supplementary Table 1).

Out of this list, 13 genes scored positive in all three cell lines and a further 60 genes were common to two cell lines, which we combine to define a core set of 73 high-confidence PARP inhibitor (PARPi)-resistance genes (Fig. 1b, Supplementary Table 1). Gene Ontology analysis of the 73- and 155-gene sets (Fig. 1c, Extended Data Fig. 1c, respectively) shows strong enrichment for biological processes related to homologous recombination, providing unbiased confirmation that the screens identified bona fide regulators of the response to PARP inhibition. Mapping the 73-gene set on the HumanMine protein–protein interaction data (Fig. 1d) generated a highly connected network consisting of DNA damage response genes that include many regulators of homologous recombination (such as BRCA1, BARD1, BRCA2 and PALB2), components of the Fanconi anaemia pathway, as well as the kinases ATM and ATR. Outside or at the edge of the network, we noted the presence of genes encoding the MUS81–EME1 nuclease, splicing and general transcription factors (such as SFB3/B and CTDP1) and the three genes coding for the ribonuclease (RNase) H2 enzyme complex (RNASEH2A, RNASEH2B and RNASEH2C), RNASEH2A, RNASEH2B and RNASEH2C were hits in all three cell lines, with RNASEH2A and RNASEH2B being the two highest-scoring genes, as determined by the mean Drug2 value from the three cell lines (Supplementary Table 1). A similar analysis of the 155-gene set generated an even denser network, with additional genes lying at the periphery of a homologous recombination and Fanconi anaemia core (Extended Data Fig. 1d).

Next, we generated RNase H2-null HeLa, RPE1, SUM149PT and HCT116 clonal cell lines using genome editing (denoted as KO; Extended Data Fig. 2a–d) and confirmed that RNase H2 deficiency caused hypersensitivity to both olaparib and a second clinical-stage PARPi, talazoparib, in all cell lines tested (Fig. 2a, b, Extended Data Fig. 2e–g, with half-maximal effective concentration (EC50) values reported in Extended Data Fig. 2h). The RNASEH2A KO and RNASEH2B KO cells also exhibited increased levels of apoptosis after PARP inhibition (Extended Data Fig. 2i–l), a phenotype that was particularly prominent with talazoparib treatment (Extended Data Fig. 2i–l). Given the strength of the PARPi-induced phenotypes in RNase H2-deficient cells, and as RNase H2 had not previously been linked to the response to PARP inhibition, we sought to determine the mechanism of PARPi sensitization in RNase H2-deficient cells.

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RNase H2 cleaves single ribonucleotides incorporated into DNA, as well as longer RNA–DNA hybrids15. To distinguish between these two functions, we carried out cellular complementation experiments with variants of RNase H2. The sensitivity of RNASEH2AKO cells to olaparib was not rescued by either a catalytically-inactive RNase H2 enzyme (RNASEH2A(D34A/D169A)), or by a separation-of-function mutant (RNASEH2A(P40D/Y210A)16) that retains activity against RNA–DNA hybrids, but not DNA-embedded monoribonucleotides (Fig. 2j, Extended Data Fig. 4). These data indicate that it is the removal of genome-embedded ribonucleotides by ribonuclease excision repair (RER), and not RNA–DNA hybrid cleavage by RNase H2, that protects cells from PARPi-induced cytotoxicity.

To determine the genetic basis of the sensitivity of RNASEH2AKO cells to PARPi, we carried out CRISPR screens to identify mutations that restored resistance to talazoparib in RNase H2-deficient HeLa and RPE1-hTERT cell lines (Fig. 3a, Extended Data Fig. 5a, Supplementary Table 2). The screens identified a single common gene, PARP1. The genetic dependency on PARP1 for talazoparib- and olaparib-induced cytotoxicity was confirmed in double mutant RNASEH2AKO/PARPi cells (Fig. 3b, Extended Data Fig. 5b–e), providing evidence that the lethality associated with PARP inhibition requires formation of trapped PARPi–DNA adducts4. Consistent with this finding, treatment with veliparib, a PARP inhibitor with poor trapping ability4 induced much less apoptosis than olaparib or talazoparib in RNASEH2AKO cells (Extended Data Fig. 5f).

Analysis of DNA content by flow cytometry revealed that RNASEH2AKO cells arrest in S phase in a PARPi-dependent manner upon talazoparib treatment (Fig. 3c, Extended Data Fig. 5g). RNASEH2AKO cells also demonstrated increased levels of talazoparib-induced γ-H2AX and these levels did not decline upon drug removal (Fig. 3c, Extended Data Fig. 5h). These observations suggest that unresolved DNA lesions induced by PARP trapping are the likely cause of cell death in PARPi-treated RNASEH2AKO cells.

Genome instability in RER-deficient yeast cells is dependent on an alternative, topoisomerase 1 (TOP1)-mediated ribonucleotide excision pathway17–19. In this process, TOP1 enzymatic cleavage3′ of the embedded ribonucleotide results in DNA lesions predicted to engage PARPi, including nicks with difficult-to-ligate 2′-3′-cyclophosphate ends 17,18,20 and covalent TOP1–DNA adducts (TOP1 cleavage complexes 21) in conjunction with single-strand DNA gaps or double-strand breaks22. Given that the mechanisms promoting genome instability in mammalian RNase H2-deficient cells remain poorly defined, we assessed whether TOP1 action on misincorporated ribonucleotides contributed to the DNA damage observed in human RER-deficient cells. Short-term depletion of TOP1 using short interfering RNAs (siRNAs) reduced the number of sister chromatid exchanges, reminiscent of the ‘hyper-rec’ phenotype observed in RNAse H2-deficient yeast23 (Fig. 2f). This phenotype was probably caused by increased levels of replication-dependent DNA damage, as determined by γ-H2AX staining (Fig. 2g, Extended Data Fig. 3e–h) and marked poly(ADP-ribosylation) of PARPi (Fig. 2h, Extended Data Fig. 3i, j), supporting previous observations of replication-associated genome instability in yeast and mammalian cells deficient in RNAse H212–14.

The increased levels of sister chromatid exchanges prompted us to test whether RNAse H2-deficient cells required homologous recombination for survival. Indeed, we observed synthetic lethality when an sgRNA against RNAseH2B was delivered into engineered BRCA1KO and BRCA2KO cell lines in the RPE1-hTERT and DLD-1 backgrounds, respectively (Fig. 2i, Extended Data Fig. 3k–o).

As deficiency in homologous recombination causes PARPi sensitivity, we first considered that RNase H2 might promote homologous recombination. Consistent with this possibility, fission yeast cells that combine mutations in RNase H2 and RNase H1 have defects in homologous recombination24. However, in RNase H2-deficient cells, RAD51 readily formed ionizing radiation-induced foci, suggesting efficient recombinase filament assembly (Fig. 2c, d, Extended Data Fig. 3a, b). Furthermore, the efficiency of homologous recombination, as assessed by the direct repeat-green fluorescent protein (DR-GFP) assay10, was at near wild-type levels in cells transduced with RNASEH2A and RNAseH2B short guide RNAs (sgRNAs) (Fig. 2e, Extended Data Fig. 3c, d). Third, rather than presenting reduced homologous recombination, RNAseH2AKO cells displayed higher levels of sister chromatid exchanges, reminiscent of the ‘hyper-rec’ phenotype observed in RNAse H2-deficient yeast23 (Fig. 2f). This phenotype was probably caused by increased levels of replication-dependent DNA damage, as determined by γ-H2AX staining (Fig. 2g, Extended Data Fig. 3e–h) and marked poly(ADP-ribosylation) of PARPi (Fig. 2h, Extended Data Fig. 3i, j), supporting previous observations of replication-associated genome instability in yeast and mammalian cells deficient in RNAse H212–14.

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**Fig. 1** | CRISPR screens identify determinants of PARPi sensitivity. a, Schematic of the screening pipeline. b, Venn diagram of all high-confidence hits (FDR ≤ 0.01 in one cell line and FDR ≤ 0.1 in at least two cell lines) in individual cell lines. c, Gene Ontology (GO) terms significantly (P < 0.05, binomial test with Bonferroni correction) enriched among hits common to at least two cell lines. d, esN network analysis of interactions between hits common to at least two cell lines. Node size represents the mean DrugZ score across cell lines. Out of 73 genes, 31 are mapped on the network. See also Extended Data Fig. 1.

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We determined RNASEH2B copy number by multiplex ligation-dependent probe amplification (MLPA) in 100 patients with CLL. RNASEH2B deletions were present in 43% of CLL samples, with biallelic loss detected in 14%. Co-deletion of the DLEU2 microRNA cluster was confirmed by comparative genomic hybridization (CGH) microarray (Fig. 4a, Extended Data Fig. 7a, b), establishing that collateral RNASEH2B loss is frequent in CLL. Furthermore, analysis of whole-exome sequencing of metastatic castration-resistant prostate cancers (CRPCs) demonstrated frequent collateral loss of RNASEH2B with RB1 gene deletion co-occurring in 34% of tumours (2% biallelic loss; Extended Data Fig. 7c).

The frequent collateral deletion of RNASEH2B prompted us to test whether RNASEH2B loss in cancer cells could be an actionable vulnerability to PARP inhibition. To do so, we performed ex vivo analysis on primary CLL cells derived from 21 of the 100 patient samples assayed above. Patient characteristics of selected samples were similar across groups (Extended Data Table 1). RNase H2 status was confirmed by immunofluorescent foci number per nucleus in 5-ethynyl-2'-deoxyuridine (EdU) or EdU' wild-type and RNASEH2AKO cells. h. Representative poly(ADP-ribose) (PAR) immunoblot of PARP1 immunoprecipitates (IP) from whole cell extracts (WCEs). Mean fold-increase in poly(ADP-riboseylation) between wild-type and RNASEH2AKO indicated. Data are from three biologically independent experiments and are normalized to immunoprecipitated PARP1 levels. Tubulin and IgG heavy chain were included as loading controls. i. Synthetic lethality in the combined absence of RNase H2 and BRCA1. Quantification of colony formation of wild-type BRCA1-proficient and BRCA1KO RPE1-hTERT Cas9 TP53KO cells transduced with constructs encoding sgRNAs targeting LacZ or RNASEH2B. Open circles, individual values normalized to the sgRNA LacZ values; red lines, mean. Data are from three biologically independent experiments. j. PARPi sensitivity is associated with RER deficiency. Survival of olaparib-treated wild-type and RNASEH2AKO HeLa cells transduced with indicated Flag-tagged constructs. Data are mean ± s.d. from three biologically independent experiments and are normalized to untreated cells. Solid lines, nonlinear least-squares fit to a three-parameter dose–response model. d–g, e, g, i are from unpaired two-tailed t-tests. See also Extended Data Figs. 2–4.

**Fig. 2** | Defective RER causes PARPi sensitivity, DNA damage and synthetic lethality with BRCA1 deficiency. a, b, Reduced survival of HeLa RNASEH2AKO cells after treatment with the indicated PARPi. Data are mean ± s.d. normalized to untreated cells. c–f, RNASEH2AKO cells are homologous-recombination proficient. c, d, Normal RAD51 focus formation in RNASEH2AKO HeLa cells after ionising radiation (IR) exposure. e, Representative micrographs of wild-type (WT) and RNASEH2AKO HeLa cells stained with the indicated antibodies. d, Quantification of the percentage of cells with more than five RAD51/γ-H2AX colocalizing foci at the indicated time points. Data are from three biologically independent experiments. Scale bar, 10 μm. e, Homologous recombination (HR) is not impaired in RNase H2-null cells. Quantification of gene conversion in DR-GFP reporter cells transduced with Cas9 and sgRNAs targeting RNASEH2A and RNASEH2B (sgRNH2A and sgRNH2B, respectively) or empty vector (EV) with or without I-SceI transfection. Values are normalized to the transfection efficiency of a control GFP vector. f, Increased sister chromatid exchanges (SCEs) in RNASEH2AKO cells. Representative micrographs of SCEs in wild-type and RNASEH2AKO metaphases. Numbers below the images indicate the numbers of SCEs per chromosome. Data are mean ± s.d. from three biologically independent experiments. Scale bars, 10 μm. g, h, Spontaneous replication-associated damage and increased PARPi activation in RNASEH2AKO cells. g, Quantification of mean γ-H2AX immunofluorescent foci number per nucleus in 5-ethynyl-2'-deoxyuridine (EdU) or EdU' wild-type and RNASEH2AKO cells. h, Representative poly(ADP-ribose) (PAR) immunoblot of PARP1 immunoprecipitates (IP) from whole cell extracts (WCEs). Mean fold-increase in poly(ADP-riboseylation) between wild-type and RNASEH2AKO indicated. Data are from three biologically independent experiments and are normalized to immunoprecipitated PARP1 levels. Tubulin and IgG heavy chain were included as loading controls. i, Synthetic lethality in the combined absence of RNase H2 and BRCA1. Quantification of colony formation of wild-type BRCA1-proficient and BRCA1KO HeLa cells transduced with constructs encoding sgRNAs targeting LacZ or RNASEH2B. Open circles, individual values normalized to the sgRNA LacZ values; red lines, mean. Data are from three biologically independent experiments. j, PARPi sensitivity is associated with RER deficiency. Survival of olaparib-treated wild-type and RNASEH2AKO HeLa cells transduced with indicated Flag-tagged constructs. Data are mean ± s.d. from three biologically independent experiments and are normalized to untreated cells. Solid lines, nonlinear least-squares fit to a three-parameter dose–response model. d–g, e, g, i are from unpaired two-tailed t-tests. See also Extended Data Figs. 2–4.

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Fig. 3 | PARPi-induced PARP1 trapping occurs in RER-deficient cells as a result of TOP1-mediated processing of genomic ribonucleotides. **a, b, PARP1 is required for PARPi-mediated toxicity in RNASEH2A	extsuperscript{KO} cells. CRISPR screens for talazoparib sensitivity suppressors in RNASEH2A	extsuperscript{KO} HeLa Cas9 and RPE1 Cas9 TP53	extsuperscript{fl} cell lines. Model-based analysis of genome-wide CRISPR–Cas9 knockout (MAGeCK) positive scores for each gene plotted. Colours indicate gene density in each hexagonal bin. **b, Percentage of cleaved caspase-3	extsuperscript{−} cells of the indicated genotype with or without talazoparib treatment as measured by flow cytometry (FACS). Open circles, individual experiments; red lines, mean from three biologically independent experiments. **c, DNA damage persists on withdrawal of PARPi in RNASEH2AKO cells. Wild-type and RNASEH2A	extsuperscript{KO} HeLa were treated with talazoparib and released into fresh medium for the indicated times before being processed for γ-H2AX immunofluorescence and propidium iodide (PI) staining. The γ-H2AX immunofluorescence (pseudocolor plots) and cell cycle (histograms) FACS profiles shown are representative of five biologically independent experiments. **d–f, Increased γ-H2AX foci formation in RNASEH2AKO cells depends on TOP1. Images are representative of five biologically independent experiments. **d, Wild-type and RNASEH2AKO HeLa cells were transfected with non-targeting (siCTRL) or TOP1-targeting (siTOP1) short interfering RNAs (siRNAs). Immunoblot of WCEs, probed for TOP1. Actin was used as a loading control. **e, Representative micrographs of wild-type and RNASEH2AKO HeLa cells transfected with siCTRL or siTOP1 immunostained for γ-H2AX. Scale bars, 10 μm. **f, Quantification of experiments shown in e. Mean number of foci per nucleus per experiment (open circles) with the mean of five biologically independent experiments (red lines). At least 100 cells were analysed per sample in each experiment. **g, TOP1 depletion alleviates PARPi-induced apoptosis in RNASEH2AKO cells. Quantification of cleaved caspase-3	extsuperscript{−} wild-type and RNASEH2AKO cells transfected with the indicated siRNAs, with or without talazoparib treatment. Data are mean ± s.d. from three biologically independent experiments normalized to untreated cells. At least 10,000 cells were analysed per sample in each experiment. **P values in **b, **f, **g, are from unpaired two-tailed t-tests. See also Extended Data Figs. 5, 6.

Fig. 4 | Talazoparib selectively suppresses growth of RNase H2 deficient tumours. **a–c, PARP inhibitors selectively kill RNASEH2B-deficient CLL primary cancer cells. **a, RNASEH2B deletion frequency in a panel of 100 primary CLL samples, determined by MLPA. **b, Reduced Rnase H2 activity in lysates from CLL samples with monoallelic and biallelic RNASEH2B deletions. Top, substrate schematic. Individual data points are the mean of technical duplicates for each sample. Red lines are the mean of individual genotypes (n = 8 wild type, 4 monoallelic- and 9 biallelic-deleted biologically independent primary CLL samples). Data are normalized to the mean of the wild-type RNASEH2B samples. **c, Reduced survival of CLL cells with monoallelic and biallelic RNASEH2B loss following treatment with talazoparib. Individual points are mean ± s.e.m. from n = 8, 4 and 9 CLL samples as in **b, each analysed in biological triplicate. **P values are from an unpaired two-tailed t-test (b) and two-way ANOVA (c). **d, Selective inhibition of RNASEH2A	extsuperscript{KO} xenograft tumour growth. Wild-type RNASEH2A (bottom) or RNASEH2AKO (top) HCT116 TP53	extsuperscript{KO} cells were injected subcutaneously into bilateral flanks of CD-1 nude mice. Mice were randomized to either vehicle or talazoparib (0.335 mg kg	extsuperscript{−1}) treatment groups (n = 8 animals per group) and tumour volumes were measured twice-weekly. Data are mean ± s.e.m. **P values are from a two-way ANOVA. **e, Model of the processing of genome-embedded ribonucleotides. Genome-embedded ribonucleotides (R) can be processed by TOP1 as an alternative to RNase H2-dependent RER. DNA lesions that engage PARP1 (black circles) are formed as a result, and PARP inhibitors induce PARP1 trapping on these TOP1-dependent lesions, causing replication arrest, persistent DNA damage and cell death. See also Extended Data Figs. 7, 8, Extended Data Table 1, Supplementary Table 3.
to RNase H2 loss as comcomitant with an RNASEH2A transgene abrogated PARPi sensitivity (Extended Data Fig. 8d). We conclude that collateral loss of RNase H2 enhances the vulnerability of cancer cells to PARP-trapping drugs.

Finally, we note that genome-embedded ribonucleotides are by far the most abundant aberrant nucleotides in the genome of cycling cells, and may thus represent a major source of the traps that mediate the cytotoxicity of PARPi alongside base excision repair intermediates. In support of this possibility, RNASEH2A KO cells are more sensitive to PARPi than isogenic cell lines with homozygous mutations in the catalytic domain of DNA polymerase-β (POLB^Δ188-190), a key enzyme in base excision repair (Extended Data Fig. 9). We therefore propose a model whereby the canonical RER pathway and TOP1 compete for the processing of genome-embedded ribonucleotides (Fig. 4e). Whereas RNase H2 cleavage initiates their problem-free removal, the action of TOP1 on ribonucleotides creates PARP-trapping DNA lesions that impair successful completion of DNA replication and the resulting burden of genomic lesions ultimately causes cell death. We propose that the manipulation of genome ribonucleotide processing could be harnessed for therapeutic purposes and this strategy may expand the use of PARP inhibitors to some tumours that are proficient in homologous recombination.

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Author contributions
M.Z. performed the initial CRISPSPR screens with the help of M.A., M.C., S.A. and J.M.; T.H. analysed the data; M.Z. and O.M. performed additional screens and A.A. helped with data analysis. D.D. supervised the project. M.Z. and O.M., with input from M.A.M.R., performed all additional experiments and A.M. helped with data analysis. Unless otherwise stated, M.Z. and O.M, with input from M.A.M.R., performed all additional experiments and data analysis. M.A.M.R. performed biochemical characterization of RDE- deficient RNase H2, and together with Z.T. and A.F. contributed to the generation of HeLa and HTCl16 RNASEH2A^Δ3^ cells line. A.A., under the supervision of T.S., conducted ex vivo CLL studies and CGH arrays. S.P. and P.M. clinically characterized CLL patients and provided CLL blood samples. R.C. performed MLPA assays. W.Y., M.C.I. and M.B.L., under the supervision of J.D.B., analysed copy-number alterations (CNAs) in the RNaseH2^Δ3^ region in CRBCs. L.P. and M.O., under the supervision of V.G.B., conducted xenograft experiments. A.P.J. and D.D. designed and directed the study. D.D. and A.P.J. wrote the manuscript with help of M.Z., O.M. and M.A.M.R. and all authors reviewed it.

Competing interests
D.D. and T.H. are advisors to Repare Therapeutics.

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METHODS

Cell culture. HeLa, RPE1-hTERT and 293T cells were purchased from ATCC and grown in Dulbecco’s Modified Eagle Medium (DMEM; Gibco/Thermo Fisher) supplemented with 10% fetal bovine serum (FBS; Wisent), 200 mM GlutaMAX, 1× non-essential amino acids (both Gibco/Thermo Fisher), 100 U ml⁻¹ penicillin and 100 μg ml⁻¹ streptomycin (Pen/Strep; Wisent). HCT116 TP53Δ32 cells, a gift from B. Vogelstein, were maintained in modified McCoy’s 5A medium (Gibco/Thermo Fisher) supplemented with 10% FBS and Pen/Strep. SUM149PT cells were purchased from Asterand BioScience and grown in a DMEM/F12 medium mixture (Gibco/Thermo Fisher) supplemented with 5% FBS, Pen/Strep, 1 μg ml⁻¹ hydrocortisone and 5 μg ml⁻¹ insulin (both Sigma). Wild-type and BRCA2Δ30 DLD-1 cells were purchased from Horizon and maintained in RPMI medium (Gibco/Thermo Fisher) supplemented with 10% FBS and Pen/Strep. All cell lines were grown at 37°C and 5% CO₂. HeLa, RPE1-hTERT (with the exception of BRCA1Δ100 and POLBΔ52–140 clones) and HCT116 cells were grown at atmospheric O₂. RPE1-hTERT BRCA1Δ100 and POLBΔ52–140 clones, as well as DLD-1 and SUM149PT cell lines were maintained at 3% O₂.

Lentiviral and retroviral transduction. To produce lentivirus, 4.5 × 10⁵ 293T cells in a 10-cm dish were transfected with packaging plasmids (5 μg pVSVG, 3 μg pMDLg/pRRE and 2.5 μg pRSV-Rev) along with 10 μg of transfer plasmid using calcium phosphate. Medium was refreshed 12–16 h later. Virus-containing supernatant was collected 36–48 h post transfection, cleared through a 0.4-μm filter, supplemented with 4 μg ml⁻¹ polybrene (Sigma) and used for infection of target cells. The TKOv1 library virus was prepared as previously described. The following antibiotics were used for selection of transductants: puromycin (HeLa, SUM149PT; HeLa, RPE1-hTERT and HCT116) 15–20 μg ml⁻¹ each for 48–72 h unless indicated otherwise) and blasticidin (5 μg ml⁻¹, 1–4 days for all cell lines). Cells stably expressing Flag–Cas9–2A–Blast were maintained in the presence of 2 μg ml⁻¹ blasticidin.

To complement the HCT116 TP53Δ30 RNASEH2AΔ50 cell line, cell lines were infected with retroviral supernatant produced in amphotropic Phoenix packaging cells11 using either pMSCVpuro empty vector (EV) or pMSCVpuro-RNASEH2A-Δ50 in the presence of 4 μg ml⁻¹ polybrene (Sigma) and 48 h later selected for stable integration using 2 μg ml⁻¹ puromycin.

RNAseH2A expression plasmids. A Flag-tagged human RNAseH2A cDNA (NM_003697.2; encoding amino acids 2–299) and the D344A/D169A double mutant12 were cloned into the PCW57.1 vector (a gift from D. Root; Addgene #41393) using the Gateway system (Life Technologies/Thermo Fisher) according to the manufacturer’s protocol. The P40D and Y210A mutations were generated by site-directed mutagenesis using the following primers (5′ to 3′): P40D forward, GGCCGACACTCGCGCCCTGC; P40D reverse, CGGGCGAG GGGACAGTTGCGTCGG and Y210A forward, GTCTTGGATCATCGGAGGCGTCCTGAC and Y210A reverse, ACTGATTATGGCTCAGGGATT.

Cells were transduced at a low (~0.3) multiplicity of infection (MOI) with either pMSCVpuro-RNAseH2AΔ50 or pMSCVpuro-RNAseH2AΔ50 plasmid DNA and the experiment was terminated at day six. Cells were trypsinized, resuspended in medium and the live cell count was determined by trypan blue exclusion on a ViCELL instrument (Beckman Coulter). Cell numbers were plotted relative to the manufacturer’s transduced samples.

Generation of CRISPR knockout cell lines. To establish HeLa and HCT116 TP53Δ30 RNAseH2AΔ50 cell lines, 0.5 × 10⁵ cells were seeded in 6-well plates and transfected with two vectors encoding both Cas9n and sgRNAs targeting RNAseH2A (derivatives of pSpCas9n(BB)-2A-GFP and pSpCas9n(BB)-2A-Puro) using Lipofectamine 2000 (Life Technologies/Thermo Fisher). Forty-eight hours after transfection, single GFP⁺ cells were sorted into 96-well plates on a BD FACSalibur instrument (BD Biosciences) and grown until colonies formed. RNAseH2AΔ50 clones were selected on the basis of the size of PCR amplimers from the target region to detect clones that underwent editing, which was subsequently confirmed by Sanger sequencing. The oligonucleotides (5′ to 3′) used for PCR amplification and sequencing of targeted RNAseH2A loci were ACGCCGTCCTGAGTATTAG and TCCCTTGTTGCACTCAGATTAC. The absence of functional RNAseH2A was confirmed by immunoblotting, an RNase H2 activity assay and alkaline gel electrophoresis as described below. Functionally wild-type RNAseH2A clones were identified in parallel and used as controls.

To generate the remaining CRISPR-edited HeLa and RPE1-hTERT cell lines, cells were electroporated with 5 μg of vectors encoding the sgRNA (lentiGuide-Puro, for cells stably expressing Cas9) or encoding both the sgRNA and Cas9 (lentiCRISPR v2) using an Ammax Nucleofector II instrument (Lonza). RPE1-hTERT cells (0.7 × 10⁵) in a 10-cm dish were electroporated using program T-23. For HeLa cells, the AmmaCell Nucleofector Kit R (Lonza) was used with program I-1 according to the manufacturer’s instructions. Cells were re-plated into antibiotic-free McCoy’s 5A medium supplemented with 10% FBS and allowed to recover for 24 h. Puromycin was subsequently added to growth medium to enrich for transfectants and removed 24 h later. Cells were then cultured for an additional 3–5 days to provide time for gene editing and eventually selected at low densities (400–1,000 cells, depending on cell line) on 15-cm dishes. Single colonies were isolated using glass cylinders to two or three weeks later. SUM149PT Cas9 RNAseH2BΔ50 cells were generated by transient transfection of parental SUM149PT Cas9 cells with a lentivirus-puro–sgRNAseH2B construct using Lipofectamine 2000 (Thermo Fisher) as per the manufacturers protocol (2 μg plasmid DNA and 2 μl of Lipofectamine 2000 was used for 1 × 10⁵ cells in a 1-well plate). Transfected cells were selected with puromycin for 24 h, grown for an additional 4 days and single clones were isolated as above.

Targeted clones were identified by immunofluorescence and/or immunoblotting and successful gene editing was confirmed by PCR and TIDE analysis (https://tide-calcator.nki.nl/). The following PCR primers (5′ to 3′) were used for amplification of targeted loci in RNAseH2A forward, AGATCTGTAGGCGCGCTGAAT GT GG, RNAseH2A reverse, AGTGGCCTGATACGTCAGGG; RNAseH2B forward, TAGATGTTGTGCTGTGTTG, RNAseH2B reverse, TGCAGCTCG TCTTCTTCTTCTTCT, BRCA1 forward, TCTGAAAAGCTTGCAGAATCG, BRCA1 reverse, TAGACGAGTTGAGAGCTGACAGG; POLB forward, TTACGTGTTCATCA CAGATCTGAG, POLB reverse, TACGTGTTACGAGATCATCGTCA CGCC. The following PCR primers (5′ to 3′) were used for amplification of targeted loci in RNAseH2A forward, AGATCTGTAGGCGCGCTGAAT GT GG, RNAseH2A reverse, AGTGGCCTGATACGTCAGGG; RNAseH2B forward, TAGATGTTGTGCTGTGTTG, RNAseH2B reverse, TGCAGCTCG TCTTCTTCTTCTTCT, BRCA1 forward, TCTGAAAAGCTTGCAGAATCG, BRCA1 reverse, TAGACGAGTTGAGAGCTGACAGG; POLB forward, TTACGTGTTCATCA CAGATCTGAG, POLB reverse, TACGTGTTACGAGATCATCGTCA CGCC.
reverse, AGCAACTCATGGAAGAAATATTAGG; TP53 forward, GCATGTATCTCTGAGGAGG; TP53 reverse, TCATGCCTAGGGAAGGC. Generation of wild-type and RNASEH2A KO HeLa FUCCI cells. To establish wild-type RNASEH2A KO HeLa cells expressing the Fucci cell cycle reporters mKO2-Cdt1 and mAG-Geminin, wild-type and RNASEH2A KO HeLa cells were transduced at a low MOI with pLent-mKO2-Cdt1 and pLent-mAG-Geminin vectors and transductants were selected with 2 μg ml−1 blasticidin. Subsequently, cells positive for both mKO2-Cdt1 and mAG-Geminin fluorescence were collected by sorting on a BD Biosciences FACS Aria II instrument, expanded and used for further experiments. Expression of mKO2-Cdt1 and mAG-Geminin was confirmed by immunofluorescence and FACS analysis.

CRISPR–Cas9 screening. CRISPR screens were performed as described. Cas9-expressing cells were transduced with the lentiviral TK0v1 library at a low MOI (~0.2–0.3) and puromycin-containing medium was added the next day to select for transductants. Selection was continued until 72 h post transduction, which was considered the initial time point (day 0). At this time point the transduced cells were split into technical triplicates. During negative-selection screens (for PARPi sensitizers), cells were subcultured at day 3 and at day 6 each of the three replicates were split into technical triplicates. During negative-selection screens (for PARPi sensitizers), cells were subcultured at day 3 and at day 6 each of the three replicates were split into technical triplicates. During negative-selection screens (for PARPi sensitizers), cells were subcultured at day 3 and at day 6 each of the three replicates were split into technical triplicates. 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Cell cycle analysis by FACS. Cells (0.5 × 10^6) were seeded on 6-cm dishes into medium with or without PARPi (doses and durations are indicated in respective figures). Cells were then collected by trypsinization, resuspended in medium and centrifuged (233g, 5 min, 4 °C). Cells were then fixed by adding 4% paraformaldehyde for 15 min at 4 °C and analysed on a BD FACSCalibur or BD LSRII Fortessa X-20 instrumentation.

Immunoprecipitation. Cells were collected by trypsinization, washed once with PBS supplemented with 1 μM ADP–HDP (PARG inhibitor; Enzo) and 4 × 10^6 cells were snap-frozen in liquid nitrogen and then lysed in 1 ml of lysis buffer (50 mM HEPES pH 8.0, 100 mM KCl, 1 mM EDTA, 0.5% NP-40, 10% glycerol, 1 mM DTT, complete protease inhibitor cocktail (Roche), 1 μM ADP–HDP). Lysates were incubated with gentle rotation at 4 °C for 15 min and then centrifuged at 15,000g for 10 min. Fifty microlitres of total cell lysates were used as input and 950 μl were incubated with 5 μl of mouse anti-PARPi antibody (Enzo; F1-23) ALX-804-211-R050 for 5 h at 4 °C. Protein G-agarose beads (60-μl slurry; Pierce) were added for an additional hour. Beads were collected by centrifugation, washed four times with lysis buffer and eluted by boiling in 60 μl 2× sample buffer. Samples were run on an 8% SDS–PAGE gel and immunoblotting was performed as described above (see ‘Immunoblotting’ section).

Immunofluorescence. Cells were transfected with either TOP1 and/or RNASEH2A KO or wild-type and RNASEH2A KO/WT HeLa FUCCI cells were trypsinized, washed once with PBS, collected in tubes with PBS supplemented with 3% FCS and 1 μM ADP–HDP, and incubated on ice for 15 min. Cells were fixed in 4% paraformaldehyde for 10 min on ice and subsequent incubation with 200 μl of 150 mM ADP–HPD). Membranes were blocked in 5% milk in TBST and immunoblotting was performed as described above (see ‘Immunoblotting’ section).

Antibodies. The following antibodies were used for immunofluorescence and immunoblotting at the indicated dilutions: sheep anti-pan-RNase H2 (raised against human recombinant RNase H2α; Sigma, immunoblotts 1:1,000, immunoprecipitation 5 μl per 1 ml lysisate); rabbit anti-RNASEH2C (Proteintech 16518-1-AP; immunoblotts 1:1,000); rabbit anti-RNASEH2A (Origene TA306706, immunoblotts 1:1,000); mouse anti-RNASEH2A (Abcam ab29827; immunofluorescence 1:500); mouse anti-RNASEH2A G-10 (Santa Cruz Biotechnologies sc-61547; western blot 1:1,000); rabbit anti-RNASEH2A (Abcam ab92876; immunofluorescence 1:500); rabbit anti-RNASEH2A (Abcam ab92876; immunofluorescence 1:500); rabbit anti-RNASEH2A H242 (HeLa Fucci cells were trypsinized, washed once with PBS, collected in tubes with PBS supplemented with 3% FCS and 1 μM ADP–HDP, and incubated on ice for 15 min. Cells were fixed in 4% paraformaldehyde for 10 min on ice and subsequent incubation with 200 μl of 150 mM ADP–HPD). Membranes were blocked in 5% milk in TBST and immunoblotting was performed as described above (see ‘Immunoblotting’ section).

Cell Titer Glo assay. Two hundred cells per condition were plated on 96-well assay plates in technical triplicates either in drug-free medium or in a range of MMS concentrations. MMS was washed out 24 h later and cells were grown in drug-free medium for another 48 h. Cell viability was analysed using the Cell Titer Glo assay kit (Promega) according to the manufacturer’s instructions. Luminescence was read on an Envision 2104 plate reader (PerkinElmer).

Detection of ribonucleosomes in genomic DNA. Total nucleic acids were isolated from 10^6 cells by lysis in ice-cold buffer (20 mM Tris-HCl pH 7.5, 75 mM NaCl, 50 mM EDTA) and subsequent incubation with 200 μg/ml 1 proteinase K (Roche) for 10 min on ice followed by addition of sarcosyl (Sigma) to a final concentration of 1%. Nucleic acids were sequentially extracted with TE-equilibrated phenol, naphtholacetic acid:isoamyl alcohol (25:24:1), and chloroform, and then precipitated with 1mM sodium acetate, 30% ethanol, air-dried and dissolved in nuclease-free water.

For alkaline gel electrophoresis 10^6 total nucleic acids were incubated with 1 pmol of purified recombinant human RNase H226 and 0.25 μg of DNase-free RNase (Roche) for 30 min at 37 °C in 100 μl reaction buffer (60 mM KCl, 50 mM Tris-HCl pH 8.0, 10 mM MgCl₂, 0.01% BSA, 0.01% Triton X-100). Nucleic acids...
were ethanol-purified, dissolved in nucleic acid-free water and separated on a 0.7% agarose gel in 50 mM NaOH, 1 mM EDTA. After electrophoresis, the gel was neutralized in 0.7 M Tris-HCl pH 8.0, 1.5 M NaCl and stained with SYBR Gold (Invitrogen). Imaging was performed on a FLA-5100 imaging system (FujiFilm), and densitometry plots generated using an AIDA Image Analyzer (Raytest).

**RNAse H2 activity assay.** Recombinant RNAse H2 was expressed in Rosetta-2 Escherichia coli cells using a polycistronic construct based on pET6Fp1 (pMAR22) and purified as previously described. Site-directed mutagenesis to introduce the D344A and D169A or P400D and Y210A mutations was performed using the Quickchange method (Agilent). To measure enzyme activity, a range of RNAse H2 concentrations (0.06–2 nM) was incubated with 2 μM substrate in 5 μL reactions (in a buffer containing 60 mM KCl, 50 mM Tris-HCl pH 8.0, 10 mM MgCl2, 0.01% BSA and 0.01% Triton X-100) at 37 °C for 30 min or 1 h. Substrate was formed by annealing a 3'-fluorescin-labeled oligonucleotide (GATCTGAAGCTTGAGGACT, DRD–DNA; or gauccagcu-gggagcu, RNA–DNA; uppercase RNA, lowercase DNA) to a complementary 5'-dabcyl-labeled DNA oligonucleotide (Eurogentec). Reactions were stopped by adding an equal volume of 96% formamide, 20 mM EDTA, and heating at 95 °C. Products were resolved by denaturing PAGE (20%, 1× TBE), visualized on a FLA-5100 imaging system (FujiFilm) and quantified using ImageQuant TL (GE Healthcare).

To assess RNAse H2 activity in WCEs a FRET-based fluorescent substrate release assay was performed as previously described. RNAse H2 specific activity was determined against a DRD–DNA substrate (described above). Activity against a double-stranded DNA substrate of the same sequence was measured and used to correct for non-RNAse H2 activity against the DRD–DNA substrate. Reactions were performed in 100 μL of buffer with 250 nM substrate in 96-well flat-bottomed plates at 25 °C. Whole cell lysates were prepared as described above, and the final protein concentration used per reaction was 100 ng/μL. Fluorescence was read for 100 ms using a VICTOR2 1420 multilabel counter (Perkin Elmer), with a 480-nm excitation filter and a 535-nm emission filter.

**Ex vivo CLL studies.** Peripheral blood mononuclear cells were isolated from blood samples collected from patients with a new or existing diagnosis of CLL, irrespective of the stage of disease or duration or type of treatment from two Birmingham hospitals (Heartlands and Queen Elizabeth). This study was approved by the South Birmingham Ethics Committee (REC number 10/H1206/58), performed according to institutional guidelines and written consent was obtained from all participants. Primary CLL cells (5 × 106) and CD40L-expressing mouse embryonic fibroblasts (5 × 107) were seeded in each well of a 96-well plate (Corning) in 100 μL RPMI medium supplemented with 10% FBS (Sigma-Aldrich, UK) and 25 ng mL−1 IL-21 (ebiSciences). After 24 h, 200 μL more medium was gently added and cells were incubated for another 48 h. The medium was then aspirated, replaced with 200 μL of medium containing talazoparib and cells were incubated for a further 72 h. The cytotoxic effect of PARPi was determined by propidium iodide exclusion by flow cytometry with a FACSCalibur (Becton Dickinson Biosystems). Only cells which entered the cell cycle upon stimulation (as determined by forward- and side-scatter FACs profiles), were considered for analysis. Data was expressed as a surviving fraction relative to untreated cells. For gating strategies, see Supplementary Fig. 2.

**MLPA assay.** Genomic DNA was isolated from primary CLL cells using the Flexigene kit (Qiagen). To identify deletions in RNASEH2B gene the MLPA assay was performed on approximately 100 ng of genomic DNA (gDNA) per sample using the P388-A2 SALSA MLPA kit (MRQ-Holland) according to the manufacturer’s protocol. Two microlitres of amplified products were separated by capillary electrophoresis on an ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems) with a GeneScan 600 LIZ Size Standard (Thermo Fisher). Data were analysed using GeneMarker software v2.4.0 (SoftGenetics). Data were normalized using gDNA from four control reference samples. Copy number changes represented as a surviving fraction relative to untreated cells. For gating strategies, see Supplementary Fig. 2.

**Cytokinesis assay.** Cells complemented either with an empty vector or a vector encoding wild-type RNASEH2B (2 × 106 cells per flank) were measured by caliper every 3–4 days and tumour volume was determined by the formula (length × width)2/2. When the tumour volumes reached approximately 100 mm3 (10 days after injection), mice were randomized into treatment and control groups (8 animals per group, 32 animals in total; sample size was determined based on previous relevant studies). Talazoparib (BNM673, 0.333 mg kg−1, pharmacological grade, a gift from T. Heffernan and N. Feng) or vehicle (10% N,N-dimethylacetamide (ACROS Organics), 5% SoluTol HS 15 (Sigma-Aldrich) in PBS (Gibco)) was administered once daily by oral gavage (0.1 ml per 10 g of body weight) for 72 h. The cytotoxic effect of PARPi was determined by propidium iodide exclusion by flow cytometry with a FACSCalibur (Becton Dickinson Biosystems). Only cells which entered the cell cycle upon stimulation (as determined by forward- and side-scatter FACs profiles), were considered for analysis. Data was expressed as a surviving fraction relative to untreated cells. For gating strategies, see Supplementary Fig. 2.

**Reporting summary.** Results of PARP inhibitor CRISPR screens, source data for mouse xenograft experiments, unprocessed images of immunoblots and examples of gating strategies for FACs experiments are provided as Supplementary Information. All other datasets generated during this study are available from the corresponding authors upon reasonable request.

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Extended Data Fig. 1 | CRISPR screens for determinants of PARPi sensitivity. This figure is related to Fig. 1. a, Cas9 immunoblot of WCEs from parental HeLa, RPE1-hTERT and SUM149PT cells and clones stably transduced with a lentiviral Flag–Cas9–2A–Blast construct. Tubulin was used as a loading control. The immunoblot is representative of two biologically independent experiments.

b, Validation of CRISPR–Cas9 gene editing efficiency in Cas9-expressing HeLa, RPE1-hTERT and SUM149PT clones. Cell proliferation was monitored after transduction with a control sgRNA construct (sgLacZ) or sgRNAs targeting essential genes PSMD1, PSMB2 and EIF3D. Solid circles, individual values. Data are mean ± s.d. from three technical replicates normalized to sgLacZ. e, Gene Ontology terms significantly (P < 0.05, binomial test with Bonferroni correction) enriched among hits from olaparib screens common to at least two cell lines. Enrichment was analysed using PANTHER. d, esyN network analysis of interactions between hits common to at least two cell lines. Node size corresponds to mean DrugZ score across cell lines; 77 out of 155 genes are mapped on the network.
Extended Data Fig. 2 | See next page for caption.
Extended Data Fig. 2 | RNase H2 deficiency leads to PARPi sensitivity.
This figure is related to Fig. 2a, b. a. CRISPR-mediated inactivation of RNASEH2A or RNASEH2B in the cell lines used in this manuscript. WCEs of indicated cell lines and genotypes were processed for immunoblotting using antibodies against RNASEH2A, RNASEH2B or RNASEH2C. Vinculin, tubulin and GAPDH were used as loading controls. Representative immunoblots of at least two biologically independent experiments. b–d. Abolished RNase H2 enzymatic activity and increased levels of genome-embedded ribonucleotides in RNASEH2A KO cells. Analysis of total nucleic acids from wild-type and RNASEH2A KO HeLa cells treated with recombinant RNase H2 and separated by alkaline agarose gel electrophoresis. Ribonucleotide-containing genomic DNA from RNASEH2A KO HeLa cells is nicked and therefore has increased electrophoretic mobility13. Data are representative of three biologically independent experiments. c. Densitometric quantification of the alkaline gel shown in b, d. Cleavage of an RNase H2-specific double-stranded DNA oligonucleotide with a single incorporated ribonucleotide (DRD-DNA; ribonucleotide position is shown in red) by wild-type and RNASEH2A KO WCEs of the indicated cell types was measured using a fluorescence quenching-based assay32. Data are individual values (open circles) with the mean (red lines) of three biologically independent experiments.

e–l. RNase H2 deficiency leads to PARPi sensitivity in multiple cell types. e–g. Clonogenic survival assays of the indicated cell lines treated with the indicated PARPi. Data are mean ± s.d. from three biologically independent experiments normalized to untreated cells. Solid lines show a nonlinear least-squares fit of the data to a three-parameter dose–response model. h, EC50 values for olaparib (left) and talazoparib (right) in the indicated cell lines as determined by nonlinear least-squares fitting of the data in e–g and Fig. 2a, b. Data are EC50 values ± 95% confidence intervals. i–l. Increased apoptosis in RNASEH2A KO HeLa, Cas9 RNASEH2B KO SUM149PT and RNASEH2A KO HCT116 cells following PARPi treatment. i, Cleaved caspase-3 immunofluorescence and flow cytometry profiles of untreated and talazoparib-treated wild-type and RNASEH2A KO HeLa. FSC, forward scatter. j–l, Percentages of cleaved caspase-3+ (caspase-3+) cells of the indicated genotypes treated with the indicated PARPi. Data are individual values (coloured symbols) with the mean (solid lines) of three biologically independent experiments. Insets and outsets, levels of cleaved caspase-3+ cells without PARPi treatment. Red lines, mean of three biologically independent experiments. P values are from unpaired two-tailed t-tests. In a, d, g, l, RNASEH2A KO HCT116 cells were transduced either with an empty vector (+EV) or a full-length RNASEH2A expression construct (+WT).
Extended Data Fig. 3 | See next page for caption.
Extended Data Fig. 3 | Functional homologous recombination, increased replication-associated DNA damage and synthetic lethality with the loss of BRCA1 or BRCA2 in RNase H2-deficient cells. This figure is related to Fig. 2. a–d. Homologous recombination is not affected by the inactivation of RNase H2. a. Micrographs of wild-type and RNASEH2AKO RPE1-lHTERT Cas9 TP53KO and cells exposed to 3 Gy of X-rays (IR) and assessed using γ-H2AX and RAD51 immunofluorescence 4 h later. Images are representative of three biologically independent experiments. b. Quantification of the experiment in a at the indicated time points after irradiation, plotted as a percentage of cells with more than five γ-H2AX and RAD51 colocalizing foci. Data are individual values (open circles) and the mean (red lines) from three biologically independent experiments. P values, unpaired two-tailed t-test. c. Quantitative image-based cytometry (QIBC) plots of DR-GFP experiments in Fig. 2e. Each point shows the mean GFP and RNASEH2A immunofluorescence intensities per nucleus of mock- or I-SceI-transfected HeLa DR-GFP wild-type and GFP− cells transduced with indicated Cas9 sgRNA constructs (EV = empty vector). Dashed lines separate RNASEH2A+ and RNASEH2A− and GFP+ and GFP− cell populations. Data are representative of three biologically independent experiments. d. Quantification of RNASEH2A+ cells in DR-GFP experiments shown in c and Fig. 2e as determined by QIBC. Data are individual values (open circles) and the mean (red lines) of three biologically independent experiments. e–h. Replication-dependent endogenous DNA damage in RNase H2-deficient cells. e. Micrographs for experiments quantified in Fig. 2g. γ-H2AX immunofluorescence in EdU+ and EdU− wild-type and RNASEH2AKO HeLa cells. Scale bars, 5 μm. f. Quantification of γ-H2AX foci per nucleus in experiments shown in e and Fig. 2g. Dots, foci number in individual nuclei; red lines, mean from three biologically independent experiments. g, h. Wild-type and RNASEH2AKO HeLa cells were treated with aphidicolin and EdU as indicated in the schematic (g, top), and immunostained with γ-H2AX antibodies. Mean number of foci per EdU+ nucleus in each experiment (g, open circles) or the number of foci in individual EdU+ nuclei (h, dots) were quantified. Red lines are the mean from three biologically independent experiments; at least 100 cells were analysed per sample in each experiment. P value, unpaired two-tailed t-test. i, j. Increased poly(ADP-ribosylation) of PARP1 in G1 as well as in S/G2/M phases in RNASEH2AKO cells. i, FACS plots of wild-type and RNASEH2AKO HeLa cells expressing the FUCCI cell cycle reporters mKO2-Cdt1 and mAG-Geminin34. Data are representative of two biologically independent experiments. j, PARP1 immunoprecipitates from WCEs of FUCCI-sorted G1 or S/G2/M wild-type and RNASEH2AKO HeLa cells, probed with the indicated antibodies. Images are representative of two biologically independent experiments. Tubulin was used as a loading control. Densitometric quantification of PAR signals normalized to immunoprecipitated PARP1 is shown as fold changes from wild-type to RNASEH2AKO cells. k–o, Inactivation of RNase H2 in BRCA1− or BRCA2− deficient backgrounds results in synthetic lethality. k, BRCA1 and BRCA2 expression, respectively, in wild-type and BRCA1KO RPE1-lHTERT TP53KO cells (top) or wild-type and BRCA2KO DLD-1 cells (bottom). WCEs were processed for immunoblotting with the indicated antibodies. Tubulin and KAP1 were used as loading controls. Immunoblots are representative of at least two biologically independent experiments. l. RNase H2 levels in cells used in m–o and Fig. 2i. Cells were transduced with the indicated sgRNA vectors and processed for RNASEH2A immunofluorescence. Each point represents mean RNASEH2A intensity per nucleus as measured by QIBC (n = 1 experiment). At least 2,000 cells were analysed per sample. Percentages of RNASEH2A+ cells in individual samples are shown above each plot. m, Clonogenic survival assays quantified in Fig. 2i. Images are representative of three biologically independent experiments. n, o, Synthetic lethality after inactivation of RNASEH2A or RNASEH2B in BRCA2-deficient cells. Clonogenic survival of wild-type and BRCA2KO DLD-1 cells was assessed after transduction with indicated Cas9 sgRNA vectors. n, Representative images of three biologically independent experiments. o, Quantification of the experiments shown in n. Data are individual values (open circles) with the mean (red lines) of three biologically independent experiments. P values are from an unpaired two-tailed t-test.
Extended Data Fig. 4 | See next page for caption.
Extended Data Fig. 4 | A separation-of-function mutant of RNase H2. This figure is related to Fig. 2j. RNASEH2A(P40D/Y210A) is a separation-of-function mutant that cannot excise single DNA-embedded ribonucleotides, but cleaves RNA–DNA heteroduplexes (similar to the yeast rnh201-P45D-Y219A mutant16). a, Schematic depicting enzymatic activity against two different RNase H2 substrates (DRD–DNA, double-stranded DNA with an embedded ribonucleotide, or RNA–DNA hybrids) in cell lines used in b–d and Fig. 2j. Wild-type and RNASEH2A<sup>KO</sup> cells were transduced with either an empty vector or the indicated RNASEH2A constructs. b, Complementation of HeLa RNASEH2A<sup>KO</sup> cells with Flag-tagged RNASEH2A variants restores RNase H2 complex protein levels. WCEs from wild-type and RNASEH2A<sup>KO</sup> HeLa and RNASEH2A<sup>KO</sup> cells stably expressing the indicated lentiviral constructs were processed for immunoblotting with the indicated antibodies. Vinculin was used as a loading control. Asterisk indicates a non-specific band. Immunoblots are representative of three biologically independent experiments. c, d, Complementation of HeLa RNASEH2A<sup>KO</sup> cells with wild-type RNASEH2A, but not with the D34A/D169A (catalytically dead) or P40D/Y210A (separation-of-function) mutants, rescues increased levels of genome-embedded ribonucleotides. c, Total nucleic acids from the cell lines shown in a, b were treated with recombinant RNase H2 and separated by alkaline agarose gel electrophoresis. Image is representative of four experiments. d, Densitometric quantification of alkaline gel shown in c. e, Purified human RNase H2 complexes consisting of RNASEH2B, RNASEH2C and RNASEH2A wild type, P40D/Y210A or D34A/D169A subunits separated by SDS–PAGE and stained with Coomassie blue. One experiment was performed. f–k, RNase H2 activity assays with fluorescein-labelled RNA–DNA substrate (f) or double-stranded DNA with a single incorporated ribonucleotide (DRD–DNA) (g) and increasing amounts of recombinant wild-type, P40D/Y210A or D34A/D169A RNase H2. Products were separated by polyacrylamide gel electrophoresis and detected by fluorescence imaging. Images are representative of three biologically independent experiments. h, k, Quantification of gels from f, g. Product signal is plotted relative to substrate signal per lane. Data are mean ± s.d. from three biologically independent experiments.
Extended Data Fig. 5 | PARP1 trapping is the underlying cause of PARPi sensitivity in RNase H2-deficient cells

This figure is related to Fig. 3a–c. a, Schematic representation of CRISPR screens for suppressors of talazoparib sensitivity in RNase H2-deficient cells. Cas9-expressing cells were transduced with the TKOv1 library, talazoparib was added on day 6 (t6; HeLa, 20 nM; RPE1-hTERT, 50 nM) and cells were cultured in its presence until day 18 (t18). Cells were subcultured once at day 12 (RPE1) or 13 (HeLa). sgRNA representations in the initial (t6) and final (t18) populations were quantified by next-generation sequencing. Gene knockouts that were enriched at day 18 over day 6 were identified by MAGeCK42. b, CRISPR-mediated inactivation of RNASEH2A and/or PARP1 in cell lines used in c–e and Fig. 3b. WCEs were processed for immunoblotting with the indicated antibodies. KAP1 was used as a loading control. Immunoblots are representative of two biologically independent experiments. c–e, Loss of PARP1 restores PARPi-resistance in RNASEH2A KO cells. c, Percentage of cleaved caspase-3+ HeLa cells of indicated genotypes with or without olaparib treatment measured by flow cytometry (FACS). Data are individual values (open circles) with the mean (red lines) of three biologically independent experiments. P value is from an unpaired two-tailed t-test. d, e, Clonogenic survival assays with HeLa (d) and RPE1-hTERT (e) cells of the indicated genotypes treated with olaparib (left) or talazoparib (right). Data are mean ± s.d. from three biologically independent experiments. Solid lines show a nonlinear least-squares fit to a three-parameter dose–response model. f, Trapping activity of PARPi correlates with the ability to induce apoptosis in RNASEH2A KO cells. Quantification of cleaved caspase-3+ wild-type and RNASEH2A KO HeLa cells without treatment or treated with the indicated PARPi. Data are individual values with the mean (black lines) of three biologically independent experiments. Note that PARP-trapping activity decreases as follows: talazoparib > olaparib > veliparib48. g, PARPi-induced S-phase arrest in RNASEH2A KO cells is alleviated in the absence of PARP1. Top, schematic of talazoparib and EdU treatment. Bottom, EdU (pseudocolor plots) and DNA content (histograms) FACS profiles of untreated and talazoparib-treated wild-type, PARP1 KO, RNASEH2A KO and PARP1 KO/RNASEH2A KO HeLa cells. DNA content was determined by propidium iodide (PI) staining. Data are representative of three biologically independent experiments. h, Quantification of mean γ-H2AX intensities in experiments shown in Fig. 3c. Data are individual values (open circles) with the mean (red lines) of three biologically independent experiments. At least 10,000 cells were analysed per sample in each experiment.
Extended Data Fig. 6 | TOP1-mediated cleavage at genome-embedded ribonucleotides leads to PARPi sensitivity in RER-deficient cells.

This figure is related to Fig. 3d–g. a, Reduced endogenous DNA damage in TOP1-depleted RNASEH2A KO cells. Quantification of γ-H2AX foci per nucleus in the experiments shown in Fig. 3e, f. Dots, foci number in individual nuclei; red lines, mean of five biologically independent experiments. b–i, TOP1 depletion alleviates PARPi-induced apoptosis and S-phase arrest in HeLa RNASEH2A KO cells (b–e) and in RNASEH2A(WT) P40D Y210A separation-of-function mutant cells (f–h). b, Cleaved caspase-3 FACS plots for experiments quantified in Fig. 3g. Data are representative of three biologically independent experiments. c, Wild-type and RNASEH2A KO HeLa cells were transfected with non-targeting (siCTRL-SP) or TOP1-targeting (siTOP1-SP) SMARTpool siRNAs. WCEs were analysed by immunoblotting with antibodies to TOP1 and actin (loading control). Images are representative of three biologically independent experiments. d, FACS plots of cleaved caspase-3 in wild-type and RNASEH2A KO HeLa cells transfected with siCTRL-SP or siTOP1-SP after talazoparib treatment. e, Quantification of the experiment shown in d. f, RNASEH2A KO HeLa cells stably expressing the indicated Flag-tagged constructs were transfected with non-targeting (siCTRL) or TOP1-targeting (siTOP1) siRNAs. WCEs were analysed by immunoblotting with TOP1, Flag and actin (loading control) antibodies. Immunoblots are representative of three biologically independent experiments. g, FACS plots of cleaved caspase-3 in RNASEH2A KO HeLa cells transfected with siCTRL or siTOP1 and expressing wild-type RNASEH2A or the P40D/Y210A mutant. Data are representative of three biologically independent experiments. h, Quantification of the experiment shown in g. i, Cell cycle profiles, before and after talazoparib treatment, of wild-type and RNASEH2A KO HeLa cells transfected with the indicated siRNAs. DNA content was assessed by propidium iodide staining and FACS. Data are representative of three biologically independent experiments.
Extended Data Fig. 7 | See next page for caption.
Extended Data Fig. 7 | Frequent collateral loss of RNASEH2B in CLL and metastatic CRPC. This figure is related to Fig. 4a–c. a, b, MLPA analysis (a) and CGH array profiles for chromosome 13q (b) of representative CLL samples carrying two wild-type RNASEH2B alleles (top), a monoallelic RNASEH2B deletion (middle) or biallelic deletion (bottom). a, For MLPA analysis, genomic DNA from reference and experimental samples was analysed using probes targeting control loci and individual RNASEH2B exons (exon 1–11). The MLPA ratio was calculated per probe and normalized to control probes and reference samples. Error bars indicate s.d. of the mean from eight control probes for each sample. Dashed lines indicate the threshold set for diploid copy number. b, For each CGH array profile the y-axes of the top and bottom plots indicate copy number probe intensity (logR ratio) and the x-axes mark the position on chromosome 13 represented by the ideogram (middle). An enlargement of the frequently deleted 13q14.2–14.3 region, including the miRNA-15A/16-1 gene cluster and the RNASEH2B gene, is shown in the bottom plot. One experiment was performed. c, RNASEH2B is frequently co-deleted with RB1 in CRPC. Copy number alterations (CNA) in the RB1–RNASEH2B region in CRPC (n = 226 cases) are shown. Horizontal lines represent the CNA profile for individual CRPC samples (dark blue, homozygous loss; light blue, heterozygous loss; grey, no change; pink, copy number gain (CNA 3–4); red, copy number amplification (CNA > 4); white, insufficient data to determine CNA). Samples are clustered on the basis of RNASEH2B gene status. CNA frequencies for RNASEH2B and the RB1–RNASEH2B region without a copy number breakpoint are shown on the right.
Extended Data Fig. 8 | PARPi sensitivity in RNase H2-deficient primary CLL cells and mouse xenograft tumours. This figure is related to Fig. 4.

**a, b,** Proliferating cells, and not quiescent cells, are the major population of viable cells in ex vivo cultured primary CLL patient samples irrespective of treatment group. Quantification of absolute (a) and relative (b) quiescent and proliferating cell numbers as determined by FACS analysis of the primary CLL samples used in Fig. 4b, c. Wild-type RNASEH2B, n = 8 individual samples; monoallelic deletion, n = 4 individual samples; biallelic deletion, n = 9 individual samples. Data are mean ± s.d. from three technical replicates. FACS gating strategy for stimulated peripheral blood lymphocytes (PBLs) from CLL patients is shown in Supplementary Fig. 2.

**c,** RNase H2-deficient primary CLL cells have reduced survival when cultured with olaparib. Data are the mean of individual samples ± s.e.m. (n = 3 biologically independent CLL samples per group, each analysed in technical triplicates). P value from a two-way ANOVA.

**d,** Talazoparib selectively inhibits the growth of RNASEH2A KO xenograft tumours. RNASEH2A KO cells complemented either with empty vector or wild-type RNASEH2A were injected subcutaneously into bilateral flanks of CD-1 nude mice. Mice were randomized to either vehicle or talazoparib (0.333 mg kg⁻¹) treatment groups (eight animals per group) and tumour volumes were measured twice weekly. Data are mean ± s.e.m. P values are from two-way ANOVA under the null hypothesis that talazoparib does not suppress the tumour growth.
Extended Data Fig. 9 | RNase H2-deficient cells are more sensitive to PARPi than DNA polymerase β mutants. a, Schematic of the POLBΔ188–190 CRISPR mutation. The Mg2+-coordinating aspartate residues (D190, D192 and D256, red triangles) are highlighted in the domain structure of the human DNA polymerase β protein. The sgRNA target site and antibody epitope are indicated by black lines. b, WCEs from parental RPE1-hTERT Cas9 TP53KO cells and two POLBΔ188–190 clones were processed for immunoblotting with DNA polymerase β and tubulin (loading control) antibodies. Immunoblots are representative of two biologically independent experiments. c, The POLBΔ188–190 mutation impairs base excision repair. Wild-type or POLBΔ188–190 RPE1-hTERT Cas9 TP53KO cells were exposed to different concentrations of MMS for 24 h, and then grown in drug-free medium for an additional 48 h. Cell viability was determined using the Cell Titer Glo assay. d, Sensitivity of wild-type, RNASEH2A-KO and POLBΔ188–190 RPE1-hTERT Cas9 TP53KO cells to indicated talazoparib concentrations in clonogenic survival assays. Data in c, d are mean ± s.d. from three biologically independent experiments normalized to untreated cells. Solid lines show a nonlinear least-squares fit to a three-parameter dose–response model.
**Extended Data Table 1 | Clinical and molecular characteristics of primary CLL samples**

| Sample | Age | Sex | Binet stage | Time from diagnosis (Months) | Treatment | Time on treatment (Days) | Response to treatment | Clinical characteristics | Molecular characteristics |
|--------|-----|-----|-------------|-----------------------------|-----------|-------------------------|----------------------|-------------------------|--------------------------|
| CLL1   | 67  | F   | A           | 35                          | Pre-treatment | 0                       | -                    |                        | Trisomy 12               |
| CLL2   | 74  | F   | A           | 24                          | Pre-treatment | 0                       | -                    |                        | Normal                   |
| CLL3   | 67  | M   | A           | 176                         | Ibrutinib   | 0                       | PRL                  |                        | Normal                   |
| CLL4   | 68  | M   | A           | 49                          | Pre-treatment | 0                       | -                    |                        | Normal                   |
| CLL5   | 76  | M   | A           | 49                          | Pre-treatment | 0                       | -                    |                        | Normal                   |
| CLL6   | 65  | F   | A           | 153                         | Pre-treatment | 0                       | -                    |                        | Normal                   |
| CLL7   | 63  | F   | A           | 199                         | Fludarabine, Cyclophosphamide + Rituximab | 37         | CR                   |                        | Trisomy 12               |
| CLL8   | 39  | M   | B           | 80                          | Pre-treatment | 0                       | -                    |                        | Normal                   |
| CLL9   | 80  | F   | A           | 33                          | Chlorambucil | 83                      | PR                   |                        | del(13q)                |
| CLL10  | 57  | F   | A           | 136                         | Pre-treatment | 0                       | -                    |                        | del(13q)                |
| CLL11  | 79  | F   | A           | 70                          | Bendamustine + Rituximab | 251        | CR                   |                        | N/A                      |
| CLL12  | 48  | M   | B           | 159                         | Ibrutinib   | 486                     | PR                   |                        | N/A                      |
| CLL13  | 62  | F   | A           | 203                         | Pre-treatment | 0                       | -                    |                        | N/A                      |
| CLL14  | 63  | M   | A           | 27                          | Pre-treatment | 0                       | -                    |                        | N/A                      |
| CLL15  | 42  | F   | A           | 414                         | Bendamustine + Rituximab + Ibrutinib | 120        | SD                   |                        | del(13q)                |
| CLL16  | 84  | F   | A           | 19                          | Pre-treatment | 0                       | -                    |                        | del(13q)                |
| CLL17  | 72  | F   | A           | 153                         | Chlorambucil | 63                      | PR                   |                        | del(13q)                |
| CLL18  | 79  | F   | A           | 36                          | Pre-treatment | 0                       | -                    |                        | del(13q)                |
| CLL19  | 48  | F   | B           | 8                           | Pre-treatment | 0                       | -                    |                        | del(17p), del(13q)     |
| CLL20  | 70  | F   | B           | 10                          | Pre-treatment | 0                       | -                    |                        | del(13q)                |
| CLL21  | 67  | M   | B           | 56                          | Pre-treatment | 0                       | -                    |                        | del(13q)                |

Data used in Fig. 4a, c, Extended Data Figs 7a, b, 8a, b. CLL samples grouped by RNASEH2B status. CR, complete response; F, female; M, male; N/A, not available; PRL, partial response with lymphocytosis; PR, partial response; SD, stable disease; dashes (-), not applicable.

1Based on MLPA and CGH array. del, deleted.
2Intact ATM status confirmed by next-generation sequencing and/or functional assays.
3TP53 status determined by sequencing. *, monoallelic TP53 alteration; #, biallelic TP53 alteration.
4Maturational status of CLL assessed by detection of hypermutation in immunoglobulin variable region heavy chain (IgVH); UM, unmutated (more than 98% sequence homology with germline sequence); M, mutated (less than 98% sequence homology with germline sequence).
Reporting Summary

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Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

| n/a | Confirmed |
|-----|-----------|
| ☑   | The exact sample size \((n)\) for each experimental group/condition, given as a discrete number and unit of measurement |
| ☑   | An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| ☑   | The statistical test(s) used AND whether they are one- or two-sided |
| ☑   | Only common tests should be described solely by name; describe more complex techniques in the Methods section. |
| ☑   | A description of all covariates tested |
| ☑   | A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| ☑   | A full description of the statistics 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 |
| ☑   | Clearly defined error bars |
| ☑   | State explicitly what error bars represent (e.g. SD, SE, CI) |

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection

Flow cytometry data were collected using CellQuest Pro v6.0, FACSDIVA v8.0.1, v6.1.3 and BD Accuri C6.

Data analysis

CRISPR/Cas9 screen data analysis: DrugZ (see supplementary manuscript); MAGeCK 0.5.3 (https://sourceforge.net/p/mageck/wiki/Home/); PANTHER (http://pantherdb.org/), esyN (http://www.esyn.org/), Cytoscape 3.4.0 (http://www.cytoscape.org) Data visualization and statistical analysis: GraphPad PRISM 6; RStudio 1.0.136; Microsoft Excel 2011 Image analysis: Fiji 1.0 (http://fiji.sc/); Columbus 2.3.2 (PerkinElmer) Flow Cytometry data analysis: Data were analysed using either FlowJo v7.6.5, FlowJo v10.2 (Tree Star) or BD Accuri C6 software MLPA: GeneMaker software v2.4.0 (SoftGenetics) CGH array: GenomeStudio software Genotyping Module v.3.1 (Illumina) Analysis of copy number alterations: BWA (0.5.9), stampy (1.0.2), ASCAT (version 2.3)

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.
Data

Policy information about availability of data
All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The results of the PARP inhibitor CRISPR screens and source data for mouse xenograft experiments are included in the on-line version of the manuscript as Supplementary Tables 1, 2 and 3. Unprocessed images of all immunoblots are presented in Supplementary Fig 1. Supplementary Fig 2 contains examples of gating strategies for FACS experiments. All other datasets generated during the study are available from the corresponding authors upon reasonable request.

Field-specific reporting

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☑ Life sciences  ☐ Behavioural & social sciences  ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

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

Sample size
No statistical methods were used to pre-determine sample size. Sample sizes (at least 3 independent experimental replicates in most experiments, unless indicated otherwise) were chosen based on the standard practices of the field.

Data exclusions
In Fig 4b,c, one primary CLL sample was excluded from the analysis. In this sample, a single exon was scored as deleted by the MLPA assay, but in subsequent functional validation performed by RNase H2 activity assay on CLL cell lysates, this sample retained substantial RNase H2 activity (117%, relative to n=5 RNASEH2B-WT samples), inconsistent with biallelic inactivation of the RNASEH2B gene, and was therefore excluded. This criterion for exclusion was chosen a priori. Also, on further investigation, this sample scored as WT based on the CGH array data, indicating this was a likely MLPA false positive result.

In the 1st xenograft experiment individual flanks that failed to grow before initiation of treatment were excluded from subsequent measurements and analysis (revised Fig. 4d). In the 2nd xenograft experiment animals with tumours that failed to grow on both flanks within the first 11 days of treatment were excluded from the analysis (revised Extended Data Fig. 8d). The exclusion criteria were set a priori. Exclusions were not confounding for the analysis.

Replication
All attempts at replication were successful.

Randomization
For xenograft studies animals were randomised at day 0, prior to talazoparib treatment. Samples used in cell biology experiments were not randomized.

Blinding
The investigator performing tumour measurements in xenograft experiments was blinded to the experimental design/identity of cells injected. In cell biology experiments the investigators were not blinded during data collection and analysis.

Reporting for specific materials, systems and methods

Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
| ☒  | Unique biological materials |
| ☒  | Antibodies |
| ☒  | Eukaryotic cell lines |
| ☒  | Palaeontology |
| ☒  | Animals and other organisms |
| ☒  | Human research participants |

Methods

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

| Antibodies used | Dilution |
|-----------------|---------|
| Sheep anti-pan-RNase H2 (raised against human recombinant RNase H2A4, IB 1:1,000, IP 5 μl / 1 ml lysate); rabbit anti-RNASEH2C (Proteintech 16518-1-AP, lot #D0007806, IB 1:1,000); rabbit anti-RNASEH2A (Origene TA306706, lot #QNC10953, IB 1:1,000); mouse anti-RNASEH2A (Abcam ab109374, lot #GR154929-1, IB 1:5,000); rabbit anti-FLAG (Cell Signaling Technologies 2368, lot #12, IB 1:1,000); rabbit anti-α-tubulin DM1A (Millipore CP06, lot #D00160163, IB 1:5,000); mouse anti-α−tubulin B512 (Sigma T6074, IB 1:5,000); mouse anti-vinculin (Sigma V9264, IB 1:1,000), rabbit anti-DNA polymerase beta (Abcam ab26343, lot #GR255464-3, IB 1:1,000); rabbit anti-cleaved caspase-3 (Cell Signaling Technologies 9661S, lot #43, IF 1:800). | |

Validation

Antibodies were validated using knockout cell lines or siRNAs where applicable. The gamma-H2AX, cleaved caspase-3 and phospho-Chk1 antibodies were validated by comparing untreated cells and cells treated with genotoxic insults (X-ray irradiation, camptothecin, PARP inhibitors etc.).

Eukaryotic cell lines

Policy information about cell lines

| Cell line source(s) | Authentication | Mycoplasma contamination |
|---------------------|---------------|-------------------------|
| HeLa, RPE1-hTERT, 293T: ATCC SUM149PT: Asterand Bioscience HCT116: a kind gift from B. Vogelstein (Johns Hopkins University School of Medicine) (PMID: 9823282) | HeLa, RPE1-hTERT and 293T cell lines were authenticated using STR DNA profiling. SUM149PT cells were validated using functional assays (PARP inhibitor sensitivity, RAD51 focus formation after DNA damage) and immunoblotting on the basis of their BRCA1 mutation status. HCT116 cells were not formally authenticated. | All cell lines tested negative for mycoplasma. |

Animals and other organisms

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

| Laboratory animals | Wild animals | Field-collected samples |
|---------------------|--------------|------------------------|
| Female athymic CD-1 Nude mice (5–7 weeks old, Charles River Laboratories) were used for in vivo xenograft studies. | The study did not involve wild animals. | The study did not involve samples collected from the field. |

Human research participants

Policy information about studies involving human research participants

| Population characteristics | Recruitment |
|----------------------------|-------------|
| Human subject characteristics documented in supplementary table. | Peripheral blood mononuclear cells were isolated from blood samples collected from patients with a new or existing diagnosis of CLL, irrespective of the stage of disease or time/type of treatment. |

Flow Cytometry

Plots

Confirm that:
- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a ‘group’ is an analysis of identical markers).
- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage (with statistics) is provided.
### Methodology

#### Sample preparation
See materials and methods sections entitled: 'Immunofluorescence/flow cytometry', 'Cell cycle analysis by FACS', 'Immunoprecipitation' and 'Ex-vivo CLL studies'.

#### Instrument
BD FACSCalibur, LSR Fortessa (BD Biosciences) X20, BD Biosciences FACS Aria II and Accuri C6.

#### Software
Data were collected using CellQuest Pro v6.0, FACS DIVA v8.0.1, v6.1.3 and BD Accuri C6. Data were analysed using either FlowJo v7.6.5, FlowJo v10.2 (Tree Star) or BD Accuri C6 software.

#### Cell population abundance
N/A

#### Gating strategy
In all cases, live cells were first selected by excluding events with low forward (FSC) and side scatter (SSC) signals. Other gates were set in the respective experiments as follows:

**Cell cycle analysis:** Single cells were selected within the live cell populations after plotting the propidium iodide (PI) pulse width (FL2-W) vs. area (FL2-A) and excluding events with high FL2-W (doublets or aggregates) as described e.g. in Wersto et al., 2001, Cytometry (DOI: 10.1002/cyto.1171). From the single cell population, a cell cycle profile was generated by plotting a histogram of the pulse height signal (FL2-H).

**Analysis of recovery from talazoparib-induced replication block (\(\gamma H2AX/PI\)):** Mean \(\gamma H2AX\) fluorescence signal (Alexa Fluor 647; FL-4) was calculated from all events in the live-cell population.

**Detection of apoptotic cells by cleaved caspase-3 IF/FACS:** In the live cell population, the cleaved caspase-3 signal (Alexa Fluor 488; FL-1) intensity was plotted against FSC and cleaved caspase-3-positive and -negative cells were discriminated based on the increase in FL-1 signal in PARPi-treated cells as compared to untreated samples; an example is shown in ED Fig 2i.

**FACS sorting of HeLa FUCCI cells for analyzing PARP1 poly(ADP-ribosylation) in G1 and S/G2/M cell cycle phases.** Following single live cell discrimination, cells were sorted based on the mKO2 (Excitation: 561 nm; Emission: 582 nm (582/15 BP); mKO2-Cdt1) and mAG (Excitation: 488 nm; Emission: 525 nm (525/50 BP); mAG-Geminin) signal intensity. Unlabeled HeLa cells were used to define the boundaries of mKO2- and mAG-negative populations. HeLa FUCCI cells that are mKO2-positive and mAG-negative represent G1 population; cells that are mKO2-negative and mAG-positive represent S/G2/M population.

**Analysis of PARPi sensitivity of ex-vivo cultured primary CLL cells:** gating strategy documented in Supplementary Figures 2.

Briefly, cellular debris was excluded by gating on SSC/FSC signal. Subsequent gating using propidium iodide (PI)-staining for cell viability was used to determine the population of non-viable PI-positive cells (Excitation: 351 nm; Emission: 617 nm; FL3) that was excluded from further analysis. Population with high FSC/SSC signal represented proliferating CLL cells, with quiescent cells having low FSC/SSC signal, and was validated by CFSE labeling (Excitation: 494 nm; Emission: 521 nm; FL1).

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