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Supplemental Information

The CST Complex Mediates End Protection at Double-Strand Breaks and Promotes PARP Inhibitor Sensitivity in BRCA1-Deficient Cells

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Figure S1

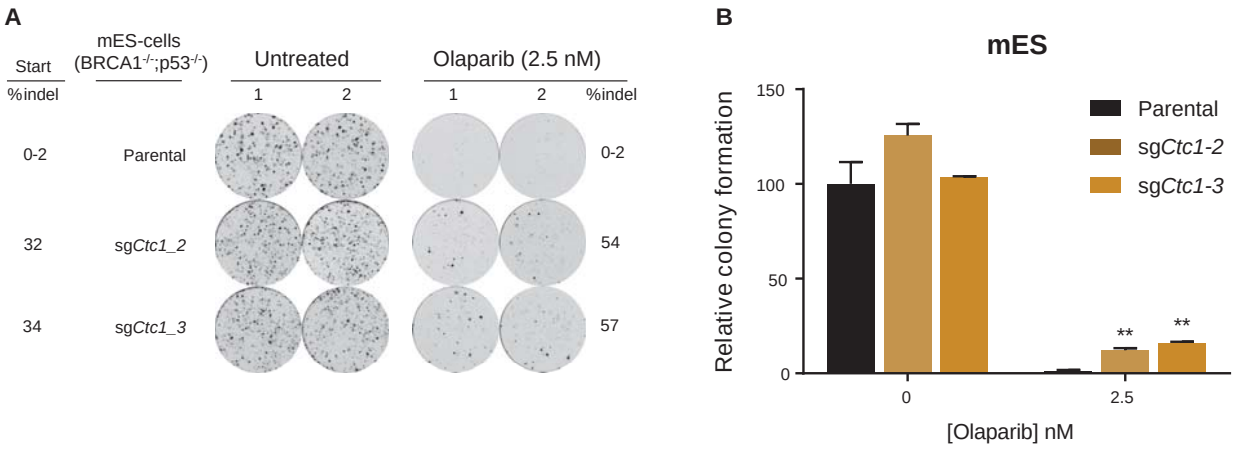

Figure S1: Depletion of CTC1 induces PARPi resistance in *Brca1-/-;p53-/-* mouse embryonic stem (mES) cells. Related to Figure 3.
(A) Parental and *Ctc1*-mutated *Brca1-/-;p53-/-* mES cells were plated for clonogenic growth in the presence or absence of olaparib (2.5 nM) for 7 days before wells were fixed and stained with crystal violet. The experiment was performed in duplicate. *Ctc1* was mutated using the pLenti-sgRNA-tetR-T2A-PuroR vector containing the indicated gRNAs. Allele distributions were determined from the starting population and the surviving population after treatment.
(B) Quantification of **A**. The number of colonies was determined using GelCount software. Data represent the relative number of colonies compared to the parental untreated mES cells. Data represent mean ± SD. P-values were determined by unpaired two-tailed students t-test (P = 0.0074 and P = 0.0017, respectively).

Figure S2

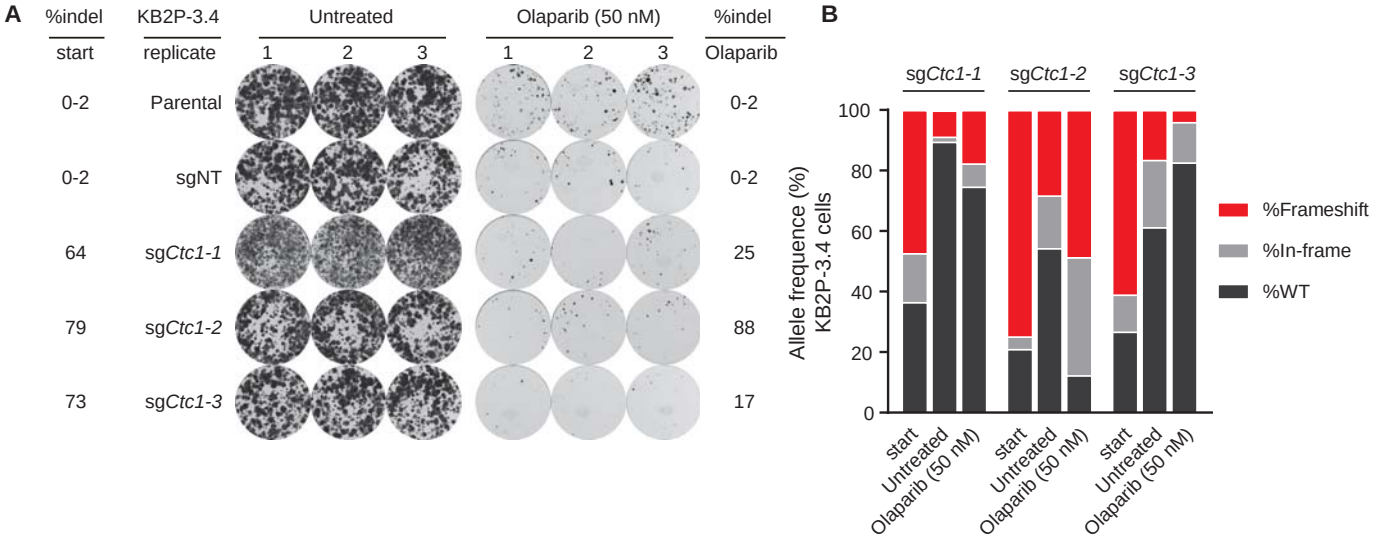

Figure S2: Depletion of CTC1 does not improve PARPi survival in BRCA2-deficient cells. Related to Figure 3.
(A-B) Parental, sgNT or *Ctc1*-mutated KB2P-3.4 SpCas9 expressing cells were plated at 2,000 cells per 6-well for clonogenic growth in the presence or absence of olaparib (50 nM) for 10 days. Then, cells were harvested and re-plated at 2,000 cells per 6-well under the same treatment for 10 days, and this was repeated one more time (total treatment duration 30 days). *Ctc1* was mutated using the pLenti-sgRNA-tetR-T2A-PuroR vector containing the indicated gRNAs. Cells were selected with puromycin (3 µg/mL) and gRNA expression was induced with doxycycline (3 µg/mL) for 5 days. Allele distributions were determined from the starting, untreated and olaparib-treated populations and the percentage indel is shown and plotted in **B**.

Figure S3

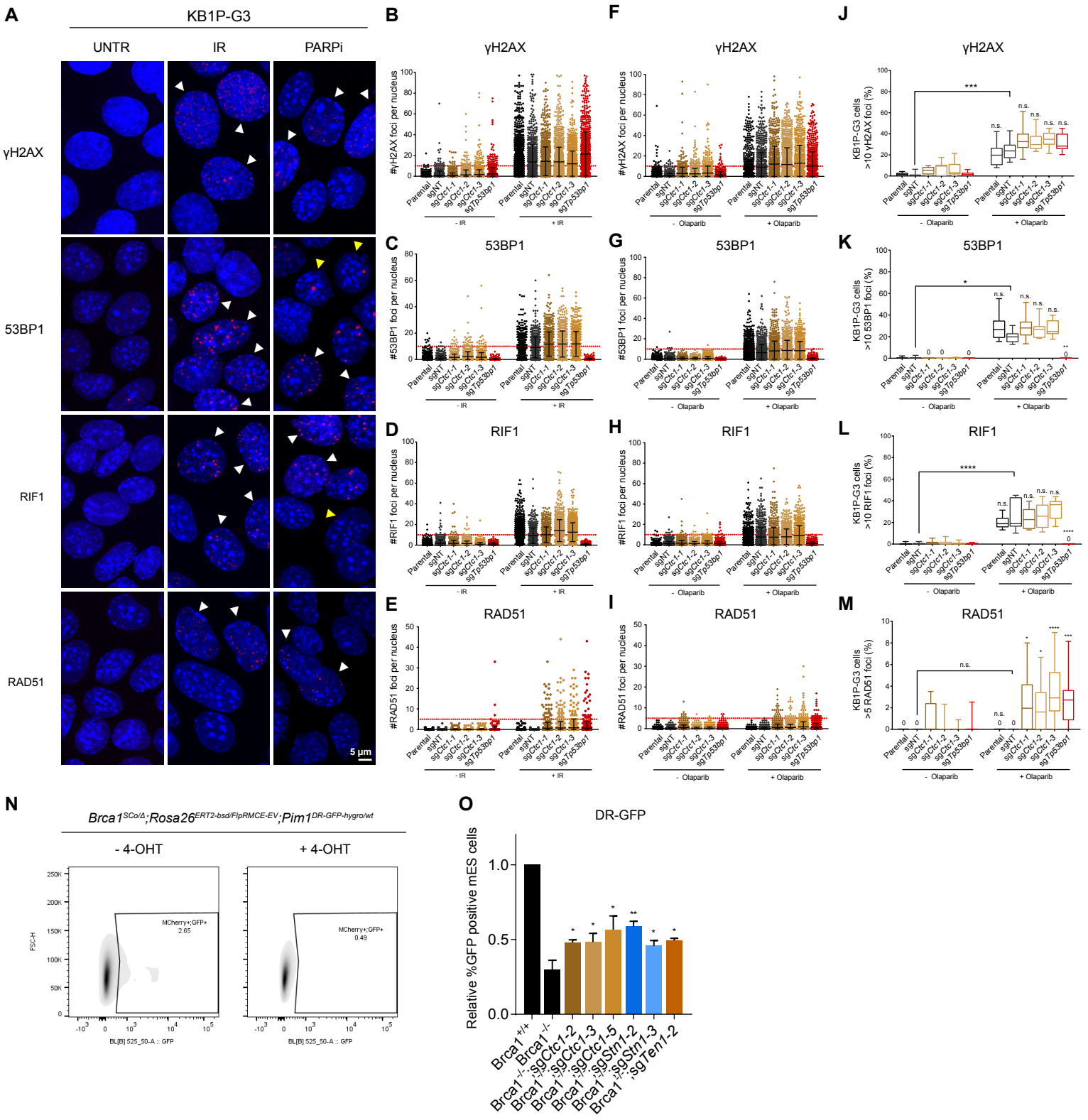

Figure S3: CTC1 functions as a resection antagonist on non-telomeric DSBs. Related to Figure 4.

(A) Overview confocal images (63x magnification) of indicated proteins and treatments. White arrowheads indicate examples of positive cells, and yellow arrowheads indicate examples of negative cells containing nuclear bodies.

(B-E) Quantifications of confocal images shown in Figure 4C. KB1P-G3 SpCas9 expressing cells of indicated genotype were irradiated (10 Gy) and harvested 3 hours later for immunofluorescence of indicated proteins. Data is plotted as #foci per nucleus for indicated proteins.

(F-M) Immunofluorescence stainings were performed after 500nM olaparib treatment for 24h to induce DSBs. Data represent two independent experiments. **F-I** show quantifications of confocal images plotted as #foci per nucleus for indicated proteins, **J-M** quantification of confocal images, plotted as box and whiskers plot. The box represents the 25th to 75th percentiles and the whiskers show the min to max values. The experiment was performed at least two times and data is plotted as percentage of γH2AX, 53BP1 or RIF1-positive cells (>10 foci) or RAD51-positive cells (>5 foci) per field. Statistics was performed by Kruskal-Wallis non-parametric test followed by Dunn's multiple comparisons test. The indicated cell lines were compared to sgNT treated cells (**** = p-value < 0.0001).

(N-O) CST complex members were depleted in *Brca1$^{SCo/Δ}$;Rosa26$^{ERT2-bsd/FlpRMCE-EV}$;Pim1$^{DR-GFP-hygro/wt}$* mES-cells using pLentiCRISPRv2 vectors containing indicated sgRNAs. Cells were treated with 4-OHT to inactivate the *Brca1^{Sco}* allele and subsequently transfected with mCherry/I-SceI constructs. HR activity was determined by flow cytometry and was calculated as the percentage of GFP+ cells in the mCherry+ population relative to BRCA1 proficient parental cells. The experiment was performed three times and the data are plotted as mean with SEM. Statistics was performed by unpaired one-tailed students t-test (* = p-value <0.05; ** = p-value <0.01).

Figure S4

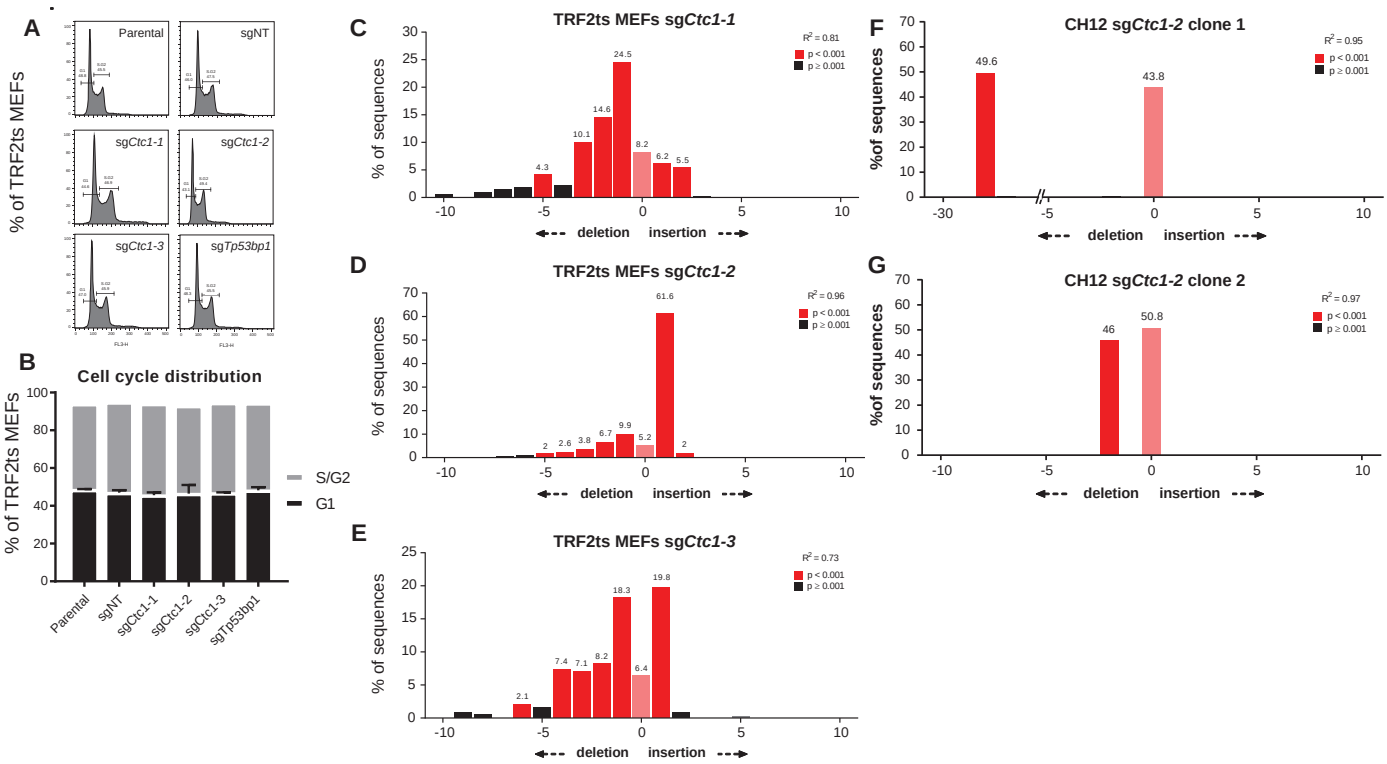

Figure S4: CTC1 facilitates cNHEJ at telomeric and non-telomeric DSBs. Related to Figure 5.
(A-B) Cell cycle distribution profiles were determined by flow cytometry from TRF2ts MEFs transfected with pX330puro vectors containing indicated sgRNAs. The experiment was performed two times.
(C-E) TIDE plots of TRF2ts MEFs targeted by indicated sgRNAs.
(F-G) TIDE plots of the two CH12 clones that were successfully targeted with sgCtc1-2.

Figure S5

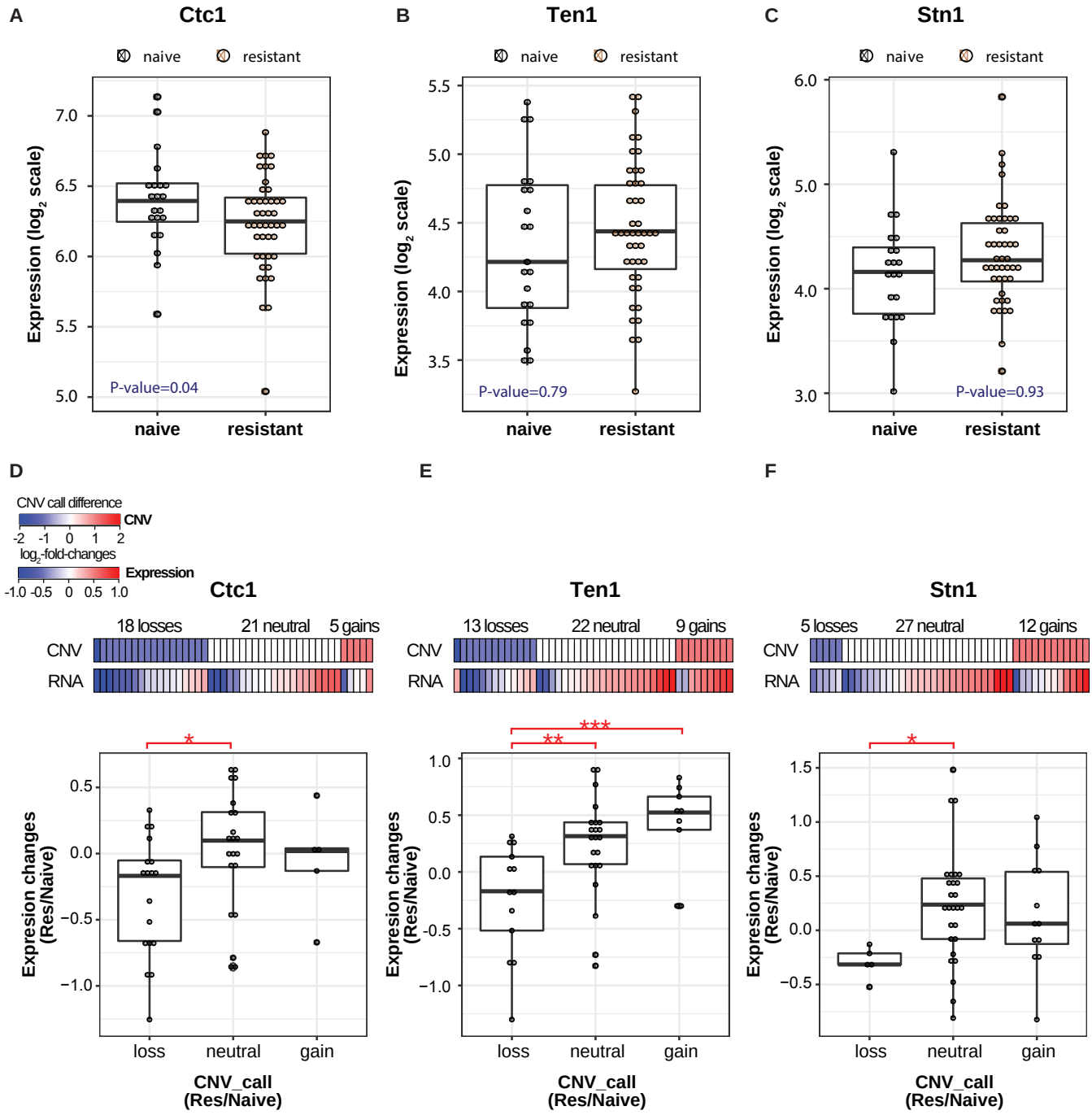

Figure S5. Expression and copy number profiles for CST complex genes (Ctc1, Ten1, and Stn1) in matched KB1P(M) naïve- and PARPi-resistant tumors for which both RNA-seq and CNV-seq were available. In total, 21 naïve and 44 resistant tumors from 21 donors were used in this analysis. Related to Figure 6.

(A-C) mRNA expression levels (log2(CPM) of Ctc1 (A), Ten1 (B), and Stn1 (C), between naïve and resistant tumors. P-values were obtained by one-tail t-test.

(D-F) Correlation between mRNA levels and CNV for CST complex genes. CNV calling and gene expression levels of 44 resistant tumors were compared to those of corresponding naïve tumors. First heatmaps indicate copy number changes (red: copy number gain, blue: copy number loss) and second heatmaps indicate gene expression changes (red: upregulation, blue: downregulation) for the same tumors. Bottom boxplots indicate correlation between copy number changes and expression changes. Adjusted P-value were obtained by one-way anova and Tukey multiple pairwise-comparison. * Adj. P-value < 0.1, ** Adj. P-value < 0.05, *** Adj. P-value <0.01.

Table S2. sgRNA and crRNA sequences. Related to Supplemental Experimental Procedures.

Mouse gene name	Mouse sgRNA name	sgRNA sequence
Non-targeting	sgNT	TGATTGGGGGTCGTTCGCCA
Ctc1	sg*Ctc1-1*	CTTGAAGCCGAACAGTGCCA
Ctc1	sg*Ctc1-2*	CACGAGTTGCTCATACAAGG
Ctc1	sg*Ctc1-3*	CTGGTCGAATAACCCGCCTG
Stn1	sg*Stn1-1*	ATGATATCTACCCGCCTTAT
Stn1	sg*Stn1-2*	CAACGGGCATCCAATAAGGC
Ten1	sg*Ten1*	CTGCGAACATTTGGCAGGTA
Tp53BP1	sg*Tp53BP1*	GAACAATCTGCTGTAGAACA
Human gene name	**Human crRNA name**	**crRNA sequence**
Non-targeting	crNT-1	AAAACACGATGACGTCTCT
Non-targeting	crNT-2	AAACGAGAAGTTTGTACTA
CTC1	cr*CTC1-1*	TGCCAACTCAATCGCCGCC
CTC1	cr*CTC1-2*	TAGGCTGTACCAGGCCGAA
CTC1	cr*CTC1-3*	ACAGACCTATCGGCAGACT
TP53BP1	cr*TP53BP1-1*	TCTAGTGTGTTAGATCAGG
TP53BP1	cr*TP53BP1-2*	GGGGGTTTTCTAACTCCAC
TP53BP1	cr*TP53BP1-3*	GACTGCTAGGAACGATAAA

Table S3. PCR primer sequences. Related to Supplemental Experimental Procedures.

sgRNA name	FW PCR primer	RV PCR primer	Source
sgNon-targeting	N.A.	N.A.	Duarte et al., 2017
sg*Ctc1-1*	TGTTCCAGACAGGGATTTTCCAA	AGGAGAGGGTTGCTTCAGGA	This paper
sg*Ctc1-2*	TGTTCCAGACAGGGATTTTCCAA	AGGAGAGGGTTGCTTCAGGA	This paper
sg*Ctc1-3*	ATTATGGTTAAGGGCGGGGGT	TGGCTACTGTTTCTCCACCAT	This paper
sg*Stn1-1*	GCATTTCAATTCTTCCACGGCT	CACTTGCCAAGGACTGACTC	This paper
sg*Stn1-2*	GCATTTCAATTCTTCCACGGCT	CACTTGCCAAGGACTGACTC	This paper
sg*Ten1*	GCCAGCTAGTCTTCCAAATGT	CAGCGTATGGTTCTCACTACC	This paper
sg*Tp53BP1*	TGAGAAATGGAGGCAACACCA	TGCAAATGTGGGCTACTGGG	This paper
Brca1-Sco allele	CACCTGCTCTGGCTGATG	AGGTCTGCCTGCCTCTACTTC	Bouwman et al., 2013
Brca1-DelSco allele	GTGGGCTTGTACTCGGTCAT	GCTGTTCTCCTCTTCCTCATC	Bouwman et al., 2013
iKRUNC sequencing primer	AAAGAATAGTAGACATAATAGC	N.A.	This paper

Supplemental Experimental Procedures

Cell culture

KB1P and KB2P mouse mammary tumor cell lines were cultured in DMEM/F-12 medium (Life Technologies) in the presence of 10% FCS, penicillin/streptomycin (Gibco), 5µg/mL insulin (Sigma), 5ng/mL epidermal growth factor (Life Technologies) and 5ng/mL cholera toxin (Gentaur) under low oxygen conditions (3% O_2, 5% CO^2 at 37^{o}C). SUM149PT cells were cultured in Ham's F12 medium (Gibco) supplemented with 5% FCS, 5 µg/ml insulin, 1 µg/mL hydrocortisone (Sigma-Aldrich, St. Louis, MI, USA). Mouse ES cells with a selectable conditional *Brca1* deletion (*R26*$^{CreERT2/wt}$*;Brca1*$^{SCo/\Delta}$ and *R26*^{CreERT2}*;Brca1*$^{SCo/\Delta}$*;Pim1*$^{DR\text{-}GFP/wt}$) (Bouwman et al., 2010; Bouwman et al., 2013) were cultured on gelatin-coated plates in 60% buffalo red liver (BRL) cell conditioned medium supplied with 10% fetal calf serum, 0.1 mM β-mercaptoethanol (Merck) and 10^3 U/ml ESGRO LIF (Millipore) under normal oxygen conditions (21% O_2, 5% CO_2, 37°C). *Trf2*$^{-/-}$*p53*$^{-/-}$ TRF2ts MEFs were grown in DMEM with 100U penicillin, 0.1 mg/ml streptomycin, 2mML-glutamine and 10%FBS. TRF2ts MEFs were maintained at the permissive temperature of 32 ^{o}C and only grown at 39^{o}C to induce telomere uncapping through inactivation of TRF2 (Peuscher and Jacobs, 2011).CH12F3 cell lines were cultured in RPMI supplemented with 5 % NCTC-109 medium, 10% FCS, 100 U/ml penicillin, 100 ng/ml streptomycin and 2 mM L-glutamine at 37°C with 5% CO_2 under ambient oxygen conditions. KB1P4 3D tumor organoid cells were seeded in Basement Membrane Extract Type 2 (BME, Trevigen) on 24-well suspension plates (Greiner Bio-One) and cultured in AdDMEM/F12 supplemented with 1 M HEPES (Sigma), GlutaMAX (Invitrogen), penicillin/streptomycin (Gibco), B27 (Gibco), 125 µM N-acetyl-L-cysteine (Sigma), 50 ng/mL murine epidermal growth factor (Invitrogen).

Transfection-based genome editing

Transfection in KB1P-G3 cells was performed using TransIT-LT1 (Mirus) reagents following manufacturer's recommendations. In brief, 150,000 cells were plated in 6-well format 1 day before transfection with 1 µg DNA. The medium was refreshed 24 hours after transfection and transfected cells were selected with puromycin for three days. CRISPR/SpCas9 targeted SUM149PT cells were generated with editR crRNA (Dharmacon, Lafayette, CO, USA).

Lentiviral transduction-based genome editing

Cell lines targeted with the pGSC_Cas9_Neo and pLenti-sgRNA-tetR-T2A-Puro system were generated by lentiviral transduction. Lentivirus was produced in HEK293FT cells as described previously (Follenzi et al., 2000) and mouse KB1P-G3 or *Brca1*$^{-/-}$*;p53*$^{-/-}$ mES cells were infected overnight using polybrene (8 µg/mL). The medium was refreshed after 12 hours and transduced cells were selected with puromycin (3 µg/mL) and blasticidin (500 µg/mL) for five consecutive days. KB1P4 tumor organoids were transduced using spinoculation as described previously (Duarte et al., 2018; Koo et al., 2011). Expression of the sgRNA was induced by incubation with 3 µg/ml doxycycline (Sigma) for at least five days.

Plasmids

pGSC_Cas9_Neo and pLenti-sgRNA-tetR-T2A-Puro were described previously (Prahallad et al., 2015). Genome-wide mouse lentiviral CRISPR sgRNA library was a gift from Kosuke Yusa (Addgene #50947). Human Improved Genome-wide Knockout CRISPR Library v1 was a gift from Kosuke Yusa (Addgene #67989). pX330-U6-Chimeric_BB-CBh-SpCas9 was a gift from Feng Zhang (Addgene plasmid # 42230). pLentiCRISPRv2 was a gift from Feng Zhang (Addgene plasmid # 52961). The MCherry/I-SceI plasmid has been described previously (Bouwman et al., 2013).

Generation of CRISPR/SpCas9 plasmids

Unless otherwise stated, KB1P-G3 experiments were performed using a modified version of the pX330 backbone (Cong et al., 2013) in which a puromycin resistance ORF was cloned under the hPGK promoter (Harmsen et al.,

2018). sgRNA sequences were cloned in the pX330puro backbone using custom DNA oligos (IDT) which were melted, annealed and subsequently ligated with quick-ligase (NEB) into BbsI-digested backbone. A similar procedure was used for cloning into the pLenti-sgRNA-tetR-T2A-Puro vector, but using BfuAI-digested backbone. All constructs were sequence verified by Sanger sequencing. sgRNA sequences are provided in Table S2.

Genomic DNA isolation, PCR amplification and TIDE analysis

Target loci were amplified by PCR using the following conditions: (1) 98 °C, 30 s, (2) 30 cycles of 98 °C for 10 s, 61 °C for 20 s and 72 °C for 30 s, (3) 72 °C, 5 min. Reaction mix consisted of 0.75 µl DMSO, 5 µl GC Phusion Buffer 5X, 0.5 µl 2 mM dNTPs, 0.125 µl 100 µM Fwd oligo, 0.125 µl 100 µM Rev oligo, 0.25 µl Phusion polymerase in 25 µl total volume. Amplified products were diluted 50X in dH_2O and 2 µl of diluted product was submitted for Sanger sequencing.

CRISPR library screens

The first PARPi resistance screen was performed in the KB1P-G3 tumor cell line, which was previously established from a KB1P tumor (Jaspers et al., 2013). This cell line is BRCA1- and p53-deficient through large intragenic deletions and shows sensitivity to PARPi treatment in the nanomolar range. The DDR sgRNA library was generated based on the gene list from Thanos Halazonetis (University of Geneva) described before (Costantino et al., 2014; Xu et al., 2015) and the NCBI search (terms: “DNA repair”, “DNA damage response”, “DNA replication”, “telomere-associated genes”) . See Table S1 for the full library details. This search resulted in a DDR-related gene list comprising a total of 1,752 genes (Table S1). *Tp53bp1* was specifically removed from this list in anticipation that loss of 53BP1 might dominate the screen results and thereby obscure the effects of other genes. sgRNAs targeting the 1,752 DDR-related genes were synthesized (MYcroarray) and cloned into the pLenti-sgRNA-tetR-T2A-PuroR vector, which allows for doxycycline inducible expression of the sgRNA (Prahallad et al., 2015). KB1P-G3-SpCas9 expressing cells were generated by transduction with the pGSC_SpCas9_Neo vector and transduced cells were selected by 500 µg/mL G418. The pLenti-sgRNA-tetR-T2A-Puro-DDR library was introduced at 100x coverage. Next, doxycycline was added to the medium for 5 days to mutagenize the population. Cells were subsequently plated in a clonogenic growth format in the presence of the PARP inhibitors olaparib or AZD2461 (Oplustil O'Connor et al., 2016) at the approximate IC90 concentration for 14 days. Cells were harvested before and after PARPi treatment for genomic DNA isolation. Subsequently, sgRNA sequences were amplified from genomic DNA by two rounds of PCR amplification as described previously (Duarte et al., 2018) and sequenced with the HiSeq 2500, using the following barcodes: GTAGCC, TACAAG, CTCTAC, GCGGAC, TTTCAC, GGCCAC. Sequencing reads were aligned to the reference sequences using edgeR software (Robinson et al., 2010). The screening data were processed by the MAGeCK algorithm (Li et al., 2014), and results were sorted on MAGeCK-based positive selected gene ranks to allow comparison across screens.

The second PARPi resistance screen was performed in BRCA1- and p53-deficient, SpCas9-expressing mouse embryonic stem (mES) cells infected with a genome-wide lentiviral sgRNA library targeting 19,150 genes (Koike-Yusa et al., 2014). The screen was performed at 75x coverage in two independent transductions (MOI 0.5) and cells were first selected with puromycin and subsequently treated with olaparib at a concentration of 15 nM for 10 days. Surviving populations were harvested and processed as described previously (Koike-Yusa et al., 2014), using the following barcodes CGTGAT, ACATCG, GCCTAA, TGGTCA, CACTGT, ATTGGC, GATCTG, TCAAGT, CTGATC, AAGCTA, GTAGCC, TACAAG, TTGACT, GGAACT. The screening data were processed similar to the screen in KB1P-G3 cells.

A third PARPi resistance screen was performed in the SUM149PT human breast cancer cell line. This cell line harbors the *BRCA1*^{2288delT} mutation and LOH (Elstrodt et al., 2006). A derivative of SUM149PT with an integrated tetracycline-inducible SpCas9 was lentivirally infected with a genome-wide sgRNA library designed to target 18,010 genes (Tzelepis et al., 2016), using a multiplicity of infection of 0.3 and infecting >1000 cells per sgRNA. After puromycin selection (3 µg/ml) to remove non-tranduced cells, a sample was removed (time or t=0); remaining cells were cultured in the presence or absence of doxycycline plus 100 nM talazoparib, a concentration which normally results in complete inhibition of the cell population. No cells survived in the absence of doxycycline. After two weeks of selection, genomic DNA from the remaining cells in the doxycycline-treated sample was recovered. The sgRNA sequences from this genomic DNA were PCR amplified using barcoded and tailed primers and deep sequenced as previously described (Koike-Yusa et al., 2014) to identify sgRNAs in the talazoparib-resistant

population. Read counts were normalized for coverage by converting to parts per ten million (pptm) reads and fold change between starting and resistant population was calculated for each guide. Fold change values were log- and Z-transformed and plotted based on z-rank (Wang et al., 2017).

Clonogenic survival assay

Ctc1, *Stn1* and *Ten1* were targeted in $R26^{CreERT2};Brca1^{SCo/\Delta}$ mouse embryonic stem (mES) cells using pLentiCRISPRv2 vectors, Cre-mediated inactivation of the endogenous mouse $Brca1^{SCo}$ allele was achieved by overnight incubation of cells with 0.5 μmol/L 4-OHT (Sigma) (Bouwman et al., 2010). Four days after switching, cells were seeded in triplicate at 10,000 cells per well in 6-well plates for clonogenic survival assay. For experiments with $R26^{CreERT2/wt};Brca1^{SCo/\Delta}$ p53-null cells, cells were plated without treatment or in the presence of olaparib 2.5 nM. Cells were stained with 0.1% crystal violet one week later, and scanned with the Gelcount (Oxford Optronix). Automated quantification of colony counts was performed using the Gelcount colony counter software.

Clonogenic survival assay with PARPi (olaparib) were performed as described previously with minor modifications (Xu et al., 2015). CRISPR/SpCas9 transfected KB1P-G3 cells were seeded in triplicate at 5 x 10^3 cells per well into 6-well plates on day 0, and then olaparib or AZD2461 was added at the indicated concentrations. On day 6, the untreated group was fixed, the other groups were fixed on day 9 and stained with 0.1% crystal violet. Plates were scanned with the Gelcount (Oxford Optronix). Quantifications were performed by solubilizing crystal violet using 10% acetic acid and the absorbance at 562nm was measured using the Tecan plate reader. The experiment was performed three times.

SUM149PT-SpCas9 cells were transfected with *CTC1* targeting crRNA and tracrRNA using the EditR system (Life Technologies), plated into 48 well plates and then treated with 50 nM talazoparib. Medium was replaced with fresh drug-containing medium as indicated. Images were taken to measure viability of cells in each well every 12 hours using the IncuCyte system. After two weeks, final viability was assessed using CellTiter Glo (Promega).

Competition assays

Competition assays were performed in KB1P-G3 SpCas9 expressing cells, transduced with pLenti-sgRNA-tetR-T2A-Puro vectors containing the indicated sgRNAs. Cells were selected with puromycin (3 μg/mL) for three days and allowed to recover from selection. A sample was harvested for gDNA isolation at t = 0, and 5,000 cells were plated in 6-well plates in triplicate per condition, with or without AZD2461 (250 nM). After 10 days of treatment, cells were harvested, counted and re-plated at 5,000 cells per 6-well two times (total treatment time of 30 days). On the last time point, each condition was plated as technical duplicate. At the end point, one technical duplicate well was fixed and stained with crystal violet and the other was used to isolate gDNA. Allele distributions were determined from gDNA samples by PCR followed by Sanger sequencing and TIDE analysis, as described above. Competition assays in KB2P-3.4 cells were performed similarly, except 2,000 cells were seeded per 6-well and cells were treated with olaparib (50 nM).

Growth curves

Growth curves were generated for CRISPR/SpCas9 transfected KB1P-G3 cells by seeding 1,000 cells per well in 96-well plates, seeding 6 technical replicates per experiment and the well confluency was recorded every 4 hours for 120 hours using an IncuCyte Zoom Live – Cell Analysis System (Essen Bioscience). The images were analyzed using IncuCyte Zoom software. Data were normalized to the confluency at 20h after seeding.

Alpha track assay

Experiments were performed as described previously (Xu et al., 2015) with small modifications. CRISPR/SpCas9 transfected KB1P-G3 cells were seeded on coverslips overnight, washed with PBS and covered with a mylar foil, allowing α-particle irradiation from above, through mylar. Irradiation was done using a ^{241}Am point- source by moving the source over the coverslip for 30s per area, cells were incubated for 1 hour at 37 °C and washed with ice-cold PBS. Subsequently, cells were extracted with cold CSK buffer (10 mM HEPES-KOH, pH 7.9, 100 mM NaCl, 300 mM sucrose, 3 mM MgCl2, 1 mM EGTA, 0.5% (v/v) Triton X-100) and cold CSS buffer (10 mM Tris, pH 7.4, 10 mM NaCl, 3 mM MgCl2, 1% (v/v) Tween20, 0.5% (w/v) sodium deoxycholate) for 5 min each before fixation in

4% PFA in PBS for 30 min at room temperature. Fixed cell were washed 2 times for 10 minutes in PBS (0.1% Triton X-100) and washed for 30 minutes in blocking solution (0.5% BSA and 0.15% glycine in PBS). Primary antibodies were diluted in blocking buffer and incubated overnight at 4 °C. Hereafter, cells were washed 2 times for 10 minutes in PBS (0.1% Triton X-100) and 1 time shortly in blocking buffer. Secondary antibodies were diluted in blocking buffer and cells were incubated for at least 1 hour at room temperature in the dark. Finally, cells were washed 2 times in PBS and coverslips were mounted using Vectashield with DAPI. Quantification was done as described previously. Primary antibodies used in this study were as follows: rabbit anti-53BP1 (NB100-304, Novus), 1:1000 dilution; mouse anti-RPA34-20 (Ab-3, CalBiochem), 1:1000 dilution; MRE11 antibody (de Jager et al., 2001), 1:250 dilution. Secondary antibodies used in this study were as follows: Alexa Fluor 594 goat anti-rabbit IgG (A 31631, Invitrogen), Alexa Fluor 488 goat anti-mouse IgG (Thermo Fisher Scientific Cat# A-11001, RRID: AB_2534069), 1:1000 dilution.

Foci formation experiments

RAD51 immunofluorescence in CRISPR/SpCas9 transfected KB1P-G3 cells was performed as described previously, with minor modifications (Xu et al., 2015). Cells were grown on 8-well chamber slides (Millipore). Ionizing-Radiation Induced Foci (IRIF) were induced by γ-irradiation (10 Gy) 3 hours prior to sample preparation. PARP inhibitor-induced foci were generated by treatment with 500 nM olaparib for 24h prior to sample preparation. Subsequently, cells were washed in PBS++ and fixed with 2% PFA/PBS++ for 20' on ice. Fixed cells were washed with PBS++ and were permeabilized for 20' in 0.2% Triton X-100/PBS++. All subsequent steps were performed in staining buffer (PBS++, BSA (2%), glycine (0.15%), Triton X-100 (0.1%)). Cells were washed 3x and blocked for 30' at RT, incubated with the 1st antibody for 2hrs at RT, washed 3x and incubated with the 2nd antibody for 1hr at RT. Antibodies were diluted in staining buffer. Last, cells were mounted and counterstained using Vectashield mounting medium with DAPI (H1500, Vector Laboratories). Primary antibodies used: rabbit-anti-RAD51; 70-001, BioAcademia, 1:1,000 dilution; rabbit-anti-53BP1; Abcam Ab21083, 1:2,000 dilution; rabbit-anti-RIF1 was a gift by Ross Chapman, 1:1,000 dilution; mouse anti yH2AX: Millipore JBW3O1, 1:1,000 dilution; Alexa fluor 568 F(ab')$_2$ Fragment goat anti-rabbit; A21069, Thermo Fisher Scientific, 1:400 dilution; Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 568 from Thermo Fisher Scientific, catalog # A-11011, RRID AB_143157; Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 568 from Thermo Fisher Scientific, catalog # A-11004, RRID AB_2534072. Z-stack images were acquired using a confocal microscope (Leica SP5, Leica Microsystems GmbH) and multiple different confocal fields were imaged per sample (63x objective). Confocal images were analyzed automatically using an ImageJ script (Xu et al., 2015). Briefly, the macro detects nuclei based on DAPI intensity and subsequently counts the number of foci within each nucleus.

DR-GFP

The DR-GFP assay was performed as described previously (Bouwman et al., 2013). Genes were targeted using the pLentiCRISPRv2 system containing indicated sgRNAs.

Assessment of telomere NHEJ

*Trf2$^{-/-}$;Trp53$^{-/-}$;*TRF2ts MEFs (TRF2ts MEFs) were described before (Peuscher and Jacobs, 2011). TRF2ts MEFs were targeted using the pX330puro system containing indicated sgRNAs at the permissive temperature of 32 ^{o}C. Following selection and recovery, they were grown for 24 h at the non-permissive temperature of 39°C to inactivate TRF2 and induce NHEJ dependent chromosome end to end fusions as a consequence of telomere uncapping. Cell harvesting, preparation of metaphase spreads and telomere FISH with an Alexa488-labeled C-rich Telomere probe (PN-TC060-005, Panagene/Eurogentec) for metaphase chromosome analysis was done as described before (Boersma et al., 2015).

Digital images of metaphases were captured using the Metafer4/MSearch automated metaphase finder system (MetaSystems, Germany) equipped with an AxioImager Z2 microscope (Carl Zeiss, Germany). After scanning metaphase preparations at 10x magnification, high-resolution images of metaphases were acquired using a 'Plan-Apochromat' 63x/1,40 oil objective.

The cell cycle distribution of TRF2ts MEFs with or without CRISPR/SpCas9 mediated disruption of *Ctc1* or *Tp53bp1* was determined by propidium-iodide staining, acquired on a FACSCalibur (Beckton Dickinson) and analyzed with FlowJo (TreeStar, Ashland, OR) software.

CSR assay

Ctc1-mutated CH12F3 cells were generated by nucleofection (Amaxa Nucleofector 2b, Lonza) with 2 μg of plasmid and Cell Line Nucleofector Kit R (Lonza), using program D-023. Isogenic cell clones were isolated by limiting dilution and mutated clones were identified by native PAGE resolution of PCR amplicons of the target site, and subsequent confirmation by Sanger sequencing. Immunoglobulin CSR was performed as described previously (Xu et al., 2015). Briefly, CH12 cells were either mock-treated or stimulated with agonist anti-CD40 antibody (0.5 mg/ml; eBioscience; HM40-3), mouse IL-4 (5 ng/ml; R&D Systems) andTGF-b1 (1.25 ng/ml; R&D Systems). Cell-surface IgA expression was determined by flow cytometry, immunostaining with biotinylated antimouse IgA antibody (eBioscience; 13-5994), and Alexa488-streptavidin conjugate (Life Technologies).

Generation of RNA sequencing data

To determine the effects of Ctc1, Ten1 and Stn1 on PARPi treatment in vivo, we used our RNASeq dataset generated from a cohort of PARPi-naive and -resistant KB1P and KB1PM tumors (Jaspers et al 2013, Gogola et al, unpublished data). Hereto, fresh-frozen tumor tissues were subjected to high-speed shaking in 2 ml microcentrifuge tubes containing 1 ml of TRIsure reagent (Bioline) and stainless steel beads (TissueLyser LT, Qiagen; 10 min, 50 Hz, room temperature). Homogenized lysates were further processed for RNA isolation following TRIsure manufacturer's protocol. Quality and quantity of the total RNA was assessed by the 2100 Bioanalyzer using a Nano chip (Agilent, Santa Clara, CA). Total RNA samples having RIN>8 were subjected to library generation. Strand-specific libraries were generated using the TruSeq Stranded mRNA sample preparation kit (Illumina Inc., San Diego, RS-122-2101/2) according to the manufacturer's instructions (Illumina, Part # 15031047 Rev. E). The libraries were analyzed on a 2100 Bioanalyzer using a 7500 chip (Agilent, Santa Clara, CA), diluted and pooled equimolar into a 10 nM sequencing stock solution. llumina TruSeq mRNA libraries were sequenced with 50 base single reads on a HiSeq2000 using V3 chemistry (Illumina Inc., San Diego). The resulting reads were trimmed using Cutadapt v.1.12 (Martin, M., 2011). The trimmed reads were aligned to the GRCm38 reference genome using STAR v.2.5.2b (Dobin et al., 2013), QC statistics from Fastqc v.0.11.5 (www.bioinformatics.babraham.ac.uk/projects/fastqc) and the above-mentioned tools were collected and summarized using Multiqc (v.0.8; (Ewels et al., 2016)). Gene expression counts were generated by featureCounts (v.1.5.2; (Liao et al., 2014))) using gene definitions from Ensembl GRCm38 version 76. The genes with counts per million (CPM) larger than one at least 20% of total number of samples were taken and used for further analysis. Then, trimmed mean of M-value (TMM) normalization was performed to obtain normalized expression using edgeR (Robinson et al., 2010).

Generation of CNV sequencing data

CNV-Seq was performed using double stranded DNA (dsDNA), quantified with the Qubit® dsDNA HS Assay Kit (Invitrogen, #Q32851). To obtain fragment sizes of 160–180 bp, 2 μg of dsDNA were fragmented by Covaris shearing and purified using 1.8X Agencourt AMPure XP PCR Purification beads according to manufacturer's protocol (Beckman Coulter, #A63881). Next, sheared DNA was quantified and qualified on a BioAnalyzer system with the DNA7500 assay kit (Agilent Technologies, #5067-1506). Library preparation for Illumina sequencing was carried out with 1 μg of DNA and KAPA HTP Library Preparation Kit (KAPA Biosystems, #KK8234). HiSeq2500 machine in one lane of a single read 65 bp run, according to manufacturer's instructions. Sequencing reads were trimmed using Cutadapt (version 1.12; Martin, 2011) and reads shorter than 30 bp were removed. The trimmed reads were aligned to the GRCm38 reference genome using BWA (version 0.7.15; (Li and Durbin, 2010)). The resulting alignments were sorted and marked for duplicates using Picard tools (version 2.5.0). Copy number calls were generated using the QDNAseq and QDNAseq.mm10 (Scheinin et al., 2014) packages from Bioconductor (versions 1.8.0 and 1.4.0, respectively). Copy number calls of the resistant tumors were subtracted to those of matched naïve tumors for downstream analysis which examined the correlation between CNV and gene expression.

***In vivo* studies**

Tumor organoids were collected, incubated with TripLE at 37^{o}C for 5', dissociated into single cells, washed in PBS, resuspended in tumor organoid medium and mixed in a 1:1 ratio of tumor organoid suspension and BME in a cell concentration of 10^4 cells per 40 µl. Subsequently, 10^4 cells were transplanted in the fourth right mammary fat pad of 6-9 week-old NMRI nude mice. Mammary tumor size was measured by caliper measurements and tumor volume was calculated (0.5 x length x width^2). Treatment of tumor bearing mice was initiated when tumors reached a size of 50-100 mm^3, at which point mice were stratified into the untreated (n = 3) or olaparib treatment group (n = 7). Olaparib was administered at 100 mg/kg intraperitoneally for 56 consecutive days. Animals were sacrificed with CO_2 when the tumor reached a volume of 1,500mm^3.

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