Supplementary Methods

Gene sequencing RT-PCR analysis

Genomic DNA was isolated from cells using the DNeasy tissue kit (Qiagen). *BRCA1* gene sequencing and BROCA sequencing was carried out as previously described (23, 24).

RNA-seq and whole genome splicing analyses

Total RNA was isolated using RNAeasy kit (Qiagen) from SUM149PT and UWB1.289 exon 11 mutant cell lines as well as MDA-MB-436 and HCC1395 cell lines that have BRCA1 mutations located outside of exon 11. Sequencing libraries were constructed from 500 ng of total RNA using the Illumina's TruSeq RNA Samplepre kit V2 (Illumina) following the manufacturer instruction. The fragment size of RNAseq libraries was verified using the Agilent 2100 Bioanalyzer (Agilent) and the concentrations were determined using Qubit instrument (LifeTech). The libraries were loaded onto the Illumina HiSeq 2500 at 6-10 pM on the rapid mode for 2x100 bp paired end read sequencing. The fastq files were generated on the Illumina's BaseSpace service or locally using the Casava software package for further analysis. 100-bp reads were aligned to human genome (hg19) using Tophat (25) (mapped reads > 15 × 10^7 reads per sample). The differential splicing analysis between the exon 11 mutant cases (SUM149PT and UWB1.289) and the mutations outside of exon 11 (HCC1395 and MDA-MB-436) samples was performed by implementing MATS (26), using the mapped read bamfiles as input. This analysis was done separately for each (exon 11, non-exon 11) pair – four comparisons in total. We calculated the total number unique splicing events by enforcing

a read cutoff (>=5 read coverage over exon-exon juction) and p-value (< 0.001). For each pairwise comparison, we estimated the number of unique events in each sample related to exon skips. After applying the *P*-value and junction read cutoff conditions, unique events for a sample were defined as an exon-skip or an exon-inclusion seen in that sample, but not at all in the second sample.

CRISPR/Cas9 gene editing

The CRISPR/Cas9 system is a kind gift from Christoph Seeger (27). SUM149PT cells were first infected with lentivirus vector pCW-Cas9 and selected with puromycin (2 μg/ml). Cas9 expression was conditionally induced by the presence of doxycycline (4 μg/ml). A Cas9 high expression clone was selected for sequential genome editing. Gene-specific sgRNA sequences were identified using the CRISPR Design Tool (http://crispr.mit.edu/). We used the below DNA oligos to generate the following sgRNAs.

sg_GFP F: caccGAGCTGGACGGCGACGTAAA

sg_GFP R: aaacTTTACGTCGCCGTCCAGCTC

sg_Exon11 F: caccGACAAATTCTTTAAGTTCAC

sg_Exon11 R: aaacGTGAACTTAAAGAATTTGTC

sg_Exon22 F: caccGAATGGATGGTACAGCTGTG

sg_Exon22 R: aaacCACAGCTGTACCATCCATTC

sgRNA oligos were cloned into sgRNA expression vector pLX-SG1 using BsmBI sites. SUM149PT-Cas9 cells were then infected with lentivirus vector pLX-SG1(27) and selected with blasticidin (10 μg/ml). After 5-day incubation of doxycycline (4 μg/ml),

cells were subject to either PARPi or cisplatin clonogenic assay or used to generate clones. For validation of targeted mutations, genomic DNA was isolated from pools or clones and followed by PCR amplification of targeted loci using F and R primers listed below and PCR products were directly sequenced (S) using primers listed below. The expression of BRCA1 was further confirmed by Western blot.

Exon11F: GAGTGGTTTTCCAGAAGTGATGA

Exon11R: TAATACTGGAGCCCACTTCATTAGT

Exon11S: TGGCTCAGTAACAAATGCTCCTAT

Exon22F: TGCTGAGTCTCTAACCAGGATAACA

Exon22R: CTCACACCTCAACTTCCCAAATAG

Exon22S: GAGAGGTCTTGCTATAAGCCTTCAT

RNA interference, generation of BRCA1 constructs and cDNA add backs

Mission shRNA non-target control (SHC002) was from Sigma, BRCA1 #1 TRCN0000018866 and #2 TRCN0000039837 were from Open Biosystems. We purchased Hs_BRCA1_FlexiTube siRNA constructs 1-2 (constructs #2 - SI00096313 and #13 - SI02654575), and AllStars Negative Control siRNA (scrambled control) from Qiagen. Custom made FOX2 siRNA sequences #1: caccuccgcagaauggaau and #2: agacacaaaguagugaaa; BRCA1-Δ11q: guaucagggugaagcagcauu (28) (Dharmacon). Transfections were carried out according to standard protocols. To generate HA-BRCA1-2594delCΔC, the pENTR HA-BRCA1 construct was cut at cDNA sites 2788 and 4439 by PpuMI as well as 5460 by BaeI to remove cDNA region 2788-5460, followed by re-ligation and site directed mutagenesis (Agilent) to introduce the 2594delC mutation. To

generate HA-BRCA1-Δ11q, the BRCA1-Δ11q was cloned by RT-PCR and gel purification, followed by restriction enzyme digests cutting both PCR product and pENTR-HA-BRCA1 with PshA1 and Stu1, followed by re-ligation of BRCA1-Δ11q PCR fragment into the pENTR1A-BRCA1. LacZ, GFP cDNA pENTR Gateway Entry vector was purchased from Open Biosystems. cDNAs were shuttled into pLX304 or pLenti-IRES-GFP Destination vectors using the LR Clonase system (Invitrogen). Lentiviral generation and infections were carried out according to standard protocols. Protein knockdown or re-expression was routinely assessed 72 hours post transfection or 96 hours post infection. Cells were either sorted for GFP positivity or maintained in 3 μg/ml puromycin or blastcitidine to generate stable cell lines.

DR-GFP and cisplatin cytotoxicity assays in mouse ES cells

Experiments in Brca1 SCo mouse embryonic stem cells were performed as described previously (29). $R26^{CreERT2/RMCE}$; $Brca1^{SCo/\Delta}$ mouse ES cells were targeted with a modified version of the p59X DR-GFP construct to generate $R26^{CreERT2/RMCE}$;$Brca1^{SCo/\Delta}$;$Pim1^{DR\text{-}GFP/wt}$ cells. These cells were used to add back human *BRCA1* exon 11 deletion variants: BRCA1- Δ11 or BRCA1- Δ11q and wild-type human *BRCA1* or empty vector controls by recombinase-mediated cassette exchange. Expression of mouse *Brca1* was switched off by overnight incubation with 0.5 μM 4-OHT. For HR reporter assays, cells were transfected with I-SceI-mCherry five days after switching, and mCherry/GFP double positive cells were monitored three days after transfection by flow cytometry. For cisplatin cytotoxicity assays, cells were seeded one week after switching in 96-well plates in triplicate at 1000-1500 cells per well. Cisplatin was added one day

after plating and cell viability was assayed three days later using resazurin (cell titer blue; Promega) and a Tecan plate reader.

Survival analysis

Mutations inside of exon 11 were determined as those from (BIC database designations) nt790-4215 where mutations resulted in frameshifts and stop codons and all other mutations were those resulting in frameshifts and or stop codons outside of exon 11. For analyses of 5-year survival we utilized patient cohorts from a previously described study(30). We used standard, time-to-event methods with a primary end point of death from all causes. Survival time was from the date of diagnosis until the date of death. Patients were censored at five years follow-up. The participants were recruited at a variable time after diagnosis – left truncation – which was allowed for in the analyses by treating time at risk from the date of recruitment. The survival functions for noncarriers and for participants with frameshift mutations inside and outside of exon 11 were estimated using the Kaplan-Meyer method and compared using the log-rank test. There were 7% of cases with missing data for stage and 10% for grade; we therefore imputed twenty complete data sets using multivariate imputation by chained equations. The imputation model included *BRCA1* mutation group, year of diagnosis, age at diagnosis, time of follow up and outcome. Parameter estimates were combined were according to "Rubin's rules" (31). The hazard ratios (HR) and 95% CI were estimated, on the imputed data, using Cox proportional hazards regression adjusted for age and stage (early and advanced) and stratified for year of diagnosis and study. Statistical analysis was conducted using STATA/SE version 13 (StataCorp).

Supplementary References

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