SUPPLEMENTARY MATERIAL

Supplementary Figures

Figure S1. BRCA1-Δ11q mRNA and protein isoform.

(**A**) The *BRCA1-Δ11q* mRNA isoform derives from the alternative choice of donor site within *BRCA1* exon 11, resulting in the majority of exon 11 nucleotides (c.788-4096) being excluded. Diagrammatic representation of intron 10, exon 11 and exon 11q region (highlighted - In-frame exon excision). The highlighted GG/GT sequence indicates the location of the 5' cryptic splice site location responsible for producing the *BRCA1-Δ11q* isoform.

(**B**) The BRCA1-Δ11q protein product is truncated but contains conserved domains found in the full-length isoform. BRCA1-Δ11q protein lacks amino acids 263 – 1365 of full-length BRCA1.

Figure S2. Genetic analyses of *BRCA1* mutant cell lines and PDX models.

(**A**) We confirmed that all cell lines and PDX models had loss of heterozygosity (LOH) for the wild-type *BRCA1* gene locus and contained the originally reported deleterious *BRCA1* mutation by Sanger sequencing. L56Br-C1 (1806C>T/Q563X), SUM149PT (2288delT/N723fsX13) and UWB1.289 (2594delC/D825fsX20) contained mutations located in exon 11(1-3). SUM1315MO2 (185delAG/E23fsX17) and HCC1395 (5251C>T/R1751X) contained mutations in exons 2 and 20, respectively(2, 4). MDA-MB-436 harbored a deleterious 5396 + 1G>A mutation in the splice donor site of exon 20(2). All mutations result in premature stop codons and are predicted to produce

truncated proteins. MDA-MB-231, MCF7, MDA-MB-468 *BRCA1* wild-type cells were used as positive controls throughout.

PDX124 is a triple negative breast cancer tumor derived from a patient skin metastasis and PDX196 is a serous ovarian cancer tumor derived from a patient with malignant pericardial effusion. Both the primary patient tumors and the derived PDX tumors harbored the germline *BRCA1*-exon11 2080delA mutation and LOH for the *BRCA1* gene locus. Both patients whose tumors were used to derive PDX124 and PDX196 initially demonstrated clinical responsiveness to olaparib therapy; while PDX124 was derived prior to the patient starting olaparib therapy, PDX196 was derived at the time of clinical tumor progression on olaparib. We Sanger sequenced PDX tumors and confirmed the presence of the *BRCA1*-exon11 2080delA mutation. Additionally, we did not detect any secondary reversion *BRCA1* mutations in any of the cell lines or PDX tumors.

(**B**) The BRCA1 protein detectable at the ~90 kDa mark on Western blots in wild-type as well as exon 11 mutant cell lines (Fig. 1A) was visible with N- and C-terminal BRCA1 antibodies. Fig.1B shows N-terminal antibody, here we show a separate membrane incubated with a C-terminal specific BRCA1 antibody. MDA-MB-231 (231), MCF7 (MCF), MDA-MB-468 (468), L56Br-C1 (L56), SUM149PT (149), UWB1.289 (UWB), SUM1315MO2 (1315), HCC1395 (1395) and MDA-MB-436 (436) cells were assessed for protein expression.

(**C**) We cloned the *BRCA1-Δ11q* mRNA junction detected in all cell lines and PDX tumors. Representative histograms show the region of exon 11 in the full-length isoform versus the *BRCA1-Δ11q* isoform.

(**D**) In addition to alternative splicing, it was possible that gene rearrangements could have accounted for the generation of *BRCA1-Δ11q* mRNA and protein. In this scenario, loss of genomic material corresponding to the exon 11 region, and an in frame fusion could produce *BRCA1-Δ11q* mRNA (c). We carried out PCR reactions on genomic DNA to test this possibility. PCR primers located in exon 10 (forward) and exon 12 (reverse) would be predicted to produce DNA fragments for full-length and exon 11 deficient genomic DNA at approximately 4.8 kb and 1.5 kb in length, respectively. PCR reactions were carried out using extension times required to capture both full-length and exon 11 deficient genes. In all cases, PCR products corresponding to full-length exon 11 containing genomic DNA were captured. No exon 11 deficient PCR products could be detected in any cell lines or PDX tumors. Therefore, we conclude that *BRCA1-Δ11q* cDNA was not produced as a result of genomic rearrangements around the exon 11 region.

Figure S3. BRCA1-minigene analyses.

Diagrammatic representation of the BRCA1-minigene. Genomic DNA derived from human blood lymphocytes that had been tested for the absence of mutations by BROCA sequencing was used to amplify the genomic region from exon 8 to exon 12 of BRCA1. HA and GFP tags were cloned on to the 5' and 3' ends, respectively, and introduced into a pcDNA6.2 vector to generate the BRCA1-minigene. BRCA1-minigene was transiently transfected into 293T cells and assessed for Δ11q-reporter construct expression 3 days post transfection by RT-PCR or Western blot (see Fig.1D and Fig. 6D). The pcDNA6.2 vector contains the Blasticidin S deaminase (BSD) gene and expression was used as a

control for transfection. A series of mutations were introduced into the minigene (mutation numbers correspond to the BIC designation or the cDNA (c.) number for full length BRCA1) and assessed for their impact on exon 11q splicing. The *BRCA1* exon 11q cryptic splice site was mutated (11q) as a negative control using the following mutation: c.T789G. Frameshift mutations (M) previously reported in patients with *BRCA1* exon 11 mutant tumors were also assessed for their effect on 11q splicing: M1: 2288delT (c.2169delT); M2: 2529C>T (c.2410C>T); M3: 3960C>T (c.3841C>T) (Fig. 1D). Additionally, both the 5' FOX binding site: c.G807A; T810C; and the 3' FOX site: c.G852A; T855C were mutated (FOX) and assessed for their impact on exon 11q splicing (Fig. 6D). Arrows indicate the approximate location of mutations in exon 11 (11q, FOX, M1, M2, M3) that were introduced into exon 11 of the minigene.

Figure S4. CRISPR/Cas9 editing of SUM149PT cells.

SUM149PT cells harbor a *BRCA1* 2288delT mutation that is predicted to result in a frameshift mutation and truncated peptide (N723fsX13). We utilized CRISPR/Cas9 to generate several SUM149PT cell line derivatives with altered BRCA1 isoform expression. We carried out DNA sequencing of genomic DNA after exposure to the below single guide RNAs (sg). DNA and peptide sequences surrounding the original 2288delT mutation-containing region of exon 11 are shown. See Fig. 1E and 3A for additional characterization of cells.

(**A**) BRCA1 wild-type sequence is shown for genomic DNA as well as the amino acids encoded by this region. SUM149PT cells expressing sg_GFP did not have any changes to the *BRCA1* gene locus, and the original *BRCA1* 2288delT mutation was detected.
(**B**) SUM149PT cells expressing sg_exon11 targeting the 2288delT mutation-containing region of *BRCA1* created additional mutations but the reading frame remained out of frame. Clones 1 and 2 had deletions or insertions that did not restore the long-form of BRCA1.
(**C**) SUM149PT cells expressing sg_exon11 targeting the 2288delT mutation-containing region of *BRCA1* demonstrated reversion mutations. We isolated 2 clones where deletions restored the reading frame. Clones 3 and 4, had deletions of 14 and 3 amino acids, respectively. Missense mutations or deletions of these amino acids have not previously been reported to result in deleterious phenotypes and the protein product is likely to behave similarly to wild-type BRCA1.
(**D**) SUM149PT cells expressing sg_exon22 targeting the BRCT encoding region of BRCA1 were generated. BRCT domain mutations disrupt protein folding and result in degradation(5-7). Similar to MDA-MB-436 and HCC1395 cell lines that harbor endogenous BRCT domain mutations **(**Fig. 1A**)**, mutations introduced into SUM149PT cells that targeted the BRCT domain resulted in loss of BRCA1-Δ11q protein expression. Cells treated with guide RNAs that induced loss of BRCA1 protein expression were slower growing and we were unable to make single cell clones so utilized pooled cells.

Figure S5. Ectopic BRCA1-Δ11q expression promotes resistance in additional cell line backgrounds.

(**A**) HCC1395 and MDA-MB-436 cells stably expressing LacZ, GFP or HA-BRCA1-Δ11q were generated. HA-BRCA1-Δ11q expressing cells were readily adapted to grow in the presence of 0.1 μM and then 1 μM rucaparib. Rucaparib resistant cultures demonstrated increased levels of HA-BRCA1-Δ11q protein compared to unselected cells. However, endogenous BRCA1 protein remained undetectable. MDA-MB-436 RR1 cells were used as a positive control for the detection of endogenous mutant BRCA1 protein as these cells were previously shown to express stabilized endogenous truncated BRCA1(5). The position of truncated BRCT BRCA1 (tr-BRCT) and BRCA1- Δ11q (11q) proteins are indicated (*).

(**B**) HCC1395 cells stably expressing LacZ (red bar) and HA-BRCA1-Δ11q (black bar) were grown in the presence of rucaparib or cisplatin and both cell lines counted when confluence was reached.

(**C**) HCC1395 cells were treated with IR and HA, RAD51 and γ-H2AX foci, as well as DAPI staining assessed, representative images and quantification of mean ± S.E.M. foci-positive cells. PARPi selected HA-BRCA1-Δ11q expressing HCC1395 formed robust HA IRIF, as well as 10- and 64-fold ($P < 0.001$, unpaired *t*-test) increased levels of RAD51 IRIF compared to LacZ expressing cells, respectively. γ-H2AX IRIF was present equally in all cell lines. Images are representative of three independent experiments.

(**D**) Empty vector (EV), full-length human BRCA1 (FL), BRCA1-Δ11 (Δ11) and BRCA1-Δ11q (Δ11q) were expressed in *R26*$^{\text{CreERT2/hBRCA1}}$*; Brca1* $^{\text{SCo/Δ}}$*;Pim1*$^{\text{DR-GFP/wt}}$ mouse ES cells and detected by Western blotting. POLII was used as a loading control, * indicates non-specific band.

(**E**) Cells from (D) expressing the indicated proteins and carrying the DR-GFP reporter were transfected with I-SceI and GFP positive cells measured by flow cytometry.

(**F**) *Brca1*- SCo ES cells expressing the indicated proteins were treated with olaparib and cell viability measured.

(**G**) Cells as for (D) were treated with cisplatin. Mean ± S.E.M. positive cells or IC50 values are shown throughout.

(**H**) MDA-MB-436 LacZ and HA-BRCA1-2594delCΔC expressing cells were subject to Western blotting with the indicated antibodies. BRCA1 exon 11 frameshift mutations potentially produce N-terminal protein containing only fragments that lack the entire C-terminal region. N-terminal proteins retain the BARD1 interaction domain as well as the nuclear localization signal (NLS), and may have hypomorphic activity that could contribute to PARPi resistance. To test this possibility, we generated stable MDA-MB-436 cells that expressed an HA-BRCA1 construct engineered to contain the 2594delC frameshift mutation. Furthermore, to ensure that reversion mutations could not occur and account for resistance, we removed the entire 3' end of the coding region using restriction enzymes to produce an HA-BRCA1-2594delCΔC construct. Cells were measured for rucaparib sensitivity by colony formation assay.

Figure S6. FOX2 binding motifs are proximal to the BRCA1-11q cryptic slice site.

FOX1 and FOX2 splice factors are recruited to (U/A)GCAUG sequences (8). Bioinformatic interrogation of the 200-nucleotide sequences flanking either side of the cryptic splice site revealed the presence of two 100% matches for FOX1/2 binding elements. FOX1/2 consensus motifs were at position +18 and +63 downstream from the

cryptic splice site location. Gene expression data indicated that FOX1 was not expressed in any of our cell lines. In contrast, FOX2 mRNA was abundant (data not shown), and FOX2 has previously been shown to play a role in breast and ovarian cancer development and progression(8), therefore we investigated the impact of FOX2 on BRCA1-Δ11q levels. *BRCA1* DNA sequences with the location of FOX1/2 binding motifs colored blue. Yellow, green and gray highlighted sequences correspond with intron 10, exon 11 and the exon 11q region that is subject to splicing, respectively.