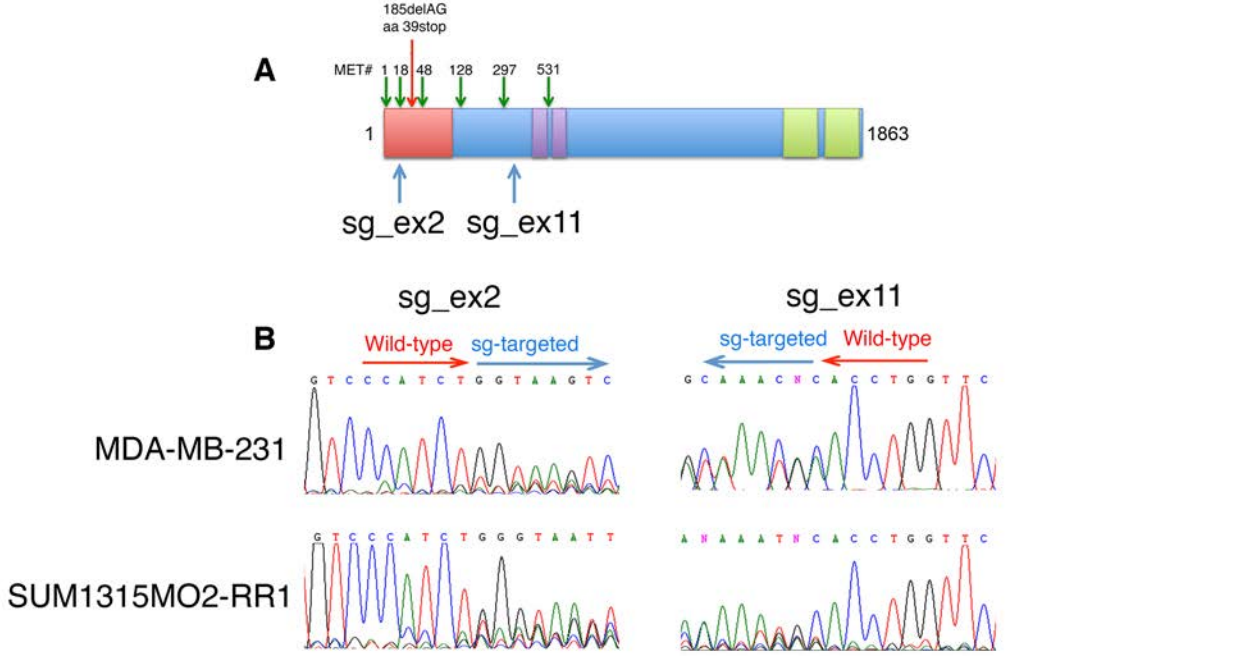
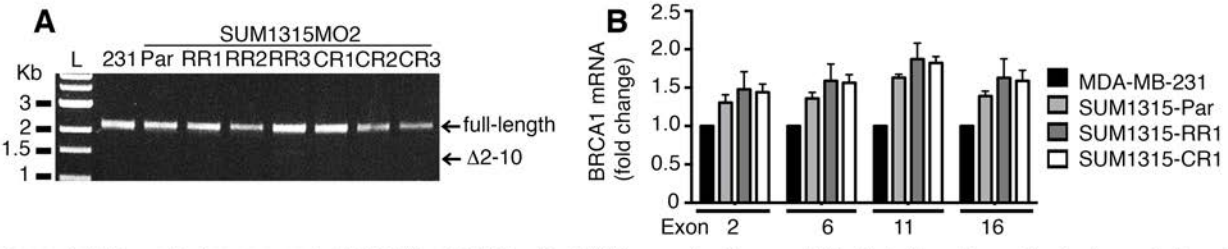
1 Met DLSALRVEEVQNVINA Met QKILECPICLELIKEPVSTKCDHIFC
KFC Met LKLLNQKKGPSQCPLCKNDITKRSLQESTRFSQLVEELLKII
CAFQLDTGLEYANSYNFAKKENNSPEHLKDEVSIIQS Met GYRNRAK
RLLQSEPENPSLQETSLSVQLSNLGTVRTLRTKQRIQPQKTSVYIEL
GSDSSEDTVNKATYCSVGDQELLQITPQGTRDEISLDSAKKAACEF
SETDVTNTEHHQPSNNDLNTTEKRAAERHPEKYQGSSVSNLHVEP
CGTNTHASSLQHENSSLLLTKDR Met NVEKAEFCNKSKQPGLARSQ
HNRWAGSKETCNDRRTPSTEKKVDLNADPLCERKEWNKQKLPCS
ENPRDTEDVPWITLNSSIQKVNEWFSRSDELLGSDDSHDGESESN
AKVADVLDVLNEVDEYSGSSEKIDLLASDPHEALICKSERVHSKSV
ESNIEDKIFGKTYRKKASLPNLSHVTENLIIGAFVTEPQIIQERPLTN
KLKRKRRPTSGLHPEDFIKKADLAVQKTPE Met INQGTNQTEQNGQ
V Met NITNSGHENKTKGDSIQNEKNPNPIESLEKESAFKTKAEPISSS
ISN Met ELELNIHNSKAPKKNRLRRKSSTRHIHALELVVSRNLSPPNC
TELQIDSCSSSEEIKKKKY NQ Met PVRHSRNLQL Met EGKEPATGAKK
SNKPNEQTSKRHDSDTFPELKLTNAPGSFTKCSNTSELKEFVNPSL
PREEKEEKLETVKVSNNAEDPKDL Met LSGERVLQTERSVESSSISL
VPGTDYGTQESISLLEVSTLGKAKTEPNKCVSQCAAFENPKGLIHG
CSKDNRNDTEGFKYPLGHEVNHSRETSIE Met EESELDAQYLQNTF
KVSKRQSFAPFSNPGNAEEECATFSAHSGSLKKQSPKVTFECEQK
EENQGKNESNIKPVQTVNITAGFPVVGQKDKPVDNAKCSIKGGSRF
CLSSQFRGNETGLITPNKHGLLQNPYRIPPLFPIKSFVKTKCKKNLL
EENFEEHS Met SPERE Met GNENIPSTVSTISRNNIRENVFKEASSSNI
NEVGSSTNEVGSSINEIGSSDENIQAELGRNRGPKLNA Met LRLGVL
QPEVYKQSLPGSNCKHPEIKKQEYEEVVQTVNTDFSPYLISDNLEQ
P Met GSSHASQVCSETPDDLLDDGEIKEDTSFAENDIKESSAVFSKS
VQKGELSRSPSPFTHTHLAQGYRRGAKKLESSEENLSSEDEELPC
FQHLLFGKVNNIPSQSTRHSTVATECLSKNTEENLLSLKNSLNDCS
NQVILAKASQEHHLSEETKCSASLFSSQCSELEDLTANTNTQDPFLI
GSSKQ Met RHQSESQGVGLSDKELVSDDEERGTGLEENNQEEQS
Met DSNLGEAASGCESETSVSEDCSGLSSQSDILTTQQRDT Met QHN
LIKLQQE Met AELEAVLEQHGSQPSNSYPSIISDSSALEDLRNPEQST
SEKAVLTSQKSSEYPISQNPEGLSADKFEVSADSSTSKNKEPGVER
SSPSKCPSLDDRWY Met HSCSGSLQNRNYPSQEELIKVVDVEEQQL
EESGPHDLTETSYLPRQDLEGTPYLESGISLFSDDPESDPSEDRAP
ESARVGNIPSSTSALKVPQLKVAESAQSPAAAHTTDTAGYNA Met EE
SVSREKPELTASTERVNKR Met S Met VVSGLTPEEF Met LVYKFARKH
HITLTNLITEETTHVV Met KTDAEFVCERTLKYFLGIAGGKWVVSYF
WVTQSIKERK Met LNEHDFEVRGDVVNGRNHQGPKRARESQDRKIF
RGLEICCYGPFTN Met PTDQLEW Met VQLCGASVVKELSSFTLGTGV
HPIVVVQPDAWTEDNGFHAIGQ Met CEAPVVTREWVLDSVALYQCQ
ELDTYLIPQIPHSHY 1863

Detected only in MDA-MB-231
Detected only in SUM1315MO2 RR3
Detected in both cell lines

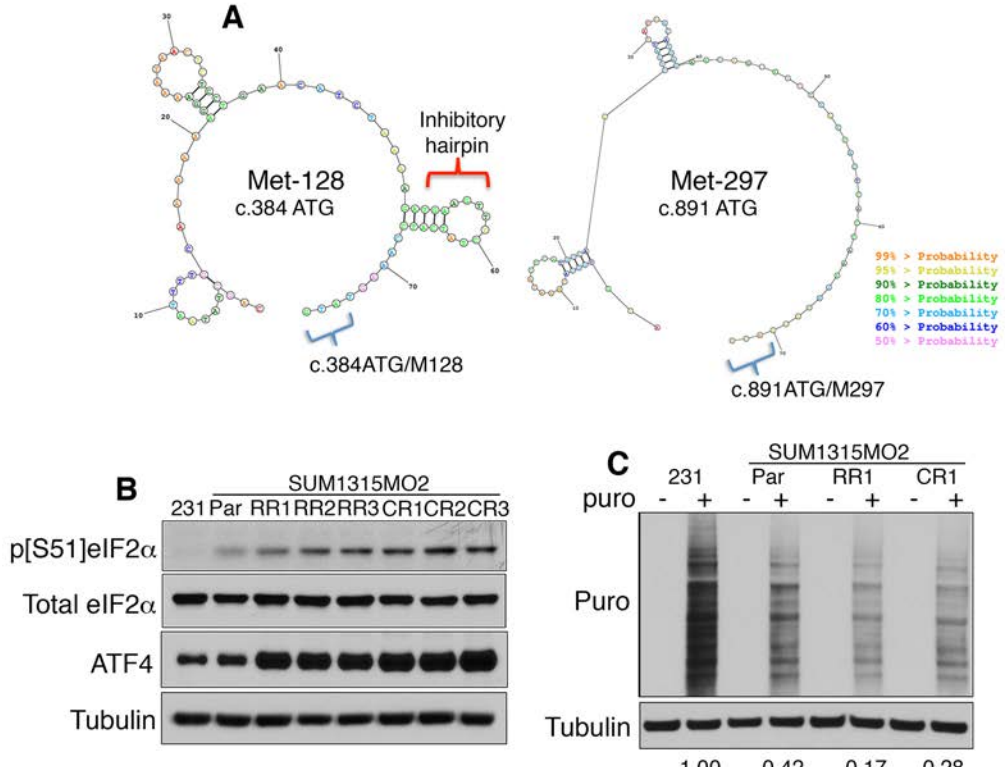
Supplemental Figure 1. BRCA1 peptides detected by mass spectrometry. Full information for peptides detected from Figure 2C. BRCA1 was immunoprecipiated from MDA-MB-231 and SUM131MO2 RR1 cells using a C-terminal specific BRCA1 antibody and subject to SDS-PAGE and analyzed by LC-MS/MS. Schematic shows the entire full-length BRCA1 amino acids 1-1863. The peptides that were only detected in MDA-MB-231 are highlighted yellow, peptides only detected in SUM131MO2RR1 cells are highlighted green, and peptides found in both cell lines are highlighted blue. Met 297 is colored red. Overall peptide coverage was greater in MDA-MB-231 cells (58% total coverage) compared to SUM1315MO2-RR1 cells (19% total coverage).



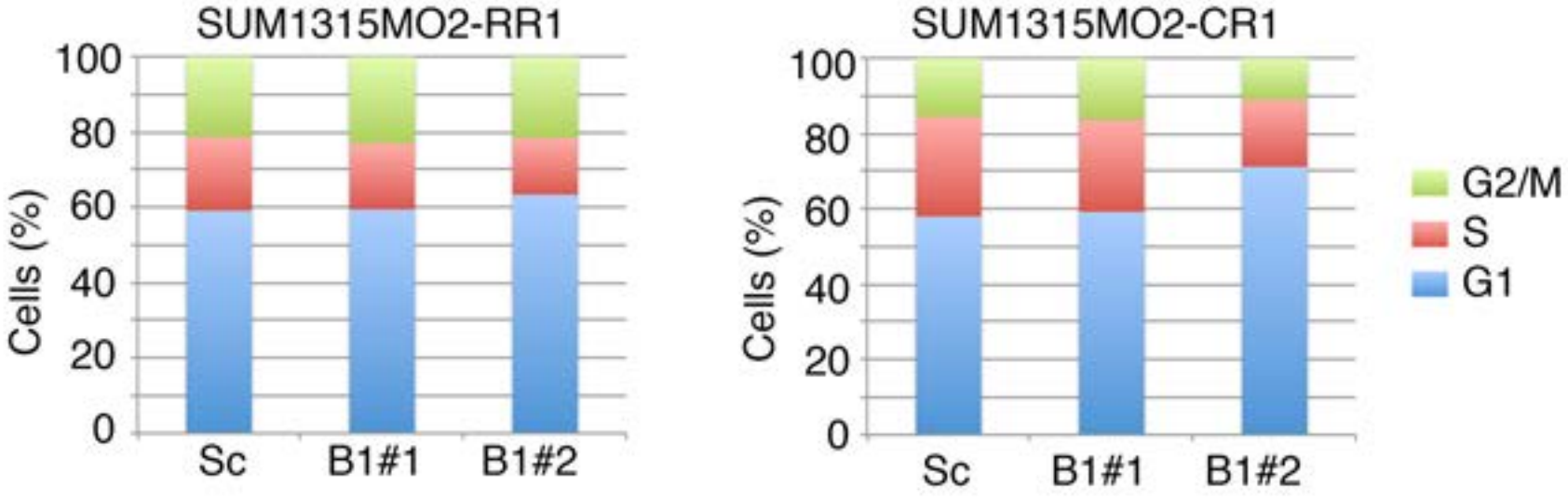
Supplemental Figure 2. CRISPR/Cas9 disruption prior to Met-297 does not impact BRCA1 expression in SUM1315MO2 cells. (**A**) Schematic of the BRCA1 gene showing methionines 1 to 531 as well as the location of the 185delAG mutation. Single guide RNA (sgRNA) targeting exon 2 (sg_ex2) disrupts the BRCA1 reading frame before Met-297, whereas an sgRNA targeting exon 11 (sg_ex11) region results in disruption of the reading frame around the Met-297 coding region. (**B**) MDA-MB-231 and SUM1315MO2-RR1 cells were treated with sg_ex2 and sg_ex11 and genomic DNA analyzed for the introduction of mutations. See Figure 2E for BRCA1 protein levels detected by Western blotting.



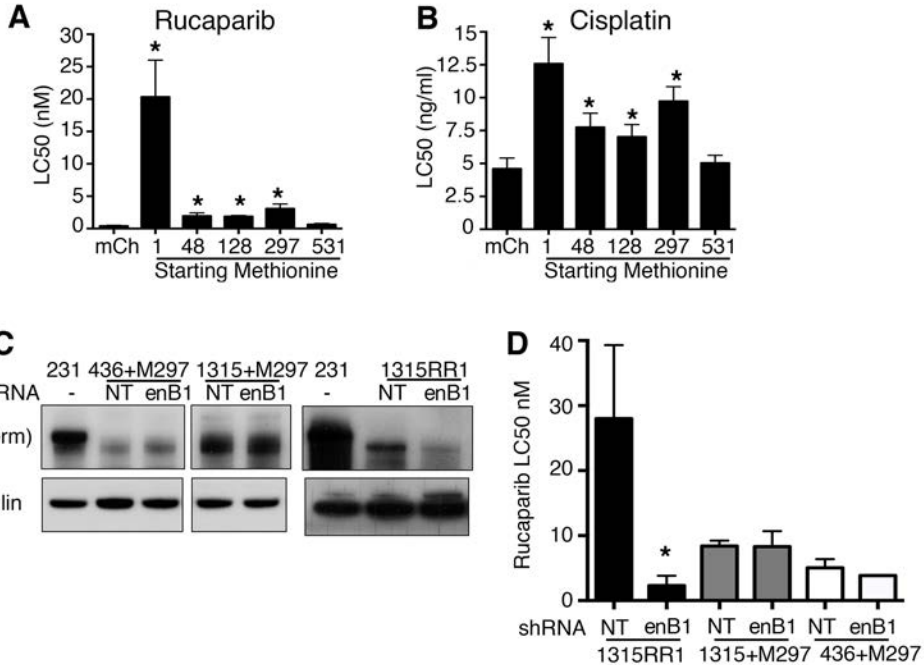
Supplemental Figure 3. Assessment of BRCA1 mRNA levels. (**A**) To examine the possibility that alternative splice isoforms lacking Met-1, -18, -48 and -128 coding exons were responsible for the generation of BRCA1-Met-297 protein, we assessed the size of BRCA1 mRNA in BRCA1 full-length expressing MDA-MB-231 cells (231) as well as SUM1315MO2 parental (Par), rucaparib resistant (RR) clones 1-3 and cisplatin resistant (CR) clones 1-3 by RT-PCR (L – molecular weight ladder). We used a forward primer targeting the BRCA1 5' UTR, and a reverse primer located in exon 11 downstream of Met-297. In this reaction, full-length BRCA1 mRNA would generate a 2.2 kb band (full-length), and BRCA1 exon deficient isoforms would migrate below 2.2 kb, specifically a BRCA1 mRNA product that lacked exons 2-10 (Δ2-10) (containing Met-1, -18, -48 and -128) would generate a 1.3 kb product. We detected a band corresponding to full-length BRCA1 in all cell lines and no lower migrating bands were detected. These data suggest BRCA1 isoforms lacking exons located between 2-10 were not abundantly expressed. (**B**) To further assess the expression of potential BRCA1 exon deficient isoforms, we carried out 4 independent quantitative RT-PCR reactions to specifically detect the levels of BRCA1 mRNA containing exon 2, 6, 11 or 16 in MDA-MB-231, SUM1315MO2 parental, RR-1 and CR-1 cells. For each reaction BRCA1 mRNA levels were normalized to POL2RF house keeping gene and expressed as a fraction of BRCA1 expression detected in MDA-MB-231 cells. We expected that if BRCA1 Δ2-10 isoforms were abundantly expressed, reactions detecting exon 11 and 16 would demonstrate relatively greater levels of BRCA1 expression compared to reactions detecting exons 2 and 6. However, the relative expression of exons 2 and 6 was similar to the levels detected for exon 11 and 16 reactions. These data indicate BRCA1 isoforms lacking either exons 2 or 6 were not abundantly expressed.



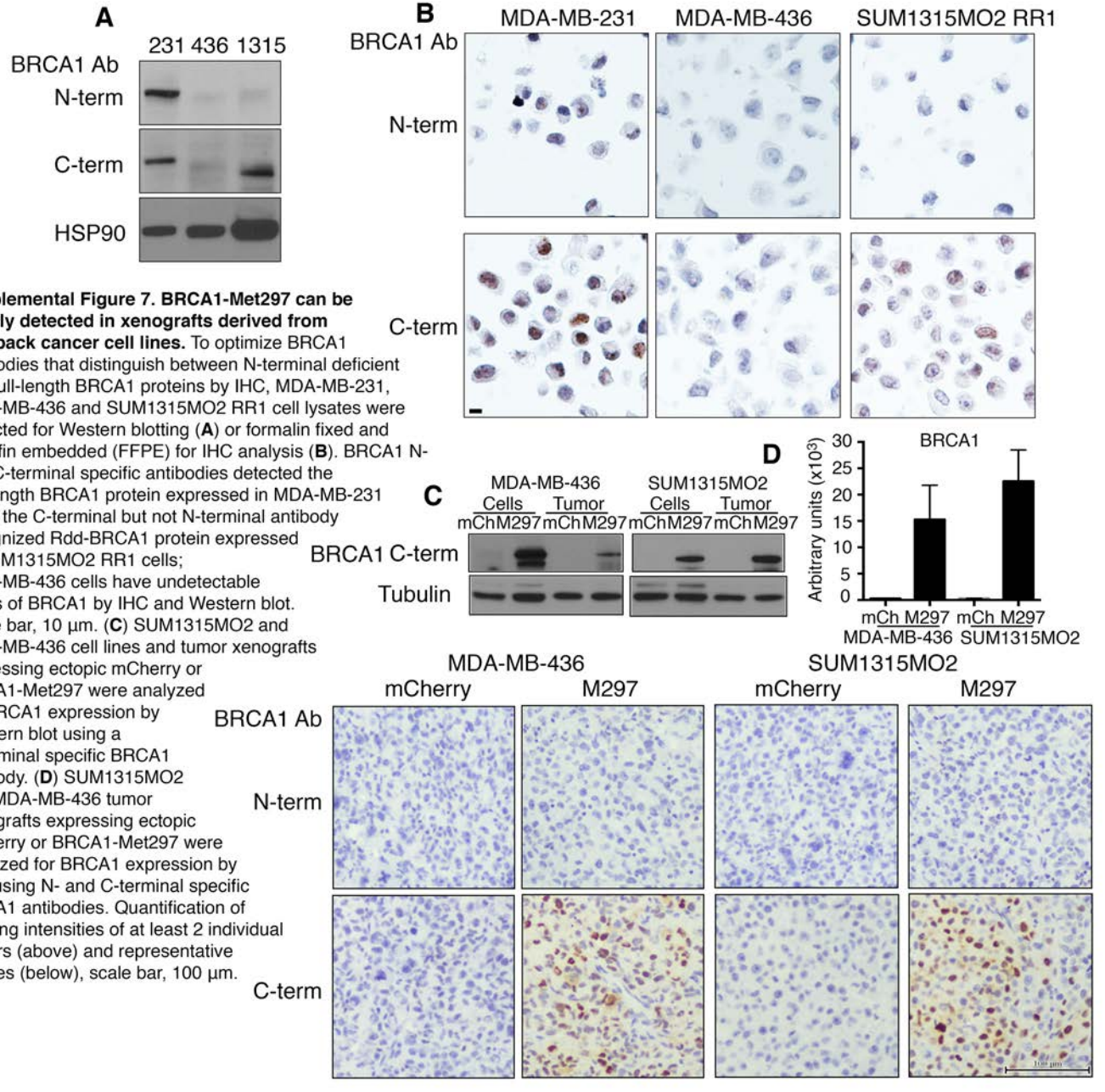
Supplemental Figure 4. Assessment of BRCA1 mRNA structure and alternative translation pathways. (**A**) We investigated the predicted secondary structure of BRCA1 mRNA at regions coding Met-128 and Met-297 using the web resource: http://rna.urmc.rochester.edu/RNAstructureWeb/Servers/Predict1/Predict1.html. Hairpins at 5' end of mRNA translation start sites commonly block initiation by preventing the 40S ribosomal subunit from binding. The probability that a hairpin structure formed immediately upstream of the Met-128 codon was >80%. However, no hairpin structures were predicted to be generated in proximity to the Met-297 codon. (**B**) Lysates were collected from MDA-MB-231 as well as SUM1315MO2 parental and resistant clones and phospho-S51 eIF2α, total eIF2α, ATF4 and tubulin levels were measured by Western blotting. (**C**) MDA-MB-231, SUM1315MO2 parental, RR1 and CR1 cells were incubated with puromycin for 30 minutes and subject to Western blotting using anti-puromycin antibody. Puromycin-protein incorporation reflects rate of translation and new protein synthesis. Lane intensities were quantified using image J software and normalized to tubulin. Numbers reflect puromycin lane intensity expressed as a fraction of MDA-MB-231 cells.



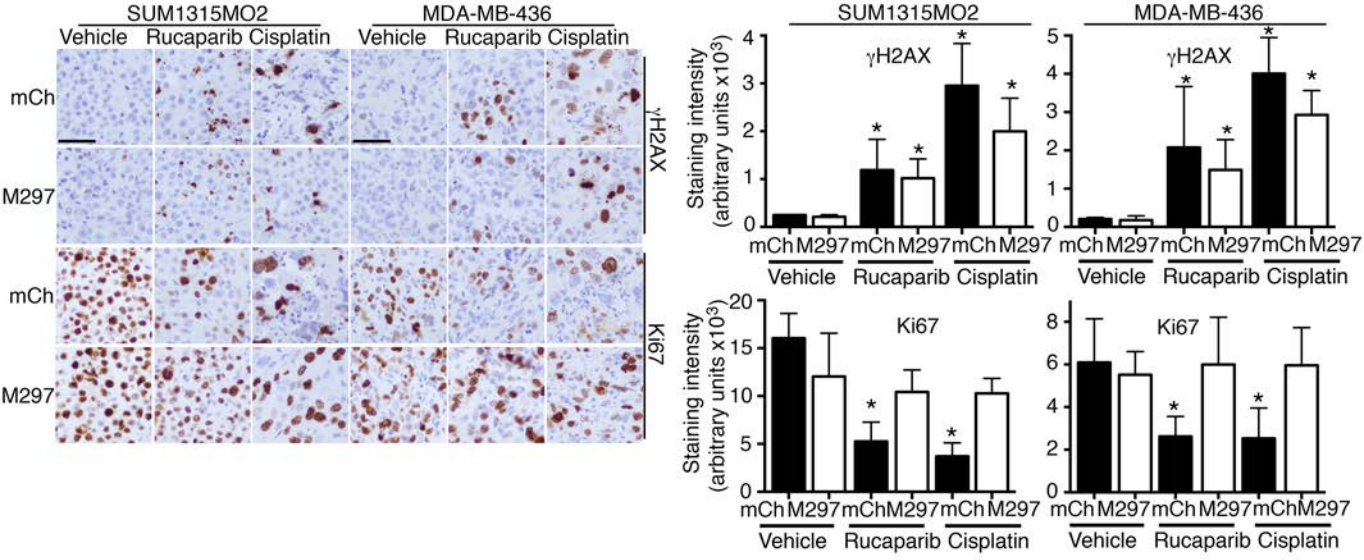
Supplemental Figure 5. BRCA1 siRNA does not dramatically impact cell cycle progression in SUM1315MO2 RR1 and CR1 cells. SUM1315MO2 RR1 and CR1 cells were treated with scrambled or 2 independent BRCA1 siRNAs (complimentary to Figure 3E), stained with propidium iodide and cell cycle position determined by flow cytometry. Changes in RAD51 foci resulting from BRCA1 siRNA treatment were not a result of diminished S/G2/M phase cells. Numbers represent mean values for 3 independent experiments.



Supplemental Figure 6. Assessment of exogenous BRCA1 protein-mediated PARPi and cisplatin rescue by colony formation assay. (**A**) MDA-MB-436 cells described in Figure 4C were treated with increasing concentrations of rucaparib or (**B**) cisplatin and colony formation assessed. Bars represent LC50 values. Met-1, -48, -128, and -297-BRCA1 expressing cells had 50.8- ($P = 0.0037$), 4.8- ($P = 0.0066$), 4.7- ($P = 0.0002$), 7.7-fold ($P = 0.0034$) increased rucaparib LC50 values compared to mCherry control cells, respectively; as well as 2.7- ($P = 0.003$), 1.6- ($P = 0.0034$), 1.5- ($P = 0.0079$), 2.1-fold ($P = 0.0008$) increased cisplatin LC50 values compared to mCherry control cells, respectively. BRCA1-Met-531 had no impact on PARPi and cisplatin rescue. (**C**) MDA-MB-231 cells were used as a control for wild-type BRCA1, MDA-MB-436 and SUM131MO2 cells expressing ectopic BRCA1-Met-297, as well as SUM1315MO2-RR1 cells were treated with non-target (NT) or 3'UTR targeting BRCA1 (enB1) shRNA. Western blotting shows endogenous targeting shRNA does not impact exogenously expressing BRCA1-Met297 protein but does reduce the levels of endogenous Rdd-BRCA1 from SUM1315MO2-RR1 cells. Samples were run on parallel gels. (**D**) Cell lines were treated with shRNA as described in (C) and assessed for colony formation with increasing concentrations of rucaparib. Bar graph shows LC50 values for rucaparib treated cells. Endogenous BRCA1 shRNA reduced the LC50 values 12.1-fold ($P = 0.0241$) compared to non-target shRNA treated SUM131MO2-RR1 cells. In contrast endogenous targeting BRCA1 shRNA had no impact on SUM1315MO2 or MDA-MB-436 cells that expressed ectopic BRCA1-M297. Colony formation assays were employed throughout and three independent experiments were performed. All in, these data indicate that residual endogenous BRCA1 protein did not contribute to resistance in BRCA1-M297 expressing cells.



Supplemental Figure 7. BRCA1-Met297 can be readily detected in xenografts derived from add-back cancer cell lines. To optimize BRCA1 antibodies that distinguish between N-terminal deficient and full-length BRCA1 proteins by IHC, MDA-MB-231, MDA-MB-436 and SUM1315MO2 RR1 cell lysates were collected for Western blotting (**A**) or formalin fixed and paraffin embedded (FFPE) for IHC analysis (**B**). BRCA1 N- and C-terminal specific antibodies detected the full-length BRCA1 protein expressed in MDA-MB-231 cells; the C-terminal but not N-terminal antibody recognized Rdd-BRCA1 protein expressed in SUM1315MO2 RR1 cells; MDA-MB-436 cells have undetectable levels of BRCA1 by IHC and Western blot. Scale bar, 10 μm. (**C**) SUM1315MO2 and MDA-MB-436 cell lines and tumor xenografts expressing ectopic mCherry or BRCA1-Met297 were analyzed for BRCA1 expression by Western blot using a C-terminal specific BRCA1 antibody. (**D**) SUM1315MO2 and MDA-MB-436 tumor xenografts expressing ectopic mCherry or BRCA1-Met297 were analyzed for BRCA1 expression by IHC using N- and C-terminal specific BRCA1 antibodies. Quantification of staining intensities of at least 2 individual tumors (above) and representative images (below), scale bar, 100 μm.



Supplemental Figure 8. Pharmacodynamic assessment of ectopic Rdd-BRCA1 proteins in vivo. Mice were treated as in Figure 5A and tumors harvested 4 days after treatment initiated and assessed for γ-H2AX and Ki67 staining by IHC, scale bars 50 μm. Quantification of staining intensities is shown for γ-H2AX and Ki67, mean and S.E.M from a minimum of 2 tumors derived from 2 mice and at least 3 images per tumor, (*$P < 0.05$).

Supplemental Material

SUPPLEMENTAL TABLES

Gene	Mutation	Effect
TP53	C.404G>T	pC135F
BRCA1	185delAG	E23fsX17

Supplemental Table 1. Loss of function gene mutations detected by BROCA analysis. BROCA analysis detected the presence of the E23fsX17 *BRCA1* frameshift and C135F *TP53* missense deleterious mutations. Neither wild-type gene sequences were identified for either *BRCA1* or *TP53* genes suggesting LOH at each locus. Both mutations were found in parental and all of the resistant cell lines. No mutations unique to either parental or resistant cell lines were detected. BROCA sequencing included the following genes: *ATM, ATR, BABAM1, BAP1, BARD1, BLM, BRCA1, BRCA2, BRCC36, BRE, BRIP1, CDK12, CHEK1, CHEK2, DCLRE1C, FAM175A, FANCC, ID4, LIG4, MLH1, MRE11A, MSH2, MSH6, NBN, PALB2, PIK3CA, PMS2, PRKDC, PTEN, RAD50, RAD51, RAD51B, RAD51C, RAD51D, RBBP8, SLX4, TOPBP1, TP53, TP53BP1, UIMC1, USP28, XRCC2, XRCC3, XRCC4, XRCC5, XRCC6.* Only clear loss of function mutations were reported.

Spec#	Tissue	Primary/Recurrent	Validation	Mutation	N-terminal	C-terminal
1014328	Breast	Primary	BRCA wt	None	**Positive**	**Positive**
3000911	Ovary	Primary	BRCA+	185delAG	Negative	Negative
1001014	Breast	Primary	BRCA+	185delAG	Negative	Negative
1003263	Breast	Recurrent	BRCA+	185delAG	Negative	**Positive**
1001020	Breast	Recurrent	BRCA+	185delAG	Negative	**Positive**

Supplemental Table 2. IHC detection of BRCA1 protein expression in *BRCA1*^{185delAG} patient carcinomas. Surgically resected patient carcinomas were stained for BRCA1 using N- and C-terminal specific BRCA1 antibodies. Breast carcinoma 1014328 was derived from a patient that did not test positive for *BRCA1* germline mutations and nuclear BRCA1 staining was detected with both N- and C-terminal antibodies. Breast carcinoma specimens 1001020 and 1003263 were negative for N-terminal but positive for C-terminal nuclear BRCA1 protein staining. Interestingly, these tumors were resected from patients with recurrent disease, and patient number 1001020 had previously received platinum therapy (treatment details for patient 1003263 were unavailable). See Figure 6E for representative images.