Supplemental Information

SI Figure legends

SI FIGURE 1: tr-BRCA1 fails to induce cytoplasmic shuttling of the NES BRCA1 mutant and subsequently does not induce persistent DNA damage following PARP inhibition. (A). The NES BRCA1 location mutant is resistant to tr-BRCA1-induced nuclear/cytoplasmic shuttling. MCF7 cells were co-transfected with NES-BRCA1 YFP (nuclear BRCA1) and tr-BRCA1. The respective controls were included as well. BRCA1 distribution 24 hours following transfection was analyzed via immunohistochemical staining. Cells were assessed as having predominantly nuclear staining, predominantly cytoplasmic staining, or mixed nuclear/cytoplasmic staining. The NES-BRCA1 mutant was predominantly located in the nucleus and failed to demonstrate tr-BRCA1-induced translocation. Shown is the representative data of three independent experiments (mean ± SEM, *p<0.01, **p < 0.001). (B). Nuclear BRCA1 abrogates tr-BRCA1 and PARP induced increase in γ-H2AX foci in human breast cancer cells. MCF7 cells were co-transfected with the NES BRCA1 mutant and tr-BRCA1. 24 hours following transfection, cells were treated with vehicle or 10μM ABT-888. 24 hours following the treatment period, cells were assessed for γ-H2AX foci. The % of foci-containing cells was quantified. A robust induction in γ-H2AX foci, indicative of DNA DSB, was observed with ABT-888 treatment in the tr-BRCA1 expressing cells, indicative of increased DNA damage, probably due to deficient DNA repair which is expected due to absence of nuclear BRCA1. On the contrary no significant induction in foci formation was observed with ABT-888 treatment in the NES-BRCA1 mutant expressing cells, indicative of lack of DNA damage probably due proficient DNA repair. Shown is the representative data of three independent experiments the % of cells (mean ± SEM) with >10 foci (**p < 0.001).
SI FIGURE 2: BRCA1 location determines PARP inhibitor induced cytotoxicity. (A). BRCA1 location mutants are resistant to radiation-induced nuclear/cytoplasmic shuttling. MCF7 BRCA1 shRNA cells were transfected with either WT-BRCA1 YFP, NLS-BRCA1 YFP (cytosolic BRCA1) mutant, or NES-BRCA1 YFP (nuclear BRCA1) mutant and exposed to 4Gy IR after 24 hours. BRCA1 distribution 24 hours following IR was analyzed via immunohistochemical staining for YFP. BRCA1 translocation following IR from the nucleus to the cytosol was observed in WT-BRCA1 expressing cells. On the other hand, the NES-BRCA1 mutant was exclusively located in the nucleus whereas NLS-BRCA1 mutant was located in the cytosol and failed to demonstrate translocation following IR exposure. Shown is the representative data of three independent experiments (mean ± SEM, *p<0.01, **p < 0.001). (B). Cytosolic BRCA1 mutant abrogates radiation-induced HR-mediated DSB repair. 16 hours following transfection with the various BRCA1 mutants, MCF7 BRCA1 shRNA cells were exposed to 4Gy radiation and 10μM ABT-888 or vehicle control. 8 hours following the drug treatment, Rad51 foci levels were analyzed via immunohistochemical staining as a surrogate marker for HR-mediated DSB repair. A robust induction in Rad51 foci was observed in WT-BRCA1 and NES-BRCA1 mutant cells which demonstrate significant nuclear BRCA1. No significant increase in radiation-induced foci formation was observed in the NLS-BRCA1 mutant, indicative of deficient HR-mediated repair. Shown is the representative data of three independent experiments the percent of cells (mean ± SEM) with Rad51 foci (**p < 0.001). (C). Cytosolic BRCA1 increases γ-H2AX foci in human breast cancer cells. 16 hours following transfection with the various BRCA1 mutants, MCF7 BRCA1 shRNA cells were exposed to 4Gy radiation treatment. 8 hours following the treatment period, cells were exposed to 10μM ABT-888 or vehicle control. 24 hours following the treatment period, cells were assessed for γ-H2AX
foci. The % of foci-containing cells was quantified. A robust induction in γ-H2AX foci, indicative of DNA DSB, was observed with ABT-888 treatment in the NLS-BRCA1 mutant expressing cells, indicative of increased DNA damage, probably due to deficient DNA repair which is expected due to absence of nuclear BRCA1. On the contrary no significant induction in foci formation was observed with ABT-888 treatment in the NES-BRCA1 mutant or WT-BRCA1 expressing cells, indicative of lack of DNA damage probably due proficient DNA repair. Shown is the representative data of three independent experiments the % of cells (mean ± SEM) with >10 foci (**p < 0.001).

**SI FIGURE 3: The observed reduction in HR-mediated repair following radiation treatment is not due to cell cycle redistribution.** MCF7 cells were seeded, exposed to 4Gy radiation treatment, transfected with ISce-1 or the empty vector, collected at (A) 8 hours, (B) 24 hours, (C) 48 hours, and (D) 72 hours following radiation treatment, fixed, and stained with propidium iodide. Cell cycle distribution was analyzed using flow cytometry. No significant redistribution of cell cycle was observed at any of the time points studied. Shown is the representative cell cycle distribution (mean +/- SEM) of at least 2 independent experiments performed in triplicate.

**SI Figure 4: Representative images of BRCA1 distribution and γH2AX foci in xenograft tissue.** Xenograft tissues were harvested and subjected to immunofluorescence staining. (A) The left panel is a representative image of cells exhibiting nuclear (N), both nuclear and cytosolic (NC), and cytosolic (C) BRCA1 (red) with the nucleus stained with DAPI (blue). (B) Inset, a representative staining of cell exhibiting γ-H2AX foci (red) with the nucleus stained with DAPI (blue).