Supplemental Figure Legends

Supplemental Figure 1. RAD51 foci formation in BRCA1-deficient cells is dependent on RAD52. (A) MCF7 cells were transfected with the indicated siRNAs and immunoblotted with the indicated antibodies. (B) Confocal images of IR-induced RAD51 foci in MCF7 cells that were transfected with the labeled siRNAs. The percentage of cells with RAD51 foci is shown (mean and SE from three independent experiments). Differences between cells transfected with BRCA1 siRNA alone compared with both BRCA1 and RAD52 siRNA are significant ($p = 0.0138$), as determined by an unpaired *t* test.

Supplemental Figure 2. RAD52 depletion does not alter cell cycle distribution. (A) Cell cycle distribution of MCF7 and H1299 cells transfected with the labeled siRNA conditions as in **Fig 3** and measured for DNA content after propidium iodide staining (REF 11 348 639 001, Roche, Mannheim, Germany) at 20ug/mL and treatment with ribonuclease A (R6513, Sigma-Aldrich, St. Louis, MO, USA) at 200 ug/mL and analyzed by flow cytometry. Cell-cycle distribution was estimated in FlowJo 7.6.1 software by Dean-Jett Fox modeling. (B) Cell-cycle distribution of MCF7 cells transfected with the labeled siRNA conditions as in **Fig 4** then stained with propidium iodide, treated with ribonuclease A and then analyzed by flow cytometry (as in A).

Supplemental Figure 3. Identical severe HR defect seen with a second RAD52 siRNA target sequence in combination with BRCA1 or PALB2 depletion. (A) Off-target effects were addressed using a second commercially available siRNA pool targeting RAD52 (siRAD52: siGENOME SMARTpool, Dharmacon) labeled as RAD52 siRNA #2. RAD52 siRNA #1 was

used in **Fig 1 – 4** (siRAD52: ID# 142431, Ambion). U2OS cells integrated with pDR-GFP reporter were transfected with the labeled siRNAs, followed by pCMV-I-*Sce*I transfection and measured by flow cytometry for GFP expression (as in **Fig 3** and **4**). RAD52 depletion by either RAD52 siRNA #1 or RAD52 siRNA #2 after BRCA1 or PALB2 depletion led to the most severe loss of HR.

Supplemental Figure 4. Two independent pathways of RAD51-dependent HR are synthetically lethal with one another. DNA double-strand breaks, one-ended DSBs and daughter-strand gaps are repaired in the S and G2 phases of the cell cycle primarily by the BRCA1-PALB2-BRCA2 pathway. In cells with deficiencies in the BRCA1-PALB2-BRCA2 pathway, the alternative RAD52 pathway can substitute to mediate RAD51-directed repair. RAD52 functions in SSA, which is independent of BRCA2 and PALB2, but dependent on BRCA1. However, RAD52 is independent of BRCA1 in mediating RAD51-directed repair.