Supporting Information

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SI Materials and Methods

Construction of GST-BRC Fusion Expression Plasmids. The BRCA2 fragments corresponding to the individual BRC repeats were amplified by PCR using AccuPrime Pfx DNA polymerase (Invitrogen) set for 35 cycles using 50 ng of a plasmid expressing full length BRCA2 gene as template. The PCR products were then purified using QiaQuick PCR purification kit (Qiagen), digested with *BamHI, XhoI* and repurified using the same kit. Then the fragments were ligated into pGEX-6-P1 vector (Amersham Biosciences) digested with *BamHI, XhoI*, using T4 DNA Ligase (Invitrogen). The clones were sequence verified using the DNA sequencing facility of UC Davis. The fragments of BRCA2 sequence amplified and corresponding to the BRC repeats are based on the Uniprot Database annotation (The Uniprot Consortium., 2010), and are the following:

BRC1: NHSFGGSFRTASNKEIKLSEHNIKKSKMFFKDIEE
BRC2: NEVGFRGFYSAHGTKLNVSTEALQKAVKLFSDIEN
BRC3: FETSDTFFQTASGKNISVAKESFNKIVNFFDQKPE
BRC4: KEPTLLGFHTASGKKVKIAKESLDKVKNLFDEKEQ
BRC5: IENSALAFYTSCSRKTSVSQTSLLEAKKWLREGIF
BRC6: FEVGPPAFRIASGKIVCVSHETIKKVKDIFTDSFS
BRC7: SANTCGIFSTASGKSVQVSDASLQNARQVFSEIED
BRC8: NSSAFSGFSTASGKQVSILESSLHKVKGVLEEFDL

Electrophoretic Mobility Shift Assay (EMSA). RAD51 was preincubated with GST-tagged BRCx (where x refers to 1, 2, 3, 4, 5, 6, 7 or 8) peptides at the indicated concentrations for 15 min, followed by addition of ssDNA (dT_{40}, labeled with ^{32}P at the 5′-end) or dsDNA (^{32}P-labeled at the 5′ end or ^{32}P-5′ end labeled duplex $dT_{40} \cdot dA_{40}$ prepared by annealing), at the concentrations indicated, in buffer containing 20 mM Tris-HCl (pH 7.5), 10 mM $Mg(OAc)_2$, 2 mM $CaCl_2$, and 2 mM ATP. The mixture was incubated for 60 min at 37 °C, as indicated. The reaction products were resolved by 6% PAGE at 4 °C in TAE, (40 mM Tris acetate (pH 7.5) and 0.5 mM EDTA). The gels were dried and analyzed on a Molecular Dynamics Storm 840 PhosphorImager using ImageQuant software. The percentage of protein-DNA complexes was quantified as the free radiolabeled DNA remaining in a given lane relative to the protein-free lane, which defined the value of 0% complex (100% free DNA). In Fig. S2, the EMSA protocol was the same except that GST-BRC5 (0.6 μM) was bound to fluorescently labeled (AlexaFluor 555) antiGST (1.5 μM) (abbreviated FL-BRC5) and supplemented with GST-BRC5 up to the concentration required prior to incubation with the DNA.

Preparation of GST-BRC Peptide Coupled with Fluorescently Labeled Anti-GST. Rabbit anti-GST (50 μl; Immunology Consultants Laboratory) was buffer exchanged using a P6 spin column (at 850 g for 4 min; BioRad, Hercules, CA) equilibrated with labeling buffer (50 mM sodium borate (pH 9.3), 140 mM NaCl, and 2.7 mM KCl). A 20-fold molar excess of AlexaFluor 555 succinimidyl ester (Molecular Probes) was incubated with anti-GST antibody at room temperature for 30 min in the dark. The sample was then applied to a P6 spin column equilibrated with 50 mM Tris acetate (pH 7.5) to remove the unreacted AlexaFluor 555. The dye and antibody concentrations were determined using the extinction coefficients ε_{552} 1.5×10^5 $M^{-1}\,cm^{-1}$ for AlexaFluor 555, and ε_{280} 1.7×10^5 $M^{-1}\,cm^{-1}$ for anti-GST. The effect of absorption by AlexaFluor 555 at 280 nm was corrected according to manufacturer specifications. The degree of labeling was calculated using the ratio of AlexaFluor 555 and antibody concentrations, and determined to be approximately seven dyes/protein. GST-BRC peptide (2 μM) was incubated with anti-GST-AlexaFluor 555 (5 μM) for 1 h at approximately 23 °C. This stock was used for the EMSA experiments shown in Fig. S2 at a final concentration of 0.6 μM GST-BRC and 1.5 μM anti-GST-AlexaFluor 555, abbreviated in the figure as FL-BRC5.

ATP Hydrolysis Assay. RAD51 (3 μM) was preincubated with the GST-tagged BRCx, for 15 min at 37 °C, followed by addition of ssDNA (9 μM nucleotides (nt) in 10 μl of buffer containing 20 mM Tris-HCl (pH 7.5), 4 mM $MgCl_2$, 1 mM DTT, 0.5 mM ATP, and 20 μCi/ml [γ^{32}P] ATP, and further incubated at 37 °C for 1 hour. Aliquots (1 μl) were spotted onto a polyethyleneimine (PEI) thin layer chromatography (TLC) plate (EMD Chemicals). The spots were air-dried and the plates were developed in 1 M formic acid and 0.5 M LiCl. The amount of ATP hydrolysed was determined from dried plates using a Molecular Dynamics Storm 840 PhosphorImager. The amount of $^{32}P_i$ and [γ^{32}P] ATP was quantified using ImageQuant software.

DNA Strand Exchange Assay. Reactions (20 μl) contained RAD51 and GST-tagged BRC peptides, at the concentrations indicated, and were incubated with ϕX174 ssDNA (15 μM, nt) for 5 min at 37 °C in buffer containing: 25 mM TrisOAc (pH 7.5); 250 mM NaCl, 2 mM ATP, 1 mM DTT, 1 mM $MgCl_2$, and 2 mM $CaCl_2$. RPA (1 μM) was then added, and incubation continued for 5 min at 37 °C. The reaction was started by the addition of *XhoI*-linearized ^{32}P-labeled ϕX174 duplex DNA (15 μM, nt). After 2 h at 37 °C, the samples were treated with Proteinase K (Roche) for 15 min at 37 °C. Products were resolved by agarose gel (1%) electrophoresis (TAE) at 40 V overnight. The gels were dried and analyzed on a Molecular Dynamics Storm 840 PhosphorImager using ImageQuant software. The amount of DNA strand exchange product at each BRC peptide concentration was calculated as a percentage of the joint molecules (JM) or nicked circular DNA (NC) products relative to the total amount of DNA in the same lane.

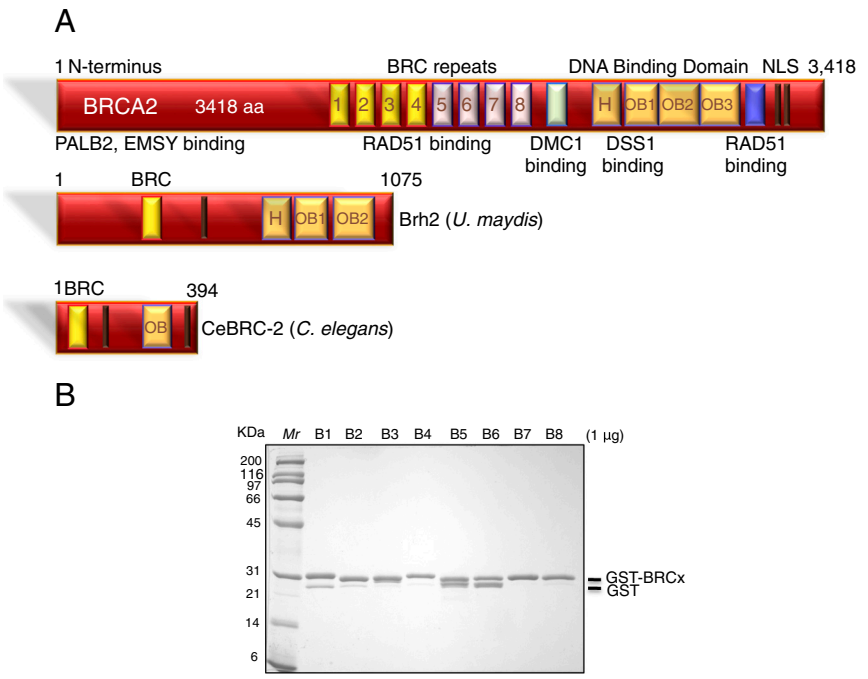

Fig. S1. (*A*) Schematic representation of human BRCA2 showing structural domains, and a comparison of the conserved domains with BRCA2 homologues from *Ustilago maydis* and *Caenorhabditis elegans*. (*B*) Purified GST-tagged BRC peptides (1 μg) analyzed by SDS/PAGE and stained with Coomassie blue: B1 (BRC1), B2 (BRC2), B3 (BRC3), B4 (BRC4), B5 (BRC5), B6 (BRC6), B7 (BRC7), and B8 (BRC8); M_r, molecular mass markers. A GST contaminant copurifies with some of the GST-BRC peptide preparations as a consequence of leaky expression from the bacterial host; the contaminating band is indicated in the figure. The concentration of GST-BRC peptide was corrected to account for the free GST.

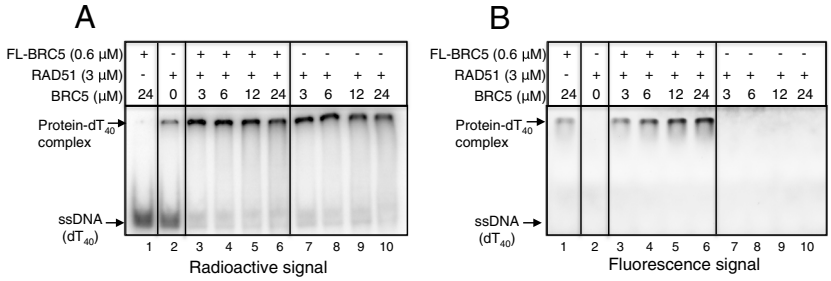

Fig. S2. BRC5 binds to ssDNA-RAD51 complexes. EMSA experiment performed as in Fig. 3C, but instead of GST-BRC5, lanes 1 and 3–6 contained a mix of fluorescent anti-GST (1.5 μM) bound to GST-BRC5 (0.6 μM) (abbreviated as "FL-BRC5"), and increasing concentrations of GST-BRC5 up to the concentration indicated in each lane. (*A*) Signal from the radiolabeled DNA substrate, dT_{40}. (*B*) Fluorescent signal from the same gel shown in *A*.

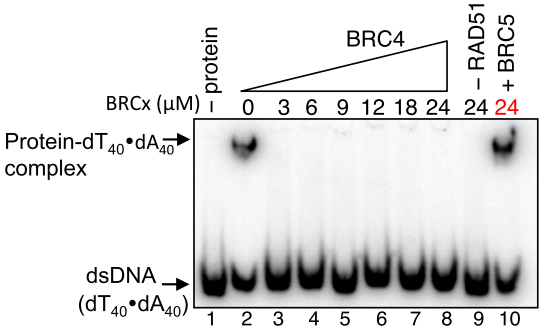

Fig. S3. BRC4 prevents formation of RAD51-dsDNA complexes. EMSA analysis showing RAD51 (3 μM) incubated with GST-BRC4 prior to incubation with ^{32}P-labeled $dA_{40} \cdot dT_{40}$ dsDNA (0.3 μM, bp) and further incubation for 1 h in the presence of ATP, Mg^{2+}, and Ca^{2+}. Protein-DNA complexes were resolved by 6% PAGE and analyzed by autoradiography. Lane 1 contains DNA alone; lane 2 contains RAD51 incubated with DNA in the absence of BRC peptide. Lanes 3–8 contain the indicated concentration of BRC4 peptide and 3 μM RAD51. Lane 9 contains the maximum concentration of BRC4 peptide and DNA in the absence of RAD51. Lane 10 contains the indicated concentration of BRC5 and RAD51 incubated with DNA under the same conditions used with BRC4. The quantification of the data is shown in Fig. 4.

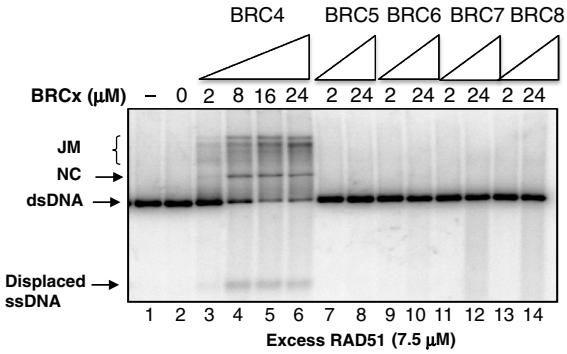

Fig. S4. BRC repeats 5, –6, –7, and –8 do not stimulate the DNA strand exchange activity of RAD51. Effect of BRC4 control (lanes 3–6), BRC5 (lanes 7–8), BRC6 (lanes 9–10), BRC7 (lanes 11–12), or BRC8 (lanes 13–14) on DNA strand exchange between ϕX174 circular ssDNA and linear dsDNA promoted by RAD51 (7.5 μM). Lane 1 is a control performed in the absence of RAD51 and BRC peptide but in the presence of RPA. Lane 2 shows the DNA strand exchange products obtained with RAD51 and RPA, but in the absence of BRC peptide.