Supporting Information

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SI Materials and Methods

Protein Expression and Purification.

DMC1 purification. 6xHis–DMC1 was produced in *E. coli* strain BLR (DE3) (Novagen) from 1 L of bacterial culture and subjected to ammonium sulfate precipitation. The ammonium sulfate pellet was resuspended in lysis buffer followed by affinity chromatography on a 1-mL HisTrap HP column (GE Healthcare). The protein was eluted with a 50–500 mM imidazole gradient in the presence of 500 mM KCl. The purest fractions were diluted 2.5-fold and applied to a 5-mL HiTrap Heparin HP column (GE Healthcare) and eluted with a 0.2–1.2 M KCl gradient. The fractions containing DMC1 were diluted to 100 mM KCl and loaded on a 1 mL Mono Q column. The purified protein was then dialyzed against a buffer containing: 50 mM Tris•HCl, pH 7.5, 50% glycerol, 350 mM KCl, 1 mM DTT, and stored at −80 °C.

Because low concentrations of DMC1 (25 nM and 75 nM) were used to facilitate experiment with BRCA2, we verified the activity of our DMC1 protein preparation by examining joint molecule formation at more standard literature conditions; i.e., in the presence Ca^{+2} alone, instead of Ca^{+2} and Mg^{+2} as used in this work. A DMC1 titration in the absence of Mg^{+2} showed, as expected, that joint formation was a maximum at 3 nt of ssDNA per DMC1 monomer, producing a yield of ∼28% (23); this yield was reduced in the presence of excess protein or excess ssDNA (Fig. S6). In the presence of Ca^{+2} and Mg^{+2}, the maximum yield was also reached at ∼3 nt of ssDNA per DMC1 monomer, but the inhibition at excess protein was less apparent (Fig. S6*B*). This phenomenon may be explained by the fact that, in the presence of Mg^{+2}, the turnover of DMC1 is faster due to a higher ATPase rate (24) and, therefore, the DMC1–ssDNA complex would be less stable, minimizing the inhibitory effect of excess protein. These results confirm that both the yield of joint molecule formation and the optimal ratio of protein to ssDNA are in agreement with the literature (23).

GFP-MBP-tagged BRCA2 purification and characterization. *Brca2*-deficient hamster cells that were stably transfected with the GFP-MBP-BRCA2 construct showed the same survival to mitomycin C as the *brca2*$^{+/+}$ parental cell line (V79), confirming the functionality of the GFP-MBP-BRCA2 construct (Fig. S5*B*).

GFP-MBP-BRCA2 was purified essentially as described (2), with a few modifications. Briefly, 10–20 15-cm plates of HEK293 cells were transiently transfected using TurboFect (ThermoFisher Scientific) following the manufacturer specifications and harvested 30 h posttransfection. Cell extracts were bound to Amylose resin (NEB), and the protein was eluted with 10 mM maltose. The eluate was then further purified by ion exchange using BioRex 70 resin (BIO-RAD) and step eluted at 250 mM, 450 mM, and 1 M NaCl. The nuclease-free 250 mM fraction shown in Fig. S5*A* was used for the experiment in Fig. 6 and Fig. S5 *C*–*F*. This GFP-MBP-BRCA2 stimulated RAD51-promoted joint molecule formation up to approximately twofold, verifying its activity in vitro (Fig. S5 *C* and *D*).

GST Pull Down Assay. Before the pull down assays, Glutathione Sepharose 4B beads (GE) were equilibrated with binding buffer B: 25 mM Tris acetate, (pH 7.5), 100 μg/mL BSA, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 1 mM DTT, including 100 mM NaCl and 0.01% Igepal CA-630. Each purified GST-BRC peptide (1 μg) was incubated in binding buffer with 0.5–2.0 μg of purified DMC1 for 15 min at 37 °C and then batch bound to 30 μL of glutathione beads for 30 min at 37 °C. The amount of DMC1 pulled down with each GST-BRC peptide in Fig. 1*B* was determined using standard curves generated from known concentrations of DMC1 (Fig. 1*B*, lanes 1–4) and GST-BRC peptides (Fig. 1*B*, lanes 11–14) run in parallel in the same gel. When present, DMC1 retained by nonspecific binding to the beads was subtracted in the quantification. The input concentration of GST-BRC peptide in each pull-down reaction was 0.4 μM and the total input amount for DMC1 ranged from 0.3 to 1.3 μM. As controls for nonspecific binding to the glutathione beads, DMC1 (2.0 μg) was incubated with glutathione beads in the absence of GST-BRC peptide. The complexes were then washed with binding buffer, resuspended in protein sample buffer, heated at 95 °C for 4 min, and loaded onto a 12% SDS-polyacrylamide gel. The gel was run for 1.5 h at 130 V and stained with SYPRO Orange (Invitrogen). The protein bands were quantified by Image Quant software on a Typhoon PhosphorImager (Amersham Biosciences). The binding data of Fig. 1*C* were fit to a hyperbola with a fixed binding stoichiometry of 1:1 by using GraphPad Prism software.

Joint Molecule (D-Loop) Assay. Oligonucleotide oAC203 (90-mer oligonucleotide complementary to pUC19) is 5′- CGGGTGTCGGGGCTGGCTTAACTATGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATGCGGTGTGAAATACCGCACAGATGCGT-3′. RAD51 reactions in Fig. S5*C* were carried out at saturating conditions [RAD51 at 75 nM, ssDNA (oAC203) at 225 nM nt], and threefold molar excess of ssDNA relative to pUC19 scDNA. The extent of joint molecule formation was determined using a Typhoon PhosphorImager (Amersham Biosciences). Quantification was performed by using Image Quant software.

DNA Strand Exchange Assay. All oligonucleotide substrates were purified by polyacrylamide gel electrophoresis (PAGE). The following oligonucleotides were used for Fig. 7: TK-167-mer (5′-CTG CTT TAT CAA GAT AAT TTT TCG ACT CAT CAG AAA TAT CCG TTT CCT ATA TTT ATT CCT ATT ATG TTT TAT TCA TTT ACT TAT TCT TTA TGT TCA TTT TTT ATA TCC TTT ACT TTA TTT TCT CTG TTT ATT CAT TTA CTT ATT TTG TAT TA TCC TTA TCT TAT TTA-3′); TK-Oligo1 (5′-TAA TAC AAA ATA AGT AAA TGA ATA AAC AGA GAA AAT AAA G-3′); TK-Oligo2 (5′-CTT TAT TTT CTC TGT TTA TTC ATT TAC TTA TTT TGT ATT A-3′). The dsDNA was generated by first radiolabeling TK-Oligo1 with ^{32}P at the 5′-end and annealing it to TK-Oligo2.

The buffer (B) contained 25 mM Tris acetate (pH 7.5), 1 mM $MgCl_2$, 2 mM $CaCl_2$, 0.1 mg/mL BSA, 2 mM ATP, and 1 mM DTT. All preincubations and reactions were at 37 °C. The DNA and proteins were at the following concentrations: DMC1 (0.22 μM), RAD51 (0.22 μM), RPA (25 nM), 2xMBP-BRCA2 (20, 40, and 80 nM in Fig. 7), ssDNA (4 nM molecules), and dsDNA (4 nM molecules). Where proteins were omitted, storage buffer substituted. The reactions were terminated by adding SDS to 0.25% and proteinase K to 0.5 mg/mL and further incubation for 10 min. The samples were analyzed by PAGE using a 6% gel and 1× TAE buffer, run at 60 V for 60 min at room temperature. The gel was then dried and exposed to PhosphorImager screen. The percentage of DNA strand exchange product was calculated as labeled product divided by total labeled input DNA in each lane using Image Quant software.

ATP Hydrolysis Assay. DMC1 (3 μM) was preincubated with each BRC peptide or BRCA2 for 15 min at 37 °C, followed by addition of the ssDNA 90-mer (oAC203; 9 μM nucleotide, nt) in a

reaction (10 μL) containing 25 mM Tris acetate, (pH 7.5), 0.1 mg/mL BSA, 1 mM $MgCl_2$, 1 mM DTT, 2 mM ATP, and 20 μCi/mL [γ^{32}P] ATP, and further incubated at 37 °C for 1 h. Aliquots (1 μL) were spotted onto a polyethyleneimine (PEI) 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 hydrolyzed was determined from dried plates using a Typhoon PhosphorImager (Amersham Biosciences). The amount of $^{32}P_i$ and [γ^{32}P] ATP was quantified by using Image Quant software.

EMSA. In the case of ssDNA binding, DMC1 (25 nM) was preincubated with each BRC peptide at the indicated concentrations for 15 min, followed by addition of ssDNA (90mer, oAC203, labeled with ^{32}P at the 5′ end at 0.2 μM nt) in D-loop reaction buffer. For dsDNA binding, DMC1 (at 0.6 or 0.3 μM in Fig. 5) was preincubated with each BRC peptide at the indicated concentrations for 15 min in a buffer containing 20 mM Tris·HCl (pH 7.5), 10 mM $Mg(OAc)_2$ followed by the addition of dsDNA (^{32}P-labeled at the 5′ end duplex dT_{40}•dA_{40} prepared by annealing to a final concentration of 0.3 μM bp) (Fig. 5). All of the reaction products were resolved by 6% PAGE in TAE. The gels were dried and analyzed on a Typhoon PhosphorImager (Amersham Biosciences) using Image Quant 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. The protein-free lane defined the value of 0% complex.

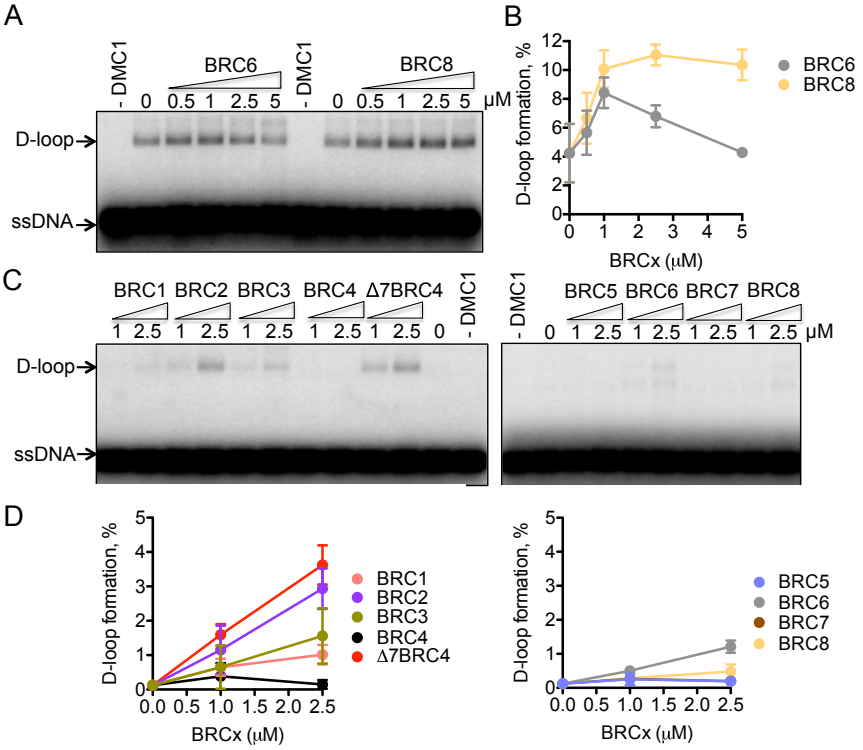

Fig. S1. Effects of BRC6 and BRC8 protein concentration on joint molecule formation by DMC1, and of BRC4, BRC7, and Δ7BRC4 on joint molecule formation at a lower concentration of DMC1. (*A*) Joint molecule reactions were carried out as in Fig. 2*A*, but at higher concentrations of BRC6; BRC8 serves as a control to show that the decrease is specific to BRC6. (*B*) Quantification of *A*. (*C*) Joint molecule formation was carried out as in Fig. 2, but with 25 nM DMC1 and 75 nM (nt) ssDNA [0.8 nM (molecule)] and pUC19 at 0.25 nM molecule. (*D*) Quantification of *C*. Error bars in *B* and *D* represent the SD for three and two independent experiments, respectively.

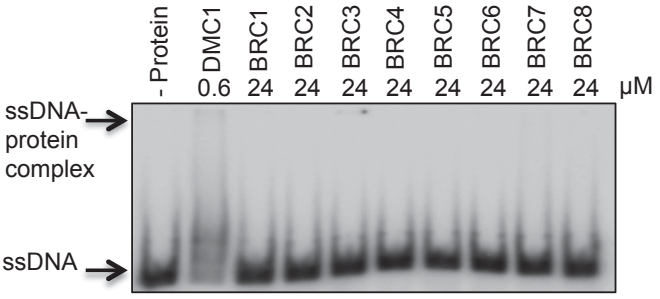

Fig. S2. The BRC repeats do not bind to ssDNA. DNA binding assay (EMSA) where DMC1 (0.6 μM) or the individual BRC repeats (24 μM) were mixed with 5′-end ^{32}P-labeled ssDNA (dT_{40}, 0.3 μM nt) and incubated for 1 h. The complexes were analyzed by PAGE and visualized by autoradiography.

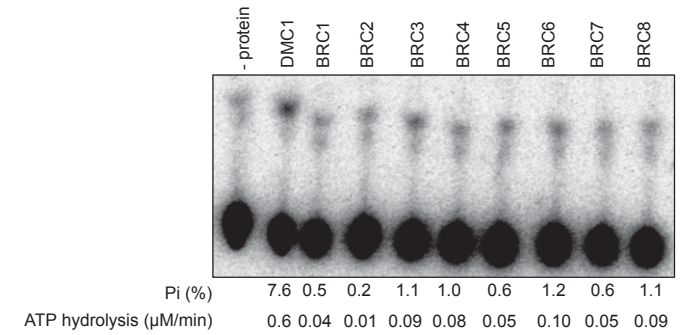

Fig. S3. The BRC repeats do not manifest ATPase activity. Autoradiography of a TLC plate showing an ATPase assay where DMC1 (3 μM) or the GST-BRC peptides (24 μM) were individually mixed with 90-mer ssDNA (9 μM nt) and incubated for 1 h in the presence of 1 mM $MgCl_2$ and 2 mM ATP. The quantification of the percentage of P_i produced, and the calculated rate of ATP hydrolysis is indicated below each lane.

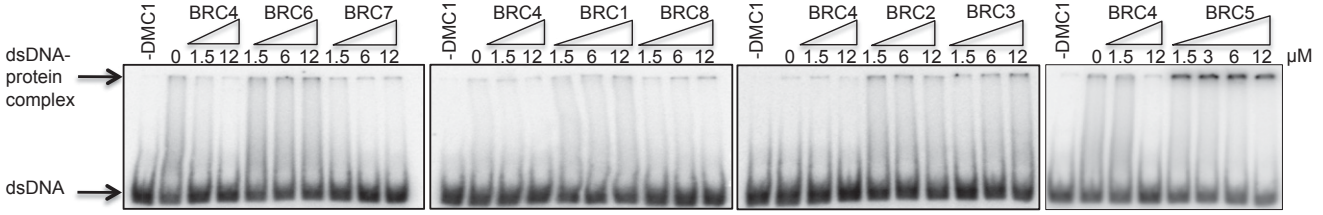

Fig. S4. Binding of DMC1 (0.3 μM) to dsDNA in the presence of the BRC repeats. Shown is the EMSA experiment that is quantified in Fig. 5*C*.

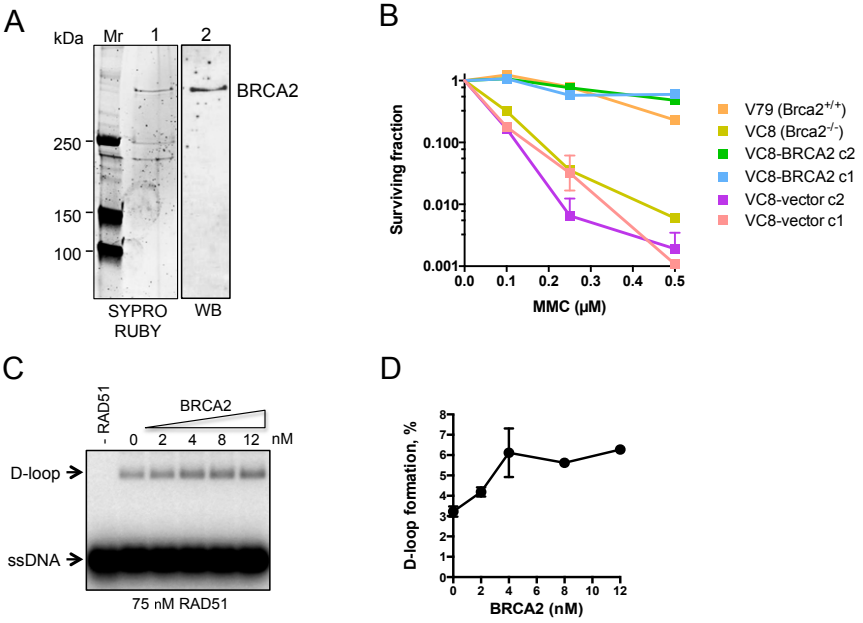

Fig. S5. Purified GFP-MBP-BRCA2 complements *Brca2*-deficient cells and stimulates RAD51-mediated joint molecule formation. (*A*) BRCA2 tagged with GFP-MBP at the N terminus was purified from human HEK293 cells and analyzed by SDS/PAGE. Lane 1: BRCA2 (0.9 μg) was loaded on a precast 7.5% SDS/PAGE gel and stained with SYPRO Ruby. Lane 2: Western blot of purified BRCA2 protein (0.5 μg) using an antibody specific for the carboxy-terminus of BRCA2 (CA1033, EMD). M_r, size markers. (*B*) Mitomycin C survival of stably transfected clones of *Brca2*-deficient hamster cells complemented with human GFP-MBP-tagged BRCA2 (green and blue), the vector containing the GFP-MBP tag (violet and pink), V79 parental cells ($Brca2^{+/+}$) (orange) and VC8 ($Brca2^{-/-}$) (gold). (*C*) RAD51 (75 nM) and the indicated concentrations of BRCA2 were preincubated with a 5′-end ^{32}P-labeled 90-mer ssDNA [2.4 nM (molecule)] for 10 min at 37 °C and scDNA [0.8 nM (molecule)] was added last to start the reaction. The mix was incubated at 37 °C for 30 min, terminated by incubation with Proteinase K, and resolved on a 1% agarose gel. (*D*) Quantification of *C*. Error bars in *D* represent the SD for three independent experiments.

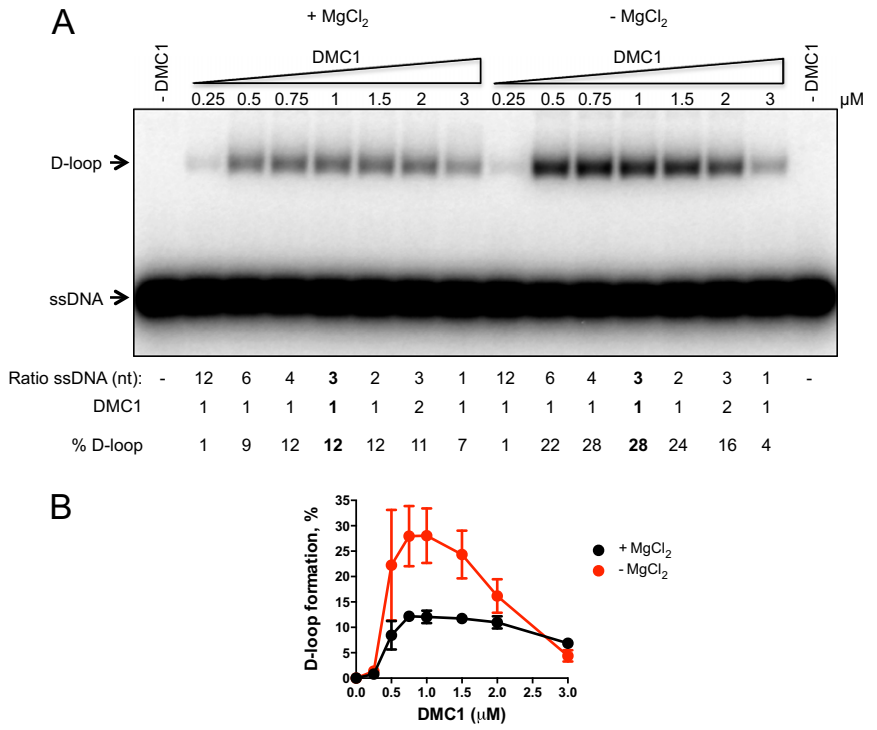

Fig. S6. Comparison of joint molecule formation by DMC1 in Ca^{+2}, in the presence or absence of Mg^{+2}. (*A*) Autoradiograph showing a D-loop assay. DMC1 at the indicated concentrations was preincubated with a 5′-end ^{32}P-labeled 90-mer ssDNA [oAC203, 3 μM (nt), 33 nM (molecule)] for 10 min at 37 °C, and supercoiled DNA (scDNA) [pUC19, 10.3 nM (molecule)] was added last to start the reaction. The mix was incubated at 37 °C for 30 min, and the products were resolved on a 1% agarose gel. (*B*) Quantification of D-loop formation from *A*. Error bars in *B* represent the SD for two independent experiments.

Table S1. Binding affinity of each BRC repeat for DMC1

BRC repeat	K_d, μM
BRC1	28 ± 2
BRC2	37 ± 3
BRC3	30 ± 2
BRC4	172 ± 14
BRC5	136 ± 28
BRC6	8.0 ± 0.8
BRC7	49 ± 2
BRC8	40 ± 4
Δ7BRC4	90 ± 15

The dissociation constants (K_d ± SD) were derived from fitting of the binding curves to a hyperbola using a fixed stoichiometry of 1 BRC repeat per DMC1.