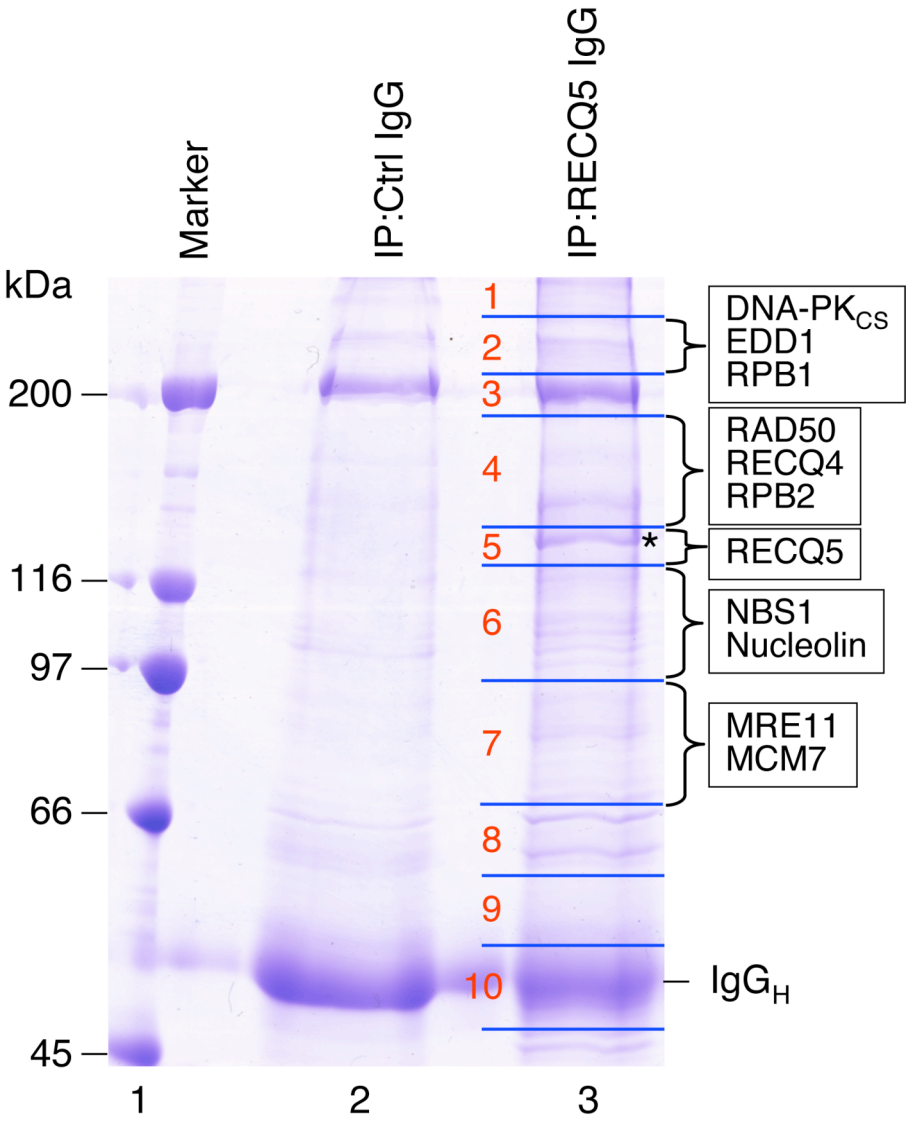

Figure S1. SDS-PAGE separation of proteins immunoprecipitated from total extract of exponentially growing 293T cells (10 mg of protein) using IgGs (10 μg) isolated from preimmune serum [control (Ctrl) IgG; lane 2] and anti-RECQ5 antiserum (RECQ5 IgG, lane 3), respectively. Proteins were visualized by Coomassie staining. The lane containing RECQ5 immunoprecipitate was cut into 10 slices as indicated and proteins present in each gel piece were identified by mass spectrometry. Selected proteins resulting from this analysis are indicated on the right of the gel and listed in Table 1. The full list of proteins identified in each gel slice is available upon request. The position of RECQ5 on the gel is indicated by an asterisk. The gel slices 3 and 10 were not analyzed.

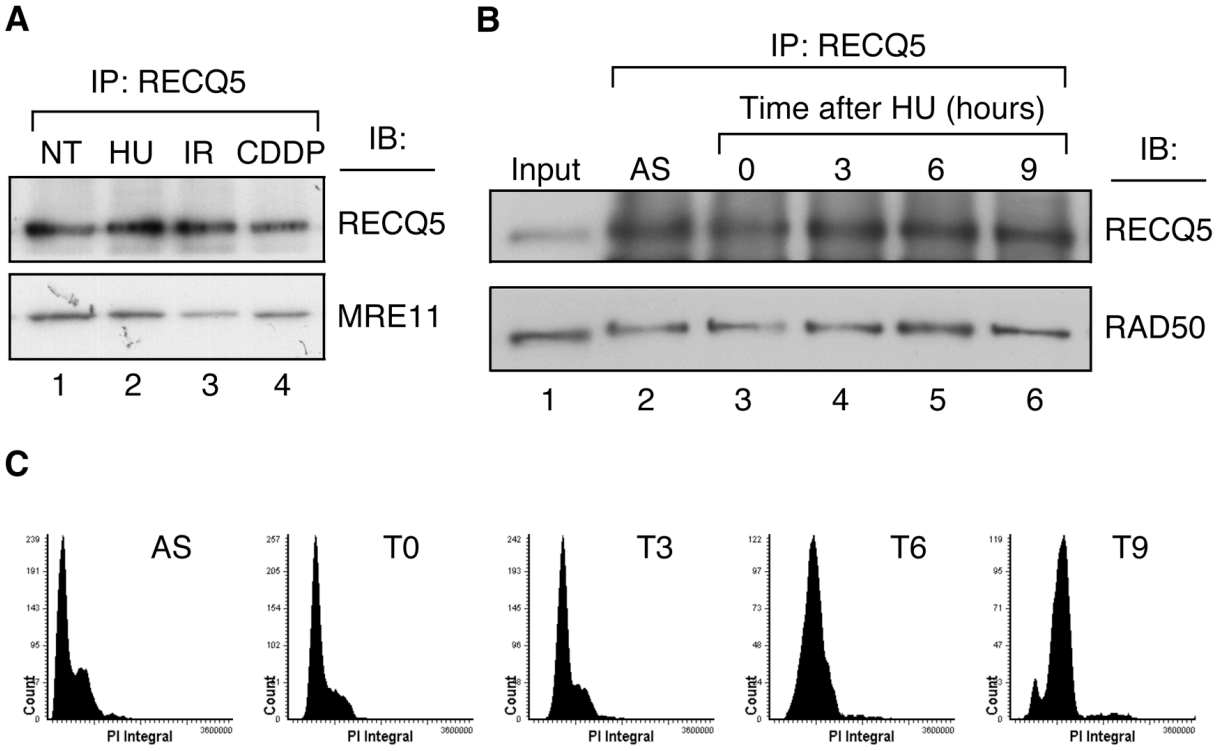

Figure S2. RECQ5 and the MRN complex associate constitutively *in vivo*. (A) Effect of various genotoxic agents on the cellular association of RECQ5 with the MRN complex. RECQ5 immunoprecipitates from treated and untreated U2OS cells were subjected to Western blot analysis to determine the level of co-precipitated MRE11 protein. NT, non-treated cells; HU, cells treated with 2 mM hydroxyurea for 16 hours; IR, cells exposed to ionizing radiation (20 Gy) followed by incubation for 8 hours; CDDP, cells treated with 20 μM cis-diamminedichloroplatinum (CDDP) for 8 hours. (B) U2OS cells were synchronized at G1/S transition by treatment with 2 mM HU for 16 hours and then released to S phase by adding fresh medium without HU. At indicated time points, the level of RECQ5-MRN complex was analysed by immunoprecipitation under conditions described in the legend of Figure 1. (C) FACS analysis. The cell cycle profiles for each time point after the release of U2OS cells from HU block (T0-9) are shown. AS, asynchronous cell population.

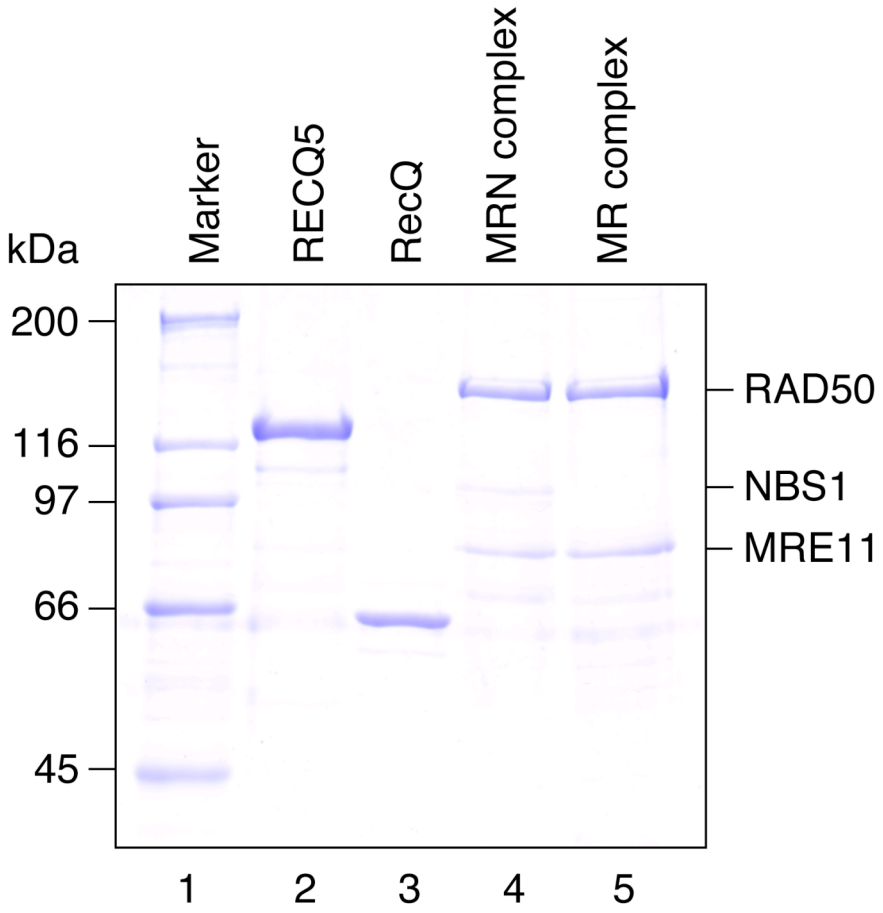

Figure S3. SDS-PAGE profile of the purified proteins used in this study. Proteins were purified as described in Materials and Methods. Lane1, protein size marker; lane 2, human RECQ5; lane 3, *E. coli* RecQ; lane 4, the MRE11-RAD50-NBS1 (MRN) complex; lane 5, the MRE11-RAD50 (MR) complex. Proteins were visualized by Coomassie staining. Approximately 1 μg of protein was loaded in each lane.

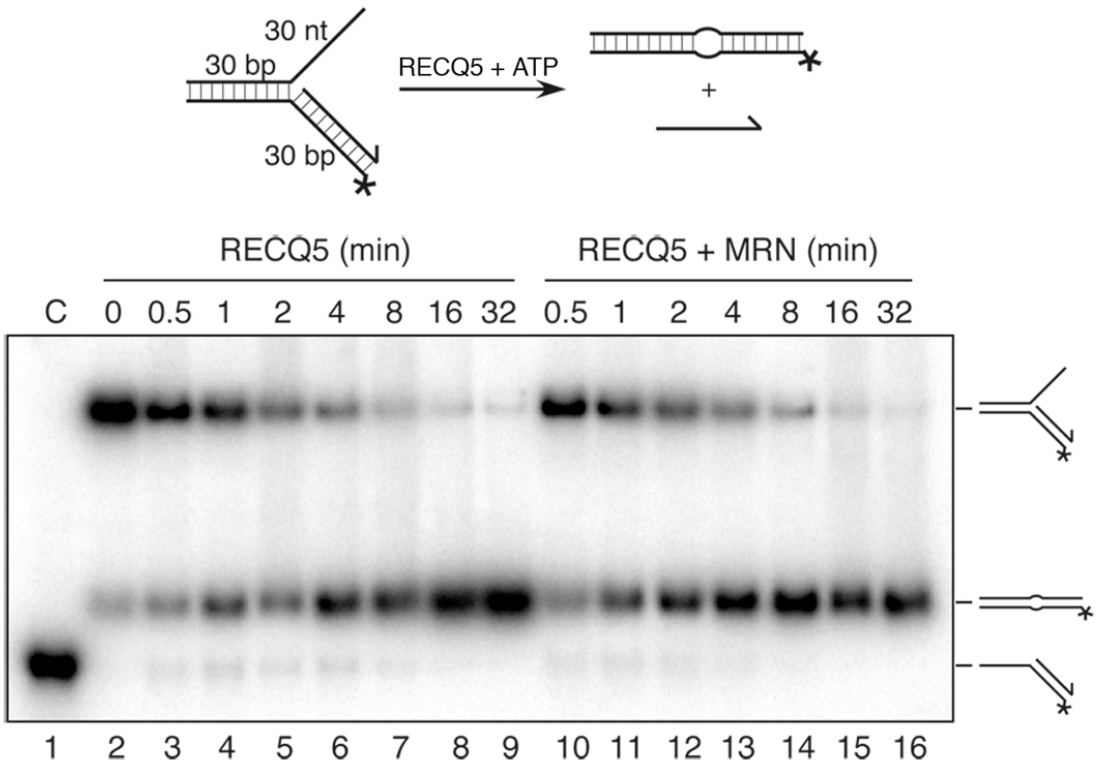

Figure S4. Effect of the MRN complex on the strand-exchange activity of RECQ5 on a synthetic forked DNA structure with homologous arms lacking the leading strand. *Top panel*: Scheme of the assay. The lengths of the oligonucleotide substrate are indicated (nt, nucleotides; bp, base pairs). The 3'-end of the lagging oligonucleotide is indicated by an arrow and the position of the 5'-^{32}P label is marked by an asterisk. The homologous leading and lagging arms have a 5-nt heterology at the fork junction to prevent spontaneous strand exchange. *Bottom panel*: 1 nM ^{32}P-labeled 30-mer/60-mer duplex was incubated with 1 nM 60-mer complementary oligonucleotide in the presence of 40 nM RECQ5 to form forked DNA structure. After 10 minutes, ATP (2 mM) was added either alone (lanes 2-9) or together with the MRN complex (40 nM) (lanes 10-16). Aliquots from different reaction time points were analyzed by non-denaturing PAGE followed by phosphorimaging. Lane 1 (marked by C), the 30-mer/60-mer duplex prior to annealing of the complementary oligonucleotide.

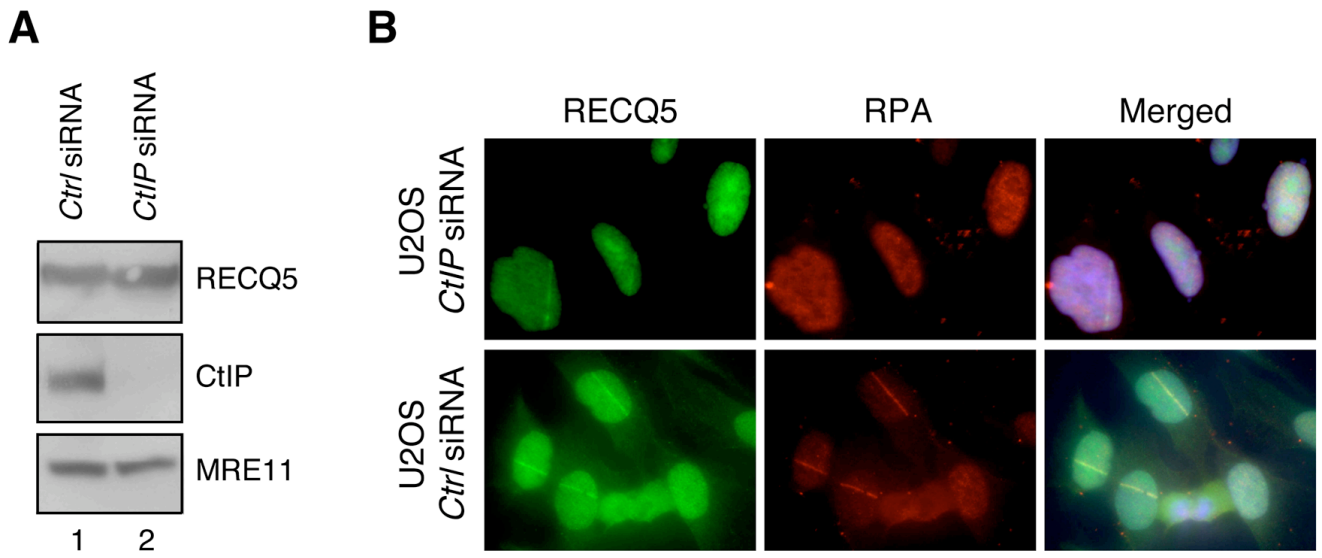

Figure S5. CtIP depletion impairs recruitment of RPA to DSBs. (A) Western blot analysis of total extracts of U2OS cells treated either with *CtIP* siRNA or control (*Ctrl*) siRNA (siRNA against Luciferase) for three days. Blots were probed with antibodies against RECQ5, CtIP and MRE11 as indicated. (B) Cells treated with indicated siRNAs for three days were microirradiated and 30 minutes later co-immunostained for RECQ5 and RPA (p34 subunit). BrdU (10 μM) was added to cell cultures at 24 hours prior to irradiation.