Supplementary Figures

Figure S1: Interactions of exogenously overexpressed proteins in vivo. (A) The interaction of BARD1 with HP1γ. HEK293T cells were transfected with plasmids expressing BARD1-StrepII or FLAG-HP1γ, as indicated. The interaction between BARD1 and HP1γ was assessed by StrepTactin pulldown followed by immunoblotting with the indicated antibodies. Inputs (1.5%) were also loaded. (B) The interaction of BARD1 mutants PEELI and PAALI with BRCA1 is similar to that of wild-type BARD1. HEK293T cells were transfected with plasmids expressing myc-tagged wild-type BARD1 or BARD1 mutants together with GFP-BRCA1 as indicated. The interaction between BARD1 and BRCA1 was assessed by anti-Myc immunoprecipitation (IP) followed by immunoblotting with the indicated antibodies. Inputs (1.5%) were also loaded.
**Figure S2:** Purified recombinant proteins used in the experiments shown in Figure 2. 
(A-C) GST-tagged fragments of BARD1 comprising ankyrin repeats (425-555) or BRCT (565-777) and His-FLAG-HP1γ (A), wild-type or the W164A mutant of His-FLAG-HP1γ (B), or wild-type or the PEELI or PAALI mutant of GST-BARD1 BRCT (C) were purified from bacteria, and 0.5 µg of each of the proteins was resolved by 15% SDS-PAGE followed by Coomassie brilliant blue staining.
Figure S3: SPR analyses of the HP1α, β, or HP1γ–chromoshadow domain binding to BARD1-BRCT. (A) Wild-type or the W164A mutant of the chromoshadow domain of His-FLAG-HP1γ was purified from bacteria, and 0.5 μg of each of the proteins was resolved by 15% SDS-PAGE followed by Coomassie brilliant blue staining. (B) Proteins from (A) (200 nM) were injected over immobilized GST-BARD1-BRCT peptides. (C) His-FLAG-HP1α, β and γ were purified from bacteria and stained as in (A). (D and E) Wild-type and the PEELI mutant of the GST-BARD1-BRCT peptides (200 nM) were injected over immobilized His-FLAG-HP1α (D) or β (E) peptides from (C).
Figure S4: SPR analyses of the BRCA1 fragments binding to HP1α, β, or γ. (A) GST-BRCA1 fragments comprising the indicated amino acids residues were purified from bacteria, and 0.5 µg of each of the proteins was resolved by 15% SDS-PAGE followed by Coomassie brilliant blue staining. The GST-BARD1-BRCT peptide was also loaded. The arrowheads indicate the aimed proteins. (B-D) Proteins from (A) (200 nM) were injected over immobilized His-FLAG-HP1γ (B), α (C) or β (D) peptides.
Figure S5: Inhibition of HP1γ only mildly decreases the stable retention of BRCA1/BARD1 at sites of DNA damage. (A and B) U2OS cells were transfected with siRNA to HP1α, HP1β, and HP1γ, as indicated and subjected to immunoblotting with the indicated antibodies (A) or were exposed to 10 Gy IR and were immunostained with anti-BARD1 or anti-BRCA1 antibodies 1 hour after IR (B). The mean numbers of BARD1 or BRCA1 foci per cell are shown. More than 100 cells were counted in each staining. (C) HP1γ+/+ and HP1γ−/− MEF cells (1) were immunostained with the indicated HP1 antibodies (upper panel) or counterstained with DAPI (lower panel). (D) HP1γ+/+ and HP1γ−/− MEF cells were exposed to 10 Gy IR and immunostained with anti-BARD1 or anti-BRCA1 antibodies 1 hour after IR. The mean numbers of BARD1 or BRCA1 foci per cell, mechanically counted using the Cellomics Image Analyzer (Thermo Fisher), are shown. More than 20 cells were counted in each staining.
Figure S6: Inhibition of HP1γ increases the interaction of BARD1 with HP1α. HeLa-shHP1γ cells were induced or not with Dox and exposed to 10 Gy IR. Cells were harvested one hour after IR, and the solubilized chromatin fractions were immunoprecipitated with anti-BARD1 antibody and subjected to immunoblotting with the indicated antibodies. HP1α in the precipitate was increased by the inhibition of HP1γ. HP1β in the precipitate was faint, but visible with the inhibition of HP1γ.
Figure S7: BARD1 is dispensable for recruitment of HP1γ at DSB sites. HeLa cells conditionally expressing shRNA to BARD1 were induced or not with Dox and subjected to immunoblotting with the indicated antibodies (A). Cells were laser-microirradiated, and immunostained for HP1γ with γH2AX 10 minutes (B) or 1 hour (C) after the microirradiation. HP1γ accumulates at the DSB loci 10 min after the microirradiation and distributes homogenously in the nucleus after 1 hour. Depletion of BARD1 did not affect the localization of HP1γ at either time point.
**Figure S8: ATM-dependent interaction of BARD1 with HP1γ/H3K9me2.**

ATM-dependent interaction of BARD1 with HP1γ/H3K9me2. HeLa cells stably expressing BARD1-EGFP were treated with 10 µM of ATM inhibitor KU55933 or DMSO and either exposed to 10 Gy IR or left unexposed. Cells were harvested one hour after IR, and the solubilized chromatin fractions were immunoprecipitated with anti-GFP antibody and subjected to immunoblotting with the indicated antibodies.
Figure S9: Ectopic accumulation of RIF1 and inhibition of RAD51 retention at DSB sites by depletion of all three isoforms of HP1 or BARD1 mutant that does not bind HP1. (A) tnHP1 cells induced or not with Dox were exposed to IR and immunostained for RAD51. (B) The percentages of the cells displaying more than ten RAD51 foci (focus-positive fraction) are shown. More than 100 cells were counted using the Cellomics Image Analyzer in each of two independent experiments, and the numbers were averaged. The error bars represent the SD. (C) tnHP1 cells were induced or not with Dox, incubated with BrdU, exposed to IR and immunostained for RIF1 and BrdU. Cells positive for the BrdU stain are highlighted with dashed lines in the RIF1 stain of the wild-type cells. (D) The percentages of the cells displaying more than ten RIF1 foci per BrdU-positive or -negative cell are shown. More than 100 cells were counted from each of two independent experiments, and the numbers were averaged. The error bars represent the SD.
Figure S10. Dissolution kinetics of $\gamma$H2AX after DSB damage in G2 cells. (A) HeLa cells induced with Dox for substitution of endogenous BARD1 with wild-type or PEELI mutant of BARD1-EGFP were exposed to 10 Gy IR, incubated for indicated time length and immunostained for $\gamma$H2AX and Cyclin B1. The mean numbers of $\gamma$H2AX foci per cyclin B1 positive interphase cell were mechanically quantified. Error bars, S.D. of two independent experiments. (B) Representative findings for 8 h time point in (A). Persistent $\gamma$H2AX IRIF in later time points indicates HR defect (2).
**Figure S11: Cell cycle progression of cells treated with UNC0638.**

HeLa cells with substitution of endogenous BARD1 with wild-type or PEEL1 mutant of BARD1-EGFP (A), or U2OS cells treated with DMSO or 3 μM UNC0638 for 24 hours (B) were subjected to cell cycle analysis. The percentage of cells in each phase of the cell cycle is shown at the bottom.
Figure S12: An H3K9-specific HKMT inhibitor UNC0638 disrupts BRCA1/BARD1 retention at sites of DNA damage. IRIF formation of BARD1 and BRCA1 in U2OS cells treated with DMSO (-) or HKMT inhibitor UNC0638 shown in the Fig. 7C are exhibited as the mean numbers of foci per cell. 1st, 2nd: Data from each independent experiment. More than 70 cells were counted in each staining.
Figure S13: A model for the role of BRCA1/BARD1/HP1 complex in the DSB repair pathways. BRCA1 and BARD1 form a stable heterodimer through their N-terminal RING domains. After DSB damage, BARD1 interacts with HP1/H3K9me2 in a manner dependent on ATM, but not on RNF168. The interaction protects the DSB site from the NHEJ effector RIF1 and mediates the retention of BRCA1, CtIP and RAD51 that promote the HDR. RNF168 antagonizes BARD1/HP1/H3K9me2 interaction while it suppresses excess DNA-end resection by assembling BRCA1/Abraxas/RAP80 complex. Thus, HKMT inhibitors that inhibit H3K9me2 abrogate the BRCA1/BARD1 retention at DSB sites and exhibit a synergistic effect on cytotoxicity with PARP inhibitors. me: methylation, Ub: ubiquitin, blue box: BRCT domains.