H4K20me0 recognition by BRCA1-BARD1 directs homologous recombination to sister chromatids

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Genotoxic DNA double-strand breaks (DSBs) can be repaired by error-free homologous recombination (HR) or mutagenic non-homologous end-joining. HR suppresses tumorigenesis, but is restricted to the S and G2 phases of the cell cycle when a sister chromatid is present. Breast cancer type 1 susceptibility protein (BRCA1) promotes HR by antagonizing the anti-resection factor TP53-binding protein 1 (53BP1) (refs. 2–5), but it remains unknown how BRCA1 function is limited to the S and G2 phases. We show that BRCA1 recruitment requires recognition of histone H4 unmethylated at lysine 20 (H4K20me0), linking DSB repair pathway choice directly to sister chromatid availability. We identify the ankyrin repeat domain of BRCA1-associated RING domain protein 1 (BARD1)—the obligate BRCA1 binding partner—as a reader of H4K20me0 present on new histones in post-replicative chromatin. BARD1 ankyrin repeat domain mutations disabling H4K20me0 recognition abrogate accumulation of BRCA1 at DSBs, causing aberrant build-up of 53BP1, and allowing anti-resection activity to prevail in S and G2. Consequently, BARD1 recognition of H4K20me0 is required for HR and resistance to poly (ADP-ribose) polymerase inhibitors. Collectively, this reveals that BRCA1–BARD1 monitors the replicative state of the genome to oppose 53BP1 function, routing only DSBs within sister chromatids to HR.

Histone H4 lysine 20 (H4K20) methylation oscillates during the cell cycle, with major implications for chromosome replication, condensation and stability. In the G1 phase, nucleosomes are fully methylated at H4K20, with >80% carrying H4K20 di-methylation (H4K20me2) (ref. 6). During S phase, new histone H4 unmethylated at K20 (H4K20me0) are incorporated on newly synthesized DNA and mixed in a 1:1 ratio with old nucleosomes methylated at H4K20. H4K20me0 thereby marks the post-replicative state of a genomic locus and thus the presence of a sister chromatid until G2/M, when a surge of SET domain-containing protein 8 (SET8) methyltransferase activity catalyses mono-methylation of H4K20 (H4K20me1), which is subsequently converted to di- and tri-methylation by suppressor of variegation 4-20 homologue 1/2 (SUV4-20h1/2) (ref. 6). The cell exploits this to regulate the recruitment of DNA repair factors. The TONSL (Tonsoku-like protein)–MMS22L (protein MMS22-like) homologous recombination (HR) complex reads H4K20me0 via its ankyrin repeat domain (ARD) to direct its function in DNA repair protein RAD51 homologue 1 (RAD51) loading to collapsed replication forks and DNA double-strand breaks (DSBs) in post-replicative chromatin. Conversely, the non-homologous end-joining (NHEJ)-promoting factor TP53-binding protein 1 (53BP1) recognizes H4K20me1/2 (ref. 7). H4K20me0 and H4K20me1/2 present on old histones throughout the cell cycle. 53BP1 accumulation at DSBs is reduced in post-replicative chromatin (ref. 7), correlating with the replication-dependent dilution of H4K20me1/2 (refs. 8,9). However, H4K20me1/2 dilution cannot explain 53BP1 suppression and the shift to HR, as breast cancer type 1 susceptibility protein (BRCA1) is required to antagonize 53BP1 accumulation at DSBs in S/G2 (refs. 11,12). DNA damage-induced ubiquitylation by RING finger proteins 8 (RNF8) and 168 (RNF168) is also required for both 53BP1 and BRCA1 recruitment, but these signalling pathways are not cell-cycle specific (ref. 11). The crucial question of how BRCA1 specifically recognizes post-replicative chromatin thus remains a missing part in the puzzle to understand DSB repair pathway choice.

Because H4K20me0 directly marks sister chromatid availability, we set out to comprehensively explore its function. Using an unbiased quantitative proteomic strategy to identify proteins specifically recognizing nucleosomes carrying H4K20me0, we identified almost exclusively three post-replication DNA repair complexes; a BRCA1–BARD1 (BRCA1-associated RING domain protein 1)-containing complex involved in HR; the RAD18 (an E3 ubiquitin-protein ligase)–SLF1 (SMC5–SMC6 complex localization factor 1)–SLF2 complex implicated in interstrand cross-link repair (ref. 13); and TONSL–MMS22L, as expected (Fig. 1a). Consistent with previous work (ref. 14), leucine-rich repeat and WD repeat-containing protein 1 (LRWD1), origin recognition complex subunit 1 (ORC1), ORC2 and ORC3 were specifically enriched on nucleosomes carrying H4K20me2 (Fig. 1a). Intriguingly, BARD1 and SLF1 both contain ARDs with high similarity to the TONSL ARD that recognizes H4K20me0 (ref. 7). The reported structure of the BARD1 ARD revealed that the histone H4-binding interface is structurally conserved (Fig. 1b). BARD1 and SLF1 showed a clear
preference for H4K20me0 over H4K20me2 marked nucleosomes in pull-down experiments (Fig. 1c) and, importantly, mutation of three residues predicted to bind H4K20me0 (ARD 3A) based on TONSL homology abrogated nucleosome binding by SLF1 and BARD1 (Fig. 1b,d). These data identify two new H4K20me0 readers and argue for a general role of H4K20me0 in promoting post-replication DNA repair. In particular, recognition of H4K20me0 by BARD1 could provide a mechanism for BRCA1 recruitment to post-replicative...
As H4K20me0 marks post-replicative chromatin irrespective of DNA lesions, BRCA1–BARD1 might bind post-replicative chromatin in unperturbed cells. Indeed, we found progressive accumulation of the BRCA1–BARD1 complex on chromatin during the S phase mirroring H4K20me0 incorporation (Fig. 2a and Supplementary Fig. 2a,b), and this was dependent on H4K20me0 recognition by the BARD1 ARD (Fig. 2b and Supplementary Fig. 2c–e). BARD1 localized to 5-ethynyl-2’-deoxyuridine (EdU) pulse-labelled replication foci in the early S phase, but co-localization decreased in the mid- and late S phase (Fig. 2c). With continuous EdU labelling of post-replicative DNA, BARD1 and EdU co-localization remained high throughout the S phase (Fig. 2c), consistent with the recruitment of BARD1 via H4K20me0 present at sites of ongoing replication, as well as in post-replicative chromatin. Depletion of SET8, which is responsible for methylation of H4K20 in G2 and mitosis, increased H4K20me0 levels and augmented BRCA1–BARD1 binding to chromatin in G2 and G1 in a manner dependent on H4K20me0 recognition by the BARD1 ARD (Fig. 2d and Supplementary Fig. 2f–h). SET8-depleted cells showed reduced NHEJ and elevated rates of HR and single-stranded DNA annealing, but due to pleiotropic defects such as chromatin decompaction and DNA damage, this system was unsuitable for testing the role of H4K20me0 recognition in supporting BRCA1–BARD1-dependent HR. Taken together, our results establish ARD-dependent recognition of H4K20me0 as a mechanism for general pre-leision recruitment of BRCA1–BARD1 to post-replicative chromatin, irrespective of DNA damage.

Next, we analysed the role of H4K20me0 binding by BARD1 ARD in DNA DSB repair. Wild-type BARD1 recruitment to ionizing radiation-induced DSBs increased during the S phase and reached a maximum in the late S phase before dropping in G2/M (Fig. 3a), mirroring H4K20me0 occupancy (Supplementary Fig. 2a) and recruitment of other HR factors. Importantly, mutation of the BARD1 ARD ablated recruitment of both BARD1 and BRCA1 to DSBs in the S phase (Fig. 3a,b). Of note, the BARD1 wild type and ARD mutants were expressed at similar levels across the cell cycle and were proficient for BRCA1 stabilization via the RING domain dimerization (Supplementary Fig. 2c,e). Furthermore, we found that ARD recognition of H4K20me0 was as important as RNF8-dependent ubiquitination for BRCA1 accumulation at DSBs (Supplementary Fig. 3a), indicating that BRCA1–BARD1 recruitment requires multivalent interactions with chromatin at DSBs, similar to 53BP1 (ref. 3). Our finding that H4K20me0 is essential for BRCA1–BARD1 recruitment explains why forced methylation of H4K20 by non-degradable SET8 blocks BRCA1 recruitment and RAD51 loading at DSBs in the S phase. Collectively, this establishes H4K20me0 recognition as the critical mechanism underlying specific recruitment of BRCA1 to DSBs in the S phase, and assigns a primary function to the BARD1 ARD.

A key essential activity of BRCA1 is to oppose the anti-resection activity of 53BP1 (refs. 4,11,12) to direct DSBs towards HR. 53BP1 accumulation at DSBs is dependent on recognition of H4K20me1/2 by its Tudor domain and RNF168-dependent mono-ubiquitination of histone H2A lysine 15 (ref. 14). However, BRCA1 can prevent 53BP1 access to repair sites11,12. We therefore hypothesized that BRCA1 recognition of H4K20me0 might target BRCA1–BARD1-dependent exclusion of 53BP1 to post-replicative chromatin. BRCA1 foci dominated in the mid and late S phase in the presence of the BARD1 wild type as expected11, but, remarkably, this pattern was reversed by the BARD1 ARD mutant (Supplementary Fig. 3b). The BARD1 ARD mutant also completely failed to suppress 53BP1, depletion of 53BP1 partly restored HR in cells expressing the ARD mutant (Supplementary Fig. 3c). Moreover, mutation of the H4K20me0 recognition site was as detrimental to HR as deletion of the full ARD or BRCA1 carboxy (C) terminus (BRCT) domain previously shown to be essential for HR by using both U-2 OS cells depleted for endogenous BARD1 and a BARD1-null mouse mammary carcinoma cell line22. Mutation of the ARD ablated BARD1 function in HR in both systems (Fig. 4a,b). Consistent with a role of the H4K20me0 recognition in antagonizing 53BP1, depletion of 53BP1 partly restored HR in cells expressing the ARD mutant (Supplementary Fig. 3c). Moreover, mutation of the H4K20me0 recognition site was as detrimental to HR as deletion of the full ARD or BRCA1 carboxy (C) terminus (BRCT) domain previously shown to be essential for HR by using both U-2 OS cells depleted for endogenous BARD1 and a BARD1-null mouse mammary carcinoma cell line22. Mutation of the ARD ablated BARD1 function in HR in both systems (Fig. 4a,b). Consistent with a role of the H4K20me0 recognition in antagonizing 53BP1, depletion of 53BP1 partly restored HR in cells expressing the ARD mutant (Supplementary Fig. 3c). Moreover, mutation of the H4K20me0 recognition site was as detrimental to HR as deletion of the full ARD or BRCA1 carboxy (C) terminus (BRCT) domain previously shown to be essential for HR by using both U-2 OS cells depleted for endogenous BARD1 and a BARD1-null mouse mammary carcinoma cell line22. Mutation of the ARD ablated BARD1 function in HR in both systems (Fig. 4a,b).
Fig. 2 | ARD recognition of H4K20me0 recruits BRCA1–BARD1 to post-replicative chromatin. a, Top, high-content microscopy of chromatin-bound BARD1 and BRCA1 in pre-extracted U-2 OS cells pulsed with EdU. Bottom, cell-cycle stages were defined by gating on EdU and DAPI (top right). Mean ± s.d. intensities are shown relative to G1 levels (n = 3 independent experiments; from left, P = 0.0203, 0.0057, 0.0076 and 0.0170 for BARD1 (left), and 0.0055, 0.0315, 0.0251 and 0.0821 (NS, not significant) for BRCA1 (middle), ratio paired two-sided t-test). Data points represent the means of >173 cells. The correlation between BARD1 and BRCA1 mean intensities (lower right) is shown with Pearson’s R² correlation (P < 0.0001). Representative cells are shown. Scale bars: 10 μm. b, Top, High-content microscopy of chromatin-bound Flag-HA-BARD1 in U-2 OS cells inducible for Flag-HA-BARD1 wild type or ARD 3A. Bottom, mean ± s.d. intensities are shown relative to wild type in G1 levels (n = 3 independent experiments; from left, P = 0.0214, 0.0036, 0.0220, 0.0204 and 0.0074, ratio paired two-sided t-test). Data points represent the means of >254 cells. c, Co-localization of chromatin-bound BARD1 and EdU in cells pulsed with EdU (left) or released into the S phase in the presence of EdU (right). S-phase stages were defined by EdU, DAPI and MCM2 staining6. Schematics illustrate the expected results based on H4K20me0 distribution6. Red lines and error bars represent means ± s.d. From left, n = 28, 22 and 18 (left), and 18, 25 and 22 cells (right). Representative cells are shown. Data were derived from two independent experiments. ****P < 0.0001, Mann–Whitney U-test (two-sided). NS results from left: P = 0.1392 and 0.4494. Scale bars: 15 μm. d, BARD1 and BRCA1 chromatin binding in the G1 (left) and G2 phase (right) in SET8-depleted cells. Cells inducible for siRNA-resistant Flag-HA-BARD1 wild type or ARD 3A were treated with BARD1 and SET8 siRNAs and analysed as in a. Mean ± s.d. intensities in SET8 and BARD1 co-depletion cells over BARD1 depletion alone are shown (n = 3 independent experiments; from left, P = 0.0048 and 0.0021 (for G1), and 0.013 and 0.0009 (for G2), ratio paired two-sided t-test). Data points represent the means of >206 cells.
and stable depletion of endogenous BARD1 following the addition of auxin indole-3-acetic acid (IAA; Fig. 5a). IAA-induced degradation of BARD1 resulted in BRCA1 destabilization (Fig. 5a) and PARPi hypersensitivity (Fig. 5b). The destabilization of BRCA1 was equally bypassed in cells expressing wild-type and mutant BARD1 transgenes (Fig. 5c). In addition to the recently reported BARD1 R99E domain mutant and BARD1 ARD 3A, we included a BARD1 BRCT-domain mutant (K619A) that abrogates poly (ADP-ribose)-dependent recruitment of BARD1 to stalled replication forks but is proficient for HR in mice. In control (glutathione S-transferase-containing) CHO cells, IAA treatment resulted in an acute hypersensitivity to PARPi (Fig. 5d). This hypersensitivity was completely reversed by the expression of wild-type BARD1, or the BARD1R99E and BARD1K619A mutants (Fig. 5d). In contrast, BARD1 ARD 3A expression failed to suppress PARPi sensitivity (Fig. 5d).

These data indicate that ARD-mediated H4K20me0 binding represents a prime function for BARD1 in HR, while the E3-ligase activity and poly (ADP-ribose) binding are dispensable, consistent with these activities not being required for tumour suppression in mice.27,28

How cells switch from mutagenic NHEJ to error-free HR during S and G2 has been a long-standing question. Our results provide a simple solution in which ARD recognition of H4K20me0 triggers BRCA1–BARD1 recruitment specifically to post-replicative chromatin to antagonize 53BP1 and promote resection only when a sister chromatid is available for HR (Fig. 5e). This pre-lesion cis-acting mechanism acts at the most upstream point in repair pathway choice before cell-cycle regulation of end resection29 and recruitment

Fig. 3 | H4K20me0 recognition is required for BRCA1–BARD1 recruitment to DSBs, 53BP1 antagonization and DNA end resection. a, Top, high-content microscopy of Flag-HA-BARD1 accumulation at ionizing irradiation-induced DNA DSBs. U-2 OS cells expressing Flag-HA-BARD1 wild type or ARD 3A were pulsed with EdU, irradiated with 1 Gy and, 45 min later, pre-extracted and analysed by immunofluorescence. Bottom, means ± s.d. foci numbers are shown relative to the BARD1 wild type in mid-S phase. S-phase stages are defined as in Fig. 2a (n = 3 independent experiments). Data points represent the means of >381 cells. Scale bars: 10 μm. b, c, BRCA1 (b) and 53BP1 (c) accumulation at ionizing irradiation-induced DSBs in U-2 OS cells treated with control or BARD1 siRNAs and induced with TET to express siRNA-resistant Flag-HA-BARD1 wild type or ARD 3A. Cells were analysed as in a, and late S phase cells are shown. Means are shown as small ‘+’ signs, and whiskers indicate the 10th–90th percentiles. From left in b, n = 1,068, 723, 1,224, 896, 1,420, 627, 902 and 597 cells. From left in c, n = 1,068, 723, 1,224, 896, 1,420, 627, 902 and 597 cells. d, BRCA1 and 53BP1 foci in single cells in the late S phase were analysed as in b and c. For the BARD1 wild type, n = 896. For BARD1 ARD 3A, n = 597. e, High-content microscopy of single-stranded DNA measured by BrdU staining under non-denaturing conditions in U-2 OS cells treated with siRNA and TET as in c. Cells with high (top 15%, left) and low (bottom 15%, right) H4K20me0 levels (defined by gating on mean intensity) are shown. Cells were fixed 3 h after ionizing irradiation (3 Gy). Means are shown as small ‘+’ signs, and whiskers indicate the 10th–90th percentiles. From left, n = 503, 442, 371, 474, 269, 381 and 330 (left), and 617, 641, 316, 327, 540, 495, 277 and 194 cells (right). f, High-content microscopy of ionizing irradiation-induced RAD51 foci analysed as in b. Means are shown as small ‘+’ signs, and whiskers indicate the 10th–90th percentiles. From left, n = 310, 384, 327, 326, 329, 295, 321 and 331 cells.
pathways with respect to ubiquitin signalling remains enigmatic. Recognition of H4K20me0 is required for HR and PARPi resistance. a, Mean ± s.d. HR efficiency measured in DR-GFP U-2 OS cells treated with control or BARD1 siRNA and complemented with siRNA-resistant BARD1 wild type, ARD 3A or empty vector. The HR efficiency is shown as the percentage of control siRNA (n = 3 independent experiments). From left, P = 0.29 and 0.0016, ratio paired two-sided t-test. b, Mean ± s.d. HR efficiency measured in BARD1-null mouse mammary carcinoma cells carrying a DR-GFP reporter and complemented as indicated. The HR efficiency relative to empty vector is indicated above each bar (n = 3 independent experiments). c, BARD1 ARD mutations identified by cancer genome sequencing (COSMIC, https://cancer.sanger.ac.uk/cosmic; TCGA Research Network, https://cancergenome.nih.gov/). Mutations H459Y, D458V and E467K (red) are predicted to disturb H4 binding, as the residues are directly involved in H4 binding. Mutations D495Y, K503T, T534V and K540T (orange) are prone to affect the structural integrity of BARD1 ARD since the wild-type residues are involved in intramolecular hydrogen bonding. Mutations buried in the core (A435V, L447F, L480S, L498I and P530S; dark green) might affect the structure of the ARD and thus indirectly disrupt H4 binding. Surface-exposed mutations (light green) are less likely to affect ARD structure and H4 binding. d, Mean ± s.d. sensitivity to olaparib analysed by colony formation in siRNA-treated U-2 OS cells induced to express Flag-HA-BARD1 wild type and ARD 3A by TET (mean, ± s.d., 4 independent experiments; *P = 0.0136, ratio paired two-sided t-test). Bars indicate mean ± s.d. HR efficiency measured in U-2 OS cells carrying a DR-GFP reporter, transfected with the indicated BARD1 constructs or empty vector along with an I-SceI expression vector (n = 4 independent experiments; *P = 0.0136, ratio paired two-sided t-test). e, HR efficiency measured in U-2 OS cells carrying a DR-GFP reporter, transfected with the indicated BARD1 constructs or empty vector along with an I-SceI expression vector (n = 4 independent experiments; *P = 0.0136, ratio paired two-sided t-test). Bars indicate mean ± s.d. HR efficiency measured in DR-GFP U-2 OS cells treated with olaparib (1 μM) treatment of U-2 OS cells induced to express Flag-HA-BARD1 wild type and ARD 3A by TET (mean, = 3 independent experiments; from left, P = 0.9865 and 0.0013, ratio paired two-sided t-test).

Fig. 4 | BARD1 recognition of H4K20me0 is required for HR and PARPi resistance. a, Mean ± s.d. HR efficiency measured in DR-GFP U-2 OS cells treated with control or BARD1 siRNA and complemented with siRNA-resistant BARD1 wild type, ARD 3A or empty vector. The HR efficiency is shown as the percentage of control siRNA (n = 3 independent experiments). From left, P = 0.29 and 0.0016, ratio paired two-sided t-test. b, Mean ± s.d. HR efficiency measured in BARD1-null mouse mammary carcinoma cells carrying a DR-GFP reporter and complemented as indicated. The HR efficiency relative to empty vector is indicated above each bar (n = 3 independent experiments). c, BARD1 ARD mutations identified by cancer genome sequencing (COSMIC, https://cancer.sanger.ac.uk/cosmic; TCGA Research Network, https://cancergenome.nih.gov/). Mutations H459Y, D458V and E467K (red) are predicted to disturb H4 binding, as the residues are directly involved in H4 binding. Mutations D495Y, K503T, T534V and K540T (orange) are prone to affect the structural integrity of BARD1 ARD since the wild-type residues are involved in intramolecular hydrogen bonding. Mutations buried in the core (A435V, L447F, L480S, L498I and P530S; dark green) might affect the structure of the ARD and thus indirectly disrupt H4 binding. Surface-exposed mutations (light green) are less likely to affect ARD structure and H4 binding. d, Mean ± s.d. sensitivity to olaparib analysed by colony formation in siRNA-treated U-2 OS cells induced for the expression of siRNA-resistant Flag-HA-BARD1 wild type or ARD 3A (n = 3 independent experiments). e, HR efficiency measured in U-2 OS cells carrying a DR-GFP reporter, transfected with the indicated BARD1 constructs or empty vector along with an I-SceI expression vector (n = 4 independent experiments; *P = 0.0136, ratio paired two-sided t-test). Bars indicate mean ± s.d. HR efficiency measured in DR-GFP U-2 OS cells treated with olaparib (1 μM) treatment of U-2 OS cells induced to express Flag-HA-BARD1 wild type and ARD 3A by TET (mean, = 3 independent experiments; from left, P = 0.9865 and 0.0013, ratio paired two-sided t-test).

of BRCA239, as well as H4K20me0-mediated RAD51 loading via TONSL–MMS2214. We propose that two complementary mechanisms control HR in mammalian cells—sister chromatid sensing via H4K20me0 (this work and ref. 1) and cell-cycle regulation4, which is well established at many levels. Consistent with this, HR is strongly impaired when the deposition of new histones and thus marking of post-replicative chromatin by H4K20me0 is attenuated31. Recognition of H4K20me0 on new histones by BARD1, contrary to recognition of H4K20me1/2 on old histones by 53BP1, explains the specific recruitment of BRCA1 to DSBs in G1 cells that lack 53BP1 (ref. 36). In this scenario, competition for ubiquitin binding at DSBs may also explain the futile recruitment of 53BP1 and BRCA1–BARD1 that converges at DSBs and is tuned to the replicative state of a genomic locus by H4K20 methylation status. Competition for ubiquitin binding at DSBs may also explain the futile recruitment of BRCA1 to DSBs in G1 cells that lack 53BP1 (ref. 36). In the absence of 53BP1, recognition of H4K20me0 provides BRCA1–BARD1 with an advantage in post-replicative chromatin, tipping the balance towards 53BP1 exclusion and induction of HR, which is then executed by downstream cell-cycle-regulated factors. Our data argue that ARD recognition of H4K20me0 mediates an essential HR function of BARD1, providing molecular insight into the function of the highly conserved ARD, and identifying the enigmatic HR function of BARD1 beyond BRCA1 stabilization37. Our work explains how the availability of a sister chromatid substrate for HR directs repair pathway choice, and the druggability of histone reader domains38 suggests that this mechanism can be exploited for targeting BRCA1 in cancers addicted to HR.
Fig. 5 | Recognition of H4K20me0 is a prime function of BARD1 in HR. a, Western blot analysis of BARD1<sup>ΔAD</sup>/ΔAD HCT116 cells at indicated time points following the addition of doxycycline to stimulate OsTIR1 expression, and auxin (IAA) to induce OsTIR1-dependent BARD1-AID proteolysis. Representatives of two biological replicates are shown (see Supplementary Fig. 5 for unprocessed blots). Asterisks indicate non-specific bands. b, IAA addition hypersensitizes BARD1<sup>ΔAD</sup>/ΔAD HCT116 cells to olaparib. Cells were seeded in the presence of doxycycline. IAA and olaparib were added 1 d later and left for 7 d before staining with crystal violet. Representatives of two biological replicates are shown. c, d, Analysis of BARD1 ARD function in PARPi resistance, as defined in BARD1<sup>ΔAD</sup>/ΔAD HCT116 cells. After 24 h in doxycycline, the indicated cell lines were treated with IAA or carrier (dimethyl sulfoxide (DMSO)) before immunoblotting whole-cell lysates (c) or measuring growth in the presence of olaparib (d), as indicated. In c, western blots show equal stabilization of endogenous BRCA1 by the expression of wild-type and all mutant BARD1 transgenes, but not the control (glutathione S-transferase (GST)) transgene. Representatives of two biological replicates are shown (see Supplementary Fig. 5. for unprocessed blots). */eBARD1 indicates a weak unspecific band and the size of exogenous BARD1. In d, mean ± s.d. olaparib sensitivity was determined by resazurin cell viability assay 7 d following incubation with olaparib (a twofold dilution range from 7.8 nM–2 μM) in the presence (left) or absence of IAA (right) (n = 3 independent experiments). e, Schematic illustrating how BARD1 recognition of H4K20me0 licenses DSBs in sister chromatids for HR. BRCA1–BARD1 identifies post-replicative chromatin by recognition of H4K20me0 on new histones, thereby enabling the complex to oppose 53BP1 function when a sister chromatid can be used for homology-directed error-free repair of DSBs.
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Any methods, additional references, Nature Research reporting summaries, source data, statements of data availability and associated accession codes are available at https://doi.org/10.1038/s41556-019-0282-9.

Received: 16 October 2018; Accepted: 14 January 2019; Published online: 25 February 2019

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Acknowledgements
We thank J. Lukas for commenting on the manuscript, researchers at the Groth laboratory for fruitful discussions, R. Bar for sharing the BRD1 plasmid and BARD1-null mouse mammary carcinoma cells, M. Kanemaki for the OxTIR1 antibody, Y. Antoku for assistance with microscopy, and J. V. Johansen for help with statistical analysis. J.R.B. is funded by a Cancer Research UK Career Development Grant (CS2690/A19270). Funding for T.B. was provided by the Medical Research Council (grant number MC_UP_1102/2) and European Research Council (ERC StG number 309952). S.L. was supported by a stipend from the Biotechnology and Biological Sciences Research Council. The Groth laboratory is supported by the Danish Cancer Society, Novo Nordisk Foundation, Lundbeck Foundation, European Research Council (ERC CoG number 724436), Independent Research Fund Denmark and Neye Foundation.

Author contributions
K.N., G.S. and A.G. conceived the study, K.N. carried out the functional analysis. G.S. performed pull-downs with endogenous BARD1 and SLF1, and established tools and reagents. J.R.B. generated and carried out experiments with BARD1null and BARD1null cells. B.M.F. prepared materials for nucleosome pull-downs. N.V.N. prepared materials, carried out SILAC nucleosome pull-down experiments and analysed the data. T.E.B. and L.C.C. performed pull-downs with BARD1 and SLF1 mutants. P.A.F. carried out mass spectrometry measurements and analysed the mass spectrometry data. S.L. performed bioinformatics analyses of the mass spectrometry data. T.F. performed structural modelling. J.R.C., T.B. and A.G. supervised the project and analysed the data. G.S. and A.G. wrote the manuscript with input from all authors.

Competing interests
G.S. and A.G. are inventors on a filed patent application covering the therapeutic targeting of ARD interactions with H4K20me0 for cancer therapy. A.G. and T.F. are co-founders of Ankin Therapeutics.

Additional information
Supplementary information is available for this paper at https://doi.org/10.1038/s41556-019-0282-9.

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Preparation of recombinant histones. Recombinant human core histones were expressed in Escherichia coli BL21(DE3)/RII cells from pET21b(+) (Novagen) vectors, and purified by denaturing gel filtration and ion exchange chromatography, essentially as described28.

Preparation of truncated histone H4 for native chemical ligation. Truncated human H4Δ1–28I29C protein for ligation of modified histone H4 was expressed from a pET24b(+) vector (Novagen) in E. coli BL21(DE3)/RII cells. The insoluble protein was extracted from inclusion bodies with unfolding buffer (20 mM Tris (pH 7.5), 7 mM guanidine hydrochloride and 100 mM dithiothreitol (DTT)) for 1 h at room temperature, and the cleared supernatant was loaded on a Sephacryl S-200 gel filtration column (GE Healthcare) in SAU-1000 (20 mM sodium acetate (pH 5.2)), 7 M urea, 1 mM NaCl and 1 mM ethylenediaminetetraacetic acid (EDTA)) without any reducing agents. H4Δ1–28I29C-containing fractions were combined and further purified on a reversed-phase chromatography column (PerkinElmer Acquity RP-300 (C8) 250 mm × 4.6 mm i.d.) using a gradient of 0–6% B (buffer A: 0.1% trifluoroacetic acid in water; B: 90% acetonitrile and 0.1% trifluoroacetic acid) over 20 column volumes. Fractions containing pure H4Δ1–28I29C were pooled and lyophilized.

Native chemical ligation. Native chemical ligations were carried out in 550 μl of degassed ligation buffer (200 mM KPO4, 2 mM EDTA and 6 M guanidine hydrochloride) containing 1 mg of modified/modified H4 peptides spanning amino acids 1–28 and containing a C-terminal thioester (Cambridge Peptides), 4 mg of truncated H4Δ1–28I29C, 20 mM reduced phosphoethylenetricarbonyl acid and 25 mg Tris(2-carboxyethyl)phosphine (TCEP) as a reducing agent at a pH of 7.5. The reactions were incubated overnight at 4 °C and quenched by the addition of 60 μl 1 M DTT and 700 μl 0.5% acetic acid. After precipitation clearance by centrifugation, the ligation reactions were directly loaded and purified on a reversed-phase chromatography column (PerkinElmer Acquity RP-300 (C8) 250 mm × 4.6 mm i.d.) using a gradient of 35–45% B (buffer A: 0.1% trifluoroacetic acid in water; B: 90% acetonitrile and 0.1% trifluoroacetic acid) over 10 column volumes. Positive fractions containing ligated full-length histone H4 were combined and directly lyophilized.

Nucleosome assembly. Histone octamers were refolded from the purified histones and assembled into nucleosomes with biotinylated DNA via salt deposition dialysis as previously described29. Biotinylated nucleosomal DNAs containing two 601 nucleosome positioning sequences separated by a 50 base pair (bp) linker (di-nucleosomes) were prepared as described39. Di-nucleosomes were assembled into nucleosomes with biotinylated DNA via salt deposition dialysis and immobilized on Streptavidin Sepharose High Performance beads (GE Healthcare) via the biotinylated DNA, washed to remove MMTV A competitor DNA and MMTV A nucleosomes, and directly used for stable isotope labeling with amino acids in cell culture (SILAC) nucleosome pull-down assays. Biotinylated di-nucleosomes carrying 1–28I29C were pooled and allowed to rotate for 1 h at 4 °C. Inputs of 2% of the extracts were taken before diluting each sample with SNAP buffer. The extracts were then added to the washed di-nucleosome-coupled beads and incubated for 4 h, rotating at 4 °C. The whole Wash Buffer (20 mM HEPES, 150 mM NaCl, 0.2 mM EDTA, 10% glycerol and 0.1% NP-40). At the same time, 320–450 μg of HeLa cell extracts were incubated with 10 μg ml−1 Herring Sperm DNA (Sigma–Aldrich) in SNAP buffer (final volume: 900 μl) and allowed to rotate for 1 h at 4 °C. Inputs of 2% of the extracts were taken before diluting each sample with SNAP buffer. The extracts were then added to the washed di-nucleosome-coupled beads and incubated for 4 h, rotating at 4 °C. These beads were then washed twice with Wash Buffer (20 mM HEPES, 150 mM NaCl, 0.2 mM EDTA, 10% glycerol and 0.25% NP-40, supplemented with 10 μg ml−1 Herring Sperm DNA) for 2 min at 4 °C. After the last wash, the beads were carefully dried and boiled in 20 μl 1x Laemmli sample buffer (LSB; 50 mM Tris–HCl pH 6.8, 100 mM DTT, 2x SDS, 8% glycerol, bromophenol blue). Pull-downs were visualized by western blotting after protein separation on a NuPAGE 12–14% Bis-Tris gradient gel (Thermo Fisher Scientific).

Peptides were eluted on line to an analytical column (Thermo Fisher Scientific Acclaim PepMap RSLC; 75 μm internal diameter, 25 cm length, C18 reversed-phase material, 2 μm diameter beads and 100 Å pore size) and separated using a flow rate of 250 μl min−1 and gradient conditions of: initial 5 min with 4% buffer B, then 90 min gradient 4–25% B, then 30 min gradient 25–45% B, then 1 min gradient 45–90% B, and finally 15 min isocratic at 100% B before returning to starting conditions for a 15 min equilibration (buffer A: 2% acetonitrile and 0.1% formic acid in water; B: 80% acetonitrile and 0.1% formic acid).

The Q Exactive instrument acquired full-scan survey spectra (m/z 300–1,650) at 70,000 resolution. An automatic gain control target value of 3 × 105 and a maximum injection time of 20 ms were used. The top 10 most abundant multiply charged ions were selected in a data-dependent manner, fragmented by higher-energy collision-induced dissociation, and data were collected over the range 200–2,000 m/z at 17,500 resolution. An automatic gain control target value of 1 × 106 with a maximum injection time of 120 ms was used. A dynamic exclusion time of 30 s was enabled.

Cell culture, transfection and drug treatment. U-2 OS (a gift from J. Bartek), HeLa S3 (a gift from P. Nakatani) and HCT116 cells (a gift from I. Tomlinson) were grown in Dulbecco’s modiﬁed Eagle’s medium (Gibco) containing 10% foetal bovine serum (HyClone), 1% penicillin/streptomycin and drugs for selection. To establish a parental cell line expressing the E3 ubiquitin ligase OsTIR1, cells were introduced by transfection with Lipofectamine 2000 (Invitrogen) according to the manufacturer’s protocol, and selection with hygromycin (200 μg ml−1). FRT/TO-Flag-HA-BARD1 plasmids with Lipofectamine 2000, according to the manufacturer’s instructions and dried with a SpeedVac.

Mass spectrometry. Samples were loaded at 8 μl min−1 onto a trap column (Thermo Fisher Scientiﬁc Acclaim PepMap 100; 100 μm internal diameter, 2 cm length, C18 reversed-phase material, 5 μm diameter beads and 100 Å pore size) in 2% acetonitrile and 0.1% trifluoroacetic acid.

Mass spectrometry data processing. Protein abundances from the Q Exactive mass spectrometer were quantified by MaxQuant 1.6.0.16 using 2-plex labeling (Arg10 and Lys18). The search was run against human UP00003640 Proteome (version GCA_000001405.26) from UniProt. Search parameters were set up to allow variable oxidation on methionines and acetylation on amino termini, as well as fixed carbamidomethylation on cysteines. Tryptic peptides with up to two missed cleavages were considered for analysis, and tolerances settings were set to Orbitrap instrument. Co-fragmented peptide identification and matching between runs was enabled. For protein quantification, the minimum ratio of spectral count ratio between two replicates was set to 1, and remaining settings were left as default. The normalized heavy/light (H/L) ratios in proteinGroups.txt file output by MaxQuant were transformed to a log scale, for further processing. Each protein in the database containing the ankyrin repeat-containing domain was identified using information from the InterPro database (IPR036779). Twenty two proteins that were marked as either ‘potential contaminant’, ‘reverse’ or ‘only identified by site’ by MaxQuant were dropped from the dataset (that is, 1.81% of the data). A further 76 proteins (5.39% of the remaining data) were dropped because they had only a forward or reverse ratio, but no complete pairs. The data presented in Fig. 1 are zoomed in to log[fold change] ratios below the value of 5, thus excluding 4 outlier proteins (SPTA1, STAG2, histone H3 and histone H4) that are not enriched in either nucleosome pull-down. The full proteomics list for H4K20me0/2me2 nucleosome pull-downs is presented in Supplementary Table 1.

Di-nucleosome pull-down assay. Biotinylated di-nucleosomes carrying H4K20me0 or H4K20me2, or biotin only as a control, were mixed with MyOne T1 Streptavidin beads (Thermo Fisher Scientiﬁc) in Coupling Buffer (10 mM Tris–HCl, 1 mM EDTA and 250 mM KCl). Di-nucleosomes and beads were incubated for 1 h at 4 °C on a rotating shaker to allow the coupling and, subsequently, washed twice with SNAP buffer (20 mM HEPES, 150 mM NaCl, 0.2 mM EDTA, 10% glycerol and 0.1% NP-40). At the same time, 320–450 μg of HeLa cell extracts were incubated with 10 μg ml−1 Herring Sperm DNA (Sigma–Aldrich) in SNAP buffer (final volume: 900 μl) and allowed to rotate for 1 h at 4 °C. Inputs of 2% of the extracts were taken before diluting each sample with SNAP buffer. The extracts were then added to the washed di-nucleosome-coupled beads and incubated for 4 h, rotating at 4 °C. These beads were then washed twice with Wash Buffer (20 mM HEPES, 150 mM NaCl, 0.2 mM EDTA, 10% glycerol and 0.25% NP-40, supplemented with 10 μg ml−1 Herring Sperm DNA) for 2 min at 4 °C. After the last wash, the beads were carefully dried and boiled in 20 μl 1x Laemmli sample buffer (LSB; 50 mM Tris–HCl pH 6.8, 100 mM DTT, 2x SDS, 8% glycerol, bromophenol blue). Pull-downs were visualized by western blotting after protein separation on a NuPAGE 12–14% Bis-Tris gradient gel (Thermo Fisher Scientiﬁc).
subclones were isolated and assessed for doxycycline inducible expression by western blot using an OsTIR1-specific antibody (a gift from M. Kanemaki at the National Institute of Genetics). Integration of Tet-OsTIR1 at the AAVS1 locus was confirmed by genomic DNA transfected and plated in the presence (0.2 mM BrdU, 3 μM BrdU) and then treated with ionizing irradiation (3 Gy). After 3 h recovery, cells were pre-extracted with nuclear extraction buffer (20 mM HEPES-NaOH (pH 7.5), 20 mM NaCl, 5 mM MgCl₂, 1 mM DTT, 0.5% NP-40, 100 μM phenylmethylsulfonyl fluoride (PMSF), 10 μg per mg protein, 0.2 μM β-glycerophosphate and 5 mM NaF) on ice for 10 min and fixed with 4% formaldehyde/phosphate buffered saline on ice for 10 min.

HR assay. DR-GFP U-2 OS or BARD1-null mouse mammary carcinoma cells (18–20) were used as described previously. BARD1, 5–5′×10⁶ cells were seeded on 6-well plates. After 24 h, the cells were transfected with 1 μg of I-scel expression vector (pcRASce), 2 μg of BARD1 expression vector or empty vector and 1 μg of monomeric red fluorescent protein (mRFP) expression vector (pcS2-mRFP). 24 h after transfection, the cells were washed and re-plated on 100 mm dishes. Flow cytometric analysis to quantify the presence of GFP-positive cells was performed 2 days later on a FACSCalibur using CellQuest Pro software (BD Biosciences). For each sample, 30,000 cells were assessed, and the percentage of GFP-positive cells in RFP-positive cells was calculated as HR repaired cells. RFP-encoding vector was used to provide a control for transfection efficiency. For 53BP1 depletion, the siRNAs were transfected 24 h before plasmid transfection.

Clonogenic assay. U-2 OS inducible for the FLAG-HA-BARD1 wild type and ARD 3A mutant were seeded in technical triplicates of 300–10,000 cells in the presence or absence of TET. After 48 h, olaparib (Selleck Chemicals; S1060) was added for 72 h. Cells were then cultured in fresh medium for 7–15 d before fixation and staining with MeOH/Crystal Violet. For the complementation experiments, control wild-type and ARD 3A mutant cells were transfected with siRNA and seeded with or without TET. The cells were trypsinized 24 h later and seeded in technical triplicates of 300–10,000 cells in the presence or absence of TET. After 24 h, olaparib was added for 24 h at the indicated concentrations. Cells were then cultured in fresh medium for 7–15 d before fixation and staining with MeOH/Crystal Violet. The colony forming efficiency was then quantified and compared to pre-existing colonies previously used to model the interaction of the histone H4 tail with BARD1. Due to the high density of the experiment, a maximum of 100 colonies was included per treatment.

Resazurin cell viability assay. Tet-OsTIR1 HCT116 cells (300 well⁻¹) were seeded in triplicate in 96-well plates in the presence of doxycycline (1 μg/ml) for each concentration of olaparib (Selleck Chemicals; S1060). Then, 24 h after plating, LAA was added to a final concentration of 1 mM. One hour after the addition of IAA, olaparib was added to indicated final concentrations. The medium was refreshed at 4 d. Seven days after the addition of olaparib, the medium was replaced with phenol-free Dulbecco’s modified Eagle’s medium (10% foetal bovine serum and 1% penicillin/streptomycin) containing 10 mg/ml −1 resazurin, and the plates were incubated for 2 h. The relative fluorescence intensity of each well was then measured using a BMG LABTECH CLARIOstar plate reader equipped with a 560 nm excitation and 590 nm emission filter set.

Antibodies. The following antibodies were used for western blot: BARD1 (Abcam A300-263A; 1:500), BARD1 (Abcam ab64164; 1:500), SF1 (Novus Biologicals NB1-88358; 1:500), HA (HA-11, BioLegend 901513 (previously Covance MMS-101P); 1:500), H4K20me2 (Diagenode C15200205; 1:1000), GFP (Roche 11 814 800 001 (mixture of clones 7.1 and 13.1); 1:500), SET8 (Invitrogen 1:400), 53BP1 (Integrated DNA Technologies; 1:400), BRCA1 (Santa Cruz Biotechnology sc-6954, clone 9; 1:400), 53BP1 (BD Biosciences). For each sample, 30,000 cells were assessed, and the percentage of GFP-positive cells in RFP-positive cells was calculated as HR repaired cells. RFP-encoding vector was used to provide a control for transfection efficiency. For 53BP1 depletion, the siRNAs were transfected 24 h before plasmid transfection.

Modelling the structure of BARD1 bound to histone H4. The Rosetta macromolecular modelling package (www.rosettacommons.org), which has proven successful in a variety of protein design applications involving protein structure predictions (predictions of protein–protein and protein–peptide interfaces) was used to model the interaction of the histone H4 tail with BARD1. Due to the high structural similarity of TONSIL (Protein Data Bank (PDB) ID: 5J4A) and BARD1 ARD (PDB ID: 3CSR) (main chain root-mean-square deviation = 0.96 Å), we chose to use the structure of TONSIL in complex with the histone H3–H4 tetramer as a seed structure to model the complex of the histone H4 tail bound to BARD1. The starting structure for the Rosetta refinement of BARD1 bound to histone H4 was prepared from the structure of the ARD domain of TONSIL (PDB ID: 5J4A) and BARD1 ARD (PDB ID: 3CSR). The imidazole side chain of H18 (in H4) is positively charged, as it is located in a cavity between the negatively charged residues E568 and D604 that is conserved (E467 and D500) in the ARD domain of BARD1. A positively charged H18 was also supported by pKa calculations using the Rosetta Modelling the structure of BARD1 bound to histone H4.
Letters

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protocol to calculate pKa values. As histidines in Rosetta are assumed to be neutrally charged by default, we prepared a parameter file to protonate both the epsilon and delta state of H18 to carry a net (+1) positive charge. The preliminary model of the BARD1 and H3–H4 complex was exhaustively refined using Rosetta 5.0.37 by subjecting it to 3,000 steps of full-atom structure relaxation (in the Rosetta force field), which samples side-chain and backbone conformational changes (similar to those suggested to underlie observed conformational heterogeneity in high-resolution crystal structures) to broadly sample the protein–protein interface and the conformational variability of the complex40. For each relax cycle, five rounds of repacking followed by gradient base minimization in torsion space (backbone φ/ψ and side chain χ torsional degrees of freedom) was performed until convergence (an absolute score change on minimization of less than one Rosetta energy unit). From all cycles performed, the best-scoring pose was selected to represent the structure of BARD1 bound to the histone H4 tail, as shown in Supplementary Fig. 4a.

Statistics and reproducibility. Statistics were analysed with GraphPad Prism 7. All statistics were evaluated by either independent or paired two-tailed Student's t-test and two-tailed Mann–Whitney U-test. Pearson’s correlation and Student’s t-test were performed under the assumption of normality. The exact sample sizes (n) used to calculate statistics are provided in the figure captions. P values are provided in the figure legends and captions. Whiskers indicate 10th–90th percentiles, and outliers are plotted as individual points in box-and-whisker plots. All experiments were reproduced with similar results a minimum two times.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Code availability
The code used to analyse the mass spectrometry data is publicly available at GitHub (https://github.com/lukauskas/publications-nakamura-2018-snap-h4k20me2).

Data availability
The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD009281, and are presented in Supplementary Table 1. Unprocessed images of all gels and blots (Figs. 1c,d and 5a,c and Supplementary Fig. 2h) are provided in Supplementary Fig. 5. Source data for all graphs are provided in Supplementary Table 2. All other data supporting the findings of this study are available from the corresponding authors on reasonable request.

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- Clearly defined error bars
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Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection | scanR acquisition software 2.6.1.0, SoftWoRX 6.5.2, Cellquest Pro 6.0.

Data analysis | GraphPad Prism7, scanR analysis software 2.6.1.0, SoftWoRX 6.5.2, Flowjo 8.8.7, MaxQuant 1.6.0.16, Rosetta 5.0.37 macromolecular modeling package (www.rosettacommons.org)

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All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

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The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD009181.
Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size
No statistical method was used to predetermine sample size. All experiments were included with multiple biological replicates based on previous experiences.

Data exclusions
No samples were excluded from analysis.

Replication
All results were tested and confirmed with at least two independent experiments

Randomization
No method of randomization was applied. Samples were organized into groups based on whether they were treated or not treated (IR/chemical treatment, over-expression by tetracyclin inducible system or transient plasmid expression, knockdown by siRNA)

Blinding
No blinding assessment was performed as microscopy and FACS data were analyzed automatically. Exactly the same gate setting was used for all samples in high-content microscopy as well as flow cytometry analysis.

Reporting for specific materials, systems and methods

Materials & experimental systems

| n/a | Involved in the study |
|-----|----------------------|
| ✗   | Unique biological materials |
| ✗   | Antibodies |
| ✗   | Eukaryotic cell lines |
| ✗   | Palaeontology |
| ✗   | Animals and other organisms |
| ✗   | Human research participants |

Methods

| n/a | Involved in the study |
|-----|----------------------|
| ✗   | ChIP-seq |
| ✗   | Flow cytometry |
| ✗   | MRI-based neuroimaging |

Unique biological materials

Obtaining unique materials
All cells and plasmids established in this study are available on reasonable request.

Antibodies

Antibodies used

| Target/Manufacturer/Catalogue number/Host/Dilution (Application)  |
|---------------------------------------------------------------|
| Immunofluorescence (IF), Western blot (WB)                     |
| HA/1867 423/Rat/1:200(IF)                                     |
| HA/BioLegend/901509/Mouse/1:100(IF)                           |
| HA/Covance/MMS-101P/Mouse/1:500(WB)                           |
| GFP/11 814 460 001/Mouse/1:500(WB)                            |
| SLF1/Novus Biologicals/NBP1-88358/Rabbit/1:500(WB)            |
| 53BP1/Santa Cruz Biotechnology/h-300/Rabbit/1:500(IF)         |
| BRCA1/Santa Cruz Biotechnology/sc-6954/Mouse/1:200(IF), 1:400(WB) |
| BARD1/Bethyl/A300-263A/Rabbit/1:500(IF)(WB)                   |
| BARD1/Abcam/ab64164/Rabbit/1:500(WB)                          |
| H4K20me3/Abcam/ab9051/Rabbit/1:250(IF), 1:10000(WB)          |
| H4K20me2/diagenode/C15200205/Mouse/1:250(IF), 1:5,000(WB)    |
| Phospho-H2AX (S139)/Cell Signaling Technology/2577/Rabbit/1:10000(IF) |
| OsTIR1/gift from Dr. Masato Kanemaki//Rabbit/1:10000 (WB)     |
| Beta-Actin/Sigma/A1978/Mouse/1:20000 (WB)                     |
**Eukaryotic cell lines**

Policy information about [cell lines](#)

| Cell line source(s) | HeLa S3: Dr. Pat Nakatani |
|---------------------|--------------------------|
|                     | U-2-OS: Dr. Jiri Bartek |
|                     | DR-GFP U2OS: Dr. Niels Mailand |
|                     | DR-GFP BARD1-null mouse mammary carcinoma cells: Dr. Richard Baer |
|                     | Flp-In T-Rex U-2-OS cells: Invitrogen |
|                     | HCT116: Dr. Ian Tomlinson |

**Authentication**

The Cell lines have been authenticated based on morphological criteria. BARD1-null mouse mammary carcinoma cell line was tested for BARD1 expression by western blot and confirmed the BARD1 deficient phenotypes can be rescued by exogenous BARD1 expression (22, and Fig. 4b and Supplementary Fig. 3d in this study). Flag-HA-BARD1 WT and ARD inducible cell lines (Flp-In T-Rex U-2-OS cells ) and HCT116 Tet-OsTIR1 cells, and BARD1 AID/ AID cell line were authenticated by western blotting and/or immunofluorescence (in this study).

**Mycoplasma contamination**

All cell lines were tested negative for mycoplasma.

**Commonly misidentified lines**

No commonly misidentified cell lines were used.

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**Flow Cytometry**

**Plots**

- Confirm that:
  - The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
  - The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a ‘group’ is an analysis of identical markers).
  - All plots are contour plots with outliers or pseudocolor plots.
  - A numerical value for number of cells or percentage (with statistics) is provided.

**Methodology**

**Sample preparation**

We used flow cytometry only for DR-GFP assay. Cells were trypsinized and suspended in PBS. Further details of the experimental procedures are provided in the Materials and Methods.

**Instrument**

FACSCalibur

**Software**

We analyzed the cells with CellQuest Pro (version 6.0) and analyzed the data with Flowjo 8.7.7.

**Cell population abundance**

N/A

**Gating strategy**

The FSC/SSC gates defined the single cell population and the percentage of GFP-positive cells in RFP positive population was calculated as HR repair efficiency. An RFP vector was used as a control for transfection efficiency. The gating strategy is provided in the Supplementary figure 3C.

- Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.