BRCA1/BARD1 site-specific ubiquitylation of nucleosomal H2A is directed by BARD1

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Mutations in the E3 ubiquitin ligase RING domains of BRCA1/BARD1 predispose carriers to breast and ovarian cancers. We present the structure of the BRCA1/BARD1 RING heterodimer with the E2 enzyme UbcH5c bound to its cellular target, the nucleosome, along with biochemical data that explain how the complex selectively ubiquitylates lysines 125, 127 and 129 in the flexible C-terminal tail of H2A in a fully human system. The structure reveals that a novel BARD1-histone interface couples to a repositioning of UbcH5c compared to the structurally similar PRC1 E3 ligase Ring1b/Bmi1 that ubiquitylates H2A Lys119 in nucleosomes. This interface is sensitive to both H3 Lys79 methylation status and mutations found in individuals with cancer. Furthermore, NMR reveals an unexpected mode of E3-mediated substrate regulation through modulation of dynamics in the C-terminal tail of H2A. Our findings provide insight into how E3 ligases preferentially target nearby lysine residues in nucleosomes by a steric occlusion and distancing mechanism.

Germline mutations in BRCA1 and BARD1 predispose carriers to breast and ovarian cancer. BRCA1/BARD1 is a large heterodimeric complex best characterized for its role in DNA double-stranded break (DSB) repair by homologous recombination. Its only known enzymatic activity is as an E3 ubiquitin (Ub) ligase, encoded by the heterodimeric N-terminal RING domains of BRCA1 and BARD1. A variety of putative cellular targets for BRCA1/BARD1-dependent E3 ligase activity have been identified, but the functional significance for most is not well understood. Furthermore, a role for the E3 ligase activity of BRCA1/BARD1 in DNA damage repair and tumorigenesis remains controversial. Nucleosomal histone H2A was recently discovered as a bona fide substrate for BRCA1/BARD1-dependent E3 ligase activity. The heterodimeric RING domains are sufficient for preferential mono-ubiquitylation of lysine residues 125, 127 and 129 on the flexible extreme C-terminal tail of canonical H2A and Lys123 in nucleosomes containing the macroH2A1 isoform. The non-E2-binding RING domain of BARD1 is required for this activity, and cancer-predisposing mutations in this domain specifically abrogate nucleosome binding and ubiquitylation. BRCA1/BARD1-dependent H2A ubiquitylation contributes to DNA DSB repair by homologous recombination and acts as a mark of transcriptional repression.

Two other RING E3 ligases, RNF168 and the Ring1b/Bmi1 complex, bind to the nucleosome via their RING domains and facilitate mono-ubiquitylation of distinct H2A lysine residues. RNF168 ubiquitylates H2A residues Lys13 and 15 in the N-terminal region of H2A, a signal that recruits the DSB repair factor 53BP1 to sites of damage to facilitate repair by non-homologous end joining. The heterodimeric E3 ligase Ring1b/Bmi1, a component of the Polycomb repressive complex 1 (PRC1), directly ubiquitylates H2A Lys119 in nucleosomes. This modification functions in transcriptional regulation and may also contribute to the regulation of DNA DSB repair. A crystal structure of the Ring1b/Bmi1 complex and an NMR-based model of the RNF168 E3-E2/nucleosome complexes reveal how these E3s direct site-specific H2A ubiquitylation. Surface-exposed polar residues in the RING domains orient the E3s on the face of the nucleosome, recognizing distinct regions of the H2A/H2B acidic patch. This directs the RING-bound E2 enzymes towards opposite ends of the nucleosome disc surface, placing the E2 active site directly over the H2A lysine residues targeted for modification. For RNF168 and Ring1b/Bmi1, the target lysines are located on the boundary of ordered regions of H2A, ~70 Å away from each other across the nucleosome disc surface (Fig. 1a,b).

The H2A lysines targeted by BRCA1/BARD1 (125/127/129) are near H2A/118/119 and reside in the fully disordered extreme C-terminal tail of H2A. The Ring1b/Bmi1 and BRCA1/BARD1 RING heterodimers are topologically similar and share several functional features. BRCA1, like Ring1b, directly interacts with E2–Ub conjugates and has a conserved nucleosome-binding ‘arginine anchor’ motif required to bind the nucleosome acidic patch. Two RINGs differ primarily at the base of their RING domains where Bmi1 has an extended loop that contacts the nucleosome surface. Structural comparison calls to question how these complexes differ in binding to nucleosomes and specifically target closely positioned H2A lysine residues (Fig. 1d) and specifically target closely positioned H2A lysine residues (Fig. 1e). In this Article, we report a cryo-EM structure of the BRCA1/BARD1 RING domain heterodimer with the E2 enzyme UbcH5c bound to the nucleosome. Together with biochemical and NMR spectroscopy data, the results reveal the basis for preferential ubiquitylation of H2A Lys125, 127 and 129. Our findings constitute the first structural analysis of the E3 ligase domain of BRCA1/BARD1 with any of its cellular targets and provide mechanistic insight into the process of site-specific ubiquitylation by RING-type E3 ligases.

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Fig. 1 | RING E3 ligases ubiquitylate H2A at distinct sites on the nucleosome. a, Location of primary target lysine residues (Cys, colored spheres) for BRCA1/BARD1 (K125/127/129, red), Ring1b/Bmi1 (K118/119, magenta) and RNF168 (K13/15, salmon) on one face of the nucleosome core particle (NCP; PDB 1KXS). b, Close-up of H2A (green) from a. The H2B α-c-helix is hidden for clarity. Residues past H2A Lys118 are disordered and generally not observed as density in X-ray or cryo-EM structures. c, Structural and sequence alignment of BRCA1/BARD1 (PDB 1JM7) and Ring1b/Bmi1 (PDB 2CKL) RING domains, with arrows pointing towards the nucleosome-binding regions of Ring1b and Bmi1. Bold residues in the sequence alignment are primary Ring1b and Bmi1 nucleosome-binding-loop residues. Asterisks denote arginine anchor motif residues. Yellow-highlighted cysteine residues are nearest analogous Zn²⁺-coordinating residues. Zinc atoms are shown in structures as colored spheres (partially overlapping), with zinc site I labeled for each RING domain. d, Native-gel electrophoretic mobility shift assays (EMSAs) measuring BRCA1/BARD1 and Ring1b/Bmi1 RING heterodimer binding to NCPs. The concentration of RING binding partner (indicated below the gels) differs between E3s. Data are representative of \( n = 2 \) (Ring1b/Bmi1) or \( n = 3 \) (BRCA1/BARD1) independent binding experiments. e, Nucleosome ubiquitylation assays (western blot for VSV-G tag on H2A) using either BRCA1/BARD1 or Ring1b/Bmi1 RING heterodimers and wild-type (WT), H2A Lys125/127/129Arg (3Kr), or H2A Lys118/119/125/127/129Arg (5Kr) NCP substrates. Data in e are representative of \( n = 2 \) independent experiments. Uncropped gel/blot images in d and e are available as source data.

Results
Reconstitution and cryo-electron microscopy structure of the BRCA1-UbcH5c/BARD1/nucleosome complex. To reconstitute the BRCA1-UbcH5c/BARD1/nucleosome complex, we generated a chimeric E3-E2 enzyme in which the RING domain of BRCA1 is genetically fused to UbcH5c by a flexible linker (referred to as the E3-E2 module; Extended Data Fig. 1b). We took this approach to circumvent the low affinity of UbcH5c for BRCA1/BARD1\(^{24}\), which could hinder the formation of a stable ternary complex with the nucleosome. The E2 enzyme UbcH5c was chosen because it has an activity in nucleosome ubiquitylation assays with BRCA1/BARD1 that is similar to that of other members of the UbcH5 (Ube2d) and Ube2e families and was used to characterize the Ring1b/Bmi1\(^{27}\) and RNF168\(^{26}\) nucleosome complexes (Extended Data Fig. 1c). Importantly, the E3-E2 module retained enzymatic activity and specificity for lysine residues 125, 127 and 129, validating its use for structural studies (Extended Data Fig. 1d,e). Consistent with a previous report\(^{12}\), Ub could not be added to the active site of UbcH5c\(^\text{Cys85Lys}\) in the context of the E3-E2 module to form a stable isopeptide-linked E3-E2-Ub moiety (Extended Data Fig. 2a). Serendipitously, we found that substitution of the E2 active-site cysteine to lysine stabilized the E3-E2/nucleosome interaction, allowing for purification of the complex by size exclusion chromatography (SEC; Extended Data Fig. 2b,c). This mutation appears to increase the association of the complex through non-specific, weak electrostatic interactions as a modest increase in ionic strength is sufficient to disrupt binding (Extended Data Fig. 2d,e). The complex was observed to have an approximate molecular weight consistent with an expected 2:1 binding stoichiometry by SEC coupled to multi-angle light scattering (Extended Data Fig. 2f). Owing to the stronger observed interaction of this complex, it was used for subsequent cryo-EM and NMR studies.

Although the complex co-purified on SEC, the E3-E2 module dissociated from the nucleosome upon cryo-EM grid preparation and was therefore stabilized by light crosslinking with glutaraldehyde immediately before vitrification. We obtained a cryo-EM reconstruction of the bound complex that refined to a global resolution of \( \approx 3.9 \) Å (3.4–7.8 Å) (Fig. 2a, Table 1 and Extended Data Figs. 3
and 4). The structure shows the E3-E2 module associated with one face of the nucleosome, consistent with recent cryo-EM structures of similar complexes expected to bind with 2:1 stoichiometry30,31. The polypeptide backbone is traceable in the density map for ordered regions of the nucleosome and the BRCA1/BARD1 RING domains. Density for the E2 enzyme UbcH5c is noisier, with missing regions in the C-terminal end furthest from the E3-E2 interface (Fig. 2a and Extended Data Fig. 4). Importantly, map quality was sufficient for unambiguous docking with full or partial side chain density for many key interface residues. Model building was accomplished by a combination of homology modeling, de novo structure prediction, rigid-body docking and all-atom refinement in Rosetta32,33 (Fig. 2b and Table 1). The resulting structure shows that the BRCA1/BARD1 RING domains straddle the H2BαC-helix, binding to both the H2A/H2B acidic patch and the H2B/H4 cleft (Fig. 2b). UbcH5c is bound to the BRCA1 RING in a canonical manner and is tilted away from the histone surface, terminating near the DNA ends without making specific interactions with the nucleosome.

**BRCA1/BARD1 RING-histone interactions.** The BRCA1 RING domain shares a common nucleosome-binding motif with Ring1b that is required for nucleosome ubiquitylation in in vitro activity assays27. Central to this motif is the BRCA1 Arg71 side chain that is observed to insert into a pocket in the nucleosome acidic patch composed of side chains from H2A residues Glu61, Asp90 and Glu92 (Fig. 3a). Our model predicts that the conserved neighboring Lys70 of BRCA1 interacts with the side chain of H2A Glu64 that is also observed in the Ring1b-histone interface. BRCA1 Arg71Ala was the only mutant tested that abrogated detectable nucleosome ubiquitylation and the Lys70Ala mutant was also severely affected (Fig. 3c). Binding to the nucleosome was also not detected for the BRCA1 Arg71Ala mutant using isothermal titration calorimetry (ITC; Fig. 3e and Extended Data Fig. 5h). These data identify Arg71 as the ‘arginine anchor’ common to many chromatin factors34. Mutation of BRCA1 RING domain residues surrounding Lys70 and Arg71 had diminishing effects on decreasing nucleosome ubiquitylation (Extended Data Fig. 5a,d,e).

To further validate the BRCA1/BARD1-histone interface, we tested the activity of BRCA1/BARD1 and Ring1b/Bmi1 with nucleosomes mutated at the H2A/H2B acidic patch. Consistent with previous reports27,28, the same canonical acidic patch surface is required for BRCA1/BARD1- and Ring1b/Bmi1-dependent nucleosome ubiquitylation (Extended Data Fig. 6a,b). Residues at the N-terminal end of the H2BαC-helix that line the nucleosome acidic patch are poised to make analogous interactions with residues on BRCA1/BARD1 and Ring1b/Bmi1 (Extended Data Fig. 6c). H2A ubiquitylation by BRCA1/BARD1 decreased similarly for H2B Glu105Ala and Lys108Ala nucleosome mutants. By contrast, nucleosome ubiquitylation by Ring1b/Bmi1 was nearly abrogated with an H2B Glu105Ala substrate, but was less affected by the Lys108Ala and His109Ala mutants (Extended Data Fig. 6d,e). These results show that BRCA1/BARD1 and Ring1b/Bmi1 have similar yet distinct interactions on the nucleosomal surface that facilitate specific H2A ubiquitylation.

Although the BRCA1 and Ring1b RINGs are structurally similar and use a common nucleosome-binding motif, the non-E2-binding RING domains of BARD1 and Bmi1 are more divergent (Fig. 1c). In the Ring1b-UbcH5c/Bmi1/nucleosome complex, Bmi1 binds to the C-terminal end of the H3α1-helix using an extended loop that is largely conserved among the six human Polycomb group RING finger proteins that form functional heterodimers with Ring1b35. Our structure reveals that, lacking this extended loop, BARD1 binds to the nucleosome H2B/H4 cleft using a novel interface in which the Trp91 side chain inserts into a pocket lined by residues H3 Phe78/Lys79, H4 Val70/Thr71/Glu74 and H2B Arg99 (Fig. 3b). This interaction is facilitated by a conformational change that extends

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**Fig. 2 | Cryo-EM structure of the BRCA1-UbcH5c/BARD1/nucleosome complex. a, Cryo-EM density of the BRCA1-UbcH5c/BARD1/NCP complex (semi-transparent surface) with an atomic model fitted. The density map is color-coded by chain using the scheme shown in b. b, Atomic model of the BRCA1-UbcH5c/BARD1/NCP complex. The Cα of H2A Lys118 and the active-site position of UbcH5c (Lys85) are depicted as spheres.**
the loop containing Trp91 towards the histone surface (Extended Data Fig. 7a). Mutation of BARD1 Trp91 to Ala sharply reduced nucleosome ubiquitylation and binding (Fig. 3d,e and Extended Data Fig. 5h). Importantly, Trp91 is not required for the structural integrity of the BARD1 RING, as the $^{1}H^{15}N$-transverse relaxation optimized spectroscopy–heteronuclear single quantum coherence (TROSY-HSQC) NMR spectrum of the BARD1 Trp91Ala RING heterodimer resembles that of wild-type BRCA1/BARD1 (Extended Data Fig. 5i). Nucleosomes with mutations in the BARD1 Trp91 binding pocket were substantially impaired toward BRCA1/BARD1 ubiquitylation (Fig. 3f), but had no or lesser effects with Ring1b/Bmi1 (Extended Data Fig. 6f,g). Our structure shows that H3 Lys79 lines the BARD1 Trp91 binding pocket and is positioned near the C-terminal cap of BARD1 helix 2 (Gly80/Thr81) (Fig. 3b and Extended Data Fig. 6h). Consistent with this, BARD1 Gly80Ala or H3 Lys79Ala mutants decreased nucleosome ubiquitylation activity (Fig. 3d and Extended Data Fig. 6i,j). Mutation of the surrounding residues in the BARD1 RING did not confer these effects (Extended Data Fig. 5b,f,g). Unexpectedly, mutation of H3 Lys79Ala enhanced Ring1b/Bmi1-dependent nucleosome ubiquitylation, indicating unique requirements for this residue between the two E3s (Extended Data Fig. 6j)).

As H3 Lys79 methylation is generally associated with actively transcribed chromatin33,34, we tested the activity of BRCA1/BARD1, Ring1b/Bmi1 and RNF168 for H3 Lys79m1 and Lys79m2 methyl-lysine analog nucleosomes. Ubiquitylation activity for these methylated substrates was significantly reduced for both BRCA1/BARD1 and Ring1b/Bmi1 compared to unmethylated nucleosomes, but unaffected for RNF168, which does not bind near H3 Lys79 (Fig. 3g and Extended Data Fig. 6k–p). This potential mode of histone-modification crosstalk suggests that BRCA1/BARD1-dependent nucleosome ubiquitylation may occur more frequently at non-transcribed genes. Histone H3 Lys79 methylation has also been implicated in recruiting 53BP1 for DNA DSB repair by non-homologous end joining47. As BRCA1/BARD1 occupancy at DSBs antagonizes 53BP1, we speculate that H3 Lys79 methylation status may further delineate chromatin substrates for these opposing DNA repair factors.

Next, we tested the nucleosome ubiquitylation activity of a series of BARD1 RING mutants found in the expert-curated Catalogue of Somatic Mutations in Cancer (COSMIC) database48. Missense mutations at BARD1 Pro89 have been reported in five patient samples. BARD1 Pro89 is in close proximity to the Trp91 interface and forms an adjacent interface with residues on the H2B α3- and αC-helices (Extended Data Fig. 5c). The BARD1 Pro89Ala mutant substantially decreased nucleosome ubiquitylation activity (Fig. 3h). A patient-derived mutant of the zinc-coordinating histidine residue His68Tyr also decreased nucleosome ubiquitylation. Other reported mutants from patients did not confer deleterious effects in vitro (Extended Data Fig. 5c,i,k). All BRCA1 and BARD1 mutants found to be highly defective in nucleosome ubiquitylation assays still retained intrinsic E3 ligase activity in E2−Ub lysine discharge assays, indicating that these residues play a critical role in the assembly of a productive RING/nucleosome complex (Extended Data Fig. 5i–o). Together, these data reveal the critical contributions of the non-E2-binding BARD1 RING domain in directing specific nucleosome ubiquitylation that is modulated by the methylation status of H3 Lys79 in vitro, and also uncover potential disease mechanisms for BARD1 RING mutations found in patients with cancer.

**BRCA1/BARD1 RING orientation keeps the UbcH5c active site away from H2A Lys119.** The Ring1b/Bmi1 and RNF168 RING complexes direct specific nucleosome ubiquitylation by binding to distinct histone surfaces and orienting the UbcH5c active site directly over H2A lysine residues 118/119 and 13/15, respectively. These lysine targets are on the boundary of ordered regions of H2A in the nucleosome. Because the H2A lysine residues (125, 127 and 129) that are modified by BRCA1/BARD1 are in a fully disordered region of the C-terminal tail that is not visible by X-ray crystallography or cryo-EM, the mechanism of site-specific ubiquitylation by this E3-E2 pair is not revealed directly.

In our structure, BRCA1-bound UbcH5c is removed from the histone surface compared to its location in the Ring1b-UbcH5c/Bmi1/nucleosome complex (Fig. 4a). Although the BRCA1 and Ring1b RINGs bind similarly, the BARD1 subunit is constrained closer to the nucleosome surface and shifted ~10 Å on an axis parallel to the H4 α2-helix compared to Bmi1 (Fig. 4b). This arrangement effectively tilts the BRCA1 E2 interaction site, orienting UbcH5c away from the nucleosome surface (Fig. 4c,d and Extended Data Fig. 7b,c). The result is that the Cx-Cx distance between the E2 active site of BRCA1-bound UbcH5c and H2A Lys118 (last ordered residue in the H2A C terminus) is increased to ~19 Å from ~9 Å for Ring1b-bound UbcH5c (Fig. 4a). Although elevated from the nucleosome surface, BRCA1-bound UbcH5c extends towards the DNA ends where the flexible region of the C-terminal tail of H2A emanates from the ordered histone surface. We propose that this increased distance from H2A Lys118 accounts for the observation

### Table 1 | Cryo-EM data collection, refinement and validation statistics

| BRCA1-UbcH5c/BARD1 nucleosome complex (EMD 22581, PDB 7JZV) |
|---------------------------------|------------------|
| **Data collection and processing** |
| Magnification | 130,000 |
| Voltage (kV) | 300 |
| Electron exposure (e−/Å²) | 90 |
| Defocus range (µm) | −0.7 to −2.7 |
| Pixel size (Å) | 1.05 |
| Symmetry imposed | C1 |
| Initial particle images (no.) | 1,072,535 |
| Final particle images (no.) | 21,479 |
| Map resolution (Å) | 3.9 |
| FSC threshold | 0.143 |
| Map resolution range (Å) | 3.4–7.8 |
| **Refinement** |
| Map sharpening B factor (Å⁻²) | −85 |
| Model composition |
| Nonhydrogen atoms | 14,339 |
| Protein residues | 1,093 |
| Nucleotides | 278 |
| Ligands | Zn⁺⁺(4) |
| R.m.s. deviations |
| Bond lengths (Å) | 0.012 |
| Bond angles (°) | 1.28 |
| **Validation** |
| MolProbity score | 0.80 |
| Clashscore | 1.02 |
| Poor rotamers (%) | 0.22 |
| Ramachandran plot |
| Favored (%) | 98.32 |
| Allowed (%) | 1.49 |
| Disallowed (%) | 0.19 |
that BRCA1/BARD1 is unable to efficiently ubiquitylate H2A Lys118 and 119 in the absence of its preferred lysine targets.

Unlike Ring1b-bound UbcH5c, our structure does not predict extensive interactions between BRCA1-bound UbcH5c and nucleosomal DNA (Extended Data Fig. 7d). Consistent with this, the presence of the E2 does not substantially alter the binding affinity of the BRCA1/BARD1 RING-nucleosome interaction measured by ITC (42 ± 12 μM versus 27 ± 5 μM in the absence and presence of UbcH5c, respectively; Extended Data Fig. 7e,f). By contrast, incorporation of UbcH5c in the Ring1b/Bmi1 nucleosome complex increased the affinity 10-fold from ~2.1 μM for the RING complex alone to ~0.23 μM for the E3-E2 module 27. Although the BRCA1/BARD1 and Ring1b/Bmi1 affinity measurements were conducted using different methods, the roughly 20-fold difference in affinity between RING/nucleosome complexes is supported by EMSA binding data (Fig. 1d). To further test the observed difference in E2 placement, we made a series of mutations in surface-exposed basic residues on UbcH5c that were shown to be important for E2 placement, we made a series of mutations in surface-exposed basic residues on UbcH5c that were shown to be important for E2 placement, we made a series of mutations in surface-exposed basic residues on UbcH5c that were shown to be important for E2 placement, we made a series of mutations in surface-exposed basic residues on UbcH5c that were shown to be important for E2 placement, we made a series of mutations in surface-exposed basic residues on UbcH5c that were shown to be important for E2 placement, we made a series of mutations in surface-exposed basic residues on UbcH5c that were shown to be important for E2 placement, we made a series of mutations in surface-exposed basic residues on UbcH5c that were shown to be important for E2 placement, we made a series of mutations in surface-exposed basic residues on UbcH5c that were shown to be important for E2 placement, we made a series of mutations in surface-exposed basic residues on UbcH5c that were shown to be important for E2 placement, we made a series of mutations in surface-exposed basic residues on UbcH5c that were shown to be important for E2 placement, we made a series of mutations in surface-exposed basic residues on UbcH5c that were shown to be important for E2 placement, we made a series of mutations in surface-exposed basic residues on UbcH5c that were shown to be important for E2 placement, we made a series of mutations in surface-exposed basic residues on UbcH5c that were shown to be important for E2 placement. These UbcH5c mutations impact Ring1b/Bmi1-dependent nucleosome ubiquitylation but have no or minimal effects on BRCA1/BARD1-dependent activity (Extended Data Fig. 7g,h). Maintenance of near WT-like activity in BRCA1/BARD1-containing nucleosome ubiquitylation assays supports our model in which UbcH5c is tilted away from the nucleosome surface. This changes the position of the BRCA1-bound UbcH5c active site compared to Ring1b-bound UbcH5c active site, preventing modification of H2A Lys119 while allowing ubiquitylation of lysine residues 125, 127 and 129 in the unstructured H2A C-terminal tail.

Lyase location in the flexible C-terminal tail of H2A dictates ubiquitylation efficiency. The H2A lysine targets of BRCA1/BARD1 are in a flexible region of the C-terminal tail that is not observed by density in our structure. This region is less conserved between isoforms than ordered regions of the histone 41 (Fig. 5a). We hypothesized that H2A residues Lys125, 127 and 129 are optimally located on the flexible C-terminal tail of H2A to sample the UbcH5c active site at high frequency. To test this, we measured the ubiquitylation efficiency of BRCA1/BARD1 for nucleosome substrates with single lysine residues placed throughout the C-terminal tail of H2A and a substrate extended by 10 residues via a flexible linker (referred to as +10-K). A sharp increase in nucleosome ubiquitylation efficiency was observed between lysines placed at positions 121 and 123 on the H2A tail (Fig. 5b,c). Ubiquitylation efficiency peaked with a single lysine at positions 125 and 127, then decreased for each subsequent position. The lysine residue at the end of a 10-residue extension was ubiquitylated with efficiency similar to that of a lysine at position 121. These data are in agreement with the linear distance of ~19 Å measured between the Cα atoms of H2A Lys118 and the active-site residue of UbcH5c (Fig. 5e,d). Together, these results indicate that a lysine residue must have an optimal reach from the ordered histone surface to sample the UbcH5c active site to be ubiquitylated efficiently. Although the native lysine at position 125 is ubiquitylated with highest efficiency in vitro, only lysine residues 127 and 129 were observed to be ubiquitylated in a previously reported cellular assay 41. This discrepancy may reflect a lack of detection by mass spectrometry or additional modes of substrate regulation that are not preserved in our unmodified recombinant nucleosome substrates.

Fig. 3 | BRCA1/BARD1 RING-histone interactions. a, Close-up view of the BRCA1-histone interface with the Arg71 side chain density shown. b, Close-up view of the BARD1-histone interface with the Trp91 side chain density shown. Relevant side chains in a and b are shown as sticks. c–d, Quantified nucleosome ubiquitylation assays using the indicated BRCA1 (c) and BARD1 (d) mutants. e, Binding of BRCA1/BARD1 (WT and indicated mutants) to NCPs measured by ITC. Data are representative of n = 2 experiments. ND, not detected. f–h, Quantified nucleosome ubiquitylation assays using BRCA1/BARD1 and the indicated H2B/H4 cleft mutant NCP substrates (f), H3 Lys79 methylation mimetic NCP substrates (g), and BARD1 COSMIC mutants (h). All quantified ubiquitylation activity data show the mean, and error bars indicate ±1 s.d. of n = 3 independent experiments for the 10 min (dark gray bars) and 30 min (light gray or colored bars) time points. Data for graphs in c, d and f–h are available as source data.
The C-terminal tail of H2A is uniquely flexible in complex with BRCA1-UbcH5c/BARD1. The altered position of UbcH5c in the BRCA1/BARD1 and Ring1b/Bmi1 nucleosome complexes suggests that the E2 may differentially impact interactions with the flexible C-terminal tail of H2A. Although the inherent flexibility of the H2A tails prevents observation in X-ray and cryo-EM structures, this property allows for detection by conventional NMR experiments that report on the chemical environment of backbone amide groups. We obtained backbone resonance assignments for human H2A residues 1–10, 120–122 and 125–129 in nucleosomes reconstituted with ²H¹⁵N¹⁵C-labeled H2A (Extended Data Fig. 8a,b). ²H¹⁵N-TROSY-HSQC spectra were collected for BRCA1-UbcH5c<sup>Cys85Lys</sup>/BARD1 binding to ²H¹⁵N-H2A-labeled nucleosomes (Fig. 6a, left). Substantial changes in chemical shifts were not observed. Instead, predominant spectral changes correspond to specific resonance broadening relative to unbound nucleosomes ($I_{\text{mm}}/I_{\text{ss}}$), primarily for H2A residues 120–122 and 125 (Fig. 6b). Resonances corresponding to residues 126–129 were relatively unaffected, with signal broadening similar to that measured for residues 1–10 in the N-terminal tail of H2A (the dashed trend line on the graph represents the mean $I_{\text{mm}}/I_{\text{ss}}$ for residues −1 to 10). Further titration of BRCA1-UbcH5c<sup>Cys85Lys</sup>/BARD1 caused uniform broadening of all observable NMR resonances, indicative of non-specific binding and formation of very high molecular weight complexes (Extended Data Fig. 8c,d). Spectra collected in the presence of BRCA1-UbcH5c/BARD1 with the WT E2 active site revealed similar trends with less resonance broadening, consistent with sub-stoichiometric binding or a shorter complex lifetime (Extended Data Fig. 8e,f).

Analogous spectra were collected for Ring1b-UbcH5c/Bmi1 binding to nucleosomes (Fig. 6a, right). Although the concentration is lower (30 μM Ring1b-UbcH5c/Bmi1 versus 60 μM BRCA1-UbcH5c<sup>Cys85Lys</sup>/BARD1) substantially greater resonance broadening was observed affecting more residues, particularly residues 120–127 (Fig. 6c). In addition, changes in chemical shifts are readily apparent for residues 121, 125 and 126, suggesting that Ring1b-UbcH5c/Bmi1 binding alters the environment of these residues to a greater extent in the nucleosome complex. Some of these effects may be attributed to tighter binding of the Ring1b-UbcH5c/Bmi1 complex. However, complementary experiments with nucleosomes reconstituted with ²H¹⁵N-labeled H3 showed robust and similar loss in intensity for both complexes due to global resonance broadening at these concentrations (Extended Data Fig. 8g,h). As the two E3-E2 complexes have approximately the same molecular weight, general signal broadening due to increases in complex molecular size was expected to be similar. These data are consistent with current models where UbcH5c in the Ring1b/Bmi1/nucleosome complex, located closer to the nucleosome surface and the emerging H2A tail, would have a greater impact on tail flexibility. Formation of the BRCA1-UbcH5c/BARD1/nucleosome complex

![Fig. 4 | Comparison to the Ring1b-UbcH5c/Bmi1/nucleosome complex. a. Close-up views of the BRCA1-UbcH5c/BARD1/NCP complex (left) and Ring1b-UbcH5c/Bmi1/NCP complex (PDB 4KBP, right) aligned by H2B on the bound face of the nucleosome. Complexes are shown side by side instead of overlaid, for clarity. Distance measurements are shown between the Cx of H2A Lys118 (green sphere) and the active-site position of the Cx Lys/Cys85 (purple sphere) of UbcH5c. All comparisons in this study are performed to the proximally bound side of the Ring1b-UbcH5c/Bmi1/NCP complex. b. View of the BARD1 and Bmi1 rING domains in the NCP complexes aligned by H2B. Only histones from the BrCA1-UbcH5c/BARD1/NCP model are depicted, BrCA1 and ring1b rING domains, with the arginine anchor residue side chains shown. c. Same alignment as in b comparing the location of the BARD1 and Ring1b rING domains, with the arginine anchor residue side chains shown. d. Same alignment as in b comparing the position of BRCA1-bound UbcH5c (dark purple) to Ring1b-bound UbcH5c (light purple). The active-site Ca position for each E2 is shown as a purple sphere, and the arrow between them highlights the shift of the active site of BRCA1-bound UbcH5c away from H2A Lys118 (green sphere). The Ring1b rING domain is hidden for clarity, and the BRCA1 rING is shown as a partially transparent red ribbon.](image-url)
Lysine position along the flexible C-terminal tail of H2A dictates ubiquitylation efficiency. **a**, Structure-based sequence alignment of H2A C-terminal tail regions from major histone H2A variants. A prime symbol denotes the C terminus of the isoform. MacroH2A and H2Ax have extensions with additional lysine residues. Lysine residues located at positions equivalent or C-terminal to His123 in H2A-2A (WT) are in bold, with an arrowhead indicating this position. Green underlined residues have been identified as targets of BRCA1/BARD1 in cells by mass spectrometry14,16. **b**, Representative nucleosome ubiquitylation assay using BRCA1/BARD1 and NCP substrates containing WT or mutant single-lysine H2A substrates. **c**, Quantification of nucleosome ubiquitylation activity assays from **b**. The sequence of the H2A C-terminal tail (118–129) is shown below the graph with the approximate distance from the Ca of H2A Lys118 to each single lysine position, assuming an inter-residue distance equivalent to a reported contour length of 3.8 Å per amino acid55. The bold and open-faced red and yellow values correspond to the radii of the mesh spheres shown in **d**. Plotted data show the mean, and error bars indicate ±1 s.d. of n = 3 independent experiments. P values were calculated using a two-tailed Student’s t-test compared to WT (∗P ≤ 0.05, **P ≤ 0.005; NS, not significant). **d**, The position of H2A Lys118 (Ca, green sphere) relative to the active-site position UbcH5c (Ca, purple sphere). Mesh spheres are centered on the Ca of H2A Lys118, with radii corresponding to the approximate linear reach of a fully disordered polypeptide to position 121 (red) or 123 (yellow) of H2A. Uncropped gels in **b** and data for the graph in **c** are available as source data.

Fig. 5 | Lysine location along the flexible C-terminal tail of H2A does somewhat restrict the flexibility of the H2A C-terminal tail closest to Lys119 on the ordered histone surface, while the extreme C-terminal region where lysine targets 127 and 129 is located retains native-like flexibility.

**Full-length BRCA1/BARD1 has increased activity and affinity for nucleosomes**. Although the RING domains of BRCA1/BARD1 are sufficient for binding and specific nucleosome ubiquitylation, the modest affinity of the BRCA1/BARD1 RING heterodimer for unmodified nucleosome substrates suggests that additional interactions may play a role. It has recently been suggested that the C-terminal region of BARD1 contributes to chromatin binding to facilitate DNA DSB repair42,43. Furthermore, both BRCA1 and BARD1 harbor DNA binding regions and interact with a variety of other chromatin-related factors44,45 (Fig. 7a). Using highly purified full-length human BRCA1/BARD1 that shows specificity for H2A Lys125, 127 and 129 in an unmodified nucleosome substrate, we observe faster H2A ubiquitylation kinetics and a greater than 100-fold increase in nucleosome-binding affinity compared to the RING heterodimer (Fig. 7b,c and Extended Data Fig. 9). This observation confirms that regions outside the RING domains of BRCA1/BARD1 contribute to interactions with nucleosomes in the context of H2A ubiquitylation. These auxiliary interactions are likely to provide additional layers of regulation and recruitment in vivo. Furthermore, it suggests that cancer-predisposing mutations outside of the RING domains may indirectly affect the nucleosome-specific E3 ligase functions of BRCA1/BARD1.

**Discussion**

We have used cryo-EM, NMR and biochemical methods to reveal the mechanism of site-specific nucleosomal H2A ubiquitylation by BRCA1/BARD1. Our structure shows that, although the BRCA1 RING binds to the nucleosome acidic patch using an arginine anchor motif nearly identical to Ring1b and other nucleosome-binding factors, BARD1 forms a distinct and novel interface with the H2B/H4 cleft that constrains the BARD1 RING closer to the histone surface than Bmi1 and causes an upward tilt of the BRCA1 E2 binding interface. The E3-E2 complex acts like a teeter-totter, elevating UbcH5c from the histone surface and distancing its active site away from H2A Lys119. This stance favors Ub transfer to Lys119 and promotes modification of lysines 125, 127 and 129 in the disordered tail of H2A, which have adequate reach distance from the histone surface to gain access to the UbcH5c active site in the complex. Another potential determinant of specificity suggested from NMR experiments is that the two heterodimeric E3-E2 complexes differentially restrict the conformation of the H2A C-terminal tail. The Ring1b-UbcH5c/Bmi1 complex, positioned closer to the nucleosome surface...
and the emerging H2A tail, limits the conformational space that can be explored by the C-terminal tail of H2A. This may inhibit H2A lysine residues 125, 127 and 129 from approaching the Ring1b-bound UbcH5c active site and actively prevent off-target modification by Ring1b/Bmi1. Conversely, the retention of native-like flexibility in the BRCA1-UbcH5c/BARD1/nucleosome complex ensures that Lys125, 127 and 129 are able to approach the E2 active site for Ub transfer. Currently, there are few data regarding the mechanism of substrate lysine approach to E2s, and especially in the case of specific mono-ubiquitylation of disordered substrate lysines. To our knowledge, all reported cases involve substrate lysine residues that are in more ordered protein regions and are observed to be positioned or stabilized near the E2~Ub active site by specific interactions between the

Fig. 6 | H2A C-terminal tail dynamics in E3-E2/nucleosome complexes. a, Overlay of 2H15N-TROSY-HSQC spectra of 2H15N-H2A in the NCP (black, both panels) with BRCA1-UbcH5cC85K/BARD1 added (red, left) or Ring1b-UbcH5c/Bmi1 added (pink, right). Binding experiments were performed using 15 μM 2H15N-H2A NCPS with 60 μM BRCA1-UbcH5cC85K/BARD1 or 30 μM Ring1b-UbcH5c/Bmi1 added. Arrows in both panels identify signals from the H2A C-terminal tail that change position or have increased signal loss in the Ring1b-UbcH5c/Bmi1/nucleosome complex spectrum. b, Quantification of H2A signal intensities from the BRCA1-UbcH5cC85K/BARD1/nucleosome complex in a, comparing the bound complex spectrum to the apo reference spectrum (Icom/Iref). The dashed trend line represents the mean broadening (Icom/Iref) from N-terminal tail residues −1 to 10. c, The same analysis as in b for the Ring1b-UbcH5c/Bmi1/nucleosome complex. Data in b and c are representative of n = 2 independent experiments. Data for graphs in b and c are available as source data.

Fig. 7 | Enhanced nucleosome ubiquitylation activity and binding of full-length BRCA1/BARD1. a, Domain illustration of full-length BRCA1 and BARD1, with a subset of putative chromatin and protein interactions indicated (ARD, ankyrin repeat domain; BRCT, BRCA1 C-terminal domain). b, Nucleosome ubiquitylation assay comparing the activity of the RING heterodimer (BRCA1RING/BARD1RING) to that of full-length BRCA1/BARD1 (BRCA1FL/BARD1FL). The E3 concentration was lowered to 100 nM to observe the ubiquitylation kinetics of BRCA1FL/BARD1FL. c, EMSA assay comparing nucleosome binding of BRCA1FL/BARD1FL to BRCA1RING/BARD1RING. The BRCA1/BARD1 concentration used in the binding experiments is reported under the gels and is ~100-fold lower for the full-length construct. Ubiquitylation activity and binding assays are representative of n = 2 independent experiments. Uncropped gels in b and c are available as source data.
E3/substrate complex or E2–Ub/substrate complex, and therefore are more similar to the mechanism of Ring1b/Bmi1 and RNF168 than BRCA1/BARD1.

In the structure, UbcH5c binds to the canonical E2 recognition surface of BRCA1. However, the quality of the cryo-EM density map for UbcH5c is better adjacent to BRCA1 subunit and diminishes toward the C-terminal end of the E2. This suggests that the E3/E2 interface is not rigidly fixed, consistent with the moderate affinity of the BRCA1–UbcH5c interaction. On binding, RING E3s, especially those that function with UbcH5c, generally promote a closed conformation of the E2–Ub conjugate to enhance Ub transfer to lysine side chains. As noted for the Ring1b/Bmi1 and RNF168 E3–E2/nucleosome complexes, the BRCA1–UbcH5c/BARD1/nucleosome complex is completely compatible with closed conformations of the E2–Ub conjugate (Extended Data Fig. 10). Indeed, tilting the UbcH5c active site away from the nucleosome surface in the BRCA1/BARD1/nucleosome complex would further remove steric restrictions that might impede formation of the closed E2–Ub conformation.

Although site-specific BRCA1/BARD1-dependent nucleosome ubiquitylation has been directly observed in vitro for canonical H2A from multiple species and macroH2A, other specialized H2A isoforms including the DNA damage specific H2A.x that colocalize with BRCA1/BARD1 at DNA DSB foci have lysine residues that are predicted to be efficiently ubiquitylated by BRCA1/BARD1 based on our model. Ubiquitylation of these different H2A isoforms in chromatin may delineate its role in various nuclear processes. Although BRCA1/BARD1 can multi-monoubiquitylate the tail of H2A in vitro, a single Ub genetically fused to the C terminus of H2A has been observed to complement defective BRCA1/BARD1 E3 ligase activity in DNA DSB repair and transcriptional regulation. Furthermore, the deubiquitylating enzyme USP48 was discovered to preferentially target the multi-ubiquitylated H2A–Ub product of BRCA1/BARD1 over Lys13/15–Ub or Lys19–Ub in vitro and to antagonize BRCA1 E3–ligase dependent activity at DSBs in cells. Further investigation is needed to better understand the biologically relevant H2A–Ub product of BRCA1/BARD1 in vivo and its signaling mechanisms. We envision that downstream chromatin factors will be specifically recruited to chromatin ubiquitylated by BRCA1/BARD1 in a manner similar to the PRC1/2 and RNF168/53BP1 systems.

Although our data explain the basis for site-specific nucleosome ubiquitylation by the BRCA1/BARD1 RING heterodimer, we show that the full-length protein has enhanced binding and activity towards a nucleosome substrate. Other regions of BRCA1 and BARD1 are implicated in binding to DNA, histone tails and histone modifications. BRCA1/BARD1 also exists as part of several discrete higher-order molecular assemblies, including with proteins known to specifically bind to nucleosomes such as PALB2. Our findings suggest that patient mutations in regions critical for these interactions could be pathogenic by indirectly affecting the nucleosome-specific E3 ligase activity of BRCA1/BARD1. Further investigation is needed to understand the chromatin landscape that recruits BRCA1/BARD1 to bind to nucleosomes to ubiquitylate H2A, and which BRCA1/BARD1 complexes are involved in these processes. Finally, our observation that a prevalent BARD1 RING mutation found in cancer patients—though not reported to be a causative agent of cancer—specifically and potentely disrupts H2A ubiquitylation supports the mounting evidence that BRCA1/BARD1-dependent nucleosome ubiquitylation is critical to its function as a tumor suppressor.

Online content
Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41594-020-00554-6.

Received: 28 August 2020; Accepted: 23 December 2020; Published online: 15 February 2021

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Methods

DNA manipulation and protein purification. The BRCA1(1–110-)(Gly/ Ser), UbC85K(2–147)-E3-E2 chimera were constructed using splicing by overlap extension (SOE) polymerase chain reaction (PCR)1. The Gly/Ser (GS) linker and an N-terminal tripeptide sequence (H3C) cleavage site were added by degenerate primers to prime sequencers. The assembled fragment was subcloned into a pCOT7n expression vector downstream of the 6×His-tag. After comparison of linker length by EMSA, a construct with sequence BRCA1(1–104)-GS-SGSGG-UbC85K(2–147) BARD1(26–140) with either the Cys85 or Cys85lys UbcH5c active site was selected for all subsequent biochemical, cryo EM and NMR experiments. An H3C cleavage site was also added onto the BRCA1(1–112) construct and cloned into the same pCOT7n expression vector. Untagged human H2A type 2A was inserted into pPET19 to make the 6×His-TEV-H2A construct (TEV, tobacco etch virus). A vesicular stomatitis virus glycoprotein (VSV-G) epitope tag was introduced on a primer to make the 6×His-TEV-H2A-GST-H3C construct. UbcH5c(2–147) was cloned into a modified pET28 vector downstream of a 6×His-small ubiquitin-like modifier (SUMO) tag. All mutants were generated by QuikChange site-directed mutagenesis (Agilent) or SOE PCR and subcloning. All constructs were verified by Sanger sequencing. Human Uba1 (E1), UbcH5a, UbcH5b, UbcH5c (untagged), Ube2c1, Ube2c2, Ube2c3, Ubc and BRCA1(1–112) BARD1(26–140) were purified as previously described3-10. GST-RNF168(1–189) was purified as previously described without clearing off the GST tag11. All BRCA1/BARD1 RING heterodimer constructs were expressed by co-transformation of two plasmids containing BRCA1 (pCOT7n vector) and BARD1 (pPET28n vector). For BRCA1(1–112)/BARD1(26–140) used in cryo-EM, ITC and NMR experiments, an additional step was taken to remove the 6×His protease on-column overnight as described for the BRCA1-UbcH5c/BARD1 E3-E2 chimera in detail below. The cleaved protein was concentrated before a final SEC step using a Superdex 75 column equilibrated in 20 mM HEPES-NaOH pH 7.5, 150 mM NaCl, 1 mM DTT on an Affi gel C protein fast liquid chromatography system (FPLC, GE Healthcare). Full-length BRCA1/BARD1 was co-expressed using the Bac-to-Bac expression system in H5 insect cells and purified as previously described in extensive detail11,12.

For E2 and E3 expression, Escherichia coli (BL21 DE3) cultures were grown to an optical density of 600 nm (OD600) of 0.6–0.8 in LB or MOPS media supplemented with 25 mM NH4Cl for NMR isotopic labeling (Cambridge Isotope Labs). The medium was supplemented with 100 μg/ml ZnCl2, for RING constructs. Protein expression was induced with 0.2 mM IPTG at 16°C for 18–18h. Bacterial pellets were collected by centrifugation, resuspended in lysis buffer (25 mM Tris-HCl pH 7.6, 500 mM NaCl, 10 mM imidazole) supplemented with SIGMAFAST EDTA-free protease inhibitor cocktail (Sigma) and 1 μM PMSF. Samples were passed through a French pressure cell, and clarified by centrifugation at 17,000 g at 4°C using a Sorvall Lynx 6000 centrifuge (Thermo Fisher). The supernatants containing 6×His-SUMO-UbcH5c or 6×His-H3C-BRCA1-UbcH5c/BARD1 were bound to a HiTrap Ni2+-NTA column (GE Healthcare). After extensive washing with lysis buffer containing 50 mM imidazole, proteins were cleaved on-column using GST-SENPI or GST-H3C protease (prepared in-house) overnight at 4°C in 25 mM Tris-HCl pH 7.6, 200 mM NaCl, 10 mM imidazole and 1 mM DTT, supplemented with protease inhibitors leupeptin, pepstatin A and aprotinin (1 μg/ml each). Cleaved protein was removed off the column, concentrated, and purified by SEC using a Superdex 75 column (GE Healthcare) equilibrated in 20 mM HEPES-NaOH pH 7.5, 150 mM NaCl, 1 mM DTT (diluted with 250 μM E2~Ub formation and lysine reactivity assays. For E2–Ub formation and lysis reactive assays. For E2 lysis discharge assays, E2–Ub was formed in a reaction consisting of 0.3 μM E1 (Uba1), 60 μM UbcH5c,
were performed using BLAST66 (NCBI). For structure-based alignment of H2A
50 mM NaCl and 0.5 mM TCEP in the presence of 10% D2O. Spectra of BRCA1/
were buffer-exchanged into 20 mM MOPS pH 7.0, 10 mM NaCl and 0.5 mM
prevent self-association in the absence of a NCP binding partner in low salt. NMR
concentration of all NMR samples was adjusted to 50 mM NaCl and supplemented
by 1:1 dilution into 4x SDS–PAGE loading dye (50 mM Tris, 1% SDS, 5% 2-mercaptoethanol) and analyzed by Coomassie-stained SDS–PAGE gel.

Isothermal titration calorimetry. For ITC analysis, all proteins were extensively
buffer-exchanged by dialysis into 20 mM HEPES-NaOH pH 7.5, 50 mM NaCl and
0.5 mM TCEP. Measurements were performed on a Microcal PEAQ-ITC system
(S.R.W., P.S.B., R.E.K. and S.R.W. and A.P. purified proteins, performed assays and analyzed data.
S.R.W., M.D.S., P.S.B. and R.E.K. conceived the project, designed the experiments and
under the supervision of C.C. S.R.W. and L.M.T. conducted and analyzed the NMR
biochemistry and is supported by the NIH (GM088055). W.Z. was supported by a V Scholar Grant
(V2019.Q13) from the V Foundation and a Young Investigator Award from the Max
and Minnie Tomerlin Voelcker Fund. F.D. was supported by the NIH (GM123089).

We thank S. Tan (Penn State University) for sharing Ring1b/Bmi1 plasmids and L. Kay
(University of Toronto) for sharing the 153-base-pair Wodin 601 repeat plasmid. We thank N. Zheng for his insightful feedback on the manuscript and sharing laboratory equipment; T. Hinds for assistance with ITC experiments; P. Hsu for advice on single-particle cryo-EM equipment; T. Hinds for assistance with ITC experiments; P. Hsu for advice on single-particle cryo-EM data processing; and L. Walls, A. Borst and D. Veesler for advice on single-particle cryo-EM and was added to a final concentration of 60 μM (more) to account for its weaker binding affinity. NMR samples with H2N–H3 NCPs were assembled identically to H2A-labeled samples. Resonance assignments for the N-terminal tail of H3 were directly transferred from a previous study47. Peak intensities were measured using NMView, and the intensity of resonances in the bound complex spectrum was compared to those in the apo reference spectrum (IM508, IM509).

Acknowledgements
We thank S. Tan (Penn State University) for sharing Ring1b/Bmi1 plasmids and L. Kay
(University of Toronto) for sharing the 153-base-pair Wodin 601 repeat plasmid. We thank N. Zheng for his insightful feedback on the manuscript and sharing laboratory equipment; T. Hinds for assistance with ITC experiments; P. Hsu for advice on single-particle cryo-EM equipment; T. Hinds for assistance with ITC experiments; P. Hsu for advice on single-particle cryo-EM and was added to a final concentration of 60 μM (more) to account for its weaker binding affinity. NMR samples with H2N–H3 NCPs were assembled identically to H2A-labeled samples. Resonance assignments for the N-terminal tail of H3 were directly transferred from a previous study47. Peak intensities were measured using NMView, and the intensity of resonances in the bound complex spectrum was compared to those in the apo reference spectrum (IM508, IM509).

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

Data availability
The cryo-EM map of the BRCA1-UbcH5c/BARD1/nucleosome complex has been deposited to the Electron Microscopy Data Bank under accession code EMDB2381 and the atomic model to the Protein Data Bank under accession code 7JZV. NMR chemical shift assignments were deposited to the Biological Magnetic Resonance Data bank under accession code 50604. Plasmid reagents generated in this study can be obtained upon request from the corresponding author. Source data are provided with this paper.

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Author contributions
S.R.W., M.D.S., F.S.B. and R.E.K. conceived the project, designed the experiments and analyzed data. S.R.W. prepared the cryo-EM sample, imaged and analyzed data with A.L.B. and J.M.H. under the supervision of J.M.K. D.P.E. built the atomic model under the supervision of F.D. with input from S.R.W. J.K. generated methylated nucleosomes under the supervision of C.C. S.R.W. and L.M.T. conducted and analyzed the NMR experiments. M.W. and W.Z. produced full-length BRCA1/BARD1 and performed binding assays. S.R.W. and A.P. purified proteins, performed assays and analyzed data. S.R.W., P.S.B. and R.E.K. wrote the manuscript with input from all co-authors.

Competing interests
The authors declare no competing interests.

Additional information
Extended data is available for this paper at https://doi.org/10.1038/s41594-020-00556-4.

Supplementary information
The online version contains supplementary material available at https://doi.org/10.1038/s41594-020-00556-4.

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Peer review information Nature Structural & Molecular Biology thanks the anonymous reviewers for their contribution to the peer review of this work. Peer reviewer reports are available. Beth Moorfield was the primary editor on this article and managed its editorial process and peer review in collaboration with the rest of the editorial team.

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120μM Ub, 10 mM MgCl2/ATP in 25 mM sodium phosphate, pH 7.0, 150 mM NaCl (PBS) at 37 °C for 30 min. The reaction was quenched by adding 50 mM EDTA, pH 7.4 for 10 min, and the lysine discharge reaction was initiated by a 1:1 dilution of the charging reaction on ice and supplemented with 50 mM lysine at pH 7.0 or 50 mM of the indicated E3 at 30 °C. Time points were taken by mixing the reaction 1:1 with 4x load dye without reducing agent, resolved on an SDS–PAGE gel and stained with Coomassie blue and visualized on a BioRad gel dock. Quantification was performed in ImageJ (NIH) by drawing a box around the E2–Ub conjugate and measuring the total area of signal and normalizing to the zero time point. E2 to the zero time points were performed using a reaction mixture of 40μM UbcH5c or BRCA1-UbcH5c/ BARD1 (WT or Cys85cys active site), 2μM E1 (UbA), 80μM Ub and 10 mM MgCl2/ ATP in 50 mM sodium phosphate pH 7.0, 150 mM NaCl (UbcH5c WT active site) or 50 mM N-Cytochexyl-3-aminopropanesulfonic acid (CAPS) pH10, 150 mM NaCl (UbcH5c Cys83lys active site). Reactions were quenched at the indicated time points by a 1:1 dilution into 4x SDS–PAGE loading dye (50 mM Tris, 1% SDS, 5% 2-mercaptoethanol) and analyzed by Coomassie-stained SDS–PAGE gel.

The cryo-EM map of the BRCA1-UbcH5c/BARD1/nucleosome complex has been deposited to the Electron Microscopy Data Bank under accession code EMDB2381 and the atomic model to the Protein Data Bank under accession code 7JZV. NMR chemical shift assignments were deposited to the Biological Magnetic Resonance Data bank under accession code 50604. Plasmid reagents generated in this study can be obtained upon request from the corresponding author. Source data are provided with this paper.

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Extended Data Fig. 1 | Specificity of BRCA1/BARD1-dependent nucleosome ubiquitylation and validation of the E3-E2 chimera. 

**a.** Western blot analysis of all four histone subunits from the same nucleosome ubiquitylation reaction. 

**b.** Native-gel EMSA measuring NCP binding of BRCA1-UbcH5c/BARD1 constructs with various Gly/Ser-repeat linker lengths. The E3-E2 chimera with a seven-residue linker was used for structure determination and all subsequent experiments. 

**c.** Nucleosome ubiquitylation assays using BRCA1/BARD1 with indicated E2 enzymes. 

**d.** Coomassie-stained gel under reducing or non-reducing conditions of an E2 charging reaction using the BRCA1-UbcH5c/BARD1 chimera. 

**e.** Nucleosome ubiquitylation activity of BRCA1/BARD1 with UbcH5c in trans or the BRCA1-UbcH5c/BARD1 chimera with wild-type (WT) or Lys125/127/129Arg (3KR) NCP substrates. Data in panels **c** and **e** are representative of n=2 independent experiments. Uncropped gels/blots in panels **a–e** are available as source data.
Extended Data Fig. 2 | Purification of a stable BRCA1-UbcH5C/BARD1/nucleosome complex. a, Coomassie-stained gel of an E2 charging reaction using UbcH5C^C85K and BRCA1-UbcH5C^C85K/BARD1. b, Size exclusion chromatography (SEC) of NCPs (black) with excess BRCA1-UbcH5C/BARD1 (purple) or BRCA1-UbcH5C^C85K/BARD1 (green) with 35 mM NaCl in SEC buffer. c, Coomassie-stained gels of fractions from SEC binding experiments shown in panel b. d, SEC of NCPs with excess BRCA1-UbcH5C^C85K/BARD1 with 100 mM NaCl (black) or 25 mM NaCl (green) in SEC running buffer. e, Coomassie-stained gels of fractions from SEC binding experiments shown in panel d. f, Size exclusion chromatography coupled to multi-angle light scattering (SEC-MALS) analysis of NCPs (black) and the BRCA1-UbcH5C^C85K/BARD1/NCP complex (green). Dashed lines report MALS molecular weight (MW) data. The MW value reported is the average MW from light scattering ± 1-s.d.

Uncropped gels in panels a, c, and e are available as source data.
Extended Data Fig. 3 | Cryo-EM processing workflow. a, Flow chart of data processing steps. Objects with dark borders represent solvent masks and are not density maps. b, Representative motion-corrected micrograph from data set. White scale bar is 100 nm.
Extended Data Fig. 4 | Cryo-EM validation and example density. a, Local resolution estimate from CryoSparc after non-uniform refinement at the FSC=0.143 cutoff plotted on the final density modified map of the whole complex (left) and close-up on the RING-histone interface (right). b, Half-map Fourier shell correlation curves from CryoSparc non-uniform refinement (red) and after density modification (blue). c, Euler angle distribution of refined particle subset used in final cryo-EM reconstruction. d, Main chain trace of the BRCA1 RING, BARD1 RING, four-helix bundle, and UbcH5c fit into the cryo-EM map. The active site position of UbcH5c (Lys85) Cα is shown as a sphere. e, Subset of important histone regions fit into the cryo-EM map with side chains shown (except for H2A C-terminal tail).
Extended Data Fig. 5 | See next page for caption.
Extended Data Fig. 5 | Extensive analysis of the BRCA1/BARD1 RING-histone interface. a, b, c, Close-up view of BRCA1 and BARD1 RING domains in the complex showing side chains of residues mutated in nucleosome ubiquitylation assays in panels d-g and j-k. d, Representative nucleosome ubiquitylation assay using the indicated BRCA1 RING mutants. e, Quantified nucleosome ubiquitylation assays for BRCA1 mutants shown in panel d. (f, g) Same experimental set-up as panels d and e using the indicated BARD1 mutants. h, ITC binding data for BRCA1/BARD1 and indicated mutants with NCPs. Summarized data are also shown in Fig. 3e. i, 1H15N-TROSY-HSQC spectra of wild-type (WT) BRCA1/BARD1 and BRCA1(WT)/BARD1(Trp91Ala). j, k, Same experimental set-up as panels d and e using BARD1 mutants reported on the COSMIC database. l, E2-Ub lysine discharge assay using the indicated BRCA1/BARD1 mutants. m, Quantified E2-Ub lysine discharge assays using the BRCA1/BARD1 mutants shown in panel l. n, o, Same experimental set-up as panels l and m using BARD1 COSMIC mutants. Quantified nucleosome ubiquitylation assay data show the mean and error bars are ±1-s.d. of n=3 independent experiments at 10-minute (gray bars) and 30-minute (colored bars) time points. Statistical analysis is compared to wild-type at the 10-minute time point. Quantified E2 lysine discharge data show the mean and error bars are ±1-s.d. of n=3 independent experiments at 2-, 6- and 10-minute time points (light, medium, and dark gray bars). Statistical comparisons are indicated with lines above the graphs. All p-values were calculated using a two-tailed Student’s t-test (* p≤0.05, ** p≤0.005, ns = not significant). Uncropped gels/blots in panels d, f, j, l, and n and data from graphs in panels e, g, k, m, and o are available as source data.
Extended Data Fig. 6 | See next page for caption.
Extended Data Fig. 6 | Comparison of nucleosome requirements for H2A-modifying E3s. a, Comparison of the conserved BRCA1 and Ring1b arginine anchor motif interactions (PDB: 4R8P). Complexes were aligned by H2B on the bound face of the NCP. H2A from the Ring1b-UbcH5c/Bmi1 nucleosome complex is shown in gray. b, Nucleosome ubiquitylation assays using the indicated H2A/H2B acidic patch NCP mutants with BRCA1/BARD1 (top) and Ring1b/Bmi1 (bottom). c, Comparison of interactions between the RING domains of BRCA1/BARD1 and Ring1b/Bmi1 with the H2B αC helix residues assayed in panels d and e. d, Representative nucleosome ubiquitylation assays using the indicated H2B αC helix NCP mutants with BRCA1/BARD1 (top) or Ring1b/Bmi1 (bottom). e, Quantified assays using the NCP mutants and E3s from panel d, f, g, Same experimental set-up as in panels d and e using H2B/H4 cleft NCP mutants. h, BARD1-histone binding interface with side chain density for H3 Phe78 and Lys79 shown as semi-transparent surface. i, j, Same experimental set-up as panels d and e using H3 Lys79Ala NCP mutant. k, l, m, Representative nucleosome ubiquitylation assays using H3 Lys79me1 and Lys79me2 mimetic NCP substrates with the indicated E3s. n, o, p, Quantified assays using H3 Lys79 methylation mimetic nucleosome substrates and the indicated E3s. Quantified ubiquitylation assays show the mean and error bars are ± 1-s.d. of n=3 independent experiments (except panel g where n=6 for wild-type) plotted for 10-minute (gray bars) and 30-minute (colored bars) time points. P-values for each mutant were calculated using a two-tailed Student’s t-test compared to wild-type ( * p≤0.05, ** p≤0.005, ns = not significant). Panels g (top), and n are also shown in Fig. 3. Uncropped blots in panels b, d, f, i, k, l, and m and data from graphs in panels e, g, j, n, o, and p are available as source data.
Extended Data Fig. 7 | See next page for caption.
Extended Data Fig. 7 | Comparison to the unbound RING heterodimer and Ring1b-UbcH5c/Bmi1/nucleosome complexes. a, Structural alignment of the BRCA1/BARD1 RING heterodimers from the NCP complex and the unbound structure (PDB: 1JM7, NMR ensemble model 1) aligned by the four-helix bundle. b, Structural alignment of the NCP from the indicated complexes by H2B on the E3-E2-bound side. c, Structural alignment of BRCA1-UbcH5c and Ring1b-UbcH5c from E3-E2/NCP complexes. d, Comparison of BRCA1-bound and Ring1b-bound UbcH5c in NCP complexes showing side chains mutated in panels g and h. Some atoms for Arg72 and Lys128 are not modelled in the Ring1b-bound structure. Green spheres are Ca of H2A Lys118. e, Analysis of NCP binding by the BRCA1/BARD1 RING heterodimer and the BRCA1-UbcH5c/BARD1 (WT UbcH5c active site) chimera by ITC. Data are representative of n=2 experiments. f, Comparison of NCP binding strength for BRCA1/BARD1 and Ring1b/Bmi1 RING heterodimer and E3-E2 chimeras. Affinities for BRCA1/BARD1 were determined using ITC while affinities for Ring1b/Bmi1 were measured using a fluorescence based binding assay27. g, Representative nucleosome ubiquitylation assays using BRCA1/BARD1 or Ring1b/Bmi1 with indicated UbcH5c mutants. h, Quantification of nucleosome ubiquitylation using the UbcH5c mutants and E3s from panel g. Data show the mean and error bars are ± 1-s.d. of n=3 independent experiments plotted for 10-minute (gray bars) and 30-minute (colored bars) time points of the reactions. P-values for each mutant were calculated using a two-tailed Student’s t-test compared to wild-type (* p≤0.05, ** p≤0.005, ns = not significant). Uncropped blots in panel g and data from graphs in panel h are available as source data.
Extended Data Fig. 8 | See next page for caption.
Extended Data Fig. 8 | NMR analysis of E3-E2/nucleosome complexes. 

a, 1H15N-TROSY-HSQC spectrum of 2H15N13C-H2A in NCP (~180 µM sample) with residue assignments plotted. Signals with asterisks were not able to be assigned. Residues 120\* and 120\*' appear to be alternate confirmations of 120 based on C\(\alpha\) chemical shifts of residues 119 and 120 in the HNCA and HNCOCA spectra. Assignment of the minor population for 6\* was based on the similar backbone H, N, and C\(\alpha\) chemical shifts as compared to the major residue 6 population. 

b, Sequence of H2A N- and C-terminal tails observable in NMR spectrum in panel a with assigned residues bolded. 

c, Overlay of 1H15N-TROSY-HSQC spectra of 2H15N-H2A in NCP, with BRCA1-UbcH5\(\text{cexp}\)/BARD1 added at increasing concentrations. 

d, Quantification of signal broadening from spectra in panel c, as a function of BRCA1-UbcH5\(\text{cexp}\)/BARD1 added, normalized to the first titration point (60\(\mu\)M addition). Signal behavior clusters into two groups, where resonances from the extreme N- and C-terminal tails lose signal at a similar rate (cluster 1). 

e, Overlay of 1H15N-TROSY-HSQC spectra of 2H15N-H2A in NCP (black, bottom), with BRCA1-UbcH5\(\text{cexp}\)/BARD1 added (red, middle), or BRCA1-UbcH5c/BARD1 (blue, top). 

f, Quantification of intensity for H2A signals from spectra in panel e comparing the bound complexes to the apo reference spectrum (I\(\text{com}/I_{\text{ref}}\)). 

g, Overlay of 1H15N-TROSY-HSQC spectra of 2H15N-H3 in NCP (black, bottom), with BRCA1-UbcH5\(\text{cexp}\)/BARD1 added (red, middle), or Ring1b-UbcH5c/Bmi1 (pink, top). 

h, Quantification of intensity for H2A signals from spectra in panel g comparing the bound complexes to the apo reference spectrum (I\(\text{com}/I_{\text{ref}}\)). Concentrations of E3-E2 complexes added as well as labelled NCPs in the experiment are indicated in the figure. Data from graphs in panels d, f, and h are available as source data.
Extended Data Fig. 9 | Biochemical purity and H2A specificity of full-length BRCA1/BARD1. a, Coomassie-stained gel of purified full-length BRCA1/BARD1. b, Nucleosome ubiquitylation assay using full-length BRCA1/BARD1 and wild-type (WT) or H2A Lys125/127/129Arg (3KR) nucleosome substrates. Uncropped gels/blots in panels a and b are available as source data.
Extended Data Fig. 10 | Compatibility of closed E2-Ub conformations. a, Structural overlay of the BRCA1-UbcH5c/BARD1/nucleosome complex with indicated RING/E2-Ub crystal structures aligned to the RING domain of BRCA1. b, Comparison of UbcH5c location in the nucleosome complex (purple) and indicated reported RING/E2-Ub structures from panel a with cryo-EM density for UbcH5c shown as gray volume. Density for the E2 is shown from a map that was locally filtered in CryoSparc to decrease excessive noise introduced from map sharpening in this region.
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Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

Leginon [automated cryoEM data collection software], TopSpin 3.2 (Bruker; NMR data collection), Malvern PEAQ-ITC control software [v1.30]

Data analysis

Cryo-EM data: MotionCorr2 (v1.0.5), CtfFind4 (v4.1.9), DoG Picker, cisTEM (v1.0.beta), Relion (v3.0), CryoSpacc (v2.15), Phenix (v1.18.2-3874); Model building: Rosetta, Rosetta/pyRosetta 2020.36+HEAD_fd1c8ced991, trRosetta 1.0, hhsuite 3.1.0, NMR data: NMRpipe (v10.9), NMRdraw, NMR View (v9.2b20); ITC: Malvern PEAQ-ITC analysis software (v1.30), Molecular visualization: UCSF chimera 1.15, U UCSF Chimera X 0.93

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The cryo-EM map is deposited to the EMBD under accession code EMD-Z2581 and the associated atomic model to the PDB with ID: 712V. NMR chemical shifts were deposited to the BMRB under accession code 50650.
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☑️ Life sciences    ☐ Behavioural & social sciences    ☐ Ecological, evolutionary & environmental sciences

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Life sciences study design

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

| Sample size | A sample size of 3,164 cryo-EM micrographs was deemed sufficient to provide enough particles to achieve a high resolution cryo-EM reconstruction. |
|-------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Data exclusions | Particles that did not contribute to a high-resolution cryo-EM reconstruction were excluded in a series of 2D and 3D classification steps, as is standard practice in single-particle cryo-EM. No other data were excluded from this study. |
| Replication | Ubiquitylation assays were performed using two or three independent replicate experiments to ensure reproducibility and facilitate quantification of an effect for a particular mutant. Isothermal titration calorimetry and electrophoretic mobility shift binding experiments were repeated at least twice to ensure reproducibility. |
| Randomization | No randomization was performed in this study. |
| Blinding | No blinding was performed in this study. |

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

| Materials & experimental systems | Methods |
|---------------------------------|---------|
| n/a | Involved in the study |
| ☐ | Antibodies |
| ☑ | Eukaryotic cell lines |
| ☑ | Paleontology and archaeology |
| ☑ | Animals and other organisms |
| ☑ | Human research participants |
| ☑ | Clinical data |
| ☑ | Dual use research of concern |
| ☑ | ChiP-seq |
| ☑ | Flow cytometry |
| ☑ | MRI-based neuroimaging |

Antibodies

Antibodies used:
- mouse anti-VSV-G (Sigma V4888, lot #058M4781V), rabbit anti-H2A (EMD millipore 07-146, lot #3023001), rabbit anti-H2B (BioRad VPA00384, lot #160309), rabbit anti-H3 (Abcam ab1791 2650, lot #GR322435-1), mouse anti-H4 (Abcam ab31830, lot #GR3204774-2), goat anti-mouse Alexa Fluor 680 (Invitrogen A21058, lot # 1834812), anti-rabbit Dylight 800 (Cell Signaling 51515, lot #I2)

Validation
- No additional validation was performed on the commercially available antibodies used in this study in addition to manufacturers validation. All antibodies were used in the context of ubiquitylation reactions using highly purified components.