Supplementary Note 1: Cryo-EM Methods

Sample preparation and image acquisition

BRCA1-UbcH5c^{C85K}/BARD1 (200 μM) was mixed with NCPs (30 μM) at a final volume of 400 μL in 20 mM HEPES-NaOH pH 7.5, 35 mM NaCl, 1 mM DTT (reconstitution buffer) and incubated at room temperature for 30 mins. The complex was purified using a Superdex 200 increase 10/300 column (GE Healthcare) equilibrated in reconstitution buffer. Pooled fractions were concentrated to ~30 μM (Amicon) and stored on ice for up to two days. The complex was crosslinked by diluting 10 μL of concentrated complex directly into 80 μL of reconstitution buffer supplemented with 0.1% glutaraldehyde (Electron Microscopy Sciences) and incubated at room temperature for 10 minutes. Crosslinking was quenched by addition of 10 μL of 500 mM Tris-HCl pH 7.5, then buffer exchanged into reconstitution buffer using a pre-equilibrated 0.5 mL Zeba desalting spin column (GE Healthcare). The crosslinked complex (3 μL at ~2.5 μM) was immediately applied to glow-discharged C-Flat 400 mesh copper 2/2 grids (Protochips), blotted for 4 seconds with an FEI Vitrobot (20 °C, 100% humidity) and plunged into liquid ethane. Grids were stored under liquid nitrogen until imaging. Data were collected in two sessions on a Titan Krios (FEI) operating at 300 kV at a nominal magnification of 130,000x and equipped with a Quantum GIF energy filter (Gatan) operating in zero-loss mode with a 20 eV slit width. Movies were recorded on a K2 Summit Direct Detection camera operating in super-resolution mode with a calibrated pixel side of 0.525 Å per super-resolution pixel (1.05 Å physical pixel) and fractionated into 50 frames over a 10 second exposure with a total fluence of 90 $e^-/Å^2$ corresponding to a dose rate of 8.9 $e^-/Å^2/s$ using a defocus range of -0.7 to -2.7 μm. Leginon software was used for automated data collection.

Image processing and reconstructions

As data were collected in two sessions, dataset 1 was first used to generate an initial model, then combined with dataset 2 to generate the final structure. For initial model generation, pre-processing was performed in the Appion framework[1]. Movie frame alignment and dose-weighted summation of all 50 frames from 675 movies was performed using MotionCor2 binning images by a factor of two (1.05 Å per pixel)[2]. CTF parameters were estimated using CtfFind4[3]. Particles were automatically picked using DoG Picker[4], extracted using a box size of 256 pixels^2 binned to 128 pixels^2, and subjected to two rounds of 2D classification in Relion 3.0[5]. A refined particle subset was un-binned, imported into CryoSparc[6], and used for ab-initio reconstruction with 4 classes. A class representing the bound complex was selected, low-pass filtered to 40 Å, and used as an initial model for initial 3D classification in Relion 3.0 using the full-dataset as described below.

For structure determination, 675 movies from dataset 1 and 2,489 movies from dataset 2 were selected after manually inspecting the motion-corrected micrographs and their respective power spectra for vitreous ice quality and isotropic frame alignment. Movie frame alignment and dose-weighted summation of all 50 frames was performed using the Relion 3.0 implementation of MotionCor2 binning images by a factor of two (1.05 Å per pixel). Motion-corrected micrographs were imported into cisTEM for the remaining pre-processing steps[7]. CTF parameters were estimated using CtfFind4. Particles were picked using the reference-free ab initio blob-picker algorithm in cisTEM and particles over carbon areas were manually de-selected. Particles were extracted in a box size of 324 pixels^2 and subjected to two rounds of reference-free 2D-classification in cisTEM that produced a refined subset of 731,086 particles. Particles were imported into Relion 3.0 and re-extracted using a box size of 256 pixels^2 binned to 128 pixels^2. 3D classification into six classes was performed using the initial model from dataset 1 described above. One class containing ~263,000 unbound nucleosome particles was selected and sub-classified into five classes. About 58,000 particles from a well-aligning class were re-extracted into a 256 pixels^2 box without binning and 3D refined with C1 symmetry to and estimated global resolution of ~3.7 Å. All particles that did not fall into the unbound nucleosome class (~468,000) were subjected to another round of 3D classification into three classes. A prominent BRCA1-UbcH5/BARD1/NCP bound class with ~199,000 particles was selected and re-extracted using a box

size of 256 pixels[2] without binning. Particles were subjected to 3D refinement without imposing symmetry using the map of the complex from 3D classification as a new initial model and a soft mask encompassing the entire bound complex. This ensured that all bound E3-E2 particles were aligned on the same side of the pseudo-symmetrical NCP. The resulting map was well-aligned on the NCP while the E3-E2 density was low-resolution and noisy. Another round of 3D classification with four classes and regularization parameter T=20 was performed without refining particle Euler angles and shifts and using a soft mask that encompassed only the E3-E2 and some interface histone density. A class containing ~29,000 particles with well-defined BRCA1/BARD1 and UbcH5c density was selected and subjected to 3D refinement with a soft mask encompassing the entire E3-E2/NCP complex. CTF-refinement and Bayesian polishing were iteratively performed in Relion 3.0. Particles were imported into CryoSparc and subjected to one round of ab initio 3D-classification, yielding 21,479 particles with better-defined density in the BARD1 RING domain. The final particle subset was subjected to non-uniform (NU) refinement in CryoSparc[8] with C1 symmetry and a soft mask encompassing the whole complex. Local resolution from the non-uniform refinement half-maps was estimated at the 0.143 FSC cut-off using CryoSparc. Global resolution was estimated using half-maps from CryoSparc NU-refinement or density modification sharpening using the 3D-FSC server[9].

To address large variations in local-resolution, post-processing was carried out using Phenix density modification for cryo-EM[10]. Local sharpening was accomplished by setting the "blur_by_resolution_factor = 25" to decrease over sharpening artifacts. The input for density modification was both half-maps, the full map, the refined solvent mask from CryoSparc non-uniform refinement, along with a molecular weight of 240,000 Da. A schematic flowchart of cryo-EM image processing strategy is outlined in Extended data Fig. 4, and additional details of cryo-EM data collection and structure determination are provided in Table I.

Model building

The structure of BRCA1-UbcH5c fusion and the nucleosome core particle were built using only comparative modeling, while the structure of BARD1 required additional de-novo modeling in order to properly accommodate the cryoEM density. To model the NCP, the Rosetta partial_thread application was used with alignments generated from hhblits and hhalign[11] to thread the histone sequences onto the template pdb 3LZ1. This threaded model was then docked unambiguously into the cryoEM density using UCSF Chimera[12]. To model the BRCA1-UbcH5c fusion, a multi-step process was used where initially comparative models were built for each domain separately without density. Hhblits was used to search for homology modeling templates for BRCA1 and UbcH5c separately, and then using the partial_thread application and the hhblits alignments the target BRCA1 and UbcH5c sequences were threaded onto each of the templates. The selected BRCA1 templates used were (PDB id, chain id), (1JM7, A), (2C2L, C), (6QU1, A) and the selected UbcH5c templates were: (PDB id, chain id), (4R8P, N), (5LBN, A), (2PE6, A), (4JUE, D), (2R0J, A), (2UCZ, A), (5A31, Q), (1AYZ, A), (2F4Z, A), (6CP0, B), (3VON, S), (3ONG, B), (4Q5E, C), (1YRV, A), (5BNB, B), (5V0R, A), (2KJH, A), (3FN1, B), (2EDI, A), (4YII, U). Then comparative models were made for both BRCA1 and UbcH5c by generating 2000 models with RosettaCM[13] using their respective templates. The top scoring model for both peptides were selected based on their Rosetta energy and each was docked into the density using UCSF Chimera.

The initial model of BARD1 was generated using a slightly modified version of the methods used on BRCA1 and UbcH5c. In addition to using hhblits to search for templates, trRosetta[14] was used in order to predict the structure of the BARD1 model. The trRosetta prediction, was then used as a template in addition to the hhblits discovered templates (PDB id, chain id) (4CFG, A), (1JM7, B), and (2C2L, A) for RosettaCM. Again, 2000 models were generated with RosettaCM, the top scoring model was selected based on the rosetta energy and then was docked into the density using UCSF Chimera. After building coarse models that approximately fit the cryoEM density RosettaES[15] was used to refine residues 75-100 of BARD1, 36-40 of BRCA1, and to extend the N-terminus of H4 on the bound side of the NCP to residue 16. Next, RosettaCM was used to refine the entire complex in addition to closing the loop

between BRCA1 and UbcH5c. 200 models were built, and the top scoring model as selected by Rosetta energy was selected.

Finally, the zinc ions were added with a custom pyRosetta script. Briefly, the script enforces the tetrahedral geometries of the cysteine and histidine residues that coordinate the Zn ions in addition to deprotonating the coordinating atoms. Histidine tautomers were selected based on Brzovic et al.[16], and then a combination of angle, distance, and dihedral constraints with values selected based on Tuow et al.[17], were added as restraints throughout Rosetta's FastRelax protocol[18]. Due to the inability of Rosetta to properly accommodate the heterogeneous density, the script also works to freeze side chains in the high/low resolutions (manually selected) so that incorrect rotamers would not be sampled separately at different interpreted densities and subsequently applies the Rosetta FastRelax. The script is available in the Rosetta macromolecular modeling package at: Rosetta/pyrosetta_scripts/apps/metal_coordination/danpf/brca1bard1-modeling/brca1bard1_model.py.

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