Supplementary Methods

Generation of transgenic lines

pSB-P_{brd-1}:brd-1::tag was generated as previously described (Polanowska et al., 2004) by inserting a 5.5 kb PCR fragment amplified from K04C2 cosmid DNA using the following primers: brd-1 forward GGGGACAAGTTTGTACAAAAAAGCAGGCTTTAATCCACACAGAGATCTGC and brd-1 reverse GGGGACCACTTTTGTAAGAAAGCTGGGTGACAAAATGCTCAATTATTAT CGGA. This PCR product was cloned by Gateway® recombination into p221 ENTRY and subsequently into pSB-GW::tag to generate pSB-P_{brd-1}:brd-1::tag, as previously described (Polanowska et al., 2004). Transgenic line were generated by microparticle bombardment of unc-119(ed3) animals with pSB-P_{brd-1}:brd-1::tag (Polanowska et al., 2004).

Purification of CeBCD complex by tandem immunoaffinity

The dwIs3 (P_{brd-1}:brd-1::tag) transgenic line was grown to high density in a 60L BioFlo5000® fermenter, harvested using a Cepa continuous centrifuge and lysed in CSK buffer [100mM Pipes (pH 6.0), 200mM NaCl, 3mM MgCl₂, 1mM EGTA, 1mM DTT, 1mM PMSF, 0.3M sucrose, 0.5% triton X 100, complete (Roche) protease inhibitor tablets, and phosphatase inhibitors: 2mM β-glycerophosphate, 5mM NaF, 1mM Na₃VO₄, 0.1μM okadaic acid]. Lysis was performed by a single freeze/thaw cycle followed by homogenization. Soluble supernatant fraction (S) was collected by centrifugation and the chromatin bound fraction (C) was extracted from the pellet by microccocal nuclease (Roche) treatment (3U/μl). Western blotting with antibodies to Histone H4 (1:5000; Alain Verrault) and tubulin (1:200; Sigma) were performed to assess the separation of extracts into soluble and chromatin fractions (Fig. S1A, B). Tandem immunoaffinity purification of the native CeBCD complex was performed using MAb12CA5 (HA) then MAb9E10 (Myc) antibody affinity columns and then eluted from the final column by cleavage of the TAG using recombinant TEV protease (Invitrogen). For detailed methods see (Polanowska et al., 2004).

Cytological preparation and immunostaining

Gravid hermaphrodites were transferred to 30μl PBS on a poly-L lysine coated slide (slides were washed in 70% ethanol, then given 2 coats of 100% poly-L-lysine, air drying between each coat). The worms were washed in PBS before transferring to 50μl 10mM levamisole. Germlines were extruded by removing the head and tail using a fine gauge needle (27G). Levamisole was replaced with 1% paraformaldehyde (PFA) in PBS for 10 minutes and germlines were permeabilised for 5 minutes in TBSBT
(TBS + 0.5% BSA + 0.1% Triton X-100), then washed in TBSB for at least 2 x 5 minutes, followed by blocking for 30 minutes. Primary antibodies were diluted in TBSB (1:200 for RAD-51 (Alpi et al., 2003), 1:50 for CeBRC-1, 1:500 for CeBRD-1 and 1:1000 for FK2 (Affiniti Research Products)) and incubated overnight at 4°C in a humid chamber. Germlines were subsequently washed at least 3x 5 minutes in TBSB before incubation with the secondary antibody for 1-2 hours at room temperature (anti-rabbit Cy3 1:10000; anti-mouse FITC 1:10000 (Sigma)). Finally germlines were washed at least 3x 5 minutes in TBSB before mounting with a cover slip on Vectashield containing DAPI (Vector Laboratories). Human cells were washed on coverslip in PBS, fixed with 4% PFA for 10 minutes, washed in PBS and then permabilized with 0.5% triton in PBS for 15 minutes. Cells were washed thoroughly in PBS before blocking with 2% BSA in PBS for 30 minutes. Primary antibodies were incubated for 1 hour at room temperature, before extensive washing in PBS, followed by addition of the secondary antibody for 1 hour at room temperature. Cells were extensively washed in PBS, washed a single time in PBS + DAPI, washed again in water before mounting on a slide with ImmuMount (Thermo, UK). Antibodies were diluted in 2% BSA in PBS (Conjugated-Ub antibody FK2 was used at 1:10000 (Affiniti, UK), mouse secondary FITC at 1:100 (Sigma); Mouse H2AX was used at 1:500 (Upstate, JBW301), mouse secondary FITC at 1:5000 (Sigma)).

**Immunoprecipitation from MCF7, Hela, Nbs1−/−, A-T and ATR-Seeckel cells**

Cultured cells were washed once in PBS and lysed in RIPA buffer (50mM Hepes pH 7.5, 150 mM NaCl, 10% glycerol, 0.1% triton X-100, 0.5mg/ml BSA, 1 mM PMSF) supplemented with complete (Roche) protease inhibitors tablets and 5U DNaseI (Sigma). 300μg of whole cell extract was incubated for 4 hours at 4°C with 1μg of BRCA1 antibody (Ab94 or Ab81 from Abcam, UK) prior to addition of 25μl of protein A sepharose beads at 4°C for 2hrs. Beads were washed four times in 1ml of RIPA buffer. Proteins bound to the beads were eluted by boiling, resolved by SDS-PAGE, transferred to nitrocellulose membrane and subjected to immunoblotting for BRCA1 (Ab9141, Abcam, UK) or UbcH5 (Affinity, UK). Signals were quantified using a Storm 860 Phosphorimager (Molecular Dynamics).

**Cell fractionation.**

MCF7 cells were resuspended in low salt buffer (10mM HEPES, pH7.4, 10mM KCl and 50μg/ml digitonin) supplemented with complete (Roche) protease inhibitors and 1mM PMSF. Nuclei were pelleted by mild centrifugation (1000rpm for 5min) and the supernatant (soluble...
fraction) was taken after high-speed centrifugation. The pellet was extensively washed with low salt buffer before treatment with DNaseI (Boehringer Ingelheim) for 30 minutes. The chromatin fraction was extracted by adding an equal volume of 2X extraction buffer (2% Triton X-100, 50mM HEPES pH7.4, 300mM NaCl, 60mM sodium pyrophosphate, 10mM NaF and 1mM EDTA). Fractionation into soluble and chromatin fraction was determined by monitoring Histone H4, a chromatin bound marker.

**Fluorescence Microscopy**

Deltavision microscopy was used to examine germlines or fixed human cells with 40x or 63x, 1.4 NA Planapochromat lens on an Olympus inverted microscope (IX71), and images captured using SoftWorx computer software (Applied Precision). Three-dimensional data sets were computationally deconvolved, and regions of interest then projected into one dimension. Merged or single colour images were recorded using GIMP software.