Supplementary data

1. BRCA1 does not localize to the midbody during cytokinesis.

Double staining for BRCA1 and Aurora B was performed. During anaphase BRCA1 staining is observed around the chromatin and at the midbody (Suppl. Fig. 1), in contrast to BARD1 which colocalizes with Aurora B at the midbody during anaphase, telophase and cytokinesis (Fig. 1C).

2. Selective repression of FL BARD1 and p82/BARD1β isoform in 293T and HeLa cells.

Generation of siRNAs that selectively target full-length (FL) BARD1 (si-34) or FL BARD1 and BARD1β (si-423 and si-78), permitted distinction of protein isoforms. BARD1 isoform p82/BARD1β is expressed more abundantly than FL BARD1 in all cancer cell lines tested (1). p82/BARD1β is repressed by si-423 and si-78, but not by si-34, and detected in different cell lines on Western blots probed with exon 4-specific anti-BARD1 antibody BL518 or H300 (Suppl. Fig. 2), as well as with other antibodies generated in our laboratory WFS (2-4). We generated a new antibody, p25, directed against an epitope encoded in the alternative ORF of exon 1, hence unique to BARD1β. This antibody only recognizes BARD1β isoform by immunoprecipitation and Western blot analysis.

3. BARD1β, but not FL BARD1 localizes to the midbody at cytokinesis

Using antibodies that distinguish FL BARD1 and BARD1β we demonstrate that BARD1β and not FL BARD1 localizes to the midbody (Suppl. Fig. 3). First we used an antibody directed against the 300 N-terminal amino acids of BARD1, which detects BARD1 staining at the midbody. PVC, an antibody directed against exon 3, which is deleted in BARD1β, does
not stain the midbody. However, p25, an antibody directed against an epitope encoded by an alternative ORF in exon 1 (Suppl. Fig. 3A), shows midbody staining. BARD1β is likely to utilize this alternative ORF. The specificity of p25 is shown in Suppl. Fig. 3C; recognizing the exogenously expressed and endogenous BARD1β.

4. Distinct phenotypes after repression of FL BARD1 only or FL BARD1 and isoforms.

Phenotype after BARD1 repression was analyzed by video enhanced microscopy. Cells were co-transduced with siRNA expression vector conceived for co-expression of GFP from the same plasmid and histone H2B-tomato (H2BT) expressing vector. This permits to observe cell shape and chromosome movements simultaneously. After depletion of FL BARD1 and BARD1 isoforms with si-78, a cell proliferation arrest is observed (Suppl. Video 1). Cells are dividing, but rarely the progenies of one division divide again, and if so, undergo apoptosis or detach, or fuse. After depletion of FL BARD1 by si-34, defects in cell division are also observed (Suppl. Video 2). Particularly, cells exiting from mitosis remain attached after division. The phenomenon of incomplete mitosis and cell fusion after mitosis is also observed after BRCA1 depletion by si-56 (Suppl. Video 3). Cells that only express control vector or GFP (Suppl. Video 4) or an unrelated siRNA do not display this phenotype (Table 1). As demonstrated in Suppl. Video 1, si-78 cells never go through 2 consecutive cycles of S-phase and mitosis and never produce 4 daughter cells. This observation was supported by monitoring of individual cells for 72 hours (Suppl. Fig. 4). A summary of cell cycle length and proliferation rate is provided in Table 1.

5. Defective mitosis in BARD1-deficient cells
Time-lapse videos of BARD1-depleted cells showed defects in maintaining the axis of a bipolar spindle and in chromosome alignment. Cells remained in metaphase for hours and days with frequent changes in the orientation of the spindle axis and/or formation of multiple poles, before undergoing apoptosis (Suppl. Fig. 5A; Suppl. Video 1). BARD1 and BRCA1-depleted cells divided into 3 or 4 cells, and cells underwent multiple consecutive divisions without the delay required for S-phase (Suppl. Fig. 5B-C; Suppl. Video 1-3). Cell fusions were also observed (Suppl. Fig. 5D), which might occur as a result of incomplete cytokinesis.

To further characterize the mitotic phenotype of BARD1-deficient cells, we performed immunofluorescence microscopy on cells transiently transfected with BARD1 si-78, expressed from a constitutive promoter. Cells were fixed 36 h after transfection, to permit sufficient BARD1 repression. Immunofluorescence staining with anti-γ-tubulin antibodies was used to determine the number of centrosomes in BARD1-depleted cells. In control transfections, two centrosomes were observed in most mitotic cells, whereas approximately 50% of BARD1-depleted mitotic cells had more than two γ-tubulin positive dots (Suppl. Fig. 6A). Compellingly, chromosome alignment on the metaphase plate was compromised in 95% of cells expressing BARD1 si-78 or si-34. This phenotype was consistent with the phenotype reported previously for BARD1 or BRCA1-depletion (5).

To further delineate the mitotic defects, we used β-tubulin antibodies to visualize MT structures. Strikingly, after BARD1 depletion, most cells were arrested in cytokinesis, with daughter cells connected by MT-bridges, indicative of defective abscission (Suppl. Fig. 6B). The number of cells connected by MT-bridges was increased ten-fold in si-34 treated cells, as compared to non-treated cells and was higher in BARD1 si-78 cells than in si-34 and BRCA1 si-56 cells (data not shown).

6. Chromosomal instability in cells depleted of BARD1
In a further step towards understanding the BARD1-induced genomic instability phenotype, HeLa and MCF-7 cells treated with si-78 siRNA were analyzed by dual-color interphase FISH (Fluorescence In Situ Hybridization) after 3 to 5 days of siRNA treatment. Using alpha-satellite probes, specific for chromosomes 7, 17, 18, and X, and locus-specific probes for chromosomal regions 13q12 and 21q12 (Suppl. Fig. 7A), we found that BARD1 repression increased the overall intra-ploidy deviation from modal chromosome number in HeLa and MCF-7 cells and elevated their propensity for polyploidization. The most striking observation was the frequent gain of chromosomes X and 18 in HeLa cells, and chromosome 18 in MCF-7 cells (Suppl. Fig. 7B). Moreover, the rates of numerical instability of centromeres 7 and 17 were twice as pronounced in the BARD1-depleted than in the untreated MCF-7 cells. These results demonstrate that impaired chromosome segregation and subsequent numerical chromosomal instability is an early event after BARD1 depletion and might be a consequence of defective chromosome alignment and midbody abscission.

Incomplete mitosis, cell fusion and defects in spindle formation should result in polyploidy and cytogenetic instability. To confirm this hypothesis, we performed cytogenetic analysis after BARD1 repression in several cell lines. This analysis demonstrated frequent polyploidy and genomic instability after 3-5 days of siRNA induction (Suppl. Fig. 7).

7. FL BARD1 depletion leads to Aurora B accumulation in cells.

Aurora B expression in untreated HeLa cells is precisely controlled during prophase, anaphase, telophase, and cytokinesis. As chromosomes align, Aurora B staining is redistributed to the midplate in anaphase. From anaphase to telophase, a decrease of Aurora B staining is observed and only two dots at the abscission point are visible at the midbody. We demonstrate that the degradation of Aurora B is enhanced in cells overexpressing exogenous
FL BARD1. On the contrary, cells that are depleted of FL BARD1 accumulate Aurora B in the cell (Suppl. Fig. 8).

**Methods**

**Histone H2B expression clone**

Histone H2B member j (HIST1H2BJ) cDNA was amplified by PCR from reverse transcribed RNAs of human fibroblasts. The dtTomato cDNA, kindly provided by R.Y. Tsien (Shaner et al., 2004), was fused to the 3’ end of H2B using the respective linker sequence (5’-CAGCGCTAAGGGATCCCAAGCTTTGCCGGGG ATGGTGAGCA-3’). To generate an entry vector, this fusion construct, named H2B-T, was then introduced into pDONR221 using the Gateway BP clonase enzyme mix. The resulting entry vector was then recombined into the 2K7bsd lentiviral vector using the Gateway LR clonase enzyme mix. A 590 bp human cytomegalovirus (CMV) promoter sequence was linked at the 5’ of H2B-T sequence using the same strategy as described (Suter et al., 2006). All of these exogenous constructs were verified by DNA sequencing.

**Dual color cytogenetic analysis**

To investigate numerical instability we applied dual-color interphase FISH with satellite probes, specific for chromosomes 7, 17, 18, and X. We also applied locus specific probes hybridizing to the Retinoblastoma region of chromosome 13 and the region critical for Down syndrome on chromosome 21. Probes were either purchased from Vysis, or CYTOCELL. Cell cultures of high mitotic index were exposed to colcemid (0.1 µg/ml) (Gibco, BRL) for 1,5 h, at 37°C and harvested according to routine cytogenetic protocols. FISH was based on pepsin pre-treatment, formamid target denaturation, over-night hybridization, and stringent post hybridization washes. To determine chromosome loss and gains, for each chromosome pair
studied, a modal number ($M_a, M_b$) was calculated as the most representative number of spots per nucleus per 100 cells/harvest (Suppl. Fig. 5). Chromosome loss was defined as $M_{n-1}$, chromosome gain as $M_{n+1}$. Overall deviation was calculated as the percentage of nuclei that did not show modal number of spots after the subtraction of polyploidy ($=2(M_a+M_b)$).

**Supplementary Figures legend**

**Supplementary Figure 1.** Double immunofluorescence staining for BRCA1 and Aurora B show partially overlapping staining at the midplate in anaphase (arrows), but not at the midbody during cytokinesis (arrows).

**Supplementary Figure 2.** Selective repression of FL BARD1 and p82/BARD1β isoform.

(A) Western blot of cell extracts from untreated 293T cells without (nt), or with induced expression of BARD1 siRNAs directed against human BARD1 exon 2 (si-34), human BRCA1 (si-56), or against human and mouse BARD1 exon 9 (si-78) were probed with anti-BARD1 antibody H300, directed against a peptide comprising the first 300 amino acids of human BARD1. Identity of FL-BARD1 (97 kDa) is confirmed by expression of exogenous mouse Bard1 (+mBard1), which lead to increase of protein of 97 kDa. Slower migrating forms are presumably phosphorylated isoforms (*). FL BARD1 is repressed by human si-34 and si-56, demonstrating that BRCA1 is required for stability of FL BARD1. p82/BARD1β is not repressed by si-34 but by si-78.

(B) BARD1 repression with different siRNAs is shown for HeLa cells in which p82/BARD1β is more abundantly expressed than FL BARD1. Repression was performed in the presence of exogenous mouse Bard1 (*), which migrates slightly faster than human BARD1. Mouse-specific si-RNA (si-12, against mouse exon 2) shows repression of this lower band only. Si -34 represses human but not mouse FL BARD1, but BARD1 p82/BARD1β is
only repressed by si-78. Si-56 shows depletion of FL BARD1, due to protein instability in the absence of BRCA1. Expression of si-78 repressed both forms.

(C) Expression of FL BARD1 and p82/BARD1β in various human cancer cell lines (HeLa, TOV-G21, SKOV-3, MCF7, MDAMB231, JEG3), fibroblasts (hs68), and mesenchymal stem cells (hMSC). In these cell lines with high proliferative activity, p82 is the predominant form. In cells that do not express FL BARD1, the repression of isoform p82/BARD1β lead to growth arrest (7). The level of BRCA1 is correlated with expression level of FL BARD1.

**Supplementary Figure 3.** Distinct antibody recognition of BARD1β at the midbody.

(A) Schematic diagram of FL BARD1 and BARD1 isoforms is shown. Positions of epitopes recognized by antibodies BL518, PVC, and p25 are indicated with blue arrows.

(B) Immunofluorescence of cells at cytokinesis using anti-BARD1 antibodies H300 (300 N-terminal amino acids, exons 1, 2, 3, and partially 4), PVC, and p25.

(C) Immunoprecipitation of BARD1 performed in HeLa cells and FL BARD1 or BARD1β over-expressing cells using BARD1 antibody BL518 or p25 directed against an epitope specific of BARD1β. As expected, p25 antibody recognizes endogenous and exogenous BARD1β isoform but not FL BARD1.

**Supplementary Figure 4.** Monitoring cell fate after BARD1 repression by si-78.

(A) Observation of individual cells in cluster of BARD1 si-78 expressing cells shows that mitotic defects are responsible for net growth of zero. Left panel shows cells numbered at the beginning of the observation. Right panel shows cells labeled A and B, if derived from first division, Aa and Ab, if derived from second division. Grey label indicates position of cells that underwent apoptosis, yellow labeling indicates cell fusions.
(B) Schematic diagram of individual cell fates of cell cluster shown in (A): mitosis (1st blue, 2nd purple), apoptosis (grey), and cell fusions (red links). Approximate time scale is indicated. Cell Nr. 3 remained with apparently fragmented chromatin for 12 hours before undergoing apoptosis. Only few cells went through 2 divisions and in no case 4 cells sustained after a second division.

**Supplementary Figure 5. Mitotic phenotype of BARD1-depleted cells.**

Video-enhanced microscopy of BARD1-depleted cells. HeLa cells transduced with lentiviral vectors, expressing inducible BARD1 siRNA and GFP with/or without second lentiviral vector constitutively expressing H2B-Tomato, were observed for 48 or 72 hours (see suppl. videos 1-4). GFP and tomato images were taken at 5 min intervals. Four categories of mitotic defects of siRNA-expressing cells lines are presented. Elapsed time is indicated in hours and minutes on all images.

(A) Abortive mitosis. Upper panel shows BARD si-78-GFP-H2B-T cell entering mitosis. Lines indicate axis of presumed midplate. Changes of axis of metaphase plate can be observed during 23 hours and finally cell undergoes apoptosis. Lower panel shows similar observations in si-78-GFP cells not expressing H2B-T. Cell A divides normally, cell B remains in metaphase for several hours. Bar represents 10 μm.

(B) Division into multiple cells. Upper panel shows BARD1 si-78-GFP-H2B expressing cells with cell A remaining in pro-metaphase for the entire time of observation, and cell B forming a tri-polar spindle and division into 3 cells. Lower panel shows BARD1 si-78-GFP cells without H2B-T that exhibit the same phenotype. Cell A divides into 3 cells, cell B divides normally. Bar represents 10 μm.
(C) Multiple consecutive divisions without S-phase. Progenies of a first division of si-78-GFP cells, A and B; continue to divide. B divides into B1 and B2. B2 continues to divide into B2a and B2b. Schematic diagram with elapsed times between divisions is shown underneath.

(D) Cell fusion and nuclear fusion. After exit of mitosis, 2 cells remain attached for hours by a cytoplasmic bridge. During a process of more than 20 hours, their cytoplasms fuse and later their nuclei.

**Supplementary Figure 6. Multiple spindle poles and defective abscission**

(A) Multiple spindle poles in BARD1-depleted cells. Immunofluorescence microscopy of BARD1-repressed cells at 48 hours after si-78 expression, applying DAPI and anti-γ-tubulin staining, is presented. Upper left panel shows control staining omitting primary antibody, lower left panel shows overview of DAPI and γ-tubulin staining in untreated HeLa cells. Most cells have 2 positive spots of γ-tubulin staining. BARD1 Si-78 expressing cells (4 examples) reveal defects in chromosome alignment and form multiple spindle poles. Aligned or mis-aligned chromosomes (short arrows) and spindle poles (long arrows) are indicated in untreated (wt) and BARD1 siRNA-expressing HeLa cells.

(B) Persistence of midbody after exit of mitosis in BARD1-depleted cells. Anti-β-tubulin staining was applied to elucidate MT structures. Representative fields of cells with elongated midbodies are shown. Midbodies show two restrictions (short arrows). Residual BARD1 staining in cells expressing si-BRCA1 is observed in the nucleus. MT staining is elevated in BARD1 repressed cells (long arrows). In untreated control cells, MT staining is less intense and BARD1 staining is at the midbody. BARD1 (H300) staining is shown in red (Alexa red 555), β-tubulin in green (FITC).
**Supplementary Figure 7.** BARD1 depletion causes numerical chromosomal instability.

Numerical chromosomal instability was assessed in HeLa and MCF-7 cells. Examples of FISH are shown for chromosomes 7, 13, 17, 18, 21, and X (A). Gains and losses of chromosomes 7, 17, 13, and 21, were assessed in HeLa (B) and chromosomes 18 and X were assessed in HeLa and MCF-7 cells (B and C). Frequency of these chromosomes is presented for untreated and BARD1 siRNA expressing HeLa and MCF-7 cells. A total of 100 cells were analyzed for each cell type. Arrows point to significant gain of chromosomes in siRNA expressing cells.

**Supplementary Figure 8.**

(A) Aurora B staining in cells over-expressing FL BARD1 (BARD1-FL) is similar to control cells, but staining in anaphase is decreased, as well as in telophase.

(B) Aurora B staining in cells repressed for FL BARD1 and BARD1β (si-78) is comparable to control cells in prophase, but up-regulated during anaphase and telophase. (C) Up-regulated and mis-located Aurora B staining is observed on long midbodies in cells depleted of FL BARD1 by si-34. Three representative examples are shown.

**Legends to videos**

**Support video 1.** HeLa cells expressing BARD1 si-78 (exon 9), co-expressing GFP, and Histone H2B-T (tomato) fusion protein, were treated with doxycyclin for 3 days to induce expression of siRNA before begin of observation.

**Support video 2.** HeLa cells expressing BARD1 si-78 (exon 9), co-expressing GFP, were treated with doxycyclin for 3 days to induce expression of siRNA before begin of observation.
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Support video 3. HeLa cells expressing BRCA1 si-56, co-expressing GFP, and Histone H2B-T fusion protein were treated with doxycyclin for 3 days to induce expression of siRNA before begin of observation.

Support video 4. Control cells expressing siRNA of unrelated dentritic cell-specific gene (DC-SIGN) and GFP.

References