SUPPLEMENTARY METHODS

Cell synchronization, Flow Cytometry, and Immunofluorescence. Cells were synchronized at the G1/S boundary with double thymidine block as described previously (11). Briefly, cells were incubated in medium containing 2mM thymidine for 12 hours, released into their complete medium for 8–10 hours and then incubated for 12 hours in medium containing 2 mM thymidine. The percentage of cells in G1, S, or G2 phase is presented as the mean of three independent experiments. Nuclear and cytoplasmic enriched lysates were collected at the same time points and analyzed by immunoblot for the indicated proteins. Intracellular localization of BRCA1 protein was determined by immunofluorescence using anti-BRCA1 antibody (EMD Chemicals, Gibbstown, NJ, USA) and confocal microscopy at the specified time points after release from cell cycle block. Greater than 500 cells were counted per slide, in triplicate. Mean intensity of BRCA1 protein in the nucleus was normalized to the total intensity utilizing the ImageJ NIH program.

Nocodazole Treatment.

To induce mitotic G2/M arrest, MCF10A-pLVX-EZH2 without or with Dox for 24h as well as CAL51 KD and control cells were treated with 50 ng/ml of Nocodazole for 20 h.

In vitro Kinase Assay

Cells were lysed in RIPA buffer (Thermo Scientific, Rockford, Il). 500 µg of cell lysates were centrifuged at 20,000 x g for 20min, and the resulting supernatants were incubated for 2h at 4ºC with Sepharose G beads conjugated with antibodies to Aurora-A. After extensive washing, the beads were incubated for 30min at room temperature, with 2µg of

histone H3- N-terminal GST tag (BPS Bioscience, San Diego, CA), in a 30µl of kinase buffer (Calbiochem, la Jolla, CA), and 10 µCi of (γ-32P)ATP. The reaction was stopped by addition of Laemmli sample buffer, and phosphorylated substrates were detected by SDS-PAGE and autoradiography.

Human breast tissue samples, immunohistochemistry and statistical analyses. A high-density tissue microarray (TMA) containing 138 primary invasive carcinomas of the breast developed and characterized by our group was employed {Kleer, 2003 #1649}. 5µ-thick sections were immunostained with antibodies against EZH2 (mouse monoclonal, BD Biosciences, Cat# 612667, 1:300), pAkt-1 (Ser473) (rabbit polyclonal, Millipore, Cat# 07-310, 1:100), and pBRCA1 (rabbit polyclonal, Abcam, Cat# AB2838, 1:700) following biotin-avidin complex technique (8). Expression of EZH2 and phospho-Akt-1 (Ser473) was evaluated as either low or high based on intensity of staining and percentage of staining cells following published literature (11, 28). Nuclear expression of pBRCA1 was scored as high or low. Cases with low nuclear staining had either cytoplasmic staining or were negative. Two tailed Fisher's exact test was performed to analyze the association between EZH2, pAkt-1 and pBRCA1 proteins. A p value <0.05 was considered significant.