Supplementary Figure Legends

Supplementary Figure 1. Doxycycline treatment of MCF10A cells containing the empty vector (pLVX) has no effect on BRCA1 localization, which confirms the specificity of the results shown in Figure 1. Shown are representative immunofluorescence images of BRCA1 protein at 3 h after release of G1/S cell cycle block. The graph shows the mean intensity of BRCA1 protein expression in the nucleus normalized to the total intensity at different times after release from double thymidine block. To measure the intensity we used the ImageJ NIH program. Experiment was repeated three times.

Supplementary Figure 2. A. Effect of EZH2 overexpression on Aurora A *in vitro* kinase activity of pLVX-EZH2-MCF10A cells. Cells without or with Dox for 24h were untreated or treated with Nocodazole (50 ng/ml for 20 h) to induce G2/M arrest. Equal amounts of cell extracts were immunoprecipitated with Sepharose G beads conjugated with antibodies against Aurora-A, and subjected to kinase assay with (γ-32P)ATP and histone H3-GST tag as exogenous substrate. Total histone H3 was used as loading control. Equal amounts of cell extracts representing 10% of the INPUT used in the immunoprecipitation were subjected to immunoblot analysis with antibodies against total histone H3. **B. Left,** Western blot of nuclear extracts of pLVX-EZH2 cells untreated and treated with Dox at indicated times after release from G1/S cell cycle block. **Right,** Western blots for the indicated proteins proteins on CAL51 cells transduced with scrambled control or EZH2 KD at indicated times after release from G1/S cell cycle block. Experiments were repeated three independent times. * $p<0.05$ (untreated vs. Dox). **C**. Real time quantitative RT-PCR shows that EZH2 expression regulates Aurora A and

B messenger RNA levels in MCF10A and CAL51 cells. Bars show mean ± SD of three independent experiments. * $p<0.05$.

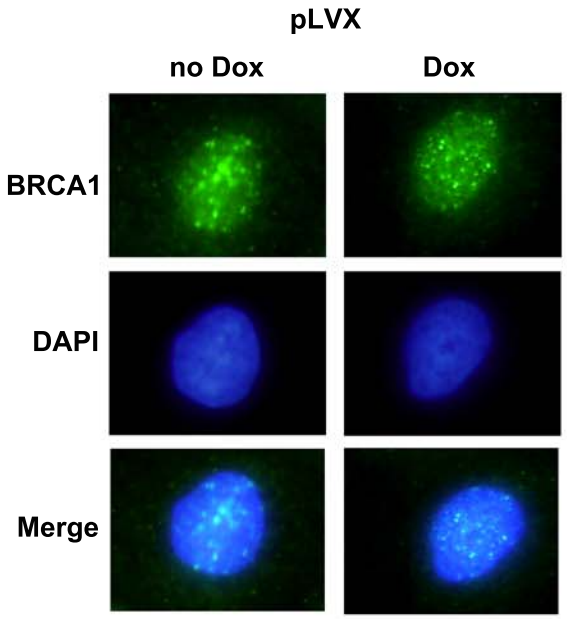
Supplementary Figure 3. Inducible synthesis of EZH2 in MCF10A cells and its effect on pAkt and Akt levels. Western blot analysis of MCF10A cells transduced with the empty vector (pLVX) and MCF10A cells transduced with EZH2-containing vector (pLVX-EZH2). Cells were untreated or treated with Dox (500 ng/ml) to induce EZH2 expression. Note that Dox treatment of the empty vector transduced cells has no effect on pAkt or total Akt protein levels, while a strong effect is seen in cells transduced with EZH2 containing plasmids.

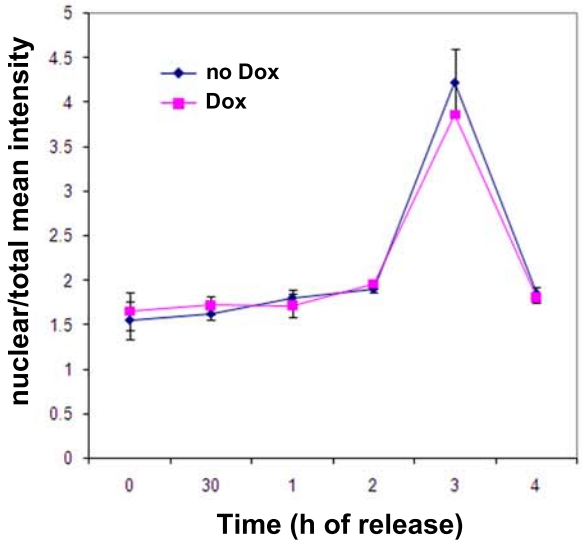
Supplementary Figure 4. EZH2 protein physically interacts with Akt-1 in MCF10A cells using an antibody that recognizes all Akt isoforms and another specific for Akt-1 isoform. MCF10A cells were infected with adenovirus containing EZH2 or adenovirus controls as previously reported (8). Immunoprecipitation of EZH2 or Akt-1 followed by reciprocal EZH2 or Akt-1 immunoblotting from whole cell lysates of MCF10A cells infected with adenovirus containing EZH2 or controls. Inputs represented 10% of extracts. IPs with IgG was used as negative control and IPs with EZH2 and Akt-1 serve as positive controls.

Supplementary Figure 5. EZH2 requires activation of PI3K/Akt to promote BRCA1 nuclear export and polyploidy. **A.** MCF10A-pLVX-EZH2 cells were treated with LY294002 (20 μM) (Left) or Wormannin (0.1 μM) (Right) and were then subjected to

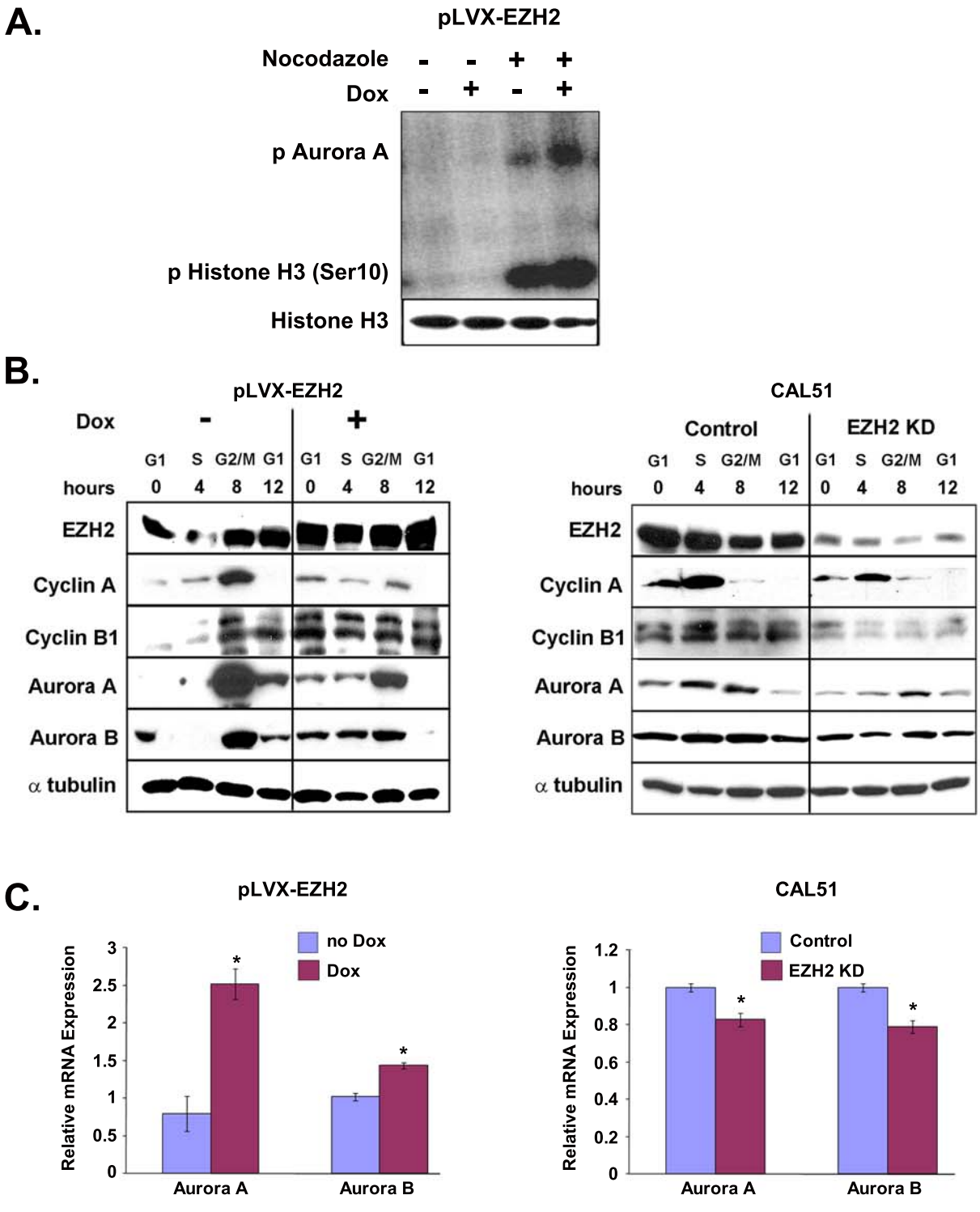
Dox or no Dox. N and C indicate nuclear and cytoplasmic-enriched fractions. Laminin B1 confirms nuclear enrichment of the fractionated samples. Lane 1 is untreated control. EZH2 overexpression upregulates pAkt Ser473 and pAkt-1 Ser473, and decreases BRCA1 and pBRCA1 in the nuclear fractions (lanes 2-3 vs 1). LY294002 (lanes 4, 5) reduces pAkt Ser473 and pAkt-1 Ser473 to nearly undetectable levels. Of note, LY294002 and Wortmannin increase BRCA1 in the nuclear fractions preventing the effects of EZH2 overexpression. **B.** PI3K/Akt inhibition prevents the effect of EZH2 overexpression on BRCA1 intracellular localization. Immunofluorescence for BRCA1 protein was carried out at the indicated time points after release from G1/S cell cycle block in the absence and presence of LY294002 (20μM) or Wortmannin (0.1 μM). Representative confocal images at 3 h after release from cell cycle block. Experiments were repeated three independent times. Error bars are the standard deviation. **C.** PI3K/Akt inhibition decreases EZH2-induced polyploidy. Chromosome counts of metaphase spreads of MCF10A-pLVX-EZH2 cells untreated or treated with Dox for 3d in the absence or presence of LY294002 (20μM) or Wortmannin (0.1 μM). Bars show the mean ± SD of three independent experiments. * $p<0.05$ (Dox vs. all of other groups).

Supplementary Figure 1

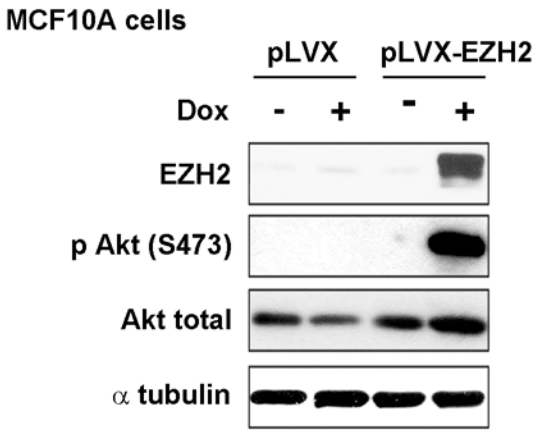




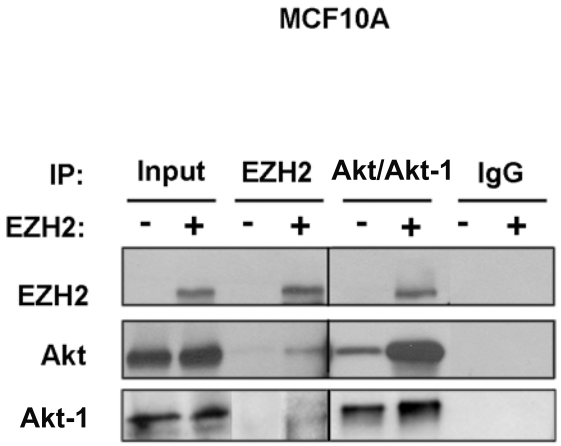
Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5