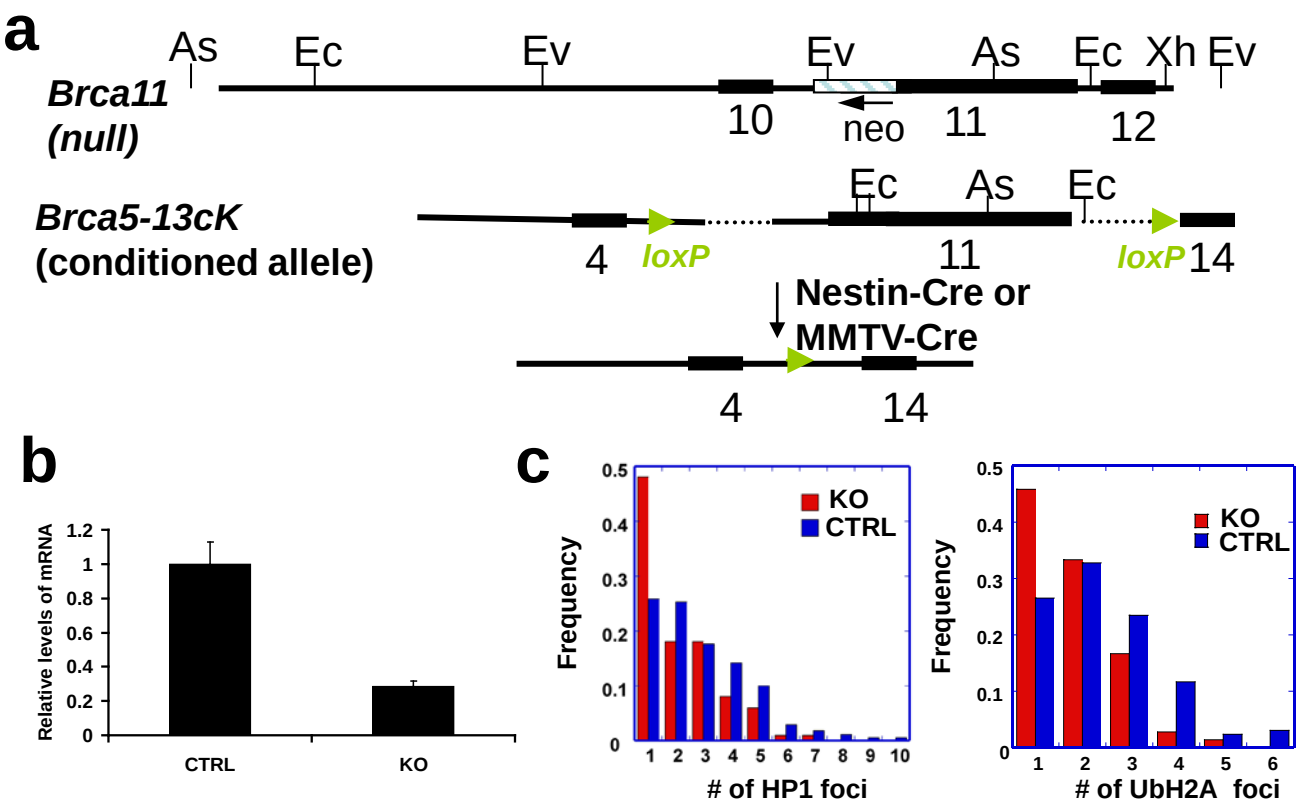
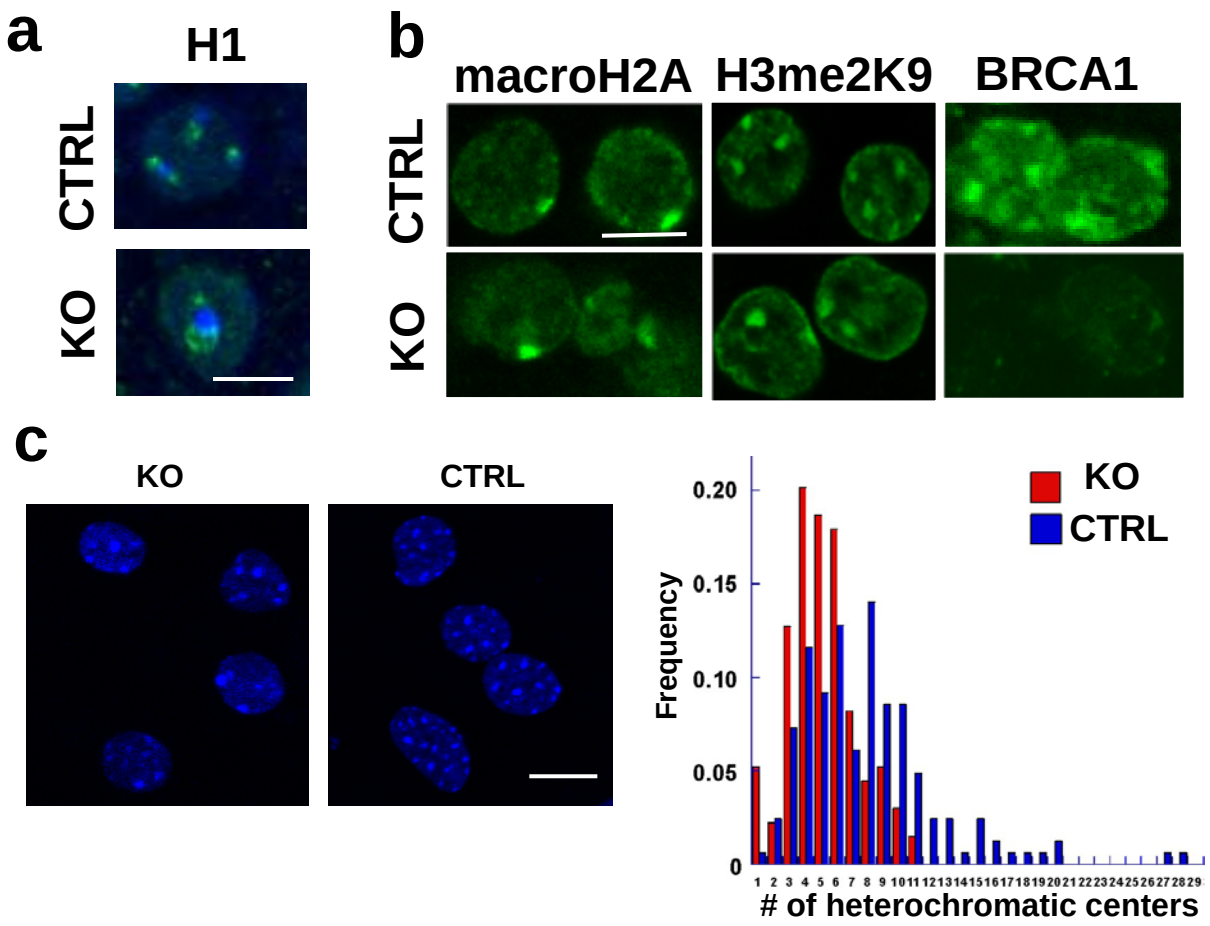
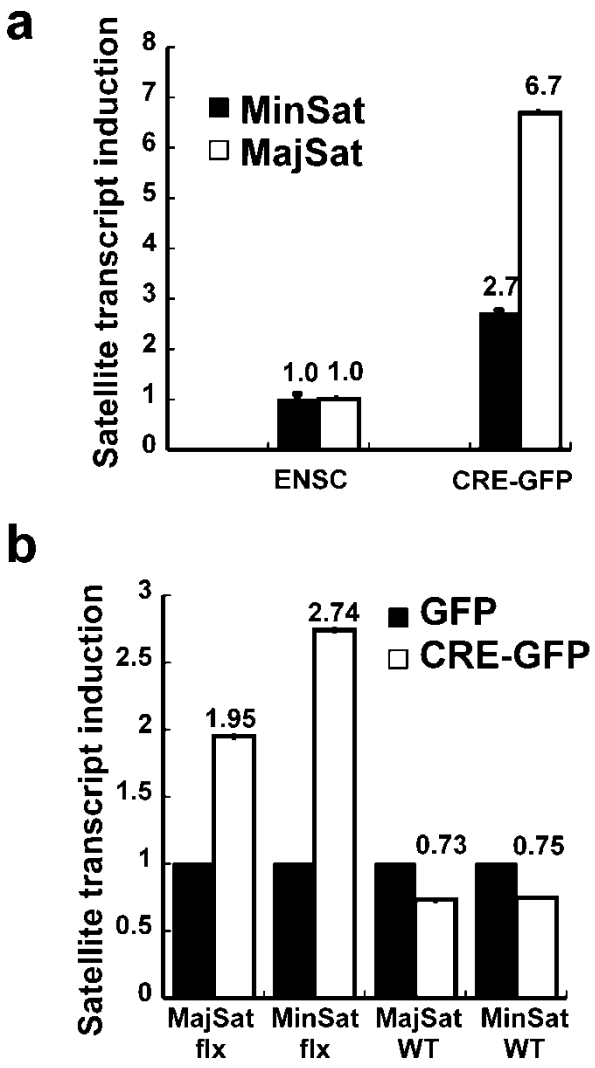


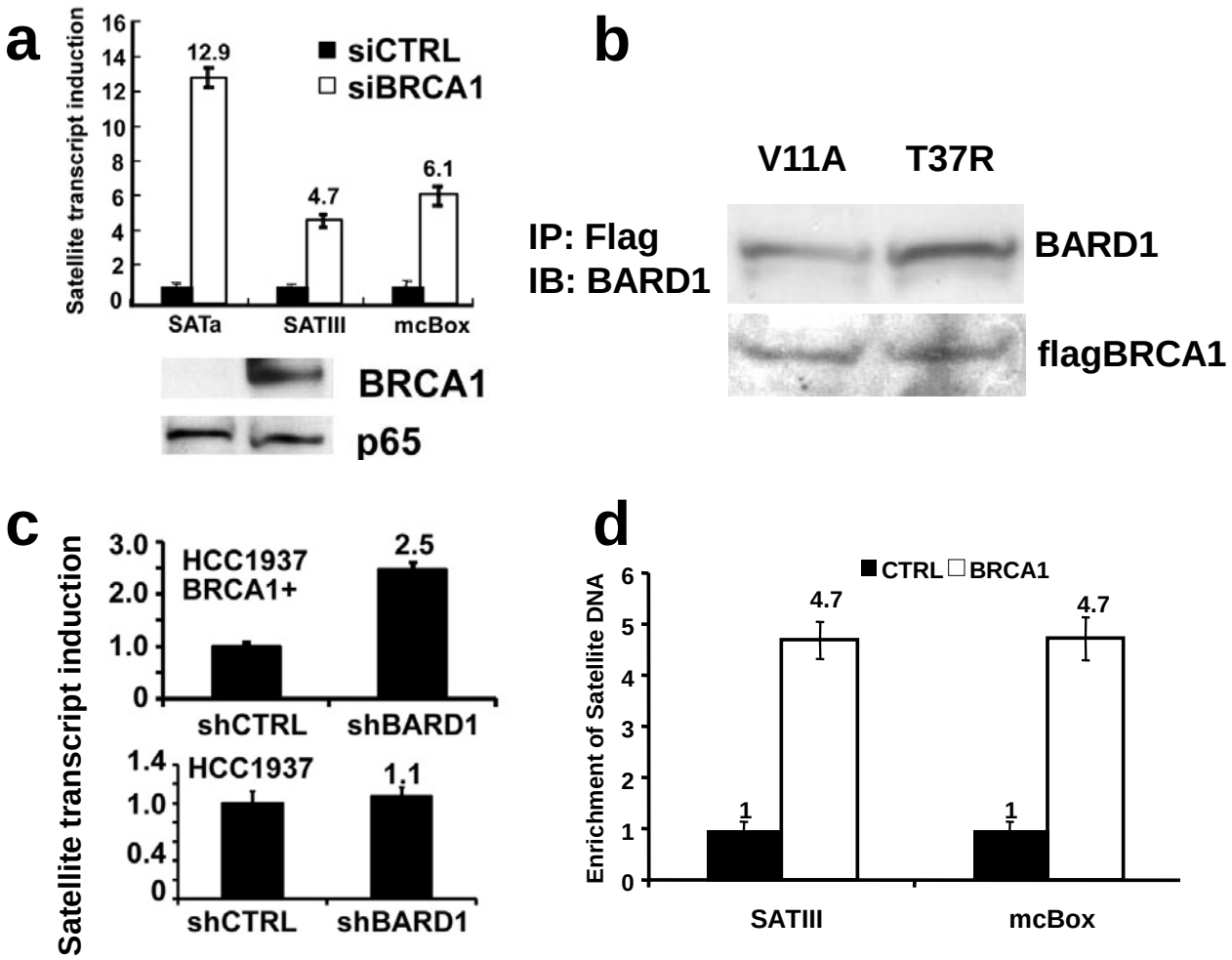
Supplementary Figure 1. Targeted deletion of BRCA1 in mice. a, Targeting of BRCA1 in the mouse CNS (Nestin-Cre) and mammary gland (MMTV-Cre). Schematic representation of the targeted loci of mouse BRCA1. *Brca11* contains a neo insertion within exon 11, which renders the allele a null. *Brca5-13*cK deletes exons 5-13 of the mouse *Brca1* gene upon exposure to Cre recombinase and is also a null. **b**, Nestin-Cre ablates mRNA expression of *BRCA1* floxed allele. Quantitative RT-PCR was performed using RNA from cerebellum of a *BRCA1* KO mouse or a control mouse. Ct values of each sample were normalized by an internal control, *cyclophilin*. **c**, Quantification of the number of *HP1* and Ub-H2A staining heterochromatic centers in the BRCA1 KO brains as shown in Fig. 1**c**, **d**, Reduced *HP1* protein levels in *BRCA1* deficiency cells. Western blotting of protein extracts from cerebellum (P2) of BRCA1 conditional knockout and control mice. The blot was probed with an antibody against *HP1*α , β, γ followed by probing with anti-*p65* serum as a protein loading control (lower panel).



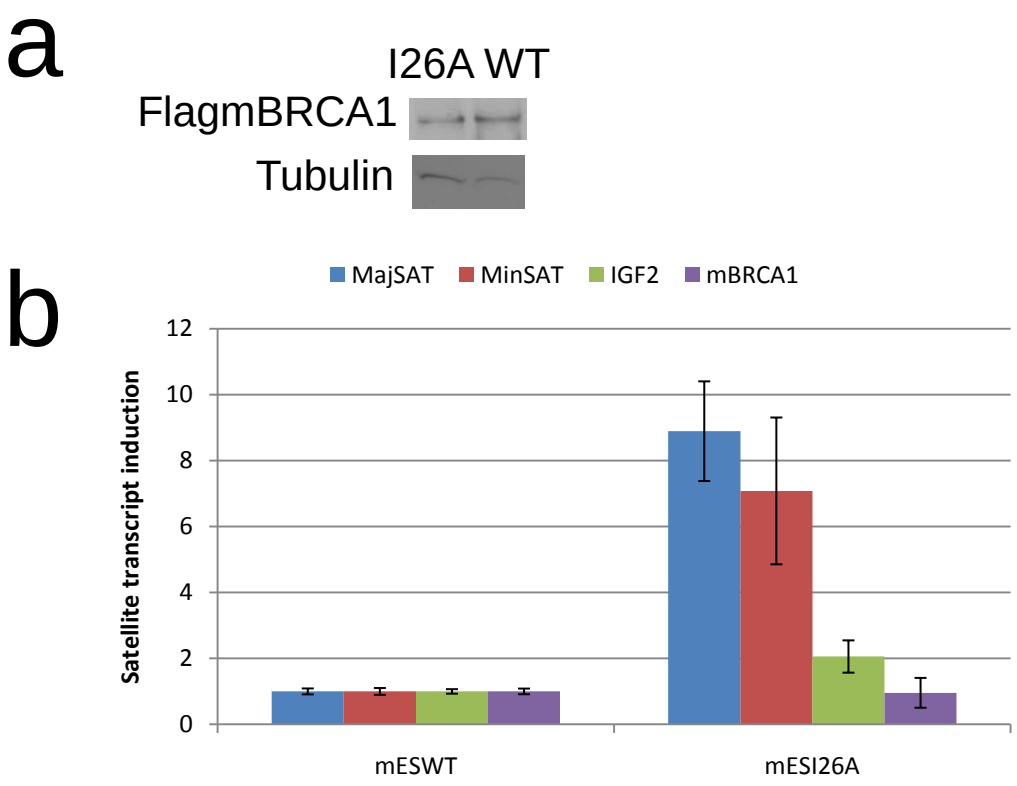
Supplementary Figure 2. Additional heterochromatic markers in *BRCA1* deficient brains. **a**, Confocal microscopic images of brain sections from *BRCA1* conditional knockout and control mice (P7) stained with antibody against histone H1. H1 staining is more diffuse in KO cortical cells. Scale bars: 10 μm. **b**, Confocal microscopic images of brain sections from *BRCA1* conditional knockout and control mice (P7) showed that staining with macroH2A and dimethyl-histone H3 lysine 9 gave similar intensities between KO and control (*Brca11+/-; Brca5-13ck+/-; nestin-Cre-*) samples. The intensity of mouse *BRCA1* staining signal was much weaker in KO brains, as expected. Scale bars: 30 μm. **c.** The number of heterochromatic foci was reduced in differentiated *BRCA1*KO NPCs in vitro. Embryonic NPCs were isolated from E14.5 *BRCA1* floxed (*Brca11+/-; Brca5-13cK+/-*) telecephelon and cultured in N2 medium supplemented with *FGF2* (100 ng/ml). Two days after virus infection, the cells were exposed to N2 medium with 1% FBS to induce differentiation for 10 days prior to DAPI staining and immunostaining with differentiation markers (data not shown). The numbers of strong DAPI staining nuclear foci/cell were counted and their frequencies plotted. CTRL: average =7.85/nucleus (n=162); KO: average =5.0/nucleus (n=70), Student *t* test, $p<0.0001$. Sale bar: 45 μm.



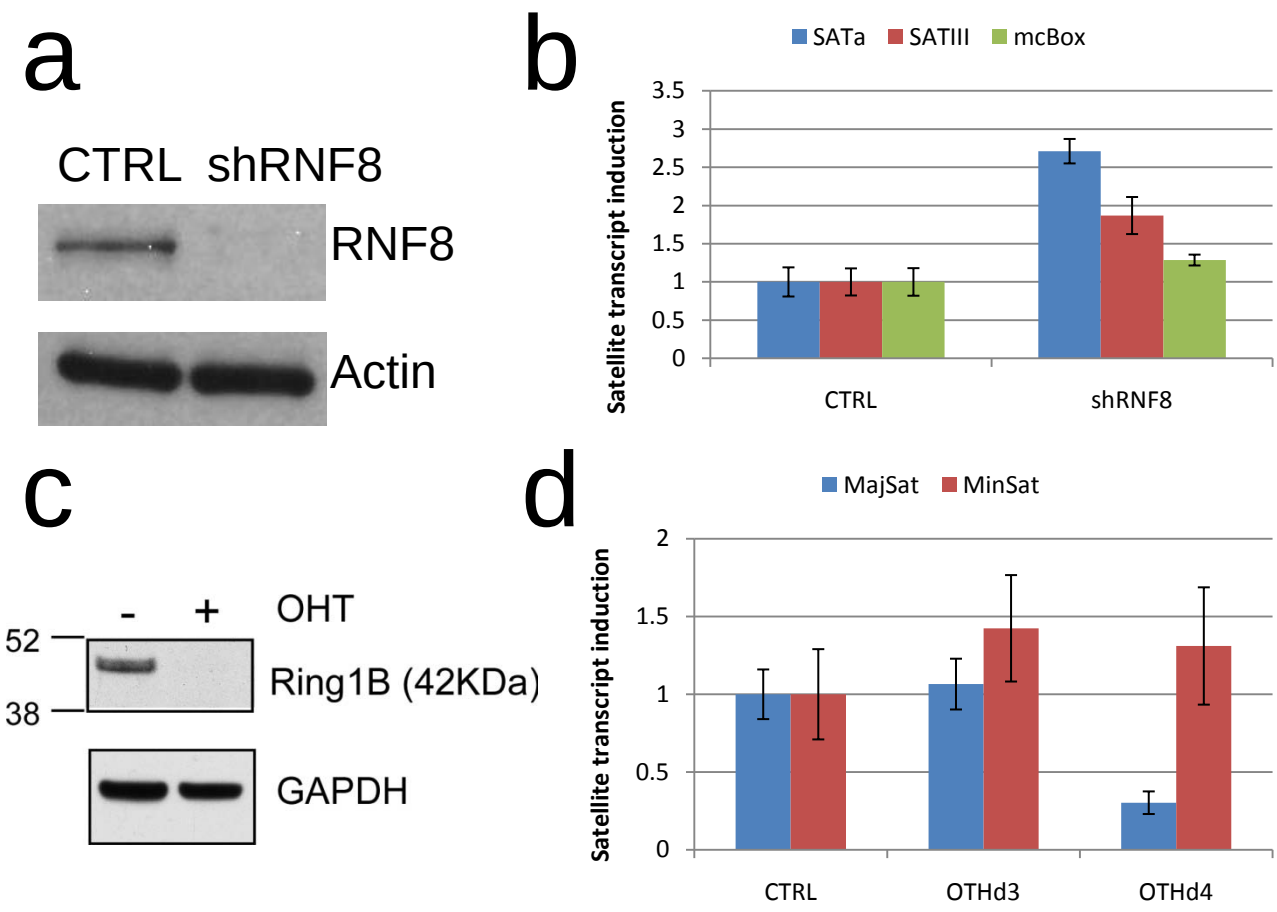
Supplementary Figure 3. *BRCA1* deficiency alters the satellite DNA expression in mouse cells. a, *BRCA1* deficiency alters the expression of MinSat and MajSat in mouse embryonic neural stem/progenitor cells (ENSCs). Quantitative RT-PCR experiments showed that expression of MinSat (flx) and MajSat (flx) was upregulated in ENSCs within 72 hours of infection by a retroviral vector expressing *CRE-GFP* (CRE-GFP) that excises the floxed *BRCA1* allele (flx) in comparison with that of cells infected with a retroviral vector expressing *GFP* (*GFP*). **b**, *BRCA1* deficiency alters the expression of MinSat and MajSat in mouse fibroblast cells. Quantitative RT-PCR experiments showed that expression of MinSat (flx) and MajSat (flx) was upregulated in mouse fibroblast cells within 72 hours of infection by a retroviral vector expressing CRE-GFP (CRE-GFP) that excises the floxed *BRCA1* allele (flx) in comparison with that of cells infected with a retroviral vector expressing *GFP* (*GFP*). Cells that contain a wildtype (WT) allele *BRCA1* did not show the same effect.



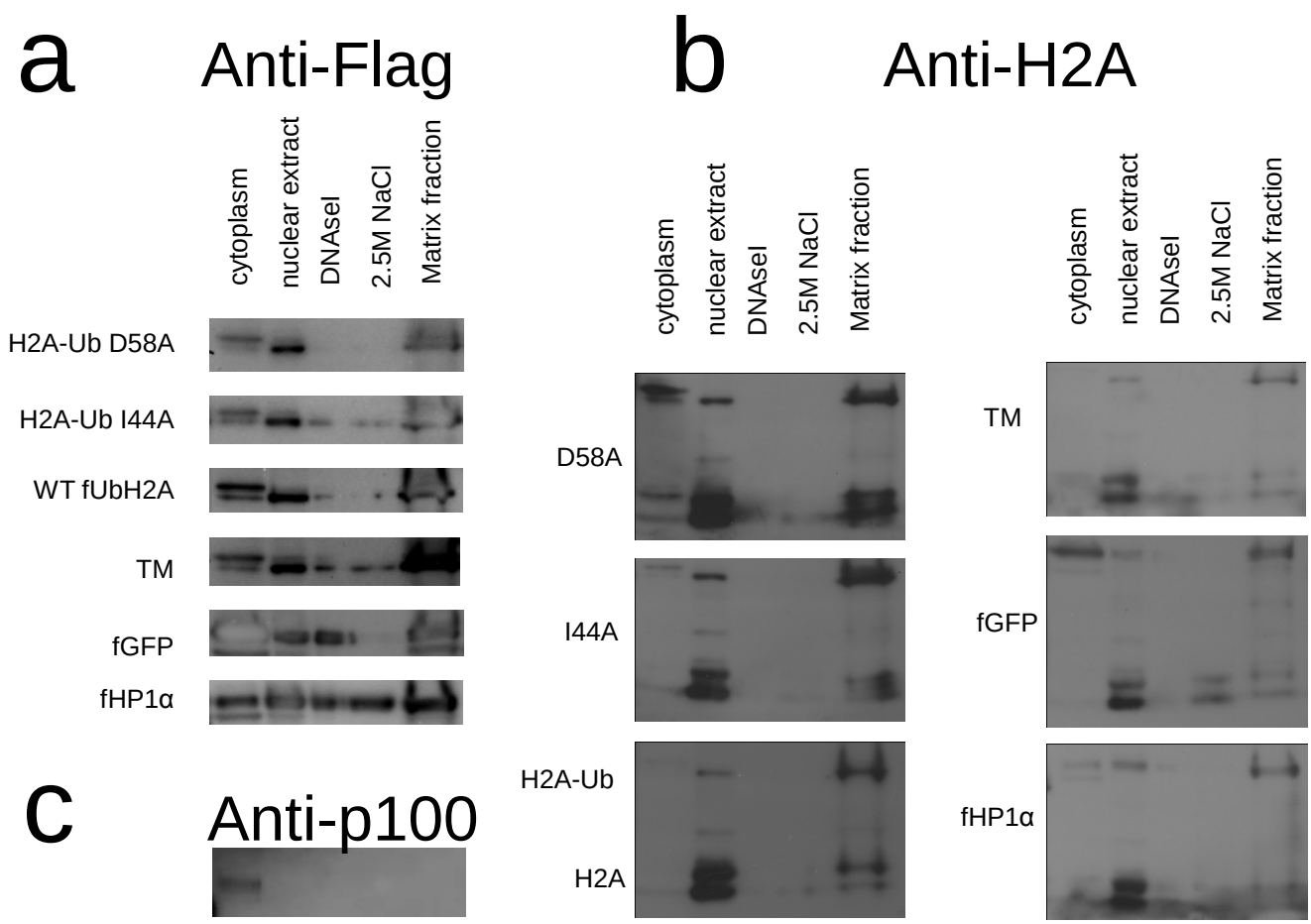
Supplementary Figure 4. BRCA1 deficiency impairs heterochromatin integrity in human cancer cells. **a**, Derepression of satellite DNA in *BRCA1* knockdown HeLa cells. HeLa cells were infected with a lentiviral vector expressing shRNA against *BRCA1* or a control shRNA. Western blotting was performed to measure the amount of protein in the infected cells (upper panel). RNA was extracted and the expression of human α-satellite DNA was measured by quantitative RT-PCR (lower panel). **b**, Ubiquityl E3 ligase mutant BRCA1 interacted with *BARD1***.** 293T cells were transfected with constructs expressing wildtype polymorphic (V11A) or mutant (T37A) flag tagged BRCA1 genes. 48h after transfection, the cells were harvested and subjected to IP experiments. Western blots were probed with anti-*BARD1* serum (upper panel) followed by flag antibody (lower panel). **c**, *BRCA1* binding partner, *BARD1*, is required for *BRCA1* mediated heterochromatin silencing. A lentiviral vector expressing shRNA against *BARD1* was used to infect either a *BRCA1* reconstituted HCC1937 cells or HCC1937 cells. The transcription of human satellite DNA (SAT III) was measured by quantitative RT-PCR. **d**, Association of *BRCA1* to heterochromatin. ChIP experiments were performed using HeLa cells and antibodies against human *BRCA1* or *VSVG* protein as a control.



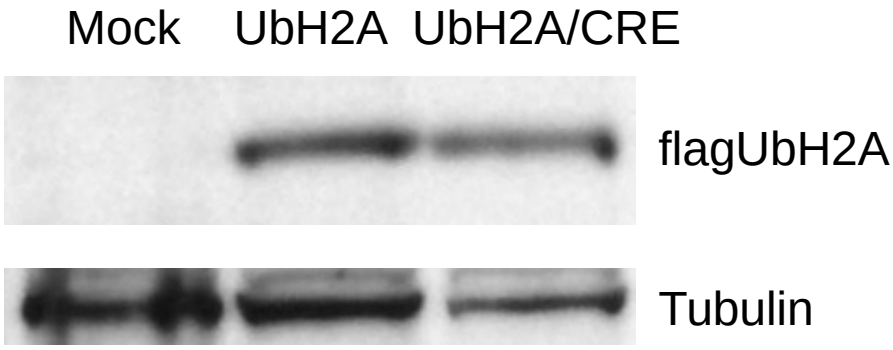
Supplementary Figure 5. Derepression of satellite DNA in *BRCA1* mutant I26A mES cells. a, Immunoprecipitation of *BRCA1* was found to pull down equivalent levels of flagBRCA1 WT and mutant I26A in mouse embryonic stem (ES) cells. The amount of *HP1*α is reduced in the mutant cells. **b**, RNA was collected from these cells and the amount of satellite DNA expression was quantified by real time RT-PCR. The result show that both major and minor satellite sequences are upregulated by the I26A mutation of *BRCA1* in ES cells. *Igf2* is upregulated two fold as expected from a defect in imprinting. The mutant *BRCA1* mRNA levels are equivalent to that of WT cells. The results support the notion that despite the lack of proliferative and apoptotic defects in mouse ES cells lacking *BRCA1* RING finger function, it still has a defect in heterochromatin formation and satellite repression suggesting a direct link between *BRCA1* ubiquityl ligase activity and satellite silencing.



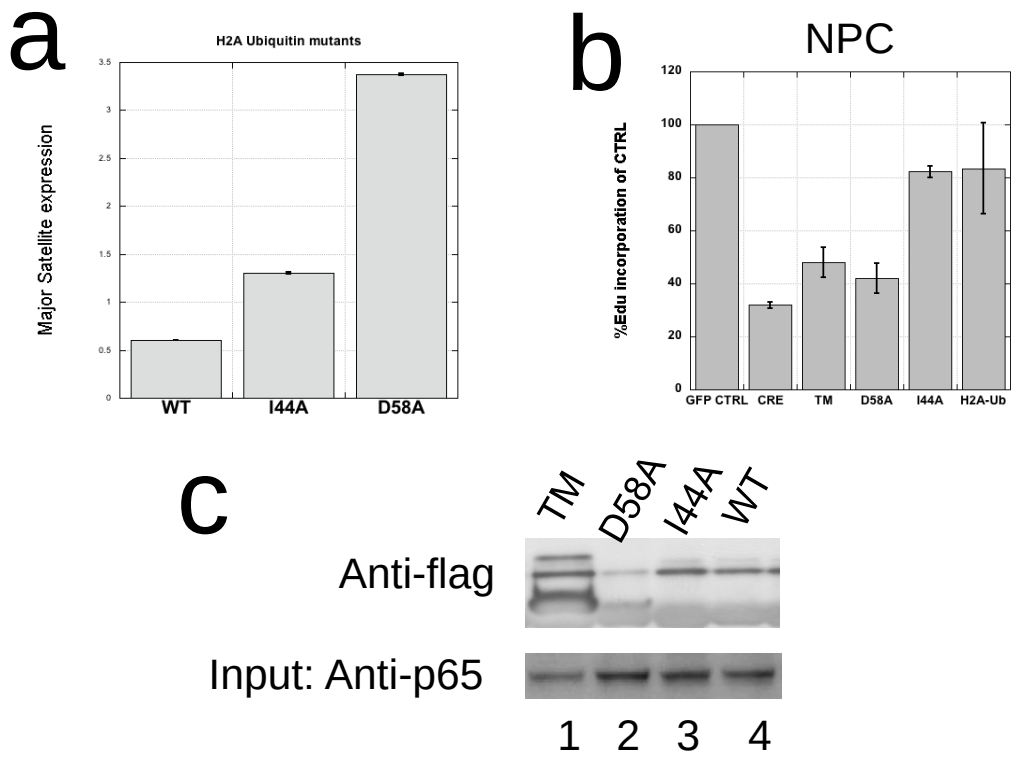
Supplementary Figure 6. *RNF8* and *Ring1B* knockdown had no significant effect on satellite DNA expression. a. HeLa cells were infected with a lentiviral vector expressing shRNA against *RNF8* or a control shRNA. Western blotting was performed to measure the amount of protein in the infected cells. Actin was used as a loading control. **b**. RNA was extracted from HeLa cells that were infected with shRNF8 or a control shRNA. The expression of human α-satellite DNA was measured by quantitative RT-PCR. **c**, Mouse embryo fibroblasts (MEFs) containing conditional deletion allele of *Ring1B* were a kind gift from Dr. Miguel Vidal. *Ring1B* was deleted by incubation of MEFs with 4OH-tamoxifen (OHT) as suggested. Western blotting was performed to measure the amount of protein in the MEF cells. *GAPDH* was used as a loading control. The amount of *Ring1B* protein was undetectable in OTH treated cells (upper panel, right lane). d. RNA was extracted from *Ring1B* knockout cells. The expression of mouse satellite DNA was measured by quantitative RT-PCR on day 3 and day 4 post tamoxifen induction. Although *Ring1B* has been reported to be a major bona fide ubiquityl ligase for histone H2A, deletion of *Ring1B* had no effect on the transcription of satellite repeats, thus obviating the specificity of *BRCA1* for satellite repeats.



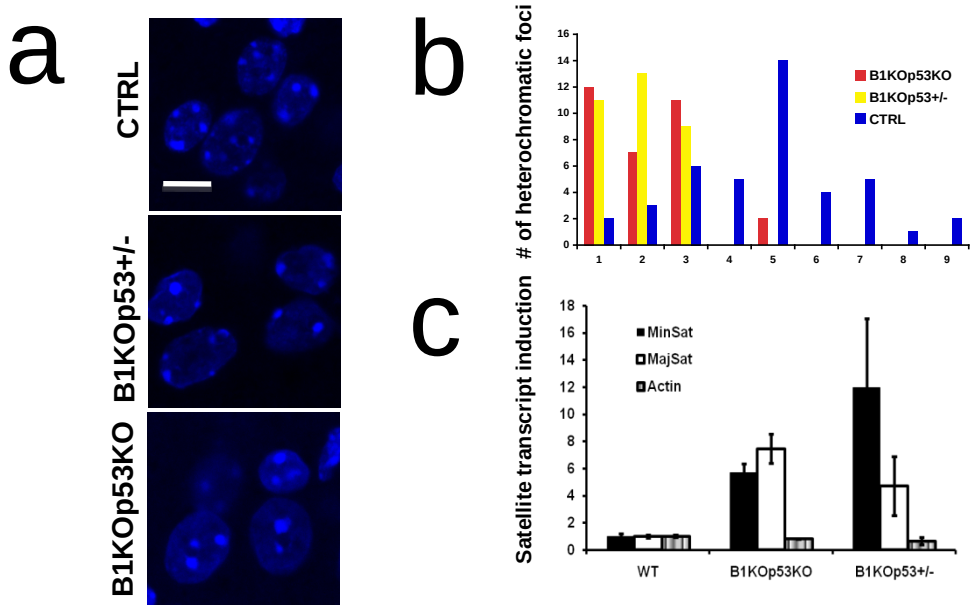
Supplementary Figure 7. The H2A-Ub mimic fusion protein incorporates into chromatin. U2OS cells were transfected with constructs expressing the wildtype H2A-Ub, various mutants, flag tagged *GFP* and flag tagged *HP1α*. Two days after transfection, subnuclear fractionation experiments were performed to isolate cytoplasmic, high salt chromatin nuclear extracts, DNAseI digestion fractions as well as nuclear matrix fractions as described by Reyes JC et al. (*J Cell Biol* 137 (2), 263-274 , 1997). The fractions were subjected to Western blots probed with **a,** an anti-flag antibody. **b,** H2A antibody, which serves as a positive control for chromatin and **c,** p100 which is a control for the cytoplasmic fraction. The results show that the exogenous H2A-Ub fusion protein incorporates into the chromatin fractions much like the endogenous histone H2A. The localization of the various mutant ubiquitin-H2A fusion proteins described in Supplementary Fig. 9 also show the same distribution within chromatin fractions despite having reduced biological activity.



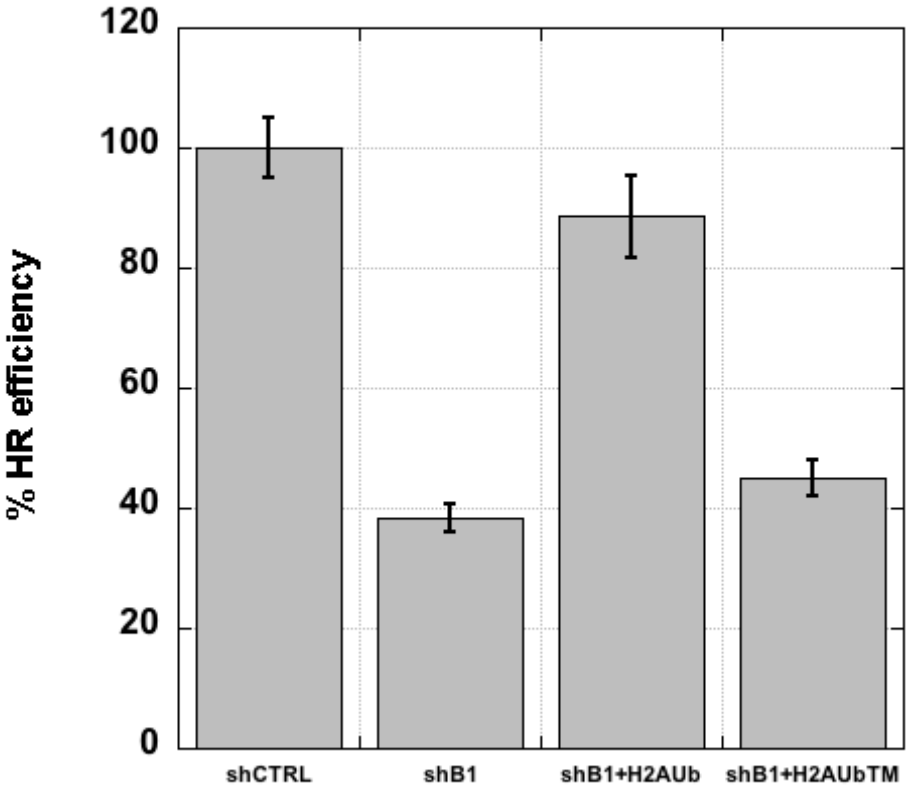
Supplementary Figure 8. Ectopic expression of ubiquitylated histone H2A in neural stem/progenitor cells. Whole cell lysates were harvested and run on a 12% Bis-Tris glycine gel and the Westerns were performed with a flag M2 antibody. α-Tubulin was used for normalization of input protein (lower panel).



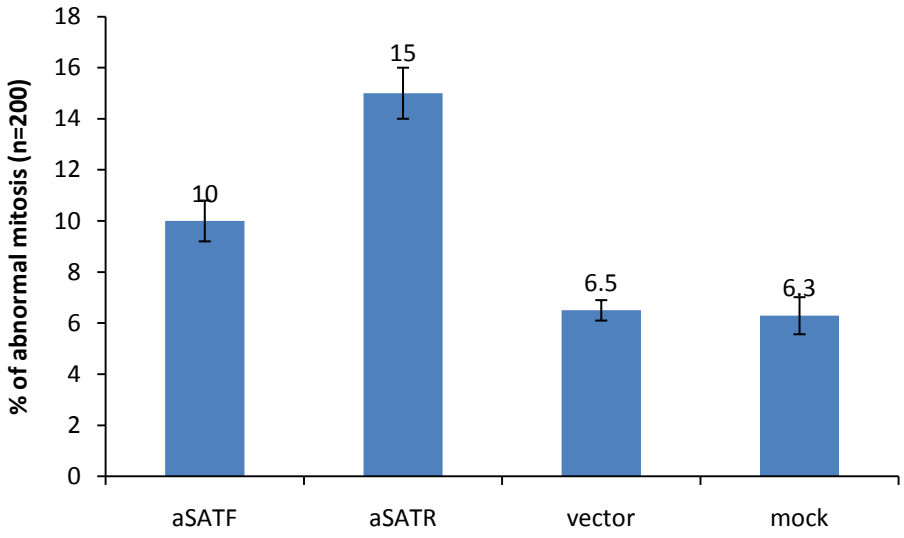
Supplementary Figure 9. Generation of mutations in H2A-Ub that impair its effect on satellite DNA silencing and proliferation rescue of *BRCA1* deficient cells. a. A D58A mutant within the ubiquitin domain of ubiquitylated histone H2A was defective in re-establishment of heterochromatin silencing in *BRCA1* deficient primary NPCs. NPCs were cultured as in Figure S2 and infected with either a *CRE-GFP* expressing retroviral vector to excise *BRCA1* (B1 -/-) or a *GFP* as control. A lenti-viral vector expressing ubiquitylated histone H2A or a mutant I44A or D58A was coinfected with either retroviral vector. The levels of satellite DNA transcription were measured by quantitative RT-PCR and graphed. The D58A mutant reduced silencing effect by 6 fold whereas the I44A mutant was still able to maintain most of the repressive activity for the major satellite repeats. **b**. H2A-Ub mutants were defective in restoring the proliferation of *BRCA1* deficient NPCs. Three days after infection with CRE-GFP to delete the floxed *BRCA1* allele (3 dpi), cells were labeled with EdU prior to fixation, immunostaining and FACS analysis. Results are representative of 3 independent experiments. Extending the hydrophobic patch mutation to include L8A, V70A and combining these with the D58A mutation (TM mutant) did not further decrease the activity of the D58A H2A-Ub mutation. **c.** IP Western shows the expression levels of Ub-H2A and mutants. Whole cell lysates were harvested and immunoprecipitated with anti Flag M2 beads. The reaction was run on a 12% Bis-Tris glycine gel and the Westerns were performed with a flag M2 antibody. P65 was used for normalization of input protein (lower panel).



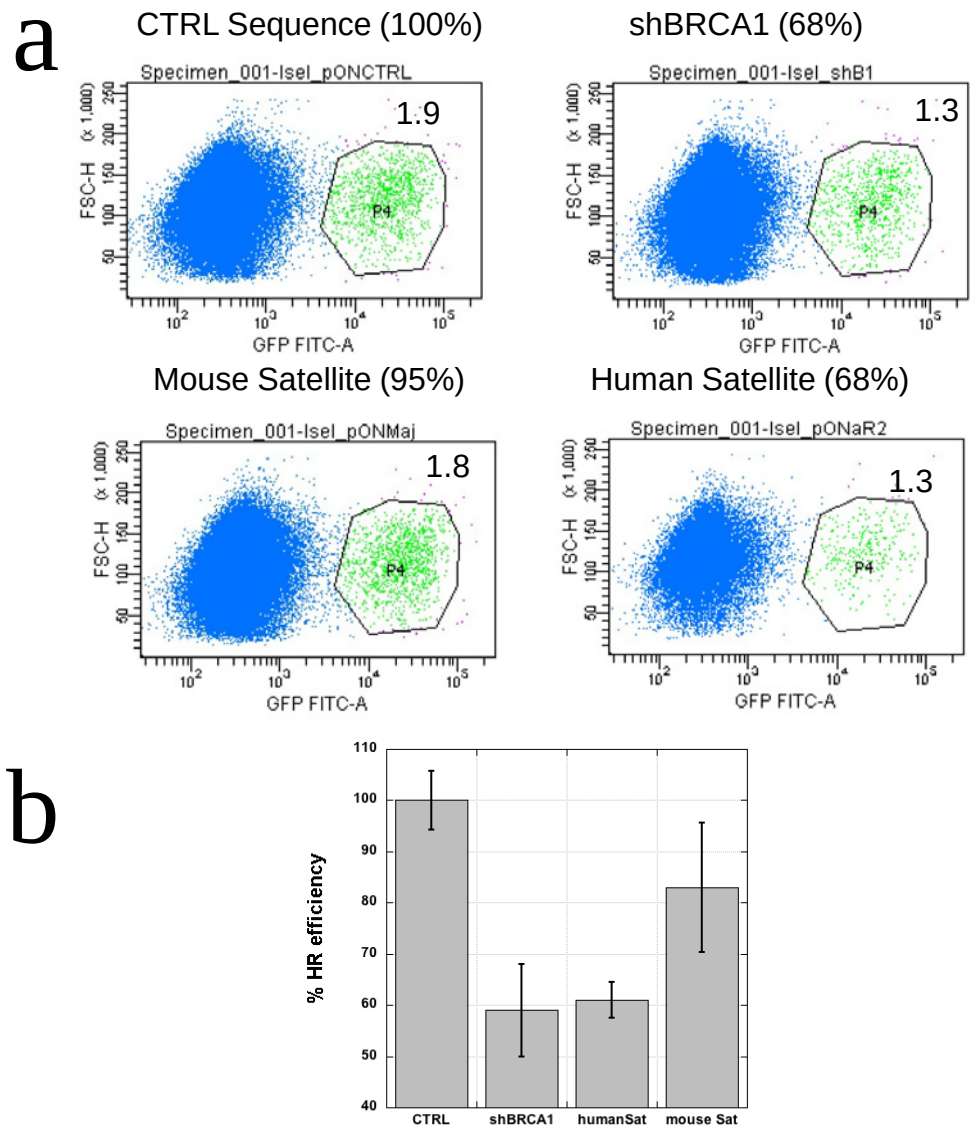
Supplementary Figure 10. Tumor suppressor p53 rescues apoptosis/proliferation but not heterochromatin defects in *BRCA1* KO brains. The BRCA1 conditional knockout mice were crossed onto a p53 knock out background. The genotypes of the animals are: *B1KOp53KO: (Brca11+/-;Brca5-13cK+/-;nestin-Cre+; p53-/-), BRCA1 KO/p53+/-: (Brca11+/-;Brca5-13cK+/-;nestin-Cre+; p53+/-)*, and control *(Brca11+/+;Brca5-13cK+/-;nestin-Cre+; p53+/-)*. **a**. DAPI staining of controls and *BRCA1* and *p53* double KO mutant *(B1KOp53KO)* cortical sections. Scale bar: 10 µm. **b**. Distributions of heterochromatic foci/cell in the brains of mutant and control animals showed that the number of heterochromatic foci/cell in *BRCA1* KO (with both *p53+/-* and *p53KO* background) mutants was significantly lower than that in the control brain (student's *t* test, $p < 0.005$). **c**. Quantitative RT-PCR experiments showed that heterochromatic region transcripts were upregulated in *BRCA1* and *p53* double KO brains. Ct values of each sample were normalized by an internal control, *cyclophilin*. *BRCA1* KO phenotypes can be largely rescued by a concomitant loss of *p53*. In the absence of *p53* DNA damage checkpoints are abrogated and *BRCA1* deficient cells regain their proliferative capacity and suppress apoptosis. Under these rescue conditions, *BRCA1*, *p53* double knockout cells still show reduced heterochromatic centers and failure to silence satellite repeat DNA regions. The result excludes the possibility that proliferative defects lead to satellite transcriptional derepression because proliferation is restored. Furthermore it also makes it unlikely that a DNA damage checkpoint signal is responsible for satellite derepression, as this too is absent in the double knockout cells.



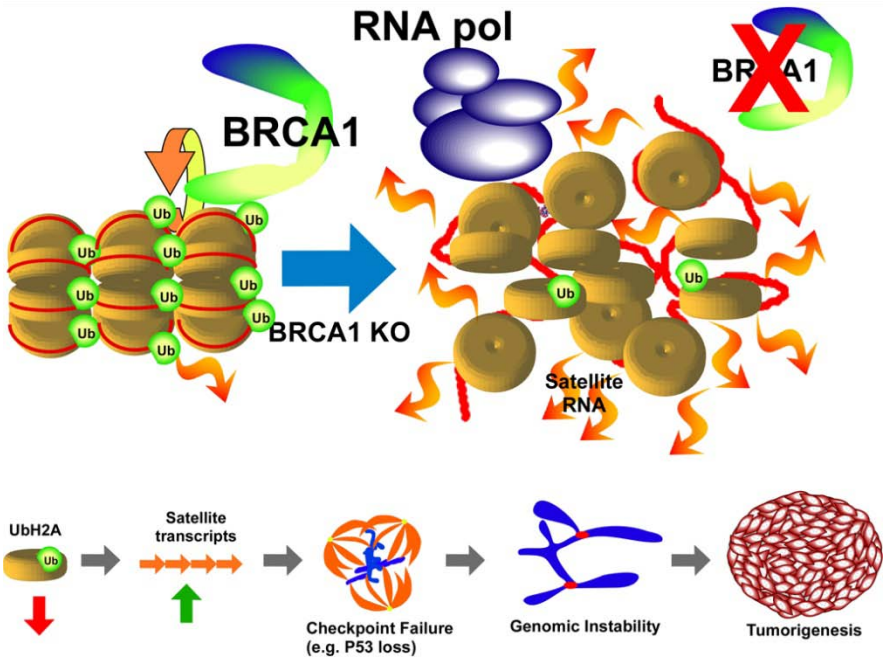
Supplementary Figure 11. UbH2A rescued HR deficiency in *BRCA1* null cells. U2OS cells expressing the homologous recombination reporter *GFP* system (Moynahan et al, 2001, *Mol Cell* 7, 263-272) were first transduced with various constructs expressing H2A-Ub or satellite RNAs. 48h later, the I-SceI expressing vector was transfected into the cells before harvesting and FACs analysis to quantify homologous recombination by the percentage of GFP positive cells. The results show that co-expression of the H2A-Ubiquitin mimic (H2A-Ub) can recover about 50% of the homologous recombination activity lost with the introduction of shBRCA1. A mutant H2A-Ub construct (H2AUbTM) that has 4 point mutations on the surface of ubiquitin, is unable to rescue more than 10% of the activity. The observation suggests that ubiquitylated H2A mediates the contribution of *BRCA1* to homologous recombination.



Supplementary Figure 12. Overexpression of satellite DNA induced mitotic catastrophe. The percentage of abnormal mitosis was plotted. 100 mitotic cells were counted for each sample. The result is an average of three different fields.



Supplementary Figure 13. Satellite RNA overexpression decreased homologous recombination efficiency but not control RNA sequences. U2OS cells expressing the homologous recombination reporter *GFP* system (same as in Supplementary Fig. 11) were cotransfected with the I-SceI expressing vector as well as the vectors expressing satellite RNA sequences. 48h after transfection, cells were harvested and subjected to FACS analysis to quantify the percentage of *GFP* positive cells. Homologous recombination activity as percentage of the wildtype unmodified cells is shown **a**. representative FACS dot plots. **b**. Bar graphs depicting the effect of satellite RNA expression on the efficiency of homologous recombination by shBRCA1, human alpha satellite or mouse Major satellite.



Supplementary Figure 14. A model for the role of *BRCA1*-mediated heterochromatin silencing in tumor suppression. BRCA1 localizes to constitutive heterochromatin and places an epigenetic repressive mark by ubiquitylating histone H2A through its E3 ubiquitin ligase (RING domain). *BRCA1* maintains the silenced state of heterochromatin thereby reducing satellite DNA transcription. However, absence of *BRCA1* leads to the reduction of the ubiquityl-histone H2A mark and destabilization of *HP1* proteins. As a result, the structure of constitutive heterochromatin is altered, silencing of satellite DNA is alleviated, and satellite RNA transcripts are elevated, which eventually leads to genomic instability and tumorigenesis.