**Supplemental Figure 1:** A) Time course of BMI1 localization to laser scissors induced DNA breaks. HeLa cells were treated with laser scissors, fixed with paraformaldehyde for IF analysis at the indicated time points. IF was performed using antibodies to pH2AX and BMI1. B) A vector encoding eGFP-BMI1 fusion proteins was introduced into HeLa cells. These cells were treated with laser scissors, and after 30 minutes fixed, and

processed for IF using antibodies to pH2AX to localize the regions of DNA breaks. C) HeLa cells were treated with BMI1 specific or control siRNA, treated with laser scissors and processed for IF using antibodies to 53BP1 and BMI1. Western blots showing protein levels of BMI1 and tubulin are shown to left.

**Supplemental Figure 2: A)** HeLa cells were incubated with 0.5 µM Aphidicolin or carrier alone for 24 hours and then processed for chromatin IP using either irrelevant IgG or BMI1 specific antibodies. PCR was performed using two different sets of primers specific for the FRA-3B locus. B) *H2AX* +/+ and *H2AX* -/- MEFs were treated with laser scissors and localization of pH2AX and 53BP1 determined by IF.

**Supplemental Figure 3:** Time course of localization of BMI1 to DNA damage in *H2AX* +/+ and *H2AX* -/- MEFs. *H2AX+/+* and *H2AX* -/- MEFs were treated with laser scissors, and IF performed for 53BP1 and BMI1 at 5min, 10min, 20 min, 30 min, 45 min and 60 min after laser treatment. Bottom panel shows quantitation of % of cells with 53BP1 stripes showing co-localized BMI1 signal in H2AX +/+ and *H2AX* -/- cells at these time points. The data are the mean of 3 separate experiments +/- SEM.

**Supplemental Figure 4:** Mouse breast cancer cell lines with either a targeted *Brca1* -/-; *p53*-/- background (top panel) or a *Brca1 +/+; p53 -/-* background were treated with laser scissors and after 30 minutes, processed for IF using antibodies to pH2AX and BMI1. Quantitation of the percent of cells treated with laser-scissor that shows co-localization of BMI1 with pH2AX are shown in the bar graph. Data are plotted as mean +/-SE of at least 3 experiments.

**Supplemental Figure 5** A) Western Blot showing level of BMI1 and tubulin in HeLa cells treated with ATMi, HelA cells treated with ATR siRNA, Seckel cells, and *Rnf8*+/+ and *Rnf8* -/- MEFs. B)HeLa cells were treated with either 0 uM or 0.5 uM ATM and localization of ATM phosphorylated on serine 1981 and 53BP1 was assayed by IF. C) HeLa cells were treated with either 0 uM or 0.5 uM ATM and localization of pH2AX and 53BP1 was assayed by IF.

**Supplemental Figure 6:** *Parp1* +/+ or *Parp1* -/- MEFs were were treated with laser scissors and after 10 or 30 minutes, processed for IF using antibodies to pH2AX and BMI1. Quantitation of the percent of cells treated with laser-scissor that shows co-localization of BMI1 with pH2AX are shown in the bar graph. Data are plotted as mean +/-SE of at least 3 experiments.

**Supplemental Figure 7:** *Rnf8* -/- MEFs were treated with laser scissors and after 30 minutes the cells were fixed and localization of pH2AX, and either RAP80 or Ubiquitinated H2A (UbH2A) was assayed by IF.

**Supplemental Figure 8.** A) HeLa cells were treated with control or RING1B specific siRNA, treated with laser-scissors, and localization of BMI1 and pH2AX determined by IF. Western blots showing protein levels of RING1B and Tubulin are shown to left. B) HeLa cells were treated with laser scissors and localization of MEL18, RING1A and pH2AX localization were determined by IF at the indicated time points. Right panel shows quantification of localization of H2AX, MEL18 and RING1A; data are mean +/-SEM of three experiments.

**Supplemental Figure 9.** Full blots of the Western blot data shown in Figure 5B are shown. Here *Bmi1-/-; Ink4a -/-; Arf-/-* MEFs, or *Bmi1+/+; Ink4a -/-; Arf-/-* MEFs were treated with 0Gy, 5 Gy, or 10 Gy IR, and after 30 minutes, chromatin extracts were prepared and subject to western blotting using either antibody to Ub-H2A-K119 (Left panel) or histone H2A (right panel).

**Table S1: Antibodies Used in this Study**

| Antibody | Species, dilution | Source | Methods |
|---|---|---|---|
| Bmi1, clone F6 | Mouse, 1:200 | Millipore, Clone F6 | IF/ChIP/IB |
| CBX2/M33 | Goat, 1:300 | sc-19295, Santa Cruz Biotech | IF/ChIP |
| pH2AX | Mouse, 1:200 | 05-678, Millipore | IF/ChIP/IB |
| pH2AX | Rabbit, 1:200 | AHP881, AbD Sero Tech | IF |
| H2A | Rabbit, 1:200 | 2578, Cell Signalling | IB |
| 53BP1 | Rabbit, 1:200 | A300-272A,Bethyl Lab | IF |
| SUMO | Goat, 1:500 | sc-6375, Santa Cruz Biotech | IF |
| BRCA1-SD118 | Mouse, 1:20 | David Livington lab | IF |
| Ub-H2A, clone E6C5 | Mouse, 1:200 | 05-678, Millipore | IF |
| FK2 | Mouse, 1:300 | SPA-205E, Assay Design | IF |
| RAP80 | Rabbit, 1:400 | Xiaochun Yu , Junjie Chen | IF |
| Ring1B | Mouse, 1:100 | Yoko Koseki | IF |
| Phospho-Chk2 (Ser19) | Rabbit, 1:250 | Cell Signalling | IB |
| Chk2 | Rabbit, 1:1000 | Cell Signalling | IB |
| Tubulin | Mouse, 1:500 | Sigma | IB |
| ATR | Rabbit, 1:500 | Abcam | IB |
| MEL-18 | Goat, 1:300 | sc-8905, Santa Cruz Biotech | IF |
| RING1B | Rabbit, 1:200 | a kind gift from Dr.Koseki | IF/IB |
| RING1A | Rabbit, 1:300 | sc-28736, Santa Cruz Biotech | IF |
| pATM (Ser1981) | Rabbit, 1:300 | abcam | IF/IB |