Supplemental Movies:

Movie S1, related to Figure 2: GFP-PRDM2 recruitment to sites of laser-induced DSBs, 7 frames per second (fps).

Movie S2, related to Figure 5: PAGFP-H2B-associated chromatin changes in sh-RFP control cells, 30 fps.

Movie S3, related to Figure 5: PAGFP-H2B-associated chromatin changes in macroH2A1 knockdown cells, 30 fps.

Supplemental Experimental Procedures

Cell culture treatments. Treatment with TSA (50 μM) was initiated 30 min prior to laser microirradation and treatment was continued throughout the course of the experiment (~ 10 min). To inhibit ATM kinase activity, cells were treated with 20 μM KU55933 (ATMi, Tocris) 1 h prior to damage induction. For topoisomerase I inhibition, cells were treated with 1-2 μM camptothecin (CPT) for the indicated time points. For I-SceI induction, cells were treated with 2 – 5 μg/ml Dox. For laser microirradiation-induced DSB induction, cells were pre-sensitized with Hoechst 33342 (0.1 μg/ml) for 60 min. induction. For clonogenic survival assays, cells were transfected with the indicated siRNAs, seeded in triplicate in 60 mm dishes (100-1000 cells/dish) and treated overnight with the indicated doses of olaparib (AZD-2281). After fifteen days, colonies were stained with crystal violet and counted. For double thymidine block, cells were treated with 2.5 mM thymidine (Sigma) for 18 h, followed by a 9 h release and a second 17 h thymidine block. For serum starvation, cells were cultured in FBS-free medium for 72 h.

RNAi screen. Individual shRNA-expressing viral supernatants were arrayed in 96 well format. Each gene was targeted by approximately five distinct shRNAs, shRNAs directed against LacZ, luciferase and RFP as well as empty LKO.1 vector served as controls. DRGFP-U2OS cells were stably transduced with individual lentiviral shRNA constructs. Cells were synchronized in S phase prior to DSB induction by double-thymidine block. DSBs were induced by transfecting the pCBASce I-SceI expression vector using LT-1 transfection reagent (Mirus Bio). Cells were analyzed for HR efficiency by determining the fraction of GFP expressing cells 48 h post DSB induction using a Cellomics Arrayscan high content imager (Thermo Scientific). DAPI was used to determine total cell counts, only shRNA clones with cell counts > 40% of average well cell density were considered for further analysis. All experiments were performed in triplicate. Hits were identified as shRNAs resulting in a reduction or increase in HR efficiency (% GFP^+ cells) outside of at least one SD of the mean of combined control shRNAs. ShRNA HR data were subsequently analyzed by RNAi gene enrichment ranking (RIGER) using GENE-E software available online at http://www.broad.mit.edu/rnai_analysis. A separate ranking was performed based on the HR score of the second-best shRNA (Luo et al., 2008). Top hits were targeted by at least two shRNAs and scored in the top 10% of both rankings (see **Table S1**).

Image analysis. To measure changes in chromatin structure, changes in the area occupied by the photo-activated PAGFP-H2B were measured using MetaMorph (v.7.7.9) or Imaris (v. 7.4) image processing and analysis software. Briefly, an intensity threshold, inclusive of the signal from the photoactivated PAGFP and above background, was applied to the cells to provide a mask of the sub-nuclear area within the cell exposed to laser microirradiation. The area covered by the mask was measured at each time point after photoactivation within the time series. The initial time point after laser microirradiation and photoactivation was normalized to 1 and the percent change in area for each subsequent time point was calculated. Changes in area were plotted as percent of maximal expansion over the duration of the time

series. To measure the recruitment of GFP-tagged proteins in laser microirradiated cells, MIPAV software (v. 5.1) was used to draw regions of interest within the laser microirradiated sub-nuclear region, within a non-laser-microirradiated region of the nucleus, and a region outside the cell (McAuliffe et al., 2001). The mean fluorescence intensity within the ROIs was measured for each time point within the time series. The intensity values were background subtracted, and the ratio of intensity within the microirradiated nuclear area to non-microirradiated area was calculated. The ratio was normalized to the time point immediately prior to laser microirradiation. Photobleaching due to imaging was less than two percent over the duration of the time series.

Immunofluorescence (IF). Primary antibodies used for IF were: α–γ–H2AX (Abcam ab11174, Milipore 05-636), α–53BP1 (Santa Cruz sc22760), α–macroH2A1.2 (Milipore 14G7), α–H3K9me2 (Abcam ab1220). Secondary antibodies were from Molecular Probes (α–mouse or α–rabbit Alexa Fluor 568 or α–mouse Alexa Fluor 488). Images were acquired using a Zeiss LSM510 or LSM710 META confocal microscope (Zeiss).

ChIP lysis and IP. Fixed cells were resuspended in cell lysis buffer (5 mM PIPES pH 8.0, 85 mM KCl, 0.5% nonidet P-40) to isolate nuclei. Nuclei were resuspended in micrococcal nuclease (MNase) digestion buffer (10 mM Tris pH 7.4, 15 mM NaCl, 60 mM KCl, 0.15 mM spermine, 0.5 mM spermidine) and 1.2 U/µl MNase was added for 30-45 min at 37°C. The reaction was stopped by adding 50 mM EDTA and nuclear pellets were resuspended in 10mM Tris-HCl (pH8.0), 100 mM NaCl, 1mM EDTA, 0.5mM EGTA, 0.1% Na-deoxycholate, 0.5% N-lauroylsarcosine. Lysates were sonicated briefly to disrupt nuclear membranes using an ultra sonicator water bath (Bioruptor, Diagenode). Diluted lysates were incubated o/n at 4°C with the indicated antibodies after addition of 1% Triton X-100. IPs were performed using 30 µl Protein A/G magnetic beads (Pierce). Eluted DNA was purified with QIAquick PCR purification (Qiagen), according to the manufacturer instructions. The following Abs were used for IP: α–macroH2A1 (Millipore 07-219), α–H3K9me2 (Abcam ab1220), α–γ–H2AX (Millipore 05-636), α–BRCA1 (Santa Cruz sc6954) normal mouse IgG (Millipore 12-371). Purified ChIP DNA was analyzed by qPCR using a LightCycler 480 II (Roche), see **Table S3** for primer sequences.

Circular chromosome conformation capture (4C) sequencing. 10 million TRI-U2OS cells were crosslinked and lysed (10 mM Tris-HCl pH 8.0, 10 mM NaCl, 0.2% NP-40, protease inhibitors (Roche)) at 4°C for 1 hour. Lysed cells were washed with restriction enzyme (RE) buffer (New England Biolabs buffer 2) and incubated at 37°C for 1 hour in RE buffer containing 0.3% SDS. SDS was sequestered by Triton X-100 (1.8%). RE digestion was performed with 400 U of HindIII (New England Biolabs) overnight. Following enzyme inactivation, the reaction was ligated using 100 U T4 DNA Ligase (Roche) at 16°C overnight. Following reversal of crosslinks, the DNA was purified by phenol extraction and ethanol precipitation. For circularization, the DNA was digested with Csp6I (Fermentas) at 37°C overnight. Following enzyme inactivation, phenol extraction and DNA re-ligation, 3.2 µg of the

4C contacts library was amplified for 30 PCR cycles. Inverted PCR primers recognized an I-SceI proximal HindIII-Csp6I fragment on the integrated DRGFP transgene (see **Table S3**).

Western blotting antibodies. The following antibodies were used for immunoblotting: α–p-RPA (S4/S8, Bethyl), α–RPA2 (Millipore NA19), α–histone H3 (Cell signaling 9715), α–histone H2A (Abcam ab15653), α–H3K9 (me)2 (Abcam ab1220), α–macroH2A1 (Millipore 07-219), α–γ–H2AX (Millipore 05-636), α–53BP1 (Santa Cruz sc22760), and α–β–tubulin (Cell signaling 2146), α–HA (Santa Cruz sc805) and α–GFP (Santa Cruz sc9996).

Supplemental References

Luo, B., Cheung, H.W., Subramanian, A., Sharifnia, T., Okamoto, M., Yang, X., Hinkle, G., Boehm, J.S., Beroukhim, R., Weir, B.A*., et al.* (2008). Highly parallel identification of essential genes in cancer cells. Proc Natl Acad Sci U S A *105*, 20380-20385.

McAuliffe, M.J., Lalonde, F.M., McGarry, D., Gandler, W., Csaky, K., and Trus, B.L. (2001). Medical image processing, analysis and visualization in clinical research. Paper presented at: Computer-based medical systems (IEEE).