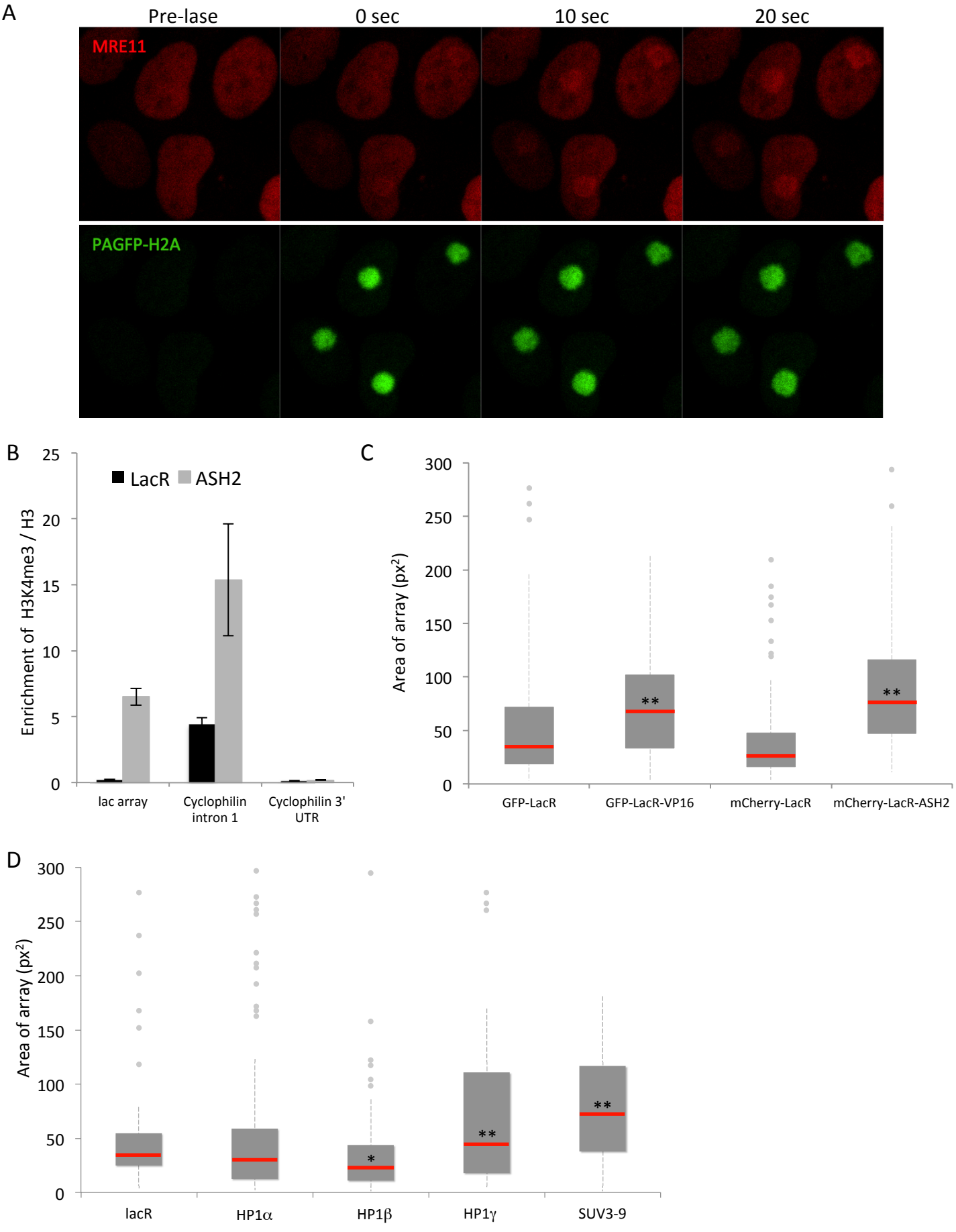
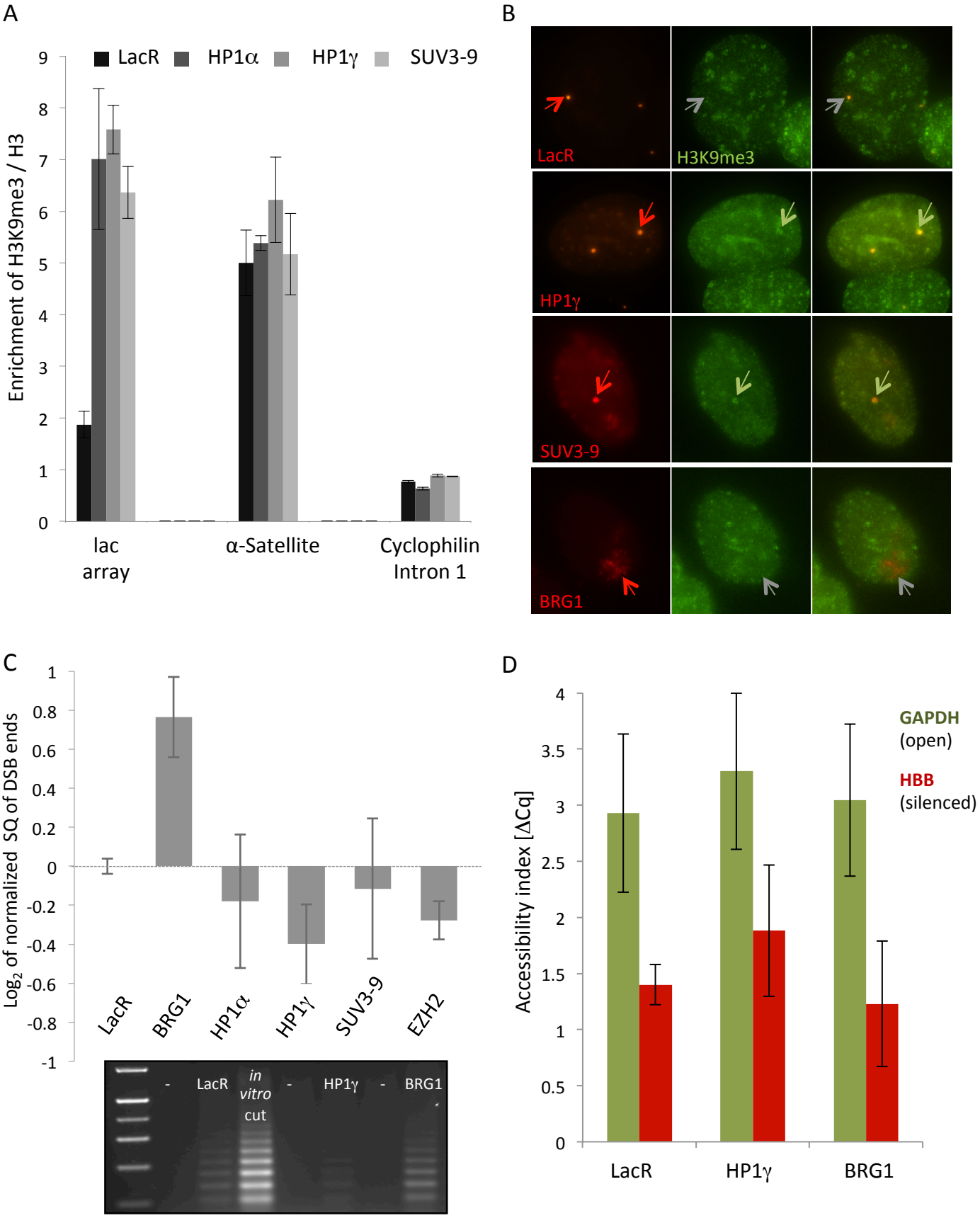
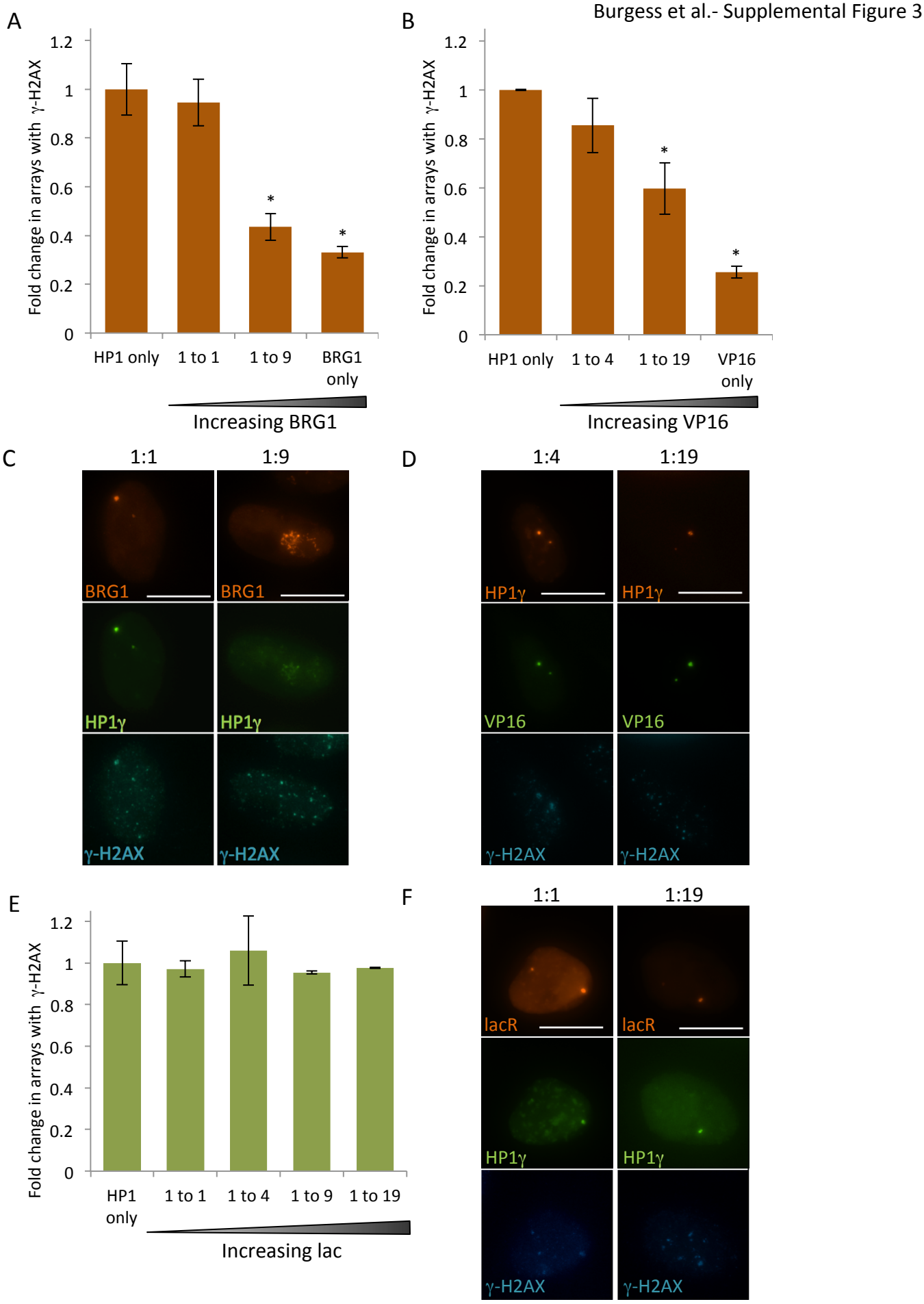
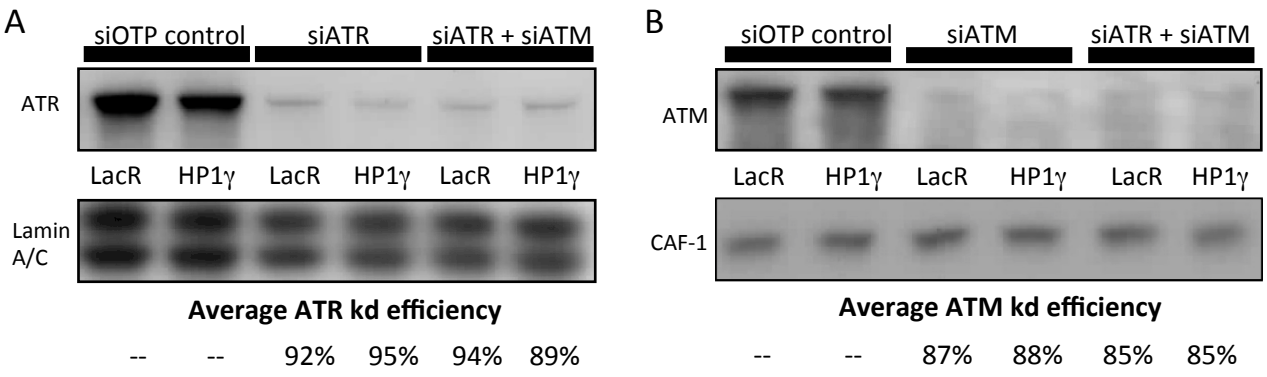
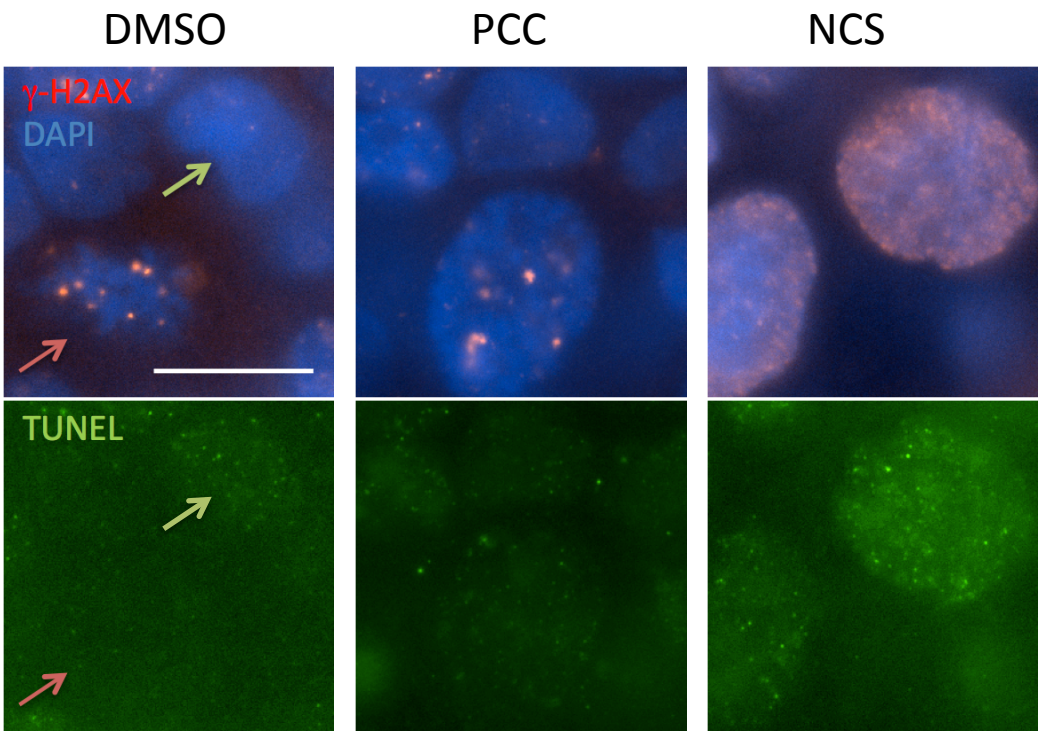
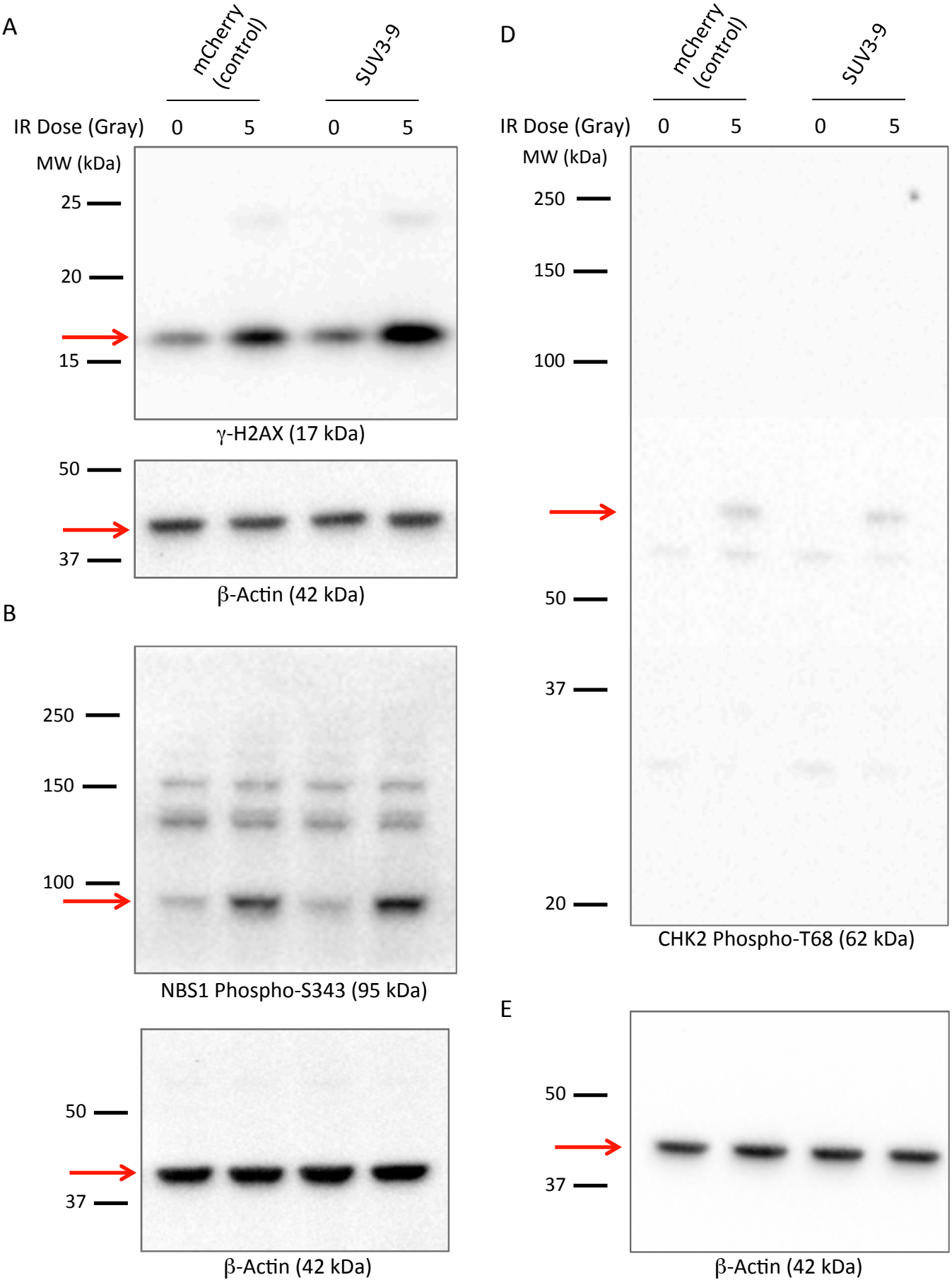

A
Pre-lase
0 sec
10 sec
20 sec
MRE11
PAGFP-H2A
B
LacR
ASH2
Enrichment of H3K4me3 / H3
lac array
Cyclophilin intron 1
Cyclophilin 3' UTR
C
Area of array (px²)
GFP-LacR
GFP-LacR-VP16
mCherry-LacR
mCherry-LacR-ASH2
D
Area of array (px²)
lacR
HP1α
HP1β
HP1γ
SUV3-9


A
LacR
HP1α
HP1γ
SUV3-9
Enrichment of H3K9me3 / H3
lac array
α-Satellite
Cyclophilin Intron 1
B
LacR
H3K9me3
HP1γ
SUV3-9
BRG1
C
Log2 of normalized SQ of DSB ends
LacR
BRG1
HP1α
HP1γ
SUV3-9
EZH2
- LacR in vitro cut - HP1γ - BRG1
D
Accessibility index [ΔCq]
GAPDH (open)
HBB (silenced)
LacR
HP1γ
BRG1


A
Fold change in arrays with γ-H2AX
1.2
1
0.8
0.6
0.4
0.2
0
HP1 only
1 to 1
1 to 9
BRG1 only
Increasing BRG1
B
Fold change in arrays with γ-H2AX
HP1 only
1 to 4
1 to 19
VP16 only
Increasing VP16
C
1:1
1:9
BRG1
HP1γ
γ-H2AX
D
1:4
1:19
HP1γ
VP16
γ-H2AX
E
Fold change in arrays with γ-H2AX
HP1 only
1 to 1
1 to 4
1 to 9
1 to 19
Increasing lac
F
1:1
1:19
lacR
HP1γ
γ-H2AX


A
siOTP control
siATR
siATR + siATM
ATR
LacR
HP1γ
LacR
HP1γ
LacR
HP1γ
Lamin A/C
Average ATR kd efficiency
-- -- 92% 95% 94% 89%
B
siOTP control
siATM
siATR + siATM
ATM
LacR
HP1γ
LacR
HP1γ
LacR
HP1γ
CAF-1
Average ATM kd efficiency
-- -- 87% 88% 85% 85%


DMSO
PCC
NCS
γ-H2AX
DAPI
TUNEL


A
mCherry (control)
SUV3-9
IR Dose (Gray) 0 5 0 5
MW (kDa)
25
20
15
γ-H2AX (17 kDa)
50
37
β-Actin (42 kDa)
B
250
150
100
NBS1 Phospho-S343 (95 kDa)
50
37
β-Actin (42 kDa)
D
mCherry (control)
SUV3-9
IR Dose (Gray) 0 5 0 5
MW (kDa)
250
150
100
50
37
20
CHK2 Phospho-T68 (62 kDa)
E
50
37
β-Actin (42 kDa)

Supplemental Figure Legends

Supplemental Figure S1. Local chromatin changes in the vicinity of DNA damage, related to Figure 1. (A) Representative timecourse of cells after DNA damage by laser micro-irradiation. mCherry-tagged MRE11 (red, top) is detectable in the earliest images capture after damage. Photo-activated GFP-H2A marks the damaged regions (green, bottom). Single z-planes are shown. Circle in t = 0 panel has a diameter of 30 pixels, 4.4 μm. (B) Chromatin immunoprecipitation of histone H3 tri-methyl lysine 4 (H3K4me3) in cells transfected with mCherry-LacR-ASH2L versus mCherry-LacR alone. Primers against the lac array were used to determine the enrichment of H3K4me3 mark after targeting chromatin modifiers to this site, while cyclophilin A intron 1 and cyclophilin A 3' UTR were used as control regions. The percentage of input was normalized to unmodified H3. Values represent means ± SD from independent experiments. (C) Array size changes when indicated chromatin factors were tethered. Box spans quartiles 1-3 and whiskers show the high and low values within 1.5 times the interquartile range (IQR). Gray circles indicate measurements that were considered outliers (outside 1.5 x IQR). Median values are marked by a red line. $N > 50$ in all conditions, $^{**}p < 0.01$. (D) Array size changes when indicated chromatin factors were tethered. Box spans quartiles 1-3 and whiskers show the high and low values within 1.5 times the interquartile range (IQR). Gray circles indicate measurements that were considered outliers (outside 1.5 x IQR). Median values are marked by a red line. $N > 25$ in all conditions, $^{*}p < 0.05$, $^{**}p < 0.01$.

Supplemental Figure S2. Local compaction of chromatin by Lac repressor fusion tethering, related to Figure 2. (A) Chromatin immunoprecipitation of histone H3 tri-methyl lysine 9 (H3K9me3) in cells transfected with mCherry-LacR-HP1α, HP1γ, and SUV3-9 versus mCherry-LacR alone. Primers for cyclophilin A intron 1 and α-satellite were used as control regions. The percentage of input was normalized to unmodified H3. Values represent means ± SD from independent experiments. (B) Enrichment of the H3K9 tri-methyl chromatin mark when mCherry-LacR-HP1γ or -SUV3-9 were tethered to arrays (red) for 20 h, then fixed and stained for the H3K9me3 chromatin mark (green). Images are maximum intensity projections of representative cells. BRG1-tethered arrays and LacR alone shown as controls. (C) Accessibility of the lac tethering array to endonucleolytic cleavage by I-SceI was tested by expressing the enzyme in cells after tethering, followed by DNA isolation and ligation-mediated real-time PCR. Shown is the $\log_2$ of starting quantities (SQ) of break ends, as determined by real-time PCR. Error bars indicate SD from 3 independent experiments. A representative gel from standard PCR amplification of these reactions is shown below the graph. (D) Analysis of nuclease accessibility of unrelated epigenetically silenced loci (*HBB*), and expressed loci (*GAPDH*). Shown is the average accessibility index [ΔCq] ± SD from 2 independent trials of triplicate quantitative PCR.

Supplemental Figure S3. Chromatin-induced DDR activation is not due to direct recruitment of DDR proteins by HP1, related to Figure 2. Tethering of HP1γ in the presence of increasing amounts of the indicated chromatin remodeling protein expression constructs: (A) BRG1 (B) VP16. Fold change of arrays showing colocalized γ-H2AX.

Values represent averages ± SD from at least 2 independent experiments. * $p < 0.05$, compared to HP1 only, $N = 75$-200 for each condition. (C, D) Representative images of cells co-transfected with HP1γ and the indicated chromatin remodeler construct at two ratios, as quantified in A and B. γ-H2AX far-red immunostaining (blue). Scale bar, 5 μm. (E) Increasing expression of LacR relative to HP1γ tethering construct does not affect phospho-H2AX at arrays. (F) Representative images of cells co-transfected with LacR alone and HP1γ at two ratios. γ-H2AX far-red immunostaining is shown in blue. Scale bar, 5 μm.

Supplemental Figure S4. Depletion of ATM and ATR kinases, related to Figure 4. Western blots demonstrating knockdown efficiency of ATR (A) and ATM (B) kinases in siRNA experiments. Shown are representative blots for ATR and ATM, while Lamin A/C and CAF-1 are used as loading controls for the two proteins, respectively. Average knockdown efficiency values are the mean of the two experiments used for quantification in Figure 4D.

Supplemental Figure S5. Absence of DNA breaks in mitotically-condensed or chemically-condensed chromosomes, **related to Figure 5.** Mitotic cells or cells undergoing premature chromosome condensation (PCC) do not show TUNEL staining (green) for DNA ends, despite the existence of γ-H2AX foci (red). Shown are single Z-plane images, with DAPI staining in blue. In the left panel of asynchronous DMSO-treated control cells, the red arrow indicates a mitotic cell, and the green arrow marks an

interphase cell. Cells treated with the radiomimetic neocarzinostatin (NCS) were included as a positive control for DNA breaks. Scale bar, 5 μm.

Supplemental Figure S6. DDR signaling from irradiation-induced DSBs is affected by chromatin compaction, related to Figure 6. Full blots from images shown in panel A. Molecular weights markers are shown on the left in kDa, and the red arrow depicts location of protein of interest. (A) NBS1 phospho-S343 (B) β-actin from the NBS1 blot (C) CHK2 phospho-T68 (D) β-actin loading control from the CHK2 blot.

Supplemental Experimental Procedures

Construction of plasmids

HP1 tethering constructs were a generous gift from the Belmont lab (University of Illinois). These were subcloned to replace GFP with mCherry using AfeI and BsrG1 sites; the functionality of these HP1 constructs was confirmed previously (Verschure et al., 2005). The mCherry-LacR-MIS18α construct was provided by Dan Foltz (University of Virginia). The mCherry-LacR-MRE11 and PAGFP-H2A constructs were obtained from Evi Soutoglou (IGBMC, Strasbourg). An EZH2-GFP plasmid from Alexander Tarakhovsky (Rockefeller University) was used for sequential subcloning to replace GFP with GFP-LacR-NLS using BsrG1 and MfeI sites, followed by replacement of the GFP-LacR with mCherry-LacR excised with AgeI and EcoRV from the original mCherry-LacR repressor construct (Dundr et al., 2007). SUV3-9H1 cDNA was amplified from a construct provided by Thomas Jenuwein (Max Planck Institute of Immunobiology and Epigenetics), using primers containing unique AfeI and XbaI sites. The digested product was then ligated in-frame into the parental mCherry-lac expression vector using the SmaI and XbaI sites. The BRG1 fusion construct was created by amplification of its cDNA with primers containing adaptamers of the restriction sites EcoRV and SpeI, which were used for in-frame ligation of the ORF downstream of lac repressor in the above-referenced mCherry-LacR expression vector. ASH2L was amplified from a construct provided by David Skalnik (Indiana University School of Medicine), using primers

containing unique SmaI and XbaI sites and ligated into the MCS of the mCherry-LacR plasmid. All constructs were confirmed by sequencing.

For experiments utilizing DNA damage an HA-I-SceI construct developed by the Jasin lab (Rouet et al., 1994) was used for constitutive cutting, whereas for inducible breaks, we produced a CFP-I-SceI-GR construct by ligation of the SacI/BamHI fragment from the RFP-I-SceI-GR plasmid (Soutoglou et al., 2007) into the SacI/BamHI site of the vector pECFP-C1 (Clontech).

Cell lines, tissue culture, transfection, and treatments

U2OS stable cell lines were generated and maintained as previously described (Soutoglou et al., 2007). Once established, stable U2OS cell lines were grown using standard tissue culture methods in DMEM, supplemented with 10% FBS, glutamine and antibiotics. Transient transfections were carried out using the Amaxa Nucleofector Kit V (Lonza), according to the manufacturer's protocol. All tethering experiments were carried out using transient expression of the constructs for 20 h before harvesting or fixation, unless otherwise noted.

To induce premature chromosome condensation (PCC), Calyculin A (CalA) was added to cells at a final concentration of 50 ng/ml for 60 min. DNA damage treatment was carried out by incubating cells with 50-200 ng/ml neocarzinostatin (NCS) for 30 min.

Prior to experiments using CFP-I-SceI-GR, cells were cultured in DMEM with 10% charcoal-dextran treated serum (Atlanta Biologicals) for 48 h. Cells were transfected first with tethering constructs using the Amaxa Nucleofector kit V (Lonza) according to the manufacturer's instructions. 12 h later, cells were trypsinized and counted using a hemocytometer and transfected with 5 μg CFP-GR-I-SceI construct per 1 million cells using the same protocol. After 12 h (24 h total), CFP-GR-I-SceI was activated with dexamethasone (Sigma) at a concentration of 100 nM for 20 min. Cells were fixed and stained as described below.

Immunofluorescence and imaging

Cells were fixed and stained as previously described (Soutoglou et al., 2007), except that images were captured on a DeltaVision workstation equipped with a CCD camera (CoolSNAP HQ; Photometrics) mounted on a microscope (IX70; Olympus) with a 60×1.42 NA oil immersion objective (Olympus). 20-50 focal planes were captured at 0.2-0.5 μm resolution, and analyzed with the softWoRx package for colocalization or total nuclear H2AX (Applied Precision), or ImageJ (NIH) for integrated density measurements of foci.

Chromatin accessibility assays

Cells were co-transfected with HA-I-SceI construct and tethering construct for 20 h before harvesting for genomic DNA with the Qiagen Blood and Cell Culture DNA Mini Kit. 1 μg of DNA was then ligated to 100 pmol of an asymmetric adaptor with I-SceI compatible ends (5'GCATCACTACGATGTAGGATG 3') and

(CATCCTACATCGTAGTGATGCTTAT) at 16 °C for 16 h. After heat inactivation, quantitative PCR was performed with the specific adaptor primer (*LM-Sce primer*: 5' CATCCTACATCGTAGTGATGC 3') and a primer from the lacO repeats (*Lac-R*: TGTGGAATTGTGAGGGGATA) using BioRad iQ SYBR Green real-time PCR mix.

For chromatin accessibility at the *GAPDH* and *HBB* loci, the BioRad EpiQ chromatin analysis kit was used according to manufacturer's instructions, but with DNA isolated from cells transfected with LacR control plasmid, HP1$_\gamma$ or BRG1 tethering constructs. Accessibility index was calculated as the absolute value of the difference in quantification cycle between the nuclease-digested and the undigested chromatin.

Chromatin immunoprecipitation (ChIP)

ChIP assays were performed as previously described (Luco et al., 2010). In brief, transfected cells were crosslinked for 10 min in 1% formaldehyde at room temperature, quenched with 125 mM glycine, and swelled on ice for 10 min. Chromatin was sonicated to an average length of 200-500 bp and incubated for 14 h with pre-coated anti-IgG magnetic beads (Dynabeads M-280 Invitrogen) previously incubated the antibodies for 6 h at 4°C. The antibodies used were: rabbit anti-H3-K9me3 (5 μg, Abcam ab8898), anti-H3-K4me3 (Millipore/Upstate 05-745) and rabbit anti-H3 (1 μg, Abcam ab1791). Control immunoprecipitations were performed with no antibody and input between samples was normalized with anti-H3 ChIP. Subsequently, the beads were washed several times and eluted in 1% SDS and 100 mM $NaHCO_3$ buffer for 15 min at 65°C. The eluates were incubated at 65°C for 7 h to reverse crosslinks. Chromatin was

precipitated with ethanol o/n, treated with proteinase K, and purified using a PCR purification kit (Qiagen). Immunoprecipitated DNA (1.5 µl) and serial dilutions of the 10% input DNA (1:4, 1:20, 1:100 and 1:500) were analyzed with SYBR-Green (iQ Supermix Bio Rad) on a Bio Rad C1000 ThermoCycler. Primer pair sequences used in real-time PCR for ChIP assay, (related to Supplemental Figures S1 and S2). Recommended annealing temperature for all pairs = 60°C.

Cyclophilin A intron 1	F: CCCCACCCCACCTATGAGTGTAGT
	R: ACCCCTCCATTCTCATCAAGACCT
α-Satellite	F: CATCGAATGGAAATGAAAGGAGTC
	R: ACCATTGGATGATTGCAGTCAA
Lac array	F: GAGTGGTAACTCGACATTACCCTG
	R: GAGGCGCCGAATTCCACAAAT
Cyclophilin A 3'UTR	F: ATTCCCTGGGTGATACCATTCAAT
	R: ATGACAACGTGGTGAGGCTATTCT

TUNEL staining

For end labeling, cells were fixed in 1:1 ice cold MeOH/Acetone for 10 min at 4°C, and incubated for 1 h at 37°C with FITC-dUTP and TdT enzyme (Roche) in a humid chamber. After labeling, cells were blocked in 10% FBS/3% BSA solution (in PBS) for 1 h, RT in the dark, followed by primary antibody incubation with 1:1000 anti-FITC AF488 (Invitrogen) in blocking buffer for 1 h at RT or 4°C overnight. If required, 1:200 anti-HA (mouse) was also added to primary antibody mix to visualize I-SceI expression. After washing, secondary antibody was added to amplify the FITC signal (1:400 donkey

anti-rabbit AF488, Invitrogen) and incubated for 1-2 h at RT in the dark. If anti-HA antibody was also used, appropriate far-red secondary antibody was also added. After the final wash, coverslips were mounted on slides with or without DAPI.

Cell synchronization

Cells were arrested in G1 by double thymidine block. Briefly, cells at 30-50% confluence were grown in normal medium supplemented with 2 mM thymidine for 17-20 hours. Cells were then released for 8 hours into medium containing 30 μM cytidine, then blocked a second time in 2 mM thymidine for 20 hours. The cells were transfected (Amaxa) with tethering constructs upon plating into the 2nd block, and fixed as described above. Cells were immunostained for proteins of interest, as well as cyclin A to exclude any cells that escaped from G1, and to ascertain the efficacy of the arrest procedure.

For experiments using cell cycle exit, cells were forced into quiescence by serum starvation (0.5% serum) for 48 hours. After transfection of tethering constructs using the above-described electroporation protocol, cells were plated in low serum medium to maintain the block. Counterstaining with Ki-67 antibody was carried out to exclude any cells that continued cycling and escaped the arrest procedure.

Fluorescence-activated cell sorting

Standard DNA content analysis for cell cycle stage was performed using FxCycle Violet stain (Invitrogen) according to the manufacturer's protocol, and sorted on a Becton-

Dickinson FACScan machine and fit to the Dean-Jett-Fox histogram fit model contained within the FlowJo software package.

Western Blotting

Protein harvested by Laemmli buffer lysis, boiled in the presence of phosphatase and protease inhibitors was run on denaturing gels using the Invitrogen Gel system, with Novex 4-12% Bis-Tris gels and the manufacturer-supplied MOPS buffer, or MES buffer for proteins smaller than 20 kDa. Blots were transferred by a standard tank transfer system (150 mA, 1 h, Tris-Glycine buffer + 10% methanol), except when blotting ATR or ATM proteins, in which case methanol was excluded from the transfer buffer, and carried out at 50 mA overnight at RT.

Southern blotting

For DNA blot analysis, 5 μg of PstI-digested genomic DNA was run overnight on a 1% agarose/TBE gel, followed by an overnight transfer onto negatively charged nylon membrane using downward capillary transfer apparatus and an alkaline buffer. The Roche DIG detection system was used for hybridization with the DIG-dUTP labeled lacO probe, prepared by nick translation as previously described (Meaburn, 2010). Washing and detection were performed according to the manufacturer's instructions.

Antibodies

Antibodies used for immunofluorescence were as follows: γ-H2AX (Millipore), 53BP1 (Novus Biologicals), NBS1 (Novus), NBS1 phospho-S343 (Cell Signaling), cyclin A

(Thermo-Fisher), MDC1 (Novus), ATM phospho-S1981 (Millipore), anti-HA (Roche), Ki-67 (BD Bioscience), Histone H3K9me3 (Millipore), SMC1 phospho-S957 (Novus). Additional antibodies used only for immunoblotting were as follows: ATM (Millipore), ATR (Santa Cruz), Lamin A/C (Santa Cruz), p53 phospho S15 (Cell Signaling), CHK2 phospho-T68 (Cell Signaling), β-actin (Sigma), NBS1 phospho-S343 (Novus).

Array size quantification

LacR array sizes were quantified with Metamorph. LacR foci were first thresholded to closely encircle the array, then individual areas calculated.

Supplemental References

Dundr, M., Ospina, J.K., Sung, M.H., John, S., Upender, M., Ried, T., Hager, G.L., and Matera, A.G. (2007). Actin-dependent intranuclear repositioning of an active gene locus in vivo. J Cell Biol *179*, 1095-1103.

Rouet, P., Smih, F., and Jasin, M. (1994). Expression of a site-specific endonuclease stimulates homologous recombination in mammalian cells. Proc Natl Acad Sci U S A *91*, 6064-6068.