SUPPLEMENTARY INFORMATION

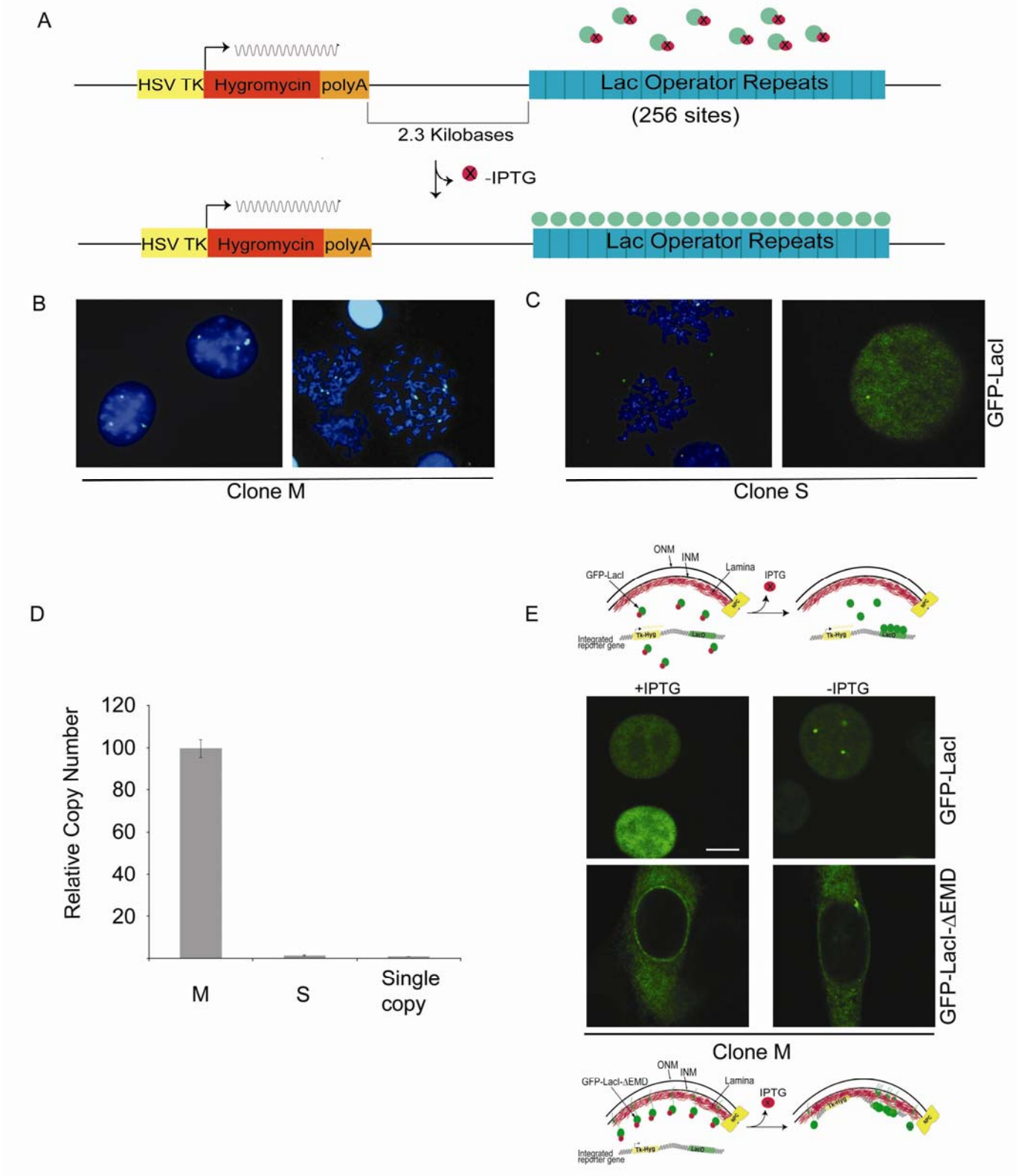

Fig. S1 Structure and analysis of integrated reporter construct. (A) Schematic depicting the *Tk-hyg lacO* reporter gene construct (17kb). The *Tk-hyg lacO* construct is comprised of a *hygromycin* gene driven by the HSV thymidine kinase promoter. The *lacO* binding sites are positioned 2.3Kb downstream of the *hygromycin* gene. Upon withdrawal of IPTG (red circles with X), the *lacO* arrays are bound by the GFP-LacI protein (green circles) and are detectable as fluorescent foci. (B) Clone M cells have multiple insertions of the reporter gene construct. 2D FISH analysis of interphase (left panel) and metaphase (right panel) with a *lacO* DNA probe shows three insertion sites. (C) Clone S cells have the reporter gene construct inserted at a single site. 2D FISH analysis with *lacO* DNA probe (left panel) and live cell imaging with GFP-LacI (right panel) (D) Quantitative PCR (Q-PCR) analysis of copy number of reporter gene construct in clone M and S cells. Copy number of the *hygromycin* reporter gene is normalized to a single copy of the β-actin gene. (E) The two schematic drawings depict the inducible visualization and tethering systems. The stably integrated reporter gene contains an array of *lac* operators. Nucleoplasmic GFP-LacI (green circles) is used to visualize the initial nuclear disposition of the reporter gene. The GFP-LacI-ΔEMD tethering protein is targeted to the inner nuclear membrane (INM) and is unable to bind *lacO* sites in the presence of IPTG (red circles with X). Upon IPTG withdrawal, the tethering protein can bind to *lacO* sites in the integrated reporter gene construct. This interaction is anticipated to result in tight association of the reporter gene with the INM. (Upper panels) Positioning of integrated reporter genes in a fibroblast nucleus (clone M) detected by GFP-LacI. Upon IPTG withdrawal (24 hours), the nucleoplasmic GFP-LacI protein forms foci by accumulating at *lacO* integration sites, thereby visualizing the initial configuration of the reporter genes. (Lower panels) Repositioning of reporter genes by GFP-LacI-ΔEMD in clone M cells. Upon IPTG withdrawal (24 hours) the tethering protein, which localizes to the nuclear membrane, forms foci at the nuclear periphery. One to three such foci were observable in 40-60% of the nuclei. Images depict single confocal optical sections of live cells. Scale bar equals 5μm. Images show non-saturated GFP foci; non-linear adjustment (gamma) was used to enable visualization of the nuclear or INM fluorescence

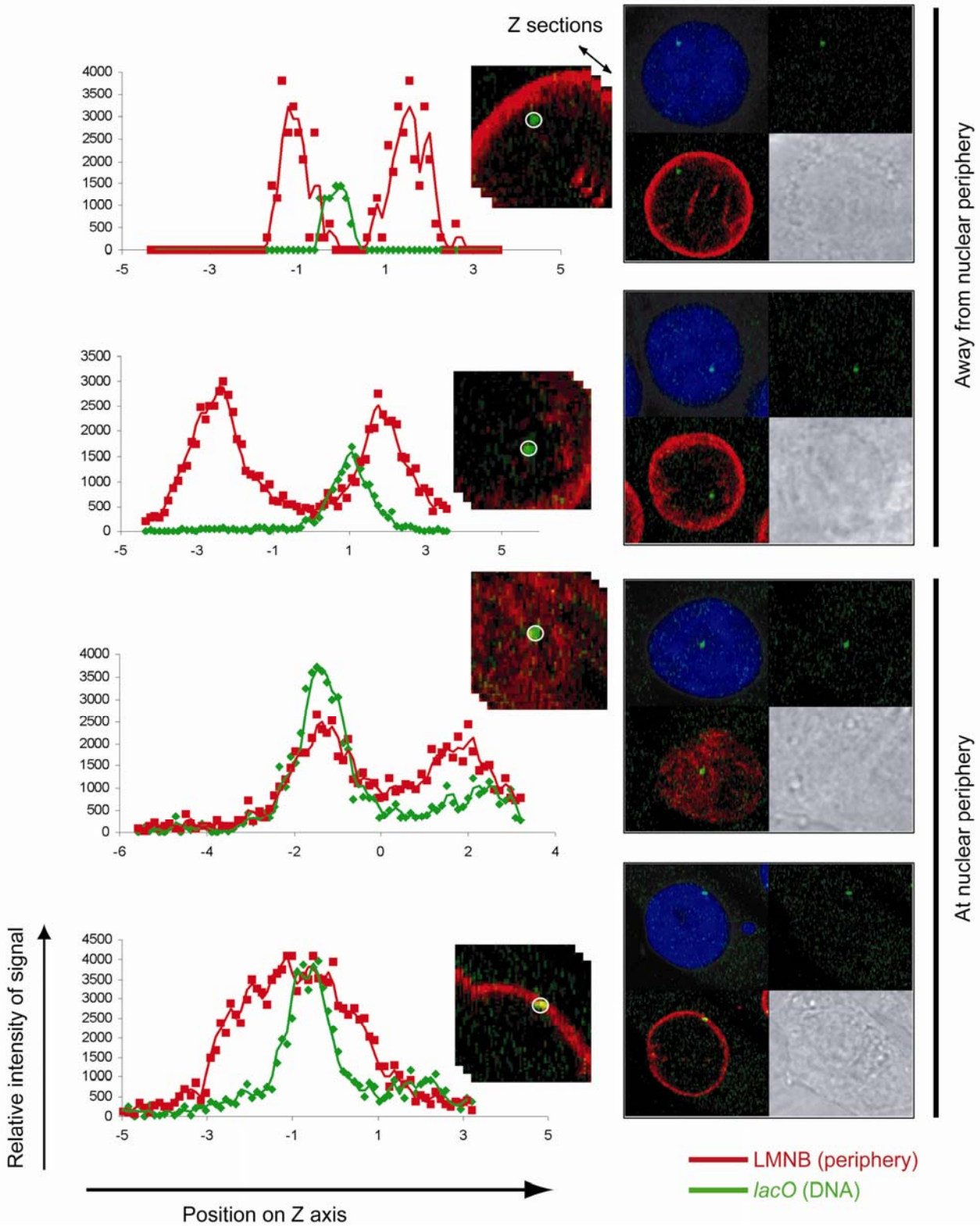

Fig S2 Image analysis used to quantitate tethered loci by 3D DNA-ImmunoFISH. Composite images of representative nuclei are shown on the right. DAPI (blue) stains DNA, *lacO* (green) detects the disposition of the integrated reporter gene construct, and LMNB1 (red) demarcates the nuclear periphery. Shown to the left of the composite images are enlarged pictures of the region around the *lacO* hybridization signal. A region of interest (white circle) was drawn around an individual *lacO* signal in the *xy* plane and the relative signal intensities of *lacO* versus LMNB1 were measured in the *z* dimension. Histograms displaying intensities of the *lacO* (green) and LMNB1 (red) signals of four different nuclear configurations are shown on the left. Overlapping peak intensities were scored as tethered *lacO* foci. Examples of non-peripheral or tethered *lacO* foci are shown.

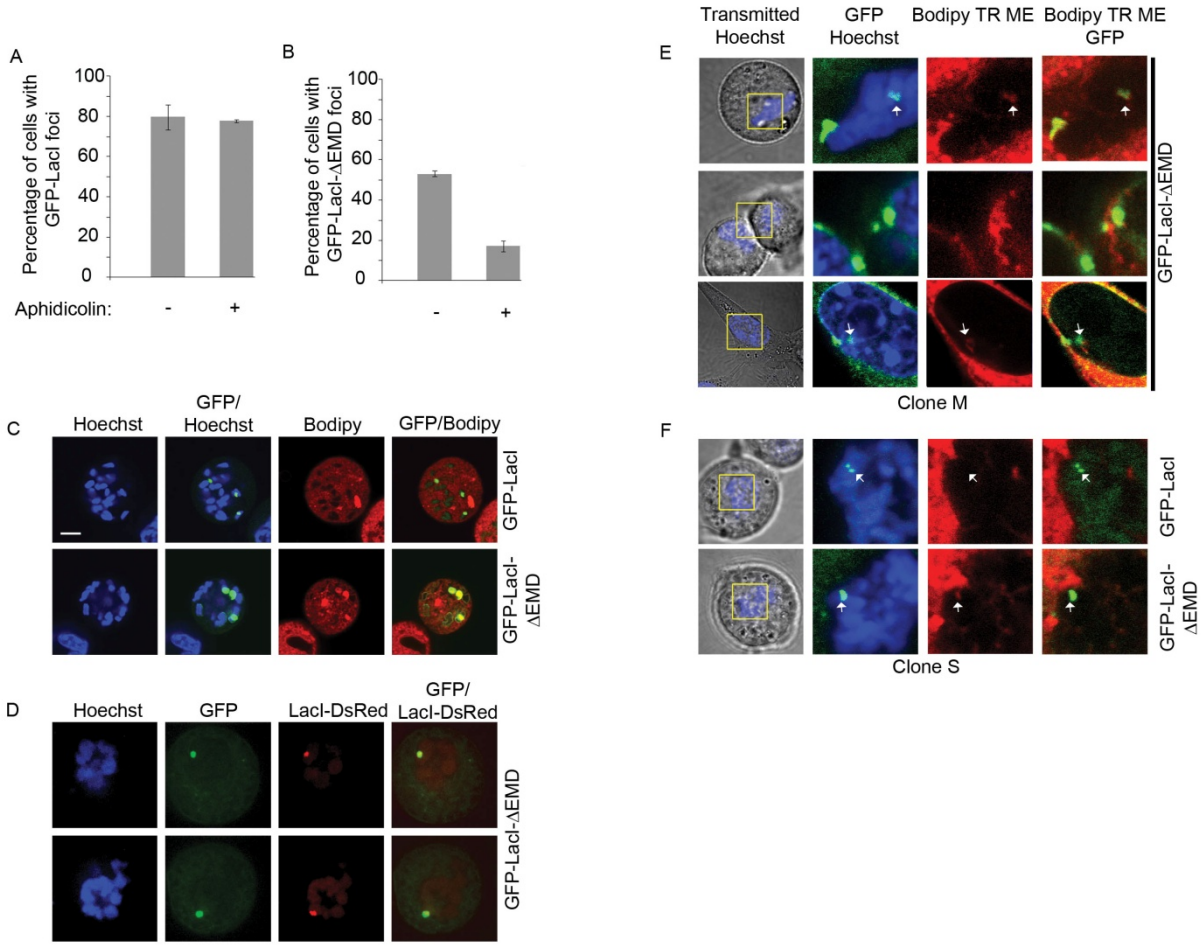

Fig. S3 GFP-LacI-ΔEMD associates with condensed chromosomes in mitosis Nuclear membrane vesicles/reticula containing the tethering protein target to mitotic chromosomes at *lacO* sites. (A,B) Aphidicolin arrested cells are unable to reposition reporter genes to the INM. Clone M cells, expressing GFP-LacI (A) or GFP-LacI-ΔEMD (B), maintained in IPTG were blocked in G1/S with aphidicolin (16h). Cells were then placed in medium lacking IPTG in the presence or absence of aphidicolin. Cells with nucleoplasmic (GFP-LacI) or membrane associated (GFP-LacI-ΔEMD) foci were quantitated by live cell fluorescence microscopy. (C) Clone M cells expressing the indicated proteins were cultured with IPTG and aphidicolin (24h). Cells were then placed into medium lacking IPTG in the presence of colcemid (8h). Live cells were analyzed by GFP fluorescence and staining with Hoechst (DNA) and Bodipy TR Methyl Ester (endomembrane). (D) Clone M cells co-expressing GFP-LacI-ΔEMD and LacI-DsRed were cultured and analyzed as in C. LacI-DsRed and GFP-LacI-ΔEMD co-localize at *lacO* insertions on mitotic chromosomes. (E) Clone M cells expressing GFP-LacI-ΔEMD maintained in IPTG were blocked in G1/S with aphidicolin (24h). Cells were then placed in medium lacking IPTG in the absence of aphidicolin and imaged through metaphase (E, upper panel), cytokinesis (E, middle panel), and the subsequent interphase (E, lower panel). Live cells were analyzed by GFP fluorescence (tethering protein) and staining with Hoechst (DNA) and Bodipy TR Methyl Ester (endomembrane). (F) Clone S cells expressing the indicated proteins were cultured with IPTG and aphidicolin as above. Cells were then placed into medium lacking IPTG in the absence of aphidicolin to allow entry into mitosis (8h). Live cells were analyzed by GFP fluorescence and staining with Hoechst (DNA) and Bodipy TR Methyl Ester (endomembrane dye). Arrows mark binding of GFP-LacI or GFP-LacI-ΔEMD to mitotic chromosomes. Two confocal sections of the same nucleus are shown. Scale bar is 5μm.

We analyzed the dynamics by which the reporter genes were repositioned to the INM. In interphase mammalian nuclei, most loci are constrained within chromosome territories and their movement in the nuclear volume is restricted[1]. Moreover, studies in resting versus cycling fibroblasts have demonstrated that specific association of a gene poor chromosome to the nuclear periphery requires an intervening mitosis[2]. Since GFP-LacI-ΔEMD is confined to the INM, it is likely to be unable to interact with chromosome regions that are positioned away from the nuclear periphery in interphase nuclei. Therefore, we hypothesized that tethering of centrally disposed *Tk-hyg lacO* insertions would require nuclear envelope breakdown and reformation that accompany mitosis[3]. This process would enable binding of nuclear membrane vesicles containing GFP-LacI-ΔEMD to *lacO* sites on condensed chromosomes resulting in attachment of these regions to the INM in the subsequent interphase. To test if cell division was required for tethering, clone M cells were cultured in the presence of aphidicolin to block the cells at the G1/S boundary. Subsequently, IPTG was removed to induce tethering and the cells were either held in (+Aphidicolin) or released (-Aphidicolin) from the cell cycle block. The frequency of GFP-LacI foci was unchanged by the aphidicolin block, indicating that progression through the cell cycle was not required for interaction of the nucleoplasmic protein with *lacO* sites (Fig. S3A). In contrast, aphidicolin treatment drastically reduced the frequency of GFP-LacI-ΔEMD foci (Fig. S3B). As noted earlier not all tethered *lacO* loci accumulate the fusion protein at levels that are discernable as fluorescent signals above the distribution in the INM and hence the frequency of GFP-LacI-ΔEMD foci underestimates the actual frequency of tethering events. We suggest that the residual tethered foci observed in the presence of aphidicolin represent *lacO* integrations that are already near the nuclear periphery and are therefore readily able to interact with GFP-LacI-ΔEMD during interphase. These data establish that cell cycle progression is required for efficient tethering of the *lacO* reporter construct.

We next investigated the stage of mitosis that GFP-LacI-ΔEMD bound to the *lacO* sites. The Emerin segment used for the GFP-LacI-ΔEMD construct lacks the LEM (Lap2, Emerin, Man1) domain and does not interact with BAF. The fusion protein was therefore not expected to target to condensed chromosomes in anaphase. GFP-LacI-ΔEMD was localized to the NE in telophase (data not shown) and interphase nuclei (Fig. S1E). If GFP-LacI-ΔEMD is interacting with the de-condensing chromosomes late in

mitosis, one would expect nuclear membrane vesicles containing the tethering protein to be associated with the *lacO* insertion sites at this stage. We probed for these interactions during mitosis using the endomembrane dye Bodipy TR Methyl Ester and GFP fluorescence. To detect *de novo* tethering interactions, clone M cells propagated in IPTG were arrested at G1/S using aphidicolin. Cells were then released from the aphidicolin block in the absence of IPTG, but arrested at pro-metaphase using colcemid. We anticipated that upon release from the colcemid block it would be possible to stage the binding of GFP-LacI-ΔEMD vesicles to the *lacO* bearing chromosomes. Surprisingly, such vesicles were seen to be already associated with condensed chromosomes in pro-metaphase as evidenced by the co-localization of the tethering protein (GFP) and the membrane dye (Bodipy) (Fig. S3C, lower panel). This association reflects *de novo* tethering as the cells were maintained in IPTG prior to their release into metaphase. Importantly, in control cells, although GFP-LacI was bound to *lacO* sites on the chromosomes, it was not associated with membrane vesicles (Fig. S3C, upper panel). To verify that the membrane vesicles containing the tethering protein were bound to *lacO* sites, we additionally expressed LacI-DsRed in clone M cells. As expected, in clone M cells arrested at pro-metaphase, GFP-LacI-ΔEMD and LacI-DsRed were co-localized (Fig. S3C). These data demonstrate that membrane vesicles containing GFP-LacI-ΔEMD are able to bind to *lacO* sites on the condensed chromosomes in pro-metaphase.

The association of nuclear membrane vesicles containing GFP-LacI-ΔEMD with metaphase chromosomes was verified using cells that were not arrested in mitosis by colcemid treatment (Fig. S3E and F). In this case the cells were arrested at G1/S by aphidicolin in the presence of IPTG. The cells were then released from the block in the absence of IPTG and monitored at metaphase. Membrane vesicles containing GFP-LacI-ΔEMD accumulated as distinct foci on the metaphase chromosomes in both clone M (Fig. S3E, upper panel) and clone S cells (Fig. S3F, lower panel). The binding of these vesicles to *lacO* sites during metaphase is likely due to bypassing with LacI the requirement for the DNA binding protein BAF, which is normally required to target Emerin to chromosomes. Importantly, chromosomes bound by GFP-LacI were not associated with membrane vesicles (Fig. S3F, upper panel). Live cell images of metaphase, late telophase, and interphase nuclei illustrate a possible sequence of events that lead to tethering of the *lacO* insertions (Fig. S3E). According to this sequence, GFP-LacI-ΔEMD vesicles or reticula associate with *lacO* sites on metaphase chromosomes and remain attached through anaphase. At

this stage they are incorporated into the reforming nuclear envelope, thereby tethering the *lacO* chromosomal segments to the INM.

Our results demonstrate that it is possible to reorient specific chromosomal domains in mammalian nuclei and attach them to the INM. However, given the apparent constraints of chromosome territories in interphase nuclei, the Emerin-mediated attachments require mitotic nuclear envelope breakdown and reformation. It remains to be determined if endogenous genes are repositioned to the INM via an intervening mitosis. In support of this possibility, association of a gene poor chromosome with the nuclear periphery in fibroblasts is dependent upon an intervening mitosis. If so, then mitosis may be used to reconfigure chromosomal domains to the INM-lamina compartment to regulate gene activity during cellular proliferation and differentiation.

1. Chubb, J.R., Boyle, S., Perry, P. & Bickmore, W.A. Chromatin Motion Is Constrained by Association with Nuclear Compartments in Human Cells. *Curr Biol* **12**, 439-445 (2002).
2. Bridger, J.M., Boyle, S., Kill, I.R. & Bickmore, W.A. Re-modelling of nuclear architecture in quiescent and senescent human fibroblasts. *Curr Biol* **10**, 149-152 (2000).
3. Hetzer, M., Walther, T.C. & Mattaj, I.W. Pushing the Envelope: Structure, Function, and Dynamics of the Nuclear Periphery. *Annu Rev Cell Dev Biol* (2005).

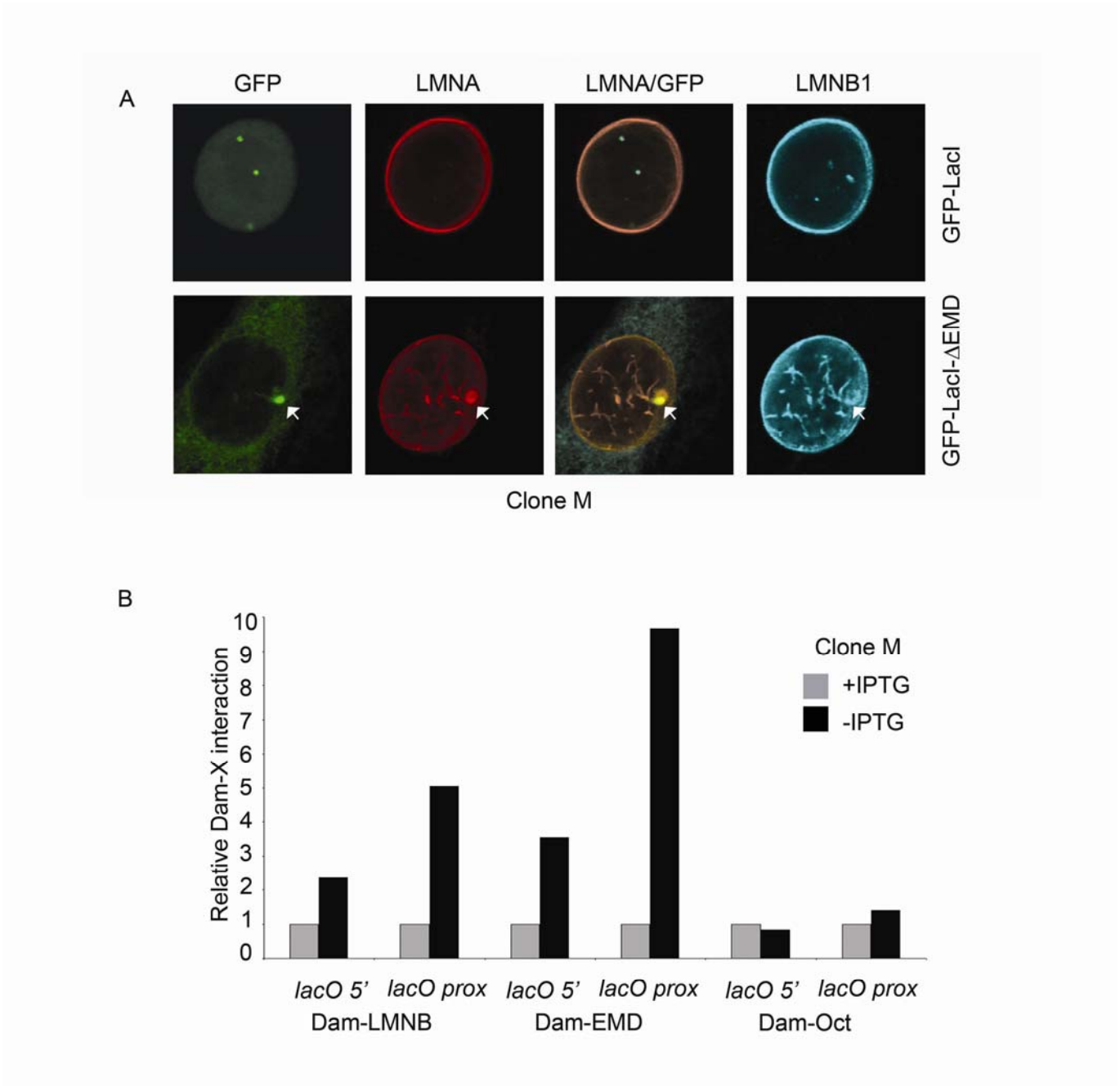

Fig. S4 Lamin A,B and Emerin accumulate at sites of tethered foci in clone M nuclei. (A) *lacO* insertions detected by GFP fluorescence in clone M cells expressing either GFP-LacI or GFP-LacI-ΔEMD upon IPTG withdrawal (24h). Antibodies directed against LMNA (A, red) and LMNB1 (A, cyan) were used to analyze accumulation of these components at sites of tethered *lacO* foci (arrows). All images shown are single confocal sections. Arrows mark tethered loci. Scale is the same as Fig. 4. (B) Molecular interactions of LMNB, Emerin and Oct1 in clone M at the reporter gene detected by Dam-ID mediated methylation. All interactions were normalized to the signal from cells tranduced with Dam alone. Untethered test genes (+IPTG) were set to one after normalization, the Y-axis indicates the fold change in detected Dam-LMNB or Dam-EMD interactions upon tethering (-IPTG). A representative experiment is shown.

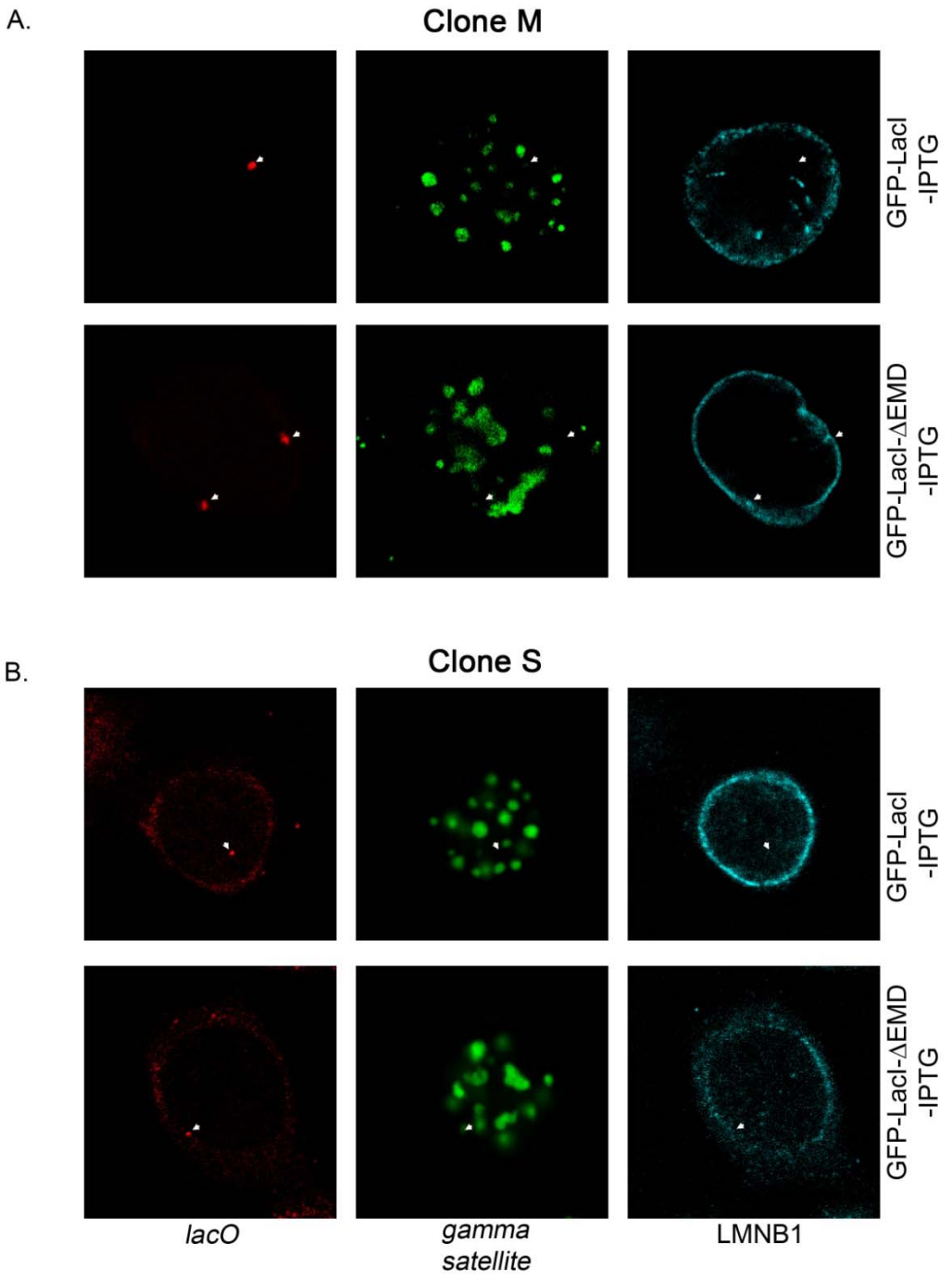

Fig. S5 Tethering to the INM does not lead to association with pericentromeric heterochromatin. Positioning of integrated reporter genes in clone M and S nuclei detected by 3D DNA-ImmunoFISH. DNA probes to *lacO* (red) and *gamma satellite* (green) were used to detect the insertions and pericentromeric heterochromatin, respectively. The nuclear lamina is shown in cyan. Nuclear distribution of *lacO* bearing reporter genes in the indicated cells expressing GFP-LacI or GFP-LacI-ΔEMD upon IPTG withdrawal (24h). Single confocal planes are shown. Arrows indicate the disposition of the *lacO* insertions.

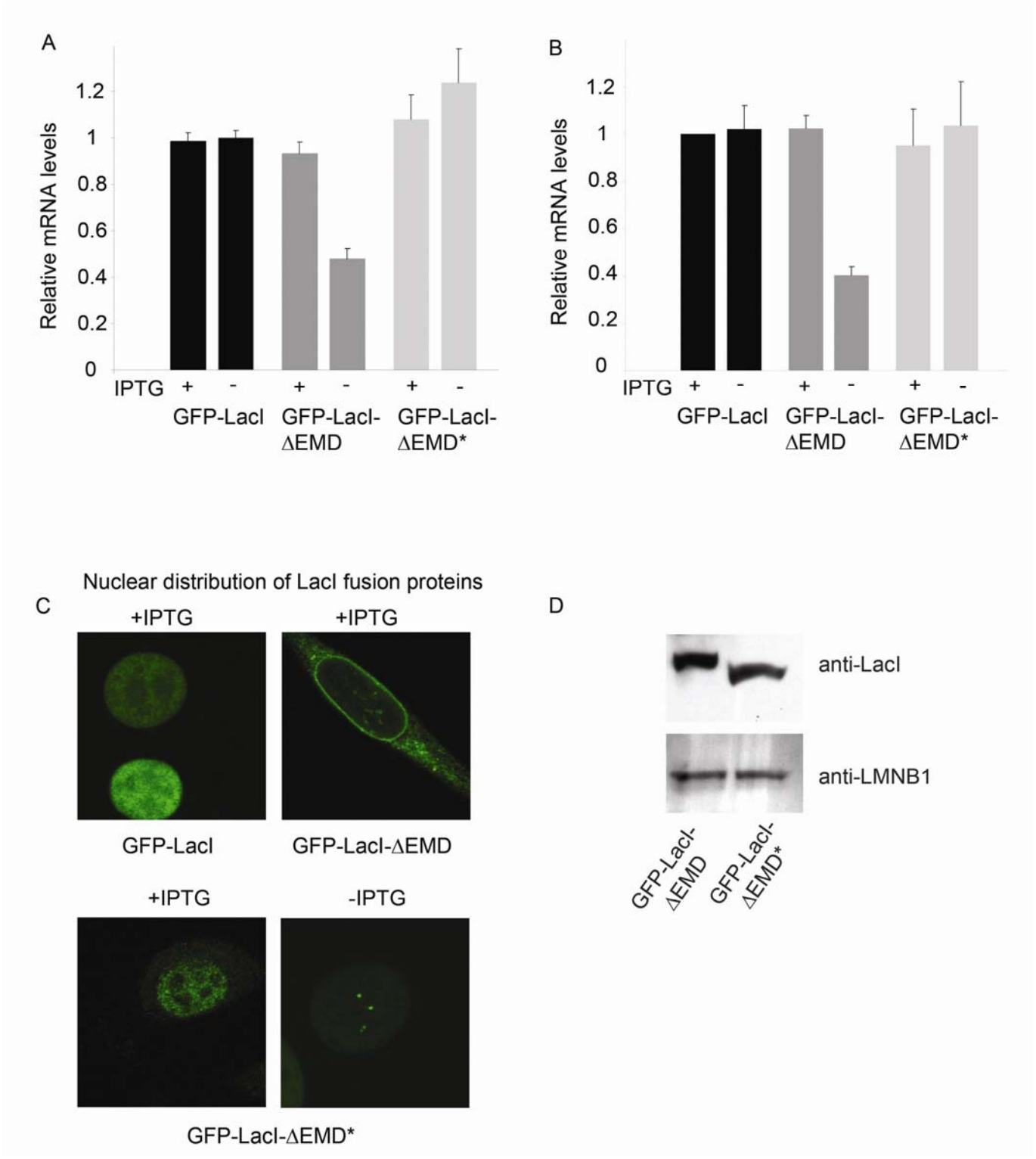

Fig. S6 Tethering to the INM leads to transcriptional repression of reporter genes. The reporter gene (*Tk-Hyg*) is repressed upon IPTG withdrawal in clone M (A) and S cells (B) expressing GFP-LacI-ΔEMD but not GFP-LacI-ΔEMD* or GFP-LacI. GFP-LacI-ΔEMD* represents a nucleoplasmic version of GFP-LacI-ΔEMD which lacks the transmembrane domain necessary for targeting to the INM. Relative transcript levels of the *hygromycin* reporter gene were measured by Q-PCR under the indicated conditions. Transcript levels were normalized to *hprt*. Error bars indicate the standard deviation between three experiments. (C) Confocal images displaying the nuclear distribution of the indicated LacI fusion proteins in clone M cells in the presence of IPTG. Also shown are the *lacO* insertions bound by GFP-LacIΔEMD* (-IPTG). (D) Western blot depicting relative levels of GFP-LacI-ΔEMD and GFP-LacI-ΔEMD*. LMNB1 was used as a loading control.

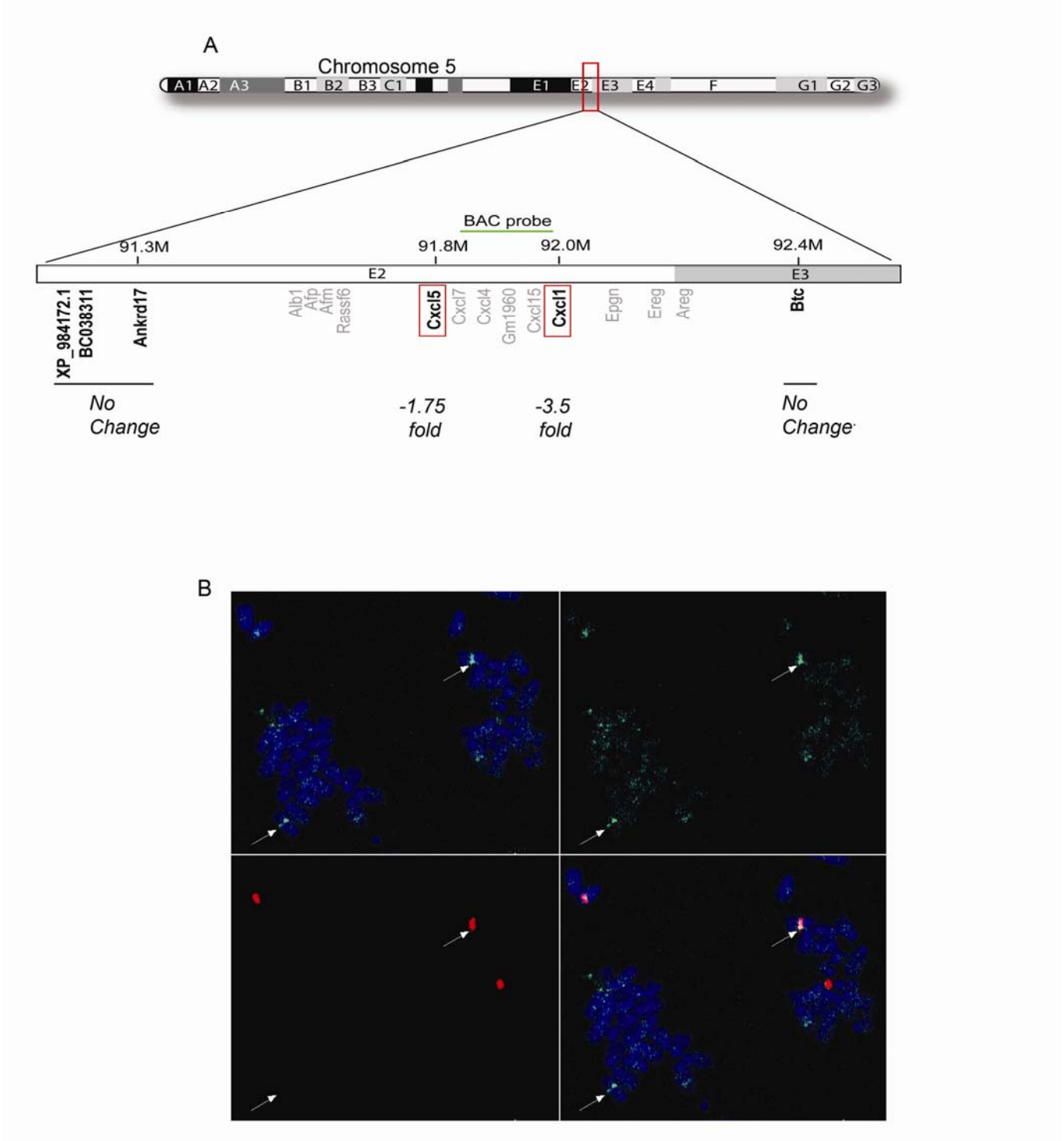

Fig. S7 One of the *lacO* insertions co-localize with the *Cxcl1* and *Cxcl5* genomic region in clone M cells (A) Schematic depicts a region of chromosome 5 that harbors a pair of chemokine genes (*Cxcl1* and *Cxcl5*) that are repressed under tethering conditions by microarray analysis. Genes in light grey are not expressed at detectable levels under any conditions (tethered or untethered). Genes in bold were expressed in the untethered cells and fold change is indicated under those genes. (B) 2D FISH analysis (BAC clone depicted in A) and a site of *lacO* insertion. The hybridization signal of the BAC clone is shown in green and the signal from the *lacO* insertions is shown in red.

Materials and Methods

Plasmid construction: *Tk-hyg lacO* was constructed by modifying pSV2-DHFR 8.32 from A. Belmont [15]. A DHFR fragment was replaced with a cassette containing pTK-Hyg (Clontech). The EGFP-LacI retroviral vector was constructed by ligating a DraI fragment containing GFP-LacI from p3′ssEGFP-LacI into an HpaI site in pMSCV-puro (Clontech) [15]. EGFP-LacI-ΔEMD was generated by PCR amplifying a portion of the region encoding emerin from cDNA using the following primers: ***5′ ΔEMD*** (5′-GGG AGA TCT GCC TCA GAC TTG GAT TCA GC-3′), ***3′ ΔEMD*** (5′-CAG AAG GGG TTG CCT TCT TC-3′). This emerin cDNA fragment was ligated in frame into the pLEGFP-C1 retroviral vector (Clontech) to generate GFP-ΔEMD. LacI was amplified with the following primers ***5′ LacI*** *(5′-*AGC CCG GGG GAT CCA TGG TG-3′); ***3′ LacI*** (5′-GGG AGA TCT AAC CTT CCT CTT CTT CTT AGG-3′). This fragment was ligated in frame into GFP-ΔEMD to generate GFP-LacI-ΔEMD. GFP-LacI-ΔEMD* was generated by using a primer (5′-CCC GGA TCC TCG ATC CTG GCC CAG AGC AGC-3′) that stopped short of the C-terminal transmembrane domain. Fusion protein constructs were confirmed by DNA sequencing. For DamID constructs, a self-inactivating retroviral construct, MSCV EGFP*, was generated by removing the 3' LTR of MSCV EGFP (Clontech derivative) and replacing with the self inactivating 3' LTR of pSM2C (Elledge lab). The minimal heatshock promoter and ecdysone response elements, DAM segment, V5 linker, and Gateway selection cassette (RFC1) from pLgw EcoDam-V5-RFC1 (Van Steensel) were cloned into MSCV-EGFP* to generate pSMGV. The Gateway cloning system (Invitrogen) was then used to PCR amplify cDNAs and transfer them into pDONR vectors, and subsequently in pSMGV. In frame fusion with the DAM-V5 domain was confirmed by sequencing. Prior to cloning into retroviral vectors, the proper nuclear localization of the fusion proteins was confirmed using immunofluorescence. The protein levels expressed from the retrovirus in transduced cells were low, consistent with observations of the Van Steensel lab in mammalian cells[18]. Transient transfection and western blot analysis in Plat E cells were used to confirm that each Dam fusion construct expressed the appropriate sized protein.

Generation and propagation of NIH3T3 cell lines: Linearized *Tk-hyg lacO* DNA was introduced into NIH3T3 fibroblast cells using FuGene (Roche). Cells were selected with HygromycinB (500μg/ml) and clones were isolated using cloning cylinders. Clone S and Clone M cells containing integrated *Tk-hyg-lacO* were transduced with the GFP-LacI retroviral vector and selected with puromycin (1μg/ml) to generate

derivatives expressing GFP-LacI. Clone S and Clone M cells were transduced with the GFP-LacI-ΔEMD retroviral vector and selected with neomycin (500μg/ml) to generate derivatives expressing GFP-LacI-ΔEMD. Cells were expanded and maintained in DMEM with 10% FCS in the presence of Hygromycin (500μg/ml) and IPTG (4mM). Importantly, all experiments were performed using cells shifted into culture medium lacking Hygromycin so as to analyze the effects of tethering on *hygromycin* gene expression in the absence of selection. To enable binding of GFP-LacI or GFP-LacI-ΔEMD, IPTG was washed out of the culture medium and cells were analyzed after 18 to 36 hours. All experiments were performed on sub-confluent cultures.

Immunofluorescence and live cell imaging: For immunofluorescence, cells were grown on sterilized 25mm round #1.5 coverslips (Deckglaser, Germany) in 6 well plates. After experimental manipulation, cells were fixed by adding paraformaldehyde to the culture medium to a final concentration of 4% and incubating at 37ºC for 12 minutes. Coverslips were then washed briefly 3 times with PBS. To permeabilize cells, the coverslips were incubated in PBS containing 0.5% Triton-X-100 detergent for 10 minutes. The coverslips were then washed briefly 3 times with PBS. To prevent non-specific binding of primary antibodies, the coverslips were blocked in 4%BSA/0.1%Triton-X-100/PBS solution for 1 hour at room temperature. Primary antibodies (see below) were added to the coverslips in the same blocking medium and were incubated in a humidified chamber for 1-2 hours at room temperature. After incubation with primary antibodies, the coverslips were washed 3 times, 15 minutes each in PBS or PBS/0.1% Triton-X-100. After these washes, secondary antibodies (see below) were added in blocking medium and allowed to incubate for 1 hour at room temperature. These antibodies were washed from the cells as above. The coverslips were then incubated for 1 minute in a 10μg/ml solution of DAPI diluted in PBS. Coverslips were washed 1 time with PBS and then mounted on slides with Vectashield (Vector Laboratories) or ProLong Gold (Molecular probes) to enable viewing and imaging. Primary antibodies used in this study include mouse anti-LacI (Upstate Biologicals, clone 9A5, 2μg/ml), mouse anti-Lap2 (BD Transduction Laboratories, 1.25μg/ml), goat anti-LaminB1 (Santa Cruz, clone M-20, 1μg/ml), and mouse anti-Nup153 (Abcam, clone QE5, 2μg/ml). Secondary antibodies were from Jackson Immunoresearch Laboratories and included donkey anti-goat-Cy5, donkey anti-goat Cy3, and donkey anti-mouse TRITC (3-4μg/ml).

Cells were prepared for live cell imaging by plating on 25mm round coverslips, as above. Coverslips were placed in chambers (kindly provided by Drs. Rainer Duden and Irina Majoul, School of Biological Sciences, Royal Holloway, University of London) with HEPES buffered medium. Temperature was maintained by heating a small metal plate that was placed upon the stage with circulating water at 37ºC. Viable cells could be maintained under these conditions for up to 6 hours. Bodipy TR methyl ester and Hoechst 33342 (Invitrogen, Carlsbad, CA) were added directly to the culture medium at a concentration of 1μM and 5μg/ml, respectively. After 10 minutes, the dye was removed by replacing with fresh media.

Images were obtained by laser scanning confocal microscopy using a Leica SP2 AOBS (University of Chicago, Digital Light Microscopy Facility) and an Olympus Fluoview 1000.

3D DNA-ImmunoFISH: Immunofluorescence coupled with fluorescent DNA *in situ* hybridization on 3D preserved nuclei (3D DNA-ImmunoFISH) was performed as described previously [21, 27]. Briefly, cells were plated on poly-L-lysine coated slides in 150mm tissue culture dishes (5 slides/dish) and allowed to adhere overnight in the presence of IPTG and Hygromycin. Cells were washed by rinsing the slides in PBS (3 times). Culture medium, lacking Hygromycin, was then added back to the slides either with or without IPTG. After 24 hours, the cells were fixed in 4% paraformaldehyde, permeabilized and subjected to five rounds of freeze/thaw in 50% glycerol. Nuclear DNA was denatured in 70% Formamide/2XSSC at 72ºC for 2-3 minutes, followed by incubation in 50% Formamide/2XSSC at 72ºC for 1 minute. Excess denaturation solution was rapidly removed from the slides and a denatured probe mixture was added immediately. The probe mixture was made by precipitating ~0.3μg *lacO* probe labeled by nick translation (Roche, Biotin Nick translation Kit) and 3μg human Cot-1 DNA (Roche), 6μg human placental DNA (Sigma) and 9μg sonicated salmon sperm DNA. The precipitate was resuspended in 10.5μl hybridization solution (50% formamide/2XSSC/10%Dextran Sulfate). The probe mixture was denatured at 85-95ºC for 5 minutes and then allowed to pre-anneal at 37ºC for 1 hour. Slides were incubated with the probe mixture in a humidified chamber at 37ºC for 36 to 48 hours. After hybridization, slides were washed in 2XSSC briefly, followed by 3 washes with 50%Formamide/2XSSC at 42ºC (7 minutes each) and then 3 washes in 0.2XSSC at 62ºC (7 minutes each). Probes were detected by incubating with avidin-FITC. Protein antigen was detected by incubating with primary antibodies, followed by secondary detection as described for immunofluorescence. Nuclei were counterstained with DAPI and prepared for imaging as above.

For quantitative image analysis, 3D DNA-ImmunoFISH samples were imaged at 100X with 2X optical zoom, with a Z step of 0.12μm between optical slices. *lacO* foci were scored as associating with the nuclear periphery by overlaying histograms of signal intensity of *lacO* signal versus LMNB1 signal (see Fig. S1F). Foci were scored as associated or not associated with the nuclear periphery. These analyses were verified using the RGB co-localization plug-in in NIH ImageJ (http://rsb.info.nih.gov/ij/).

2D-FISH: For 2D-FISH (fluorescent *in situ* hybridization) experiments, metaphase chromosomes were prepared by mitotic shake-off followed by methanol-acetic acid fixation. 2D FISH procedures were as described previously [3]. A probe to the *lacO* sites was generated by nick translation (Roche) with Alexafluor-568 nucleotides (Molecular Probes). A biotinylated probe to the *Cxcl5* gene on chromosome 5 was generated as described above using a BAC clone (RP23-307P18, CHORI BACPAC Resources, http://bacpac.chori.org/). Metaphase spreads were counterstained in DAPI and mounted for microscopic observation as above.

3D RNA ImmunoFISH: Cells were plated, fixed and permeabilized as for 3D DNA-ImmunoFISH [28]. All reagents were treated with DepC to ensure RNAse free conditions. Secondary structure of the nascent RNA was reduced by incubation of the fixed and permeabilized cells at 55°C in 50% formamide/2X SSC for 5 minutes. Such RNA denaturation did not denature the cellular DNA since a DNA specific probe was unable to hybridize to its target (data not shown). Dig-labeled DNA probes covering the hygromycin expression cassette were hybridized to the cells overnight at 46°C. This higher hybridization temperature enabled formation of RNA-DNA duplexes, but was unfavorable for DNA/DNA duplexes (in 50% formamide hybridization buffer containing vanadyl ribonucleoside complexes). Washes were done as for 3D DNA-ImmunoFISH. Mouse α-LacI was used to detect accumulated LacI protein. Only high concentrations of LacI (i.e. on LacI/*lacO* foci) could be detected since the antigen recognized by this antibody does not survive the FISH procedures well.

RNA and Q-PCR: RNA was prepared using TRIzol reagent (Invitrogen) following manufacturer's guidelines. All RNA samples were treated with DNaseI (Invitrogen) to ensure no genomic DNA contamination. cDNA was generated using SuperScriptII reverse transcriptase (Invitrogen) following manufacturer's instructions using random hexamer primers. Q-PCR experiments were performed on a

Stratagene Mx4000 machine using SYBR green dye master mix (Stratagene). All reactions were compared to a standard curve to enable determination of relative copy number. All amplifications were normalized to *hprt*. Primer pairs used in this study include: ***QhygW*** (5′-TCG GTC AAT ACA CTA CAT GG-3′), ***QhygC*** (5′-TCA CAG TTT GCC AGT GAT AC—3′), ***QHPRTW***: (5′-GTA ATG ATC AGT CAA CGG GGG AC-3′), ***QHPRTC*** (5′-CCA GCA AGC TTG CAA CCT TAA CCA-3′), ***QCxcl5W*** (5′-CGG TGG AAG TCA TAG CTA AA-3′), ***QCxclC*** (5′-AGC CCT TTC TTC TCT TCA CT-3′), ***QCxcl1W*** (5′-CAC CCA AAC CGA AGT CAT AG-3′), and ***QCxcl1C*** (5′-ACT TGG GGA CAC CTT TTA GC-3′).

Microarray analysis: Total RNA was isolated from clone M cells either expressing GFP-LacI (-IPTG) or GFP-LacI-ΔEMD (±IPTG). Probe preparation, hybridization and initial analyses were carried out by the Functional Genomics Facility at the University of Chicago. Labeled cRNA was hybridized to the Mouse Genome 430 2.0 Array from Affymetrix. Microarray data quality evaluation was performed using Affymetrix Microarray Suite 5.0 and Chip analyzer 1.3. Analysis of clustering of genes was performed by using the Entrez Biomart data mining program for Affy 430.2 probes (http://www.ensembl.org/Mus_musculus/martview) on all differentially expressed genes and analyzing chromosome position. Two or more genes within 500Kb were scored as a cluster. Two independent experiments involving each condition were performed.

Chromatin Immunoprecipitation: Chromatin immunoprecipitation was performed as previously described (3). Chromatin was sonicated to an average length of 600 base pairs and was diluted 1:4 before immunoprecipitation with 2 μg of the relevant antibody (described below). The TK primers are specific for the HSV-TK promoter in the reporter construct. Control IgG (sc-2027; Santa Cruz), and anti−acetylated H4 (06-866; Upstate Biotechnology/Millipore) were used.

DamID: DamID was performed as described with modifications[18]. Briefly, retroviral constructs were transfected into Plat E cells and and supernatants were collected at 48 and 72 hours post-transfection. NIH3T3 fibroblast cells were incubated with the pooled supernatant overnight. 38B9 pro-B cells were transduced by spin infection for 2hrs at 37ºC. NIH3T3 fibroblasts and 38B9 pro-B cells were sorted for GFP fluorescence 36-48 hrs after transduction and cultured no more than 2 additional days. Clone M GFP-LacI-ΔEMD and S GFP-LacI-ΔEMD cell lines were transduced and sorted as above (gating on high GFP

fluorescence) in the presence of IPTG. Cells were then trypsinized and split into media either with or without IPTG. Cells were incubated for 48-72 hrs before genomic DNA was isolated. Following the ligation mediated PCR (LM-PCR), PCR products were purified on Qiagen columns and diluted 1:100 in sterile dH2O for Q-PCR analysis. 12ul of this diluted sample was used with 12.5 ul Syber Green (Stratagene), and .5 ul 50um premixed primer pairs. Primer pairs were as follows: ***LacO 5′*** (5′-GAT GTG GGA AGC TGT TAC TG-3′, 5′-GAC CCT TGA ATG GGT TTT CCA -3′), ***LacO Prox*** (5′-TTA ACA GGA GGA CAC AGA GG-3′, 5′-TGC ATT CTA GTT GTG GTT TG-3′), **U7** (5′-TTA GCT CCA AGC CTT TAA TC-3′, 5′-AAT CAC AAA GCA CAG TTC CT-3′), **VH J558** 5′-AGT GCA GGG CTC ACA GAA AA-3′, 5′-CAG CTC CAT CCC ATG GTT AGA-3′ **VH intergenic** (5′-GGC TAG AAC ACC ATT TAG-3′, 5′-CTT CAT GTG CTT CTT CCT-3′), **VH J558*** (5′-TAG CCT CAA CAC AAG GTT-3′, 5'-ATG AGG ATG ATA CAG CTC-3′)

1. Belmont, A.S., Li, G., Sudlow, G. & Robinett, C. Visualization of large-scale chromatin structure and dynamics using the lac operator/lac repressor reporter system. *Methods Cell Biol* **58**, 203-222 (1999).
2. Vogel, M.J., Peric-Hupkes, D. & van Steensel, B. Detection of in vivo protein-DNA interactions using DamID in mammalian cells. *Nat Protoc* **2**, 1467-1478 (2007).
3. Bertolino, E. *et al.* Regulation of interleukin 7-dependent immunoglobulin heavy-chain variable gene rearrangements by transcription factor STAT5. *Nat Immunol* **6**, 836-843 (2005).
4. Solovei, I. *et al.* Spatial Preservation of Nuclear Chromatin Architecture during Three-Dimensional Fluorescence in Situ Hybridization (3D-FISH). *ExpCell Res* **276**, 10-23 (2002).
5. Kosak, S.T. *et al.* Subnuclear compartmentalization of immunoglobulin loci during lymphocyte development. *Science* **296**, 158-162 (2002).
6. Jolly, C., Mongelard, F., Robert-Nicoud, M. & Vourc'h, C. Optimization of nuclear transcript detection by FISH and combination with fluorescence immunocytochemical detection of transcription factors. *J Histochem Cytochem* **45**, 1585-1592 (1997).