Supplemental Experimental Procedures, Supplemental Tables, and Supplemental References.

Table of Contents

Extended Experimental Procedures

Cell lines

KBM7 cells were cultured in IMDM 1X (Gibco) supplemented with 10% Fetal Bovine Serum (Thermo Scientific HyClone) and 1% penicillin and 1% streptomycin.The Shield1-inducible Dam-LmnB1 and Dam-only stable clonal KBM7 cell lines were derived by lentiviral transductions. Briefly, PGK-DD-Dam-LmnB1 or PGK-DD-Dam constructs were cloned into the pCCL.sin.cPPT.hPGK. Δ LNGFR.Wpre lentiviral construct (Amendola et al., 2005) by standard cloning procedures. Lentivirus was produced as previously described (Amendola et al., 2005) and concentrated approximately 50 fold using Amicon Ultra-15 (UFC910024) columns. Cell lines were transduced with lentivirus in the presence of 8ug/ml of polybrene, followed by single-cell FACS sorting into 96-well plates. Selection of the clones #14, #5.5 (Dam-LmnB1) and #5.8 (Dam) was based on methylation levels as determined by DpnII-qPCR assays as previously described (Kind et al., 2013) (Figure S1A and S3D). The DNA content of the KBM7 clones was confirmed by propidium iodide staining followed by FACS analysis. Next, all cell lines were transduced with lentiviral Fucci (Sakaue-Sawano et al., 2008) constructs and clonally selected based on the expression of both Fucci markers.

The diploid derivative of #14 was generated by FACS sorting of the 5% biggest cells - according to forward and side scatter profiles - that were positive for both Fucci markers and seeding them as single cells in a 96 well plate. Clonal lines were expanded and subsequently analyzed for DNA content by FACS analysis.

Immunofluorescence labeling.

KBM7 cells were cultured on poly-lysine coated coverslips for 12 hours and HT1080 cells were cultured for 24 hours on ultra clean coverslips and Willco dish prior to fixation with 2% formaldehyde/PBS for 10 minutes. Next the cells were washed twice with PBS and permeabilized with 0.5% NP40/PBS for 20 minutes followed by a 1 hour incubation in PBS/1%BSA. Incubations with the primary antibodies were performed in PBS/1%BSA for 1 hour, and 30 minutes with the secondary fluorophore-conjugated antibody, with 3 times 5 minute PBS washes between the incubations. Antibody incubations were performed at room temperature. 4′ ,6-Diamidino-2-phenylindole (DAPI) was included in VECTASHIELD mounting medium (Vector Laboratories). Imaging was performed on a Leica TCS SP5 confocal laser-scanning microscope. The following primary antibodies were used: LmnB1 (Abcam 16048), H3K9me2 (Abcam 1220), GFP (Roche 11814460001). Secondary antibodies used for GSD

microscopy: anti-mouse Alexa 488 (A-11001) and anti-rabbit Alexa 647 (A-21244) from Life Technologies.

GSD microscopy.

Super-resolution microscopy was performed with a Leica SR GSD microscope (Leica Microsystems, Wetzlar, Germany) with a Sumo Stage (#11888963) for drift free imaging. Images were collected with an EMCCD Andor iXon camera (Andor Technology, Belfast, UK) and an oil immersion objective (PL Apo 160X, NA 1.46). Lasers used are 405 nm/30 mW (back-pumping only), 488 nm/300 mW and 647 nm/500 mW. Glass bottom dishes (Willco wells B.V., Amsterdam, The Netherlands) and coverslips cleaned and washed with base and acid overnight were used. Between 10,000 to 50,000 frames were collected at 100 Hz for each SR image. The data were analyzed with the Image J ThunderStorm analysis module (Ovesny et al., 2014); image reconstruction was using a detection threshold of 70 photons, sub pixel localization of molecules, uncertainty correction option, and a pixel size of 10 nm. Images were corrected for slight chromatic aberrations. For analysis of label density profiles (Fig. 6B and Fig. S6B) the outline of the lamina was delineated manually by drawing a spline along the outer (sharp) border of the bright lamin layer using Image J software (4-7 cells for each condition). Subsequently, the curved spline and surrounding image area was linearized using the straightening (affine transformation) routine in ImageJ. The resulting images were precisely oriented vertically by rotation and total intensity was projected onto the x-axis to arrive at the intensity profile. Full-width, half-maximum of this intensity profile is taken do describe the width of the lamina (Fig. S6). To quantitate overlap between LaminB1 and Tracer, we first selected the area just next to the nuclear envelope that is positive for both Lamin and Tracer. For this, both channels were smoothed (gaussian smoothing, radius 250 nm) and thresholded, and the area of overlap between the channels was detected using logical AND. Within this ROI, overlap was detected by scoring the % of pixels positive for both labels. The expected overlap value was based on the assumption of independence (Expected = % pixels with Lamin X % pixels with ^{m6}A-Tracer).

DNA-FISH.

KBM7 cells were plated on poly-L-lysine-coated cover glasses and fixed the day after. The fixation as well as three-dimensional FISH procedures were adapted from (Solovei and Cremer, 2010). After washes, the cover glasses were incubated with 50 ng/ml DAPI/2xSSC for 5 min at RT and mounted in the mounting solution containing 2xSSC, 10 mM Tris, 0.4% glucose, 100 μ g/ml catalase, 37 μ g/ml glucose oxidase, 2 mM Trolox. The probes were designed using the

www.hdfish.eu database and prepared by PCR as previously described (Bienko et al., 2013). All images were acquired at 100× magnification (oil immersion, high numerical aperture Nikon objective) on an inverted epi-fluorescence microscope (Nikon) equipped with a high-resolution charge-coupled device (CCD) camera (Pixis, Princeton Instruments) and controlled by MetaMorph software. DNA spots were identified by thresholding local background–subtracted images using custom-made software in Matlab, which was also used for the distance analysis. First, distances of every FISH signal to the centroid of the nucleus (d_F) were measured. Second, they were normalized to account for different sizes of individual nuclei. This was done by first calculating the average distance of the nuclear periphery points to the centroid ($\bar{d}$) and dividing each d_F by $\bar{d}$. For chr1, n = 713; for chr17, n = 1017. Probe positions in base pairs according to the numbering in Figure 2F: 1=chr1:22002154-22054597, 2=chr1:82038123-82071977, 3=chr1:172092100-172124742, 4=chr17:11119845-11156460, 5=chr17:36059791-36094686, 6=chr17:51209978-51249286.

3D image stacks (xy pixel size 125 nm) were acquired by scanning every field of view every 200 nm in the axial (z) direction. FISH dots were localized using custom Matlab scripts and the position was calculated as the 3D center of mass of the dot. In order to locate every FISH signal in the nucleus in 3D we first performed 2D segmentation of the sum projections of DAPI images. We then extended the 2D demarcation of the cells into the z direction. For each segmented nucleus the medial axis transform was calculated. Then the volumetric extent of the nuclei was inferred by a 3D reconstruction. The resulting shapes are models of the true nuclei shape with enforced medial symmetry in 3D. Since the 2D segmentation was done based on sum projection of the DAPI images, it included no positioning in the axial direction. Hence, before measuring the radial positioning of each dot, the midplanes of 3D nuclei shapes were aligned to match midplanes in the corresponding cells in which FISH signals were imaged. The midplanes in the dots images were calculated as average z-values of all dots in the image, assuming random positioning of the dots around cell centers. The distance transform was calculated from the edges of the nuclei models. Finally, for the measurement of dots positioning inside nuclei, the distance transform was interpolated at the dots.

Flow cytometry.

To check the ploidy of cells prior to single-cell DamID mapping, 0.25 x 10^6 cells were collected, resuspended in 250µl Nicoletti buffer (0.1% sodium citrate pH 7.4, 0.1% Triton X-100, 50 µg/ml PI) and immediately analyzed on the FACS using a BD LSR Fortessa flow cytometer (Becton Dickinson). To analyse the DNA content of the different Fucci populations, 1 x 10^6 cells were collected in PBS, resuspended in 1 ml PBS plus 1 ml fix buffer I (BD Bioscience #557870) and incubated 10' at 37 degrees. Next 10ml PBS/10%FBS was added and the fixed cells were

centrifuged, the pellet was resuspended in 300 µl staining buffer (PBS; 1.5 µg/ml DAPI; 50 µg/ml RNAse) and analyzed on a BD LSR Fortessa flow cytometer (Becton Dickinson). All FACS flow cytometry data were analyzed using FlowJo software (Treestar).

Gene expression analysis and CEL-seq.

Gene expression profiles from pools of KBM7 cells were obtained from Gene Expression Omnibus (GEO) accession GSE56465. For CEL-seq, KBM7 cells were stimulated with Shield1 15 hours prior to FACS. Individual cells in the G1 phase of the cell cycle were sorted based on the Fucci system into single wells of a 96-well plate containing 3 µL of cell culture media. Immediately after single-cell sorting, 100 µL of Trizol® (Life Technologies) and 0.2 µL of 1:500,000 diluted ERCC spike-in RNA mix 1 (Life Technologies) were added to each well. Next, total RNA was extracted from single cells using the manufacturer's protocol with some modifications. To enable visualizing the small RNA pellets easily, 0.25 µL of GlycoBlue™ (Life Technologies) was added to each sample. RNA was precipitated using 50 µL of 100% isopropanol by overnight incubation at -20^{0}C. The RNA pellets were washed with 100 µL of 75% Ethanol and resuspended in first strand buffer (MessageAmp II, Life Technologies) after air drying the pellets for approximately 10 minutes. mRNA was then reverse transcribed using previously described primers that contain 4-bp random barcodes that serve as unique molecule identifiers (UMI) (Grun et al., 2014). The cDNA is then amplified and Illumina libraries are prepared as previously described in the CEL-Seq protocol (Hashimshony et al., 2012). The libraries were sequenced on the Illumina High-seq 2500 platform using 50 bp paired-end sequencing. The paired end reads were mapped to RefSeq gene models based on the human genome release hg19 using Burrows-Wheeler Aligner (BWA) with default parameters. Reads mapping to multiple regions were distributed uniformly among the genes. The right mate was mapped to the transcriptome and ERCC spike-in sequences while the left mate was used to identify the cell-specific barcode and UMI. We detected 8559.4 ± 4504.9 (mean ± s.d.) individual mRNA molecules per cell, representing 2622.5 ± 1154.2 genes per cell.

Hi-C.

Hi-C was performed as described (Belton et al., 2012). Hi-C libraries were generated from two independent KBM7 cell preparations. Hi-C libraries were sequenced on an Illumina HiSeq2000 platform. Reads were mapped as described (Imakaev et al., 2012; Lajoie et al., 2015). Reads from the two replicates were then pooled, mapped and binned at 100 Kb intervals. Binned data was then corrected for intrinsic biases such as mappability and restriction site density as described before (Imakaev et al., 2012; Lajoie et al., 2015). Compartment profiles were calculated on a by-

chromosome basis as the first eigenvectors of the observed/expected Hi-C matrix (i.e. after removing the distance dependence) as in (Naumova et al., 2013).

Conventional DamID

DamID of KBM7 cells and hybridization to Nimblegen genomic tiling arrays was performed as described (Vogel et al., 2007). Quantitative measurement of ^{m6}A levels at single individual GATC sites was done with DpnII digestion and qPCR as described (Kind et al., 2013), using the following primers: iLAD1_for (GAAGGTTCCCCCACAGAAAT), iLAD_rev (CTGAGGCAAAGACAGGGAAG); iLAD2_for (ACAGCAGGAAGTACTTGAGATCC), iLAD2_rev (ATTAATCTGGCCCGGAGAGT); LAD1_for (CATTGGCTTCTTTGGTGCCAGGT), LAD1_rev (ACGGTGGAGGCAGTCAAAAGGC); LAD2_for (ACAGCAGGAAGTACTTGAGATCC), LAD2_rev (ATTAATCTGGCCCGGAGAGT).

Single cell DamID.

Expression of Dam or Dam-LmnB1 protein was induced by treating the cells with 0.5 nM Shield1 (ClonTech #632189). Fifteen hours later the cells were collected in low serum medium (1%) and sorted on a BD FACSAria I (Becton Dickinson) based on cell size and expression of both Fucci markers (Sakaue-Sawano et al., 2008). Based on a growth curve of cells counted every 24 hours for three days, the generation time of clone #14 is 30.7 hours. Considering that 48.5 % of the cells are Fucci red (G1), the estimated time these cells reside in G1 is 14.9 hours (30.7 x 0.485). Fucci green and red were excited by a 488nm argon laser, and the fluorescence signals were collected using filters 530/30 and 585/42 respectively. Based on the FCS and SSC profiles the 2% smallest cells that were double positive for the Fucci markers (Figure 1A) were single cell sorted in a PCR plate (Thermowell; Corning #6509) containing lysis buffer and proteinase K. Each well contained 1 µl of pick buffer (50mM Tris-HCl pH 8.3; 75mM KCl; 3mM $MgCl_2$; 137mM NaCl) and 2 µl of lysis buffer with proteinase K (10mM TRIS acetate pH 7.5 (Sigma #T1258); 10mM magnesium acetate (Sigma #63052); 50mM potassium acetate (Sigma #95843); 0.67% Tween-20 (Sigma #P2287); 0.67% Igepal (Sigma #I8896) and 0.67mg/ml proteinase K (Roche #03115828001). Proteinase K digestion was performed at 42 °C for 4 hours in a thermoblock with heated lid, followed by heat inactivation for 10 minutes at 80 °C.

In the following steps, reagents were added with an Eppendorf Multipipette Plus mounted with a 0.1 ml Combitip (Eppendorf #0030089405). The surface of the reaction volume was never touched by the pipette tip. Genomic DNA (gDNA) was digested for 4 hours by the addition of 7 µl of DpnI reaction mix (0.1 µl DpnI (10U/µl, New England Biolabs #R0176L); 0.7 µl 10x One-Phor-all-buffer plus (100mM TRIS acetate pH7.5; 100mM magnesium acetate;

500mM potassium acetate) and 6.2 µl nuclease free H_2O) and incubation at 37 °C in a PCR machine, followed by heat inactivation at 80 °C for 20 minutes. Adaptor ligation was performed by the addition of 10 µl ligation mix (2 µl 2x T4 ligation buffer; 0.5 µl T4 ligase (5U/ul, Roche #10799009001); 0.2 µl 50µM double-stranded DamID adapter (Vogel et al., 2007) and 7.3 µl nuclease free H_2O) and incubation in a PCR machine at 16 °C overnight. Heat inactivation at 65 °C for 10 minutes the next day was followed by PCR amplification by the addition of 30 µl PCR mix (5 µl 10x Clontech Advantage cDNA reaction buffer, 4 µl dNTPs (2.5mM each), 1.25 µl PCR primer (50 µM) NNNNGTGGTCGCGGCCGAGGATC , 1 µl Advantage enzyme mix 50x (Clontech #639105) and 18.75 µl nuclease free H_2O). The PCR primer carries 4 random nucleotides at the 5' end to meet the Illumina software requirements of generating reads with diverse starting sequences. The thermal cycling scheme is as follows:

Step	Denature	Anneal	Extend
1			68 ^{0}C for 10 min
2	94 ^{0}C for 1 min	65 ^{0}C for 5 min	68 ^{0}C for 15 min
3-6	94 ^{0}C for 1 min	65 ^{0}C for 1 min	68 ^{0}C for 10 min
7-27*	94 ^{0}C for 1 min	65 ^{0}C for 1 min	68 ^{0}C for 2 min

*29 for Dam-only expressing cells

Of the resulting PCR product 8 µl was used for standard 1% agarose gel electrophoresis for analytical purpose and the remainder was purified by column purification (Qiagen PCR purification kit #28106) and eluted in 26 µl nuclease free H_2O. Next, the samples were prepared for Illumina sequencing.

Single-cell DamID Illumina library preparation.

Of 1 µg purified PCR product the 3' or 5' overhanging ends were blunted in a 50 µl reaction following the manufacturers instructions (End-It DNA End-Repair Kit, Epicentre #ER81050). The blunted DNA samples were again purified using the PCR purification colomns of Qiagen and eluted with 26 µl nuclease free H_2O. Next, a 3' adenine was added by incubation for 30 minutes at 37 °C in a 50 µL reaction mix (1x New Engeland Biolabs restriction buffer 2, 200 µM dATP (Roche #11051440001) and 25 units of Klenow 3′ → 5′ exo$^-$ (New Engeland Biolabs #M0212M). After heat inactivation at 75 °C for 20 minutes, the DNA was purified with Agencourt AMPure XP beads (Beckman Coulter #A63881). A 1.8 x volume of beads over DNA sample was used, manufacturers instructions were followed and the DNA was eluted with 20 µl of nuclease free H_2O.

To 250 ng of purified DNA the Illumina Y-shaped adapters were then ligated for two hours at room temperature in a 10 µl reaction mix (1µl 10x T4 ligation buffer, 0.5 µl T4 ligase (5U/ul) Roche #10799009001, 0.5 µl 50 µM Y-adapter, with nuclease-free H_2O added to 10 µl final volume). Next, the T4 ligase reaction was heat inactivated at 65 °C for 10 minutes followed

by DNA purification with AMPure beads as described for the previous step. For the addition of the Illumina index primers a PCR reaction was performed with 100 ng DNA from the previous step in a 20 µl MyTaq red DNA polymerase PCR reaction mixture (10 µl 2x MyTaq reaction mixture (Bioline #BIO21110), 1 µl 5.0 µM Illumina P5 primer ATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT, 1 µl 5.0 µM Illumina indexing primer (Table S2), nuclease-free H_2O till a final volume of 20 µl). The DNA was amplified for 9 PCR amplification cycles (94 °C 1 minute; 94 °C 30 seconds, 58 °C 30 seconds and 72 °C for 30 seconds for 9 cycles and 72 °C for 2 minutes) after which 5 µl of each sample was analysed by agarose gel electrophoresis. For Illumina multiplex sequencing typically between 40 and 50 samples were mixed in approximate equimolar ratios as judged from the agarose gel image. The pooled sample was subjected to a Qiagen PCR column purification and subsequent AMPure bead purification with 1.6 x volume of beads over DNA sample before it was used for sequencing.

Processing of single-cell DamID sequencing reads.

The 51 bp reads are first parsed (using custom scripts and *cutadapt* (Martin, 2011)) to extract the different parts, i.e. the first 4 random bases (included to make cluster calling on the Illumina Hi-seq 2000 possible), 15 bp of adapter sequence, and a stretch of gDNA starting with the GATC site (Figure S1E). The gDNA sequences are aligned to the reference genome (hg19, chromosomes 1-22, X, Y, and M) using bowtie2 (Langmead and Salzberg, 2012) version 2.1.0, using the *--very-sensitive* mode and otherwise default parameters. Reads that did not align or aligned more than once were discarded. The remaining reads are assigned to GATC-fragments using a custom R-script (Lawrence et al., 2013). Only reads that precisely flanked a GATC site were associated with GATC-fragments, all other reads were discarded. Because single haploid cells have only one copy of any GATC fragment, multiple reads aligning to the same position must have originated from the same molecule and are therefore counted as one. These reads were then aggregated in genomic segments of 100kb.

Next, for each 100 kb segment i in each cell j the observed over expected read count ($OE_{i,j}$) was calculated as:

$$OE_{i,j} = \frac{n_{i,j}}{m_i} \cdot \frac{\sum m_i}{\sum n_{i,j}}$$

where $n_{i,j}$ is the number of uniquely mapped reads in segment i; $\Sigma n_{i,j}$ is the total number of uniquely mapped reads in cell j genome-wide; m_i is the maximum possible number of unique reads in segment i; and Σm_i is the maximum possible number of unique reads genome-wide. To determine m_i we performed an *in silico* DnpI digest of the complete genomic reference sequence (chromosomes 1-22, X, Y, and M), defined the complete set of potential DamID-seq reads of 32bp

length, and aligned these reads back to the reference genome using the same method and filter criteria as we used for the experimental reads (see above). m_i is the total number of simulated reads that could be uniquely mapped to segment *i.*

CF scores were calculated by binarization of the OE scores using a cutoff OE>1, followed by summation of the binary scores across all cells for each genomic segment. The OE cutoff of 1 was chosen for 2 reasons: (i) By definition, OE>1 represents read counts that are higher than expected by chance under the null hypothesis that the entire genome randomly contacts the NL; (ii) as shown in SupFig 3A, we observe a bimodal distribution of the OE values with a dip around OE==1. We also tested OE cutoffs of 0.5, 0.75, 1, 1.5, and 2. The resulting CF values change accordingly, as may be expected. However, this change is largely linear, because CF values with other cutoffs remain tightly correlated (Pearson's r 0.966-0.996) with the CF values obtained with cutoff 1. Thus, in the downstream analyses other OE cutoffs would not lead to different conclusions.

Definition of facultative and constititive (inter)LADs.

Conventional LmnB1 DamID profiles were collected from nine human cell lines: human embryonic stem cells and HT1080 fibrosarcoma cells (Meuleman et al., 2013); KBM7 cells (this study); and Tig3 lung fibroblasts, retinal pigment epithelial cells, K562 erytholeukemia cells, Jurkat T lymphocytes, Sup-T1 lymphoblasts and LS174T intestinal epithelial cancer cells (CAdG and BvS, manuscript in preparation). All samples were hybridized to 2.1M NimbleGen tiling arrays with a median probe spacing of 1 kb. Two independent replicates were averaged. In order to obtain the same resolution as the single-cell DamID data, the resulting data were binned into 100kb segments by averaging of all array probes within each segment. We then applied a Hidden Markov Model to classify each 100kb segment in each cell type as either LAD or inter-LAD (iLAD). Then, using the LAD / iLAD classification of each segment, we identified constitutive LADs (cLADs) as regions which are LAD in all nine cell types; constitutive inter-LADs (ciLADs) as regions which are inter-LAD in all cell types, facultative LADs (fLADs) and facultative inter-LADs (fiLADs). The last two classifications are regions that do not have the same NL interaction status in each cell type, but are associated with the NL in KBM7 cells (fLADs) or not associated with the lamina in KBM7 cells (fiLADs). This resulted in the following coverage of the 30,365 genome-wide segments: cLAD: 22.1%; ciLAD: 18.9%; fLAD: 15.9%; fiLAD: 36.9%; not assigned (segments not covered by conventional DamID data): 6.2%.

Haploid vs diploid comparison.

Single-cell DamID data from diploid cells were processed exactly as from haploid cells. Because

the homologous chromosomes in the diploid cells are identical, reads from the two homologs cannot be discriminated. This could lead to systematic biases in the comparison to haploid cells, because in diploid cells each Dam-methylated fragment has two chances of being detected. For a balanced comparison, we therefore generated pseudo-diploid reference maps by pooling equal numbers of sequence reads from two single haploid cells. We generated as many pseudo-diploid data sets as we have diploid data sets (N=51 in case of clone #14), with the exact same distribution of GATC-flanking read counts per data set (forward and reverse). We generated pseudo-diploid data sets from haploid data sets such that the sum of GATC-flanking reads of the two haploid data sets was as similar as possible to twice the number of GATC-flanking reads of the diploid data set (using a dynamic-programming approach), where each haploid data set was used at most once. Forward and reverse oriented reads were sampled (without replacement) from the haploid data separately, according to the number of forward/reverse reads in the diploid data set. After sampling the GATC-flanking reads from haploid data sets the pseudo-diploid data sets where processed exactly as the haploid data sets.

Run length analysis and randomization control.

Lengths of contact and no-contact were determined for each cell genome-wide based on the binarized NL contact maps. For no-contact run analysis we first removed all 100 kb segments with CF==0 and joined the two flanking regions. This is done because a substantial fraction of the genome consists of large domains that never contact the NL; in the quantitative analysis (Figure 4D) these would obscure the incidental but non-random occurrence of long no-contact runs in regions with CF>0. As a consequence of this removal of CF==0 regions, the lengths of no-contact runs are underestimated. Random shuffling of the entire 118 single-cell data matrix was done such that both marginals (i.e., the CF for each 100 kb segment as well as the total number of NL-contacting segments per cell, genome-wide) are conserved, using a previously reported algorithm (Strona et al., 2014)

Analysis of NL contact coordination.

For all possible pairs of 100 kb segments *i* and *j* within a chromosome, NL contact coordination was calculated as the Pearson correlation between the vectors b_i and b_j that contain the binary NL contact scores of segments *i* and *j* across the 118 clone #14 cells. Figure 5B shows intra-chromosomal correlations for all chromosomes (except chr8) combined. Randomization was done as for run length analysis. To compare the coordination matrix with TAD boundaries, the latter were called from Hi-C data by computing the upstream / downstream ratio for regions within 2Mb of a given locus (Lajoie et al., 2015) and then taking only those boundaries that have

strengths above that of the 99th percentile in permuted data and around which the NL contact correlation maps had fewer than ¼ of NaN entries, giving 980 TAD boundaries.

Comparisons to epigenome data.

We used ChIP-seq mapping data of histone modifications and H2A.Z in K562 cells from the ENCODE consortium (Consortium, 2012). We downloaded the previously determined coordinates of gapped peaks from http://www.broadinstitute.org/~anshul/projects/roadmap/peaks/consolidated/gappedPeak/ and plotted the average number of gapped peaks per 100 kb segment as a function of CF. Spearman's correlation coefficients were then calculated.

Supplemental Tables

Table S1.

Gene ontology analysis of genes located in segments with CF>80% – Related to Figure 3.

	GOterm	P-value
1	Sensory perception of smell	1.9^{-39}
2	Neurological system process	9.4^{-38}
3	Sensory perception of chemical stimulus	9.4^{-38}
4	G-protein coupled receptor protein signaling pathway	1.9^{-32}
5	Cognition	3.6^{-29}
6	Sensory perception	5.6^{-29}
7	Cell surface receptor linked signal transduction	8.2^{-29}
8	Transmission of nerve impulse	1.9^{-5}
9	Synaptic transmission	1.8^{-4}
10	Gamma-aminobutyric acid signaling pathway	5.2^{-4}

Table S2.

List of indexing primers used for multiplexing of single-cell DamID samples – Related to Experimental Procedures. Positions of the Illumina sequencing primer (red) and the 8-nt index sequence (cyan) are highlighted in the first sequence entry.

	Index	Oligo sequence (5' to 3')
1	TAGCTTGT	CAAGCAGAAGACGGCATACGAGTAGCTTGTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
2	CGATGTTT	CAAGCAGAAGACGGCATACGAGCGATGTTTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
3	GCCAATGT	CAAGCAGAAGACGGCATACGAGGCCAATGTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
4	ACAGTGGT	CAAGCAGAAGACGGCATACGAGACAGTGGTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
5	ATCACGTT	CAAGCAGAAGACGGCATACGAGATCACGTTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
6	GATCAGCG	CAAGCAGAAGACGGCATACGAGGATCAGCGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
7	CAGATCTG	CAAGCAGAAGACGGCATACGAGCAGATCTGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
8	TTAGGCAT	CAAGCAGAAGACGGCATACGAGTTAGGCATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
9	GGCTACAG	CAAGCAGAAGACGGCATACGAGGGCTACAGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
10	CTTGTACT	CAAGCAGAAGACGGCATACGAGCTTGTACTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
11	ACTTGATG	CAAGCAGAAGACGGCATACGAGACTTGATGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
12	TGACCACT	CAAGCAGAAGACGGCATACGAGTGACCACTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
13	TGGTTGTT	CAAGCAGAAGACGGCATACGAGTGGTTGTTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
14	TCTCGGTT	CAAGCAGAAGACGGCATACGAGTCTCGGTTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
15	TAAGCGTT	CAAGCAGAAGACGGCATACGAGTAAGCGTTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
16	TCCGTCTT	CAAGCAGAAGACGGCATACGAGTCCGTCTTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
17	TGTACCTT	CAAGCAGAAGACGGCATACGAGTGTACCTTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
18	TTCTGTGT	CAAGCAGAAGACGGCATACGAGTTCTGTGTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
19	TCTGCTGT	CAAGCAGAAGACGGCATACGAGTCTGCTGTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
20	TTGGAGGT	CAAGCAGAAGACGGCATACGAGTTGGAGGTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
21	TCGAGCGT	CAAGCAGAAGACGGCATACGAGTCGAGCGTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
22	TGATACGT	CAAGCAGAAGACGGCATACGAGTGATACGTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
23	TGCATAGT	CAAGCAGAAGACGGCATACGAGTGCATAGTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
24	TTGACTCT	CAAGCAGAAGACGGCATACGAGTTGACTCTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
25	TGCGATCT	CAAGCAGAAGACGGCATACGAGTGCGATCTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
26	TTCCTGCT	CAAGCAGAAGACGGCATACGAGTTCCTGCTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
27	TAGTGACT	CAAGCAGAAGACGGCATACGAGTAGTGACTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
28	TACAGGAT	CAAGCAGAAGACGGCATACGAGTACAGGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
29	TCCTCAAT	CAAGCAGAAGACGGCATACGAGTCCTCAATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
30	TGTGGTTG	CAAGCAGAAGACGGCATACGAGTGTGGTTGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
31	TAGTCTTG	CAAGCAGAAGACGGCATACGAGTAGTCTTGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
32	TTCCATTG	CAAGCAGAAGACGGCATACGAGTTCCATTGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
33	TCGAAGTG	CAAGCAGAAGACGGCATACGAGTCGAAGTGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
34	TAACGCTG	CAAGCAGAAGACGGCATACGAGTAACGCTGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
35	TTGGTATG	CAAGCAGAAGACGGCATACGAGTTGGTATGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
36	TGAACTGG	CAAGCAGAAGACGGCATACGAGTGAACTGGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
37	TACTTCGG	CAAGCAGAAGACGGCATACGAGTACTTCGGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
38	TCTCACGG	CAAGCAGAAGACGGCATACGAGTCTCACGGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
39	TCAGGAGG	CAAGCAGAAGACGGCATACGAGTCAGGAGGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
40	TAAGTTCG	CAAGCAGAAGACGGCATACGAGTAAGTTCGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
41	TCCAGTCG	CAAGCAGAAGACGGCATACGAGTCCAGTCGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
42	TGTATGCG	CAAGCAGAAGACGGCATACGAGTGTATGCGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
43	TCATTGAG	CAAGCAGAAGACGGCATACGAGTCATTGAGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
44	TGGCTCAG	CAAGCAGAAGACGGCATACGAGTGGCTCAGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
45	TATGCCAG	CAAGCAGAAGACGGCATACGAGTATGCCAGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
46	TCAGATTC	CAAGCAGAAGACGGCATACGAGTCAGATTCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
47	TACTAGTC	CAAGCAGAAGACGGCATACGAGTACTAGTCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
48	TTCAGCTC	CAAGCAGAAGACGGCATACGAGTTCAGCTCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
49	TGTCTATC	CAAGCAGAAGACGGCATACGAGTGTCTATCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
50	TATGTGGC	CAAGCAGAAGACGGCATACGAGTATGTGGCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
51	TTACTCGC	CAAGCAGAAGACGGCATACGAGTTACTCGCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
52	TCGTTAGC	CAAGCAGAAGACGGCATACGAGTCGTTAGCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
53	TACCGAGC	CAAGCAGAAGACGGCATACGAGTACCGAGCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT

54	TGTTCTCC	CAAGCAGAAGACGGCATACGAGTGTTCTCCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
55	TTCGCACC	CAAGCAGAAGACGGCATACGAGTTCGCACCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
56	TTGCGTAC	CAAGCAGAAGACGGCATACGAGTTGCGTACGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
57	TCTACGAC	CAAGCAGAAGACGGCATACGAGTCTACGACGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
58	TGACAGAC	CAAGCAGAAGACGGCATACGAGTGACAGACGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
59	TAGAACAC	CAAGCAGAAGACGGCATACGAGTAGAACACGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
60	TCATCCTA	CAAGCAGAAGACGGCATACGAGTCATCCTAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
61	TGCTGATA	CAAGCAGAAGACGGCATACGAGTGCTGATAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
62	TAGACGGA	CAAGCAGAAGACGGCATACGAGTAGACGGAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
63	TGTGAAGA	CAAGCAGAAGACGGCATACGAGTGTGAAGAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
64	TCTCTTCA	CAAGCAGAAGACGGCATACGAGTCTCTTCAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
65	TTGTTCCA	CAAGCAGAAGACGGCATACGAGTTGTTCCAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
66	TGAAGCCA	CAAGCAGAAGACGGCATACGAGTGAAGCCAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
67	TACCACCA	CAAGCAGAAGACGGCATACGAGTACCACCAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
68	TGCGTGAA	CAAGCAGAAGACGGCATACGAGTGCGTGAAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
69	GGTGAGTT	CAAGCAGAAGACGGCATACGAGGGTGAGTTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
70	GATCTCTT	CAAGCAGAAGACGGCATACGAGGATCTCTTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
71	GTGTCCTT	CAAGCAGAAGACGGCATACGAGGTGTCCTTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
72	GACGGATT	CAAGCAGAAGACGGCATACGAGGACGGATTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
73	GCAACATT	CAAGCAGAAGACGGCATACGAGGCAACATTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
74	GGTCGTGT	CAAGCAGAAGACGGCATACGAGGGTCGTGTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
75	GAATCTGT	CAAGCAGAAGACGGCATACGAGGAATCTGTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
76	GTACATCT	CAAGCAGAAGACGGCATACGAGGTACATCTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
77	GAGGTGCT	CAAGCAGAAGACGGCATACGAGGAGGTGCTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
78	GCATGGCT	CAAGCAGAAGACGGCATACGAGGCATGGCTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
79	GTTAGCCT	CAAGCAGAAGACGGCATACGAGGTTAGCCTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
80	GTCGCTAT	CAAGCAGAAGACGGCATACGAGGTCGCTATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
81	GGAATGAT	CAAGCAGAAGACGGCATACGAGGGAATGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
82	GAGCCAAT	CAAGCAGAAGACGGCATACGAGGAGCCAATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
83	GCTCCTTG	CAAGCAGAAGACGGCATACGAGGCTCCTTGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
84	GTAAGGTG	CAAGCAGAAGACGGCATACGAGGTAAGGTGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
85	GAGGATGG	CAAGCAGAAGACGGCATACGAGGAGGATGGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
86	GTTGTCGG	CAAGCAGAAGACGGCATACGAGGTTGTCGGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
87	GGATTAGG	CAAGCAGAAGACGGCATACGAGGGATTAGGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
88	GATAGAGG	CAAGCAGAAGACGGCATACGAGGATAGAGGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
89	GTGTGTCG	CAAGCAGAAGACGGCATACGAGGTGTGTCGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
90	GCAATCCG	CAAGCAGAAGACGGCATACGAGGCAATCCGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
91	GACCTTAG	CAAGCAGAAGACGGCATACGAGGACCTTAGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
92	GCCTGTTC	CAAGCAGAAGACGGCATACGAGGCCTGTTCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
93	GCACTGTC	CAAGCAGAAGACGGCATACGAGGCACTGTCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
94	GCTAACTC	CAAGCAGAAGACGGCATACGAGGCTAACTCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
95	GATTCATC	CAAGCAGAAGACGGCATACGAGGATTCATCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
96	GTCTTGGC	CAAGCAGAAGACGGCATACGAGGTCTTGGCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT

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