Supplemental Methods

ChIP experiments

Cells were cross-linked in 1 % formaldehyde for 10 minutes, quenched with glycine for 5 minutes and harvested in 2 mL of PBS with protease inhibitors. The cells were then lysed, first in cell lysis buffer (5 mM Pipes pH 8, 85 mM KCl, 0.5% Nonidet P-40) and then in nuclei lysis buffer (50 mM Tris–HCl pH 8.1, 1 % SDS, 10 mM EDTA), after which sonication was performed (15x30 seconds, intervals of 30 seconds). Subsequently DNA was immunoprecipitated with the indicated antibody overnight at 4 ºC with IP buffer (16.7 mM Tris–HCl pH 8, 167 mM NaCl, 1.2 mM EDTA, 0.01% SDS, 1.1 % Triton X-100). Pre-blocked beads (50 µL magnetic (Dynabeads from Invitrogen) or 50 µL of Agarose (Diagenode) beads) were incubated with samples for 1 hour at 4 ºC, and were then washed with 2x low salt (20 mM Tris–HCl pH 8, 0.1 % SDS, 1 % Triton x100, 2 mM EDTA, 150 mM NaCl), 2x high salt (20 mM Tris–HCl pH 8, 0.1 % SDS, 1 % Triton X-100, 2 mM EDTA, 500 mM NaCl), 2x LiCl (10 mM Tris–HCl pH 8, 250 mM LiCl, 1 % Nonidet-P40; 1 % Na deoxycholate; 1 mM EDTA) and 2x TE (10 mM Tris pH 8, 1 mM EDTA). Beads were eluted with 1 % SDS, 0.1 M $NaHCO_3$ for 10 minutes at 65 ºC and were then de-crosslinked overnight at 65 ºC with NaCl (200 mM). The following day, RNAse A digestions were performed for 2 hours at 37 ºC (only for ChIP-seq experiments) and proteinase K digestions were performed for 1 hour at 45 ºC. Finally, DNA was extracted using phenol /chloroform /isoamyl precipitation and was eluted in 30 to 45 µL of water.

Immunofluorescence

T47D grown on coverslips and treated as described above were fixed with 4 % paraformaldehyde in PBS for 15 minutes and permeabilized with PBS 0.2 % Triton X-100 at room temperature. Blocking was performed with 3 % BSA for 1 hour at room temperature and coverslips were incubated overnight with primary antibodies (Rabbit anti-CTCF: 07-729, Millipore; Rabbit anti-RAD21: ab992, Abcam; Goat anti-Lamin B: sc6216,Santa-Cruz; Rabbit anti-H3K9me3: 07-442; Mouse anti-RNA Pol II : Millipore:05-623) diluted at 1/500 in PBS 1 % BSA at 4 ºC. After washes with PBS, samples were incubated with convenient secondary antibodies (anti-Rabbit AlexaFluor 488 or anti-Goat AlexaFluor 647; Invitrogen-Molecular Probes) for 1 hour at room temperature. After washes with PBS and DNA staining with DAPI, samples were mounted with Mowiol. Images were acquired with a Leica TCS SP5 CFS confocal microscope.

Protein extraction and Western blots

For protein extraction cultured cells were harvested by scraping, washed once with phosphate-buffered saline and homogenized in 25 mM Hepes (pH 7.4), 150 mM NaCl, 0.1 % Triton X-100, 1 mM EDTA, 1 mM DTT, 50 mM NaF, 80 mM β-lycerophosphate, supplemented with complete TM protease inhibitor cocktail (Roche). Protein concentration was determined by Bradford assay (Bio-Rad), and between 25 and 50 μg of protein were loaded into Acrylamide gel. For immunoprecipitation 500 μg of protein were incubated with 5 μg of CTCF antibody overnight at 4 ºC with IP buffer (16.7 mM Tris–HCl pH 8, 167 mM NaCl, 1.2 mM EDTA, 0.01% SDS, 1.1 % Triton X-100). Pre-blocked beads (50 µL of Agarose (Diagenode) beads) were incubated with samples for 1 hour at 4 ºC, and were then washed with 5X extraction buffer high salt (NaCl 500 mM) and eluted with 2X Laemmli buffer. Immunoblot antibodies were:

phospho-MSK1 Cell Signaling 9591S, total MSK1 Cell Signaling, total p38 Santa Cruz sc-535, phospho-p38 Cell Signaling 9215S and PAR Trevigen 4335-AMC-050.

Chromatin fractionation

Cells were resuspended in buffer A (10 mM HEPES, pH 7.9, 10 mM KCl, 1.5 mM $MgCl_2$, 0.34 M sucrose, 10 % (v/v) glycerol, 1 mM DTT, 0.1 % (v/v) Triton X-100, supplemented with protease inhibitors) and incubated on ice for 10 minutes. After centrifugation at 1,300 g for 5 minutes at 4 °C, the supernatant was conserved as cytoplasmic extract and the nuclei washed once with buffer A depleted of Triton X-100 and subsequently lysed in buffer B (3 mM EDTA, 0.2 mM EGTA, 1 mM DTT). Soluble and insoluble (chromatin) fraction were separated via centrifugation at 1,700 g for 4 minutes at 4 °C.

Motif identification and TAD meta-analysis for CTCF peaks

Motif analyses were performed using HOMER's "findMotifsGenome.pl" with default settings for hg38. Constitutive CTCF sites were defined by overlapping narrow peaks of 9 ENCODE CTCF ChIP-seq samples processed on hg38 via BEDTools' "multiinter" (ENCFF850DQJ, ENCFF646TUX, ENCFF535MZG, ENCFF396BZQ, ENCFF785NTC, ENCFF723LVE, ENCFF730MQM, ENCFF675JFN, ENCFF753HNR; https://www.encodeproject.org/) obtained from 8 different immortalized cell lines (LNCAP, A549, K562, MCF-7, H54, 22Rv1, C4-2B, PanC1 (Heinz et al. 2010; Quinlan et al. 2010; Sloan et al. 2016). Significant association of CTCF and RAD21 / constitutive CTCF was assessed by overlapping using the corresponding peaks using BEDTools "intersect" and then testing for enrichment of sites with decreasing / increasing compared to unchanged sites via Fisher's exact test.

Meta-analysis of TADs was performed by splitting each TAD into 100 bins using BEDTools “makewindows -n 100” and manually adding the corresponding adjacent TAD borders (always 50 kb size). CTCF peaks per TAD / TAD border were counted by using BEDTools “intersect-F 0.5” requiring that at least 50 % or a peak had to overlap an interval to avoid assigning one peak to multiple intervals. CTCF peak counts were normalized by bin size and scaled by the number of peaks exhibiting the same behavior (e.g. decreased binding after hyperosmotic stress). Binding profiles were visualized using “geom_smooth()” of the ggplot2 R-package.

Identification of Pol II run-off locations

Broad Pol II peaks in each sample were merged into larger regions using BEDOPS (Neph et al. 2012) by allowing gaps up to 2.5 kb between adjacent peaks (merge, range 2500). Next, broad peak regions were merged between biological replicates of the same condition (bedops-merge), retaining only regions that were at least 30 kb in size. To determine which broad peak pairs corresponded to each other before and after treatment, we utilized the BEDTools “closest” command and only kept regions whose start coordinates were between 100 kb and 300 kb apart from each other in a stranded fashion (based on the position of the run-off peak in hyperosmotic stress, assuming it has to be located downstream of the peak in untreated cells). Lastly, we overlapped the identified regions with known GENCODE genes using BEDTools “intersect” and identified all genes that could be associated with a peak pair with the correct orientation. To produce normalized Pol II coverage tracks we utilized the deepTools' “bamCompare” command (scaleFactorsMethod SES; extend fragment length) using ChIPQC estimates of fragment length (Ramirez et al. 2014; Wartmann et al. 2015).

HiC data analysis

Reads were mapped according to a fragment-based strategy: each side of the sequenced read was mapped in full length to the reference genome Human Dec. 2013 (GRCh38/hg38). In the case reads were not mapped, they were split when intra-read ligation sites were found and individual split read fragments were then mapped independently. We used the TADbit filtering module to remove non-informative contacts and to create contact matrices as previously described (Sloan et al. 2016). PCR duplicates were removed and the Hi-C filters applied corresponded to non-digested fragments, non-ligated fragments (dandling-ends), self-circles and random breaks (Supplemental summarize the number of reads mapped and the number of valid pairs used to generate the matrices for the different samples used in this study). The matrices obtained were further normalized for sequencing depth and genomic biases using ICE. To assign A/B status, we calculated the total number of genes in 100 kb windows for each chromosome and then correlated it the corresponding eigenvalues calculated for 100 kb resolution. In case of a negative correlation coefficient, all corresponding eigenvalues were multiplied by -1 to ensure that gene-rich regions are indeed identified as A compartment.

To calculate compartment strength, we assigned a compartment (A or B) to each 100 kb bin in the genome. Then we gathered the observed and expected (considering distance decay) contacts between any pair of bins. Compartment strength is defined as the tally of contacts between bins with the same type (A with A or B with B) over the tally of contacts between bins of a different type (A with B). The higher the value, the more compartments interact with those of the same kind.

To generate saddle plots, we discretized the eigenvalues (bin size = 100 kb) into 50 categories based on quantiles for each sample and chromosome. Here, the lower

categories correspond to B compartments and the higher ones to A compartments. Then we gathered the observed and expected (considering distance decay) contacts between any pair of categories. Saddle plots represent the aggregated observed vs. expected contacts (log_2 transformed) for each of the 50 x 50 possible combinations. The diagonal pattern of contact enrichment is the consequence of same-to-same compartment interactions.

To generate aggregate contact profiles, we focused on TADs between 0.5 and 1.5 Mb of size. For each TAD, we gathered the observed and expected (considering distance decay) contacts in a 1 Mb x 1 Mb square centered on the interaction of the corresponding start and end borders at 10 kb resolution. Then we aggregated contacts grouped by the relative position to their corresponding start and end and computed the enrichment of contacts as log_2 (observed / expected). Intra-TAD contacts are those corresponding to positive values relative to the start border and negative values relative to the end border.

5. References

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