Supplemental Methods

Embryonic Stem (ES) Cell Culture

V6.5 ES cells from Novus Biologicals (NBP1-41162) were cultured as previously described (Beagan et al. 2016) under standard pluripotent (serum/LIF) conditions on Mitomycin-C inactivated MEFs. To generate the 2i/LIF condition, ES cells were transitioned to serum-free media containing 3 uM CHIR99021 (Axon Medchem #1386), and 1 uM PD0325901 (Axon Medchem #1408) (as described in (Beagan et al. 2016)) and propagated for 2 passages on feeder cells. Before fixation, both ES cell conditions were passaged onto 0.1% gelatin coated plates to remove the feeder layer, and fixed at ~60% confluency. Thus, the 2i/LIF ES cells were cultured for 3 passages under 2i/LIF conditions before fixation.

Primary Neural Progenitor Cell (NPC) Culture

Neural progenitor cells were cultured as previously described (Beagan et al. 2016). Briefly, NPCs were cultured as neurospheres for two passages to purify the population of non-adherent NPCs. Neurospheres were then dissociated and passaged onto Poly-D-Lysine Hydrobromide (100 ug/mL, Sigma P7280), and laminin (15 ug/mL, Corning 354232) coated plates, and fixed next day.

CTCF ChIPseq

Chromatin immunoprecipitation was performed as previously described (Beagan et al. 2016). Libraries were prepared for sequencing using the NEBNext Ultra Library Prep Kit (NEB #E7370) and following the manufacturer's protocol for ChIP-seq library preparation. No size selection step was performed following adapter ligation. The libraries were amplified over 18 PCR cycles using NEBNext Multiplex Oligos for Illumina (NEB #E7335). The final ChIP libraries were eluted in 30 uL 0.1x TE from the Agencourt AMPure XP beads, at which point we confirmed the library

contained DNA fragments ranging from 250 to 1200 bp, including the adapters, by running a High-Sensitivity DNA assay on an Agilent Bioanalyzer. The concentration of these libraries was assayed via the KAPA Illumina Library Quantification Kit (#KK4835), diluted to equivalent concentrations and pooled, and finally sequenced with 75-cyles per paired-end on the Illumina NextSeq500.

ChIP-seq peakcalling

Published ChIP-seq data was downloaded from GEO (http://www.ncbi.nlm.nih.gov/geo/) and reanalyzed according to (**Table S4**). Reads were aligned to mouse genome build mm9 using Bowtie with default parameters (Langmead 2010). Reads were considered if they had two or fewer reportable alignments. To facilitate the comparison of ChIPseq libraries across cell types, the mapped reads were filtered to remove optical and PCR duplicates and then downsampled to equivalent read numbers across cellular states. The CTCF ChIP libraries for ES 2i, ES serum and pNPC were downsampled to 11 MM reads and the whole cell extract libraries were downsampled to 15 MM reads. For YY1 ChIPseq libraries, the ES serum, ProB, and pNPC samples and inputs were downsampled to just over 7 MM reads. The H3K27ac ChIP libraries for ES serum and pNPC were downsampled to 7 MM reads and the whole cell extract libraries were downsampled to 7 MM reads. Peaks were identified using Model-based Analysis for ChIP Sequencing v2.0 (MACS2) (Zhang et al. 2008). For CTCF ChIPseq, default parameters were used with a p-value cutoff of $p < 1E-8$ (peaks provided in **Tables S5-7**). For YY1, we modified the parameters to facilitate accurate detection of broad peaks (--broad --broad-cutoff 1E-4 -p 1E-8) (peaks provided in **Tables S12-13**). For histone modification H3K27ac ChIPseq, the same broad peak calling approach was utilized.

Parsing Cell Type-Specific CTCF Occupancy Sites

CTCF ChIP-seq peaks ($p < 1x10^{-8}$) were utilized to parse CTCF sites into cell type specific occupancy classes with Galaxy. 'ES 2i only' CTCF peaks were defined as CTCF sites that were present in ES cells under 2i/LIF conditions and the absence of CTCF in ES cells in serum/LIF conditions and in NPCs (provided in **Table S8**). This class was generated using Galaxy to subtract ES serum and NPC CTCF peaks ($p < 1x10^{-8}$) from ES 2i CTCF peaks. Similarly, 'ES serum only' CTCF was defined by the presence of CTCF in serum/LIF ES cells and the absence of CTCF in ES cells in 2i/LIF conditions and in NPCs; 'NPC only' CTCF was defined by the presence of CTCF in NPCs and the absence of CTCF in ES cells in 2i/LIF and serum/LIF condition (provided in **Table S9**). '2i+serum' CTCF was defined by the presence of CTCF in ES cells in 2i/LIF and serum/LIF conditions and the absence of CTCF in NPCs (provided in **Table S10**). This class was generated via the intersection of ES 2i CTCF sites with ES serum CTCF sites, followed by the subtraction of NPC CTCF sites. 'Serum+NPC' and '2i+NPC' CTCF sites were similarly parsed. Finally, 'Constitutive' CTCF was defined by the presence of CTCF in ES cells in 2i/LIF and serum/LIF and in NPCs (provided in **Table S11**).

siRNA Knockdown of YY1 in pNPCs

pNPCs were cultured as described above. After two passages in suspension, cells were seeded adherently at a density of 20,000 cells/cm2. In order to allow cells to reach a critical density before the start of transfection, 40 hours were allowed to pass between seeding and the application of siRNA. The following siRNA pools were purchased from Dharmacon: YY1 (# L-050273-00-0005), Non-targeting Pool (# D-001810-10-05). Cells were transfected with a final concentration of 20 nM siRNA. RNAimax (Lifetech #13778-075) was used as a transfection reagent at 1/3 the recommended concentration (2.5 uL per well of 6 well plate, 14.5 uL per 10 cm dish). Reagents were prepared in Optimem according to RNAimax manufacturer's instructions and then added dropwise to culture well/dish. Transfection continued for 78 hours,

with media and transfection reagents replaced at hours 24 and 48 after start of transfection. After 78 hours, cells were harvested for RT-qPCR, Western blot, and 3C/5C.

In situ 3C

pNPCs subjected to siRNA transfection were fixed with formaldehyde for 3C as previously described (Beagan et al. 2016). 4 million cells were utilized per replicate and subjected to an *in situ* 3C protocol adapted from (Rao et al. 2014). Cell pellets were resuspended in lysis buffer consisting of 10 mM Tris-HCl (pH 8.0), 10 mM NaCl, 0.2% Igepal CA630 and 1x protease inhibitor and incubated with frequent agitation on ice for 20 minutes. Nuclei were washed twice with 1.2X NEBuffer. SDS was added to a final concentration of 0.3% and the homogenate was incubated for 1 hr at 37°C. SDS treatment was inactivated by the addition of 20% Triton X-100 to a final concentration of 1.8% and incubation at 37°C for 1 hr. Chromatin was digested with HindIII (300U) overnight at 37°C then 65°C for 30 minutes. Chromatin was then ligated upon the addition of ligation buffer components at final concentrations of: 0.83% Triton X-100, 1X BSA, 1mM ATP, 50mM Tris-HCl, 50mM NaCl, 10mM $MgCl_2$, 1mM DTT and 15 uL of T4 DNA ligase (Invitrogen). The ligation reaction occurred at 16°C for 4 hours and then at room temperature for 30 minutes. Finally, samples were Proteinase-K digested, RNase treated, phenol-chloroform extracted with ethanol precipitation and resuspended in 1X TE buffer. 600 ng of 3C template was utilized for 5C as described in (Phillips-Cremins et al. 2013; Beagan et al. 2016).

Gene expression quantification via RT-qPCR

RNA isolation was done using the mirVana miRNA isolation kit (Lifetech #AM1560), following manufacturers protocol for total RNA isolation. Cells were lysed in mirVana supplied lysis buffer and stored temporarily at -20°C until all samples were collected. Volume of lysate utilized in organic extraction was adjusted to contain the lysate from 500,000 cells. Manufacturer's

protocol was then followed precisely. cDNA was prepared for each sample using the SuperScript First-Strand Synthesis System (Lifetech #11904-018) according to manufacturer's specifications. 100 ng of RNA, quantified via Qubit, was loaded into each reaction. The following primers were designed to query relevant gene expression:

YY1: F: CACGCTAAAGCCAAAAACAACC ; R: ATTCCCAATCACACTCCTGAAG

Sox2: F: GCACATGAACGGCTGGAGCAACG ; R: TGCTGCGAGTAGGACATGCTGTAGG

Olig2: F: GCAGCGAGCACCTCAAATC ; R: GATGGGCGACTAGACACCAG

Nestin: F: AGGCCACTGAAAAGTTCCAG ; R: TAAGGGACATCTTGAGGTGTGC

Zfp462: F: CAAAGCCCATGCTGGTGAAC; R: TTTGCCATGGACCTTGAGGG

Klf4: F: AGACCAGATGCAGTCACAAGTC ; R: TTTTGCCACAGCCTGCATAG

Standard curves for each primer set above were generated by quantifying the product of a conventional PCR reaction and serially diluting the amplicon to create 200 – 0.0002 pM standards. qPCR reactions were performed on the Applied Biosystems StepOnePlus system using the Power SybrGreen PCR Master Mix (Applied Biosystems #4364659). For each qPCR reaction, primers were added to a final concentration of 400 nM and 1 uL of each standard and sample cDNA was loaded. The resulting CT values of the standards were used to generate a standard curve and calculate the concentration of transcript cDNA per 100 ng of RNA loaded into the first strand reaction.

Western blotting

Cells for each condition were washed with ice-cold PBS and lysed in RIPA buffer (Sigma R0278, ~100 uL per 1 million cells). Cells in RIPA were scraped off of the dish and rotated for 30 min at 4°C. Samples were then spun for 20 minutes at 12,000 rpm and 4°C, after which the supernatant was stored at -20°C until further use and the pellet was discarded. Total protein content was estimated by BCA assay (Thermo scientific #23227) in order to target equal total protein loading. Sample to be loaded was then diluted in 4X Laemmli buffer (BioRad #161-0747)

and 2-mercaptoethanol (final concentration 355 mM). Samples were run through a BioRad TGX 4-15% gel (#456-8084) and transferred to an LF-PVDF membrane using the BioRad TransBlot Turbo transfer system. After transfer, membranes were washed twice with TBS, then blocked for 1 hour in 3% BSA in TBS at room temperature. The membrane was incubated with primary antibodies (CTCF=Cell Signaling #3418 at 1:200, YY1= Santa Cruz #sc-1703 at 1:50, Gapdh=Cell Signaling #2118 at 1:1000) in 3% BSA in TBS/T overnight at 4°C under constant agitation, then at room temperature for 10 minutes. 3 washes in TBS/T were performed before incubation with secondary antibody (anti-Rabbit Dylight 650, abcam #ab96894) in 3% BSA in TBS/T at room temperature for 1 hour. Finally, blots were imaged on the ChemiDoc MP Imaging system after 3 washes in TBS/T.

5C data analysis

Technical note on preliminary processing of two analysis groups

Two sets of 5C data, group1 and group2, were processed independently for this study. Group1 represents a re-analysis of raw reads from previously published 5C experiments (Beagan et al. 2016) and consists of ES 2i (n=2 replicates), ES Serum (n=2 replicates) and pNPC (n=2 replicates) conditions. Group2 5C libraries were generated in the present study and consist of YY1 siRNA treated pNPCs (n=2 replicates) and scrambled siRNA treated pNPCs (n=2 replicates). These 5C replicates were sequenced on the Illumina NextSeq 500 with 37 bp paired-end reads and then aligned to a pseudo-genome of the 5C primer set using Bowtie with default parameters (Langmead 2010). To be considered a count for downstream processing, reads were required to: (i) have only one unique alignment, (ii) have both paired-ends map to the pseudo-genome, (iii) represent an interaction between one forward and one reverse primer. Before downstream analyses, mapped 5C reads were trimmed of entire primers if the total counts sum of that primer was less than 10 or the primer was visually identified as low quality. Group1 data were high quality/high complexity. Preliminary analysis of Group2 revealed a high

level of spatial noise likely due to technical artifacts caused by suboptimal ligation for these particular libraries. Although we provide sequencing reads for all our queried 5C regions for Group2, we only publish downstream processing and analysis in Group2 for the Sox2 and Klf4 regions, as these were highest complexity regions resembling our high quality NPC maps obtained from Group1. Thus, for Group2 data sets, the 5C primers for all regions other than Sox2 and Klf4 were removed before assembling primer-primer junction counts files. Group1 5C libraries were processed separately from Group2 5C libraries. 5C libraries were analyzed as detailed below. Custom scripts for all of the analysis steps are provided as supplemental material for full reproducibility of figures.

Quantile normalization

To account for sequencing depth and technical complexity differences among libraries, 5C replicates were conditionally quantile normalized. Briefly, the GC content of each 5C primer was calculated. Each primer-primer pair could then be assigned a pair of GC content values based on the two constituent primers. Primer-primer pairs with the *same* GC content pair were grouped. Within each group, counts for primer-primer pairs were quantile normalized across replicates as previous described (Beagan et al. 2016). Counts of the same starting value (i.e. a tie) were assigned the average value of the *lowest* rank in the set of tied counts. Group1 and group2 data were quantile normalized separately.

Primer correction

To account for known primer-specific biases in our 5C data, we applied a modification of the published Express algorithm in which we computed joint bias factors by using counts data from all replicates (Sauria et al. 2015). Group1 and group2 data were primer corrected separately.

Removal of low confidence primer-primer pairs

Primer-primer pairs were removed from downstream analyses if they did not register at least 10 normalized reads in at least 3 of the replicates (group 1) or if they did not register at least 5 normalized reads (group 2).

Interaction matrix binning

We divided each of our queried regions into adjacent 4 kb bins because 4 kb is roughly the average restriction fragment size after HindIII digestion. Each entry of the binned interaction frequency matrix represents the relative frequency with which two 4 kb bins interact. The relative interaction frequency in each bin was set as the arithmetic mean of the normalized, logged primer-primer pair reads that mapped to within a 16 kb (Group1) or 20 kb (Group2) square smoothing window surrounding the coordinates of the midpoints of the two bins.

Removal of low information content bins

Interaction frequency matrix entries were set to 'NaN' and thus removed from downstream processing if the number of primer-primer pairs within the smoothing window of that matrix entry that were 'NaN' or zero exceeded 80% of the possible primer-primer pairs.

Expected background modeling

To evaluate looping interactions, we employed slight modifications of the donut and lower left background models recently developed by the Aiden group (Rao et al. 2014). This approach requires a global distance-dependence model, which we generated by first computing the arithmetic mean of the interaction frequency matrix entries that represent interactions of equivalent genomic distance. For the shortest 1/3 of interaction distances queried we used the empirical mean as the distance-dependent expected; for the remaining interaction distances we calculated a lowess fit to the empirical means and utilized each fit value as the distance-dependent expected. Global expected values were 'corrected' for local background interaction

frequencies through the use of donut and lower left background filters specific to each entry in the binned interaction frequency matrix. The 'Donut' correction was applied according to (1):

$$E_{ij}^{d} = \frac{D_F(i,j)}{D_E(i,j)} \times E_{ij} \tag{1}$$

where E_{ij} is the global distance-dependence expected interaction frequency of bins i and j, and $D_F(i,j)$ and $D_E(i,j)$ are evaluations of a function 'D' over the interaction frequency matrix F and the global distance-dependence expected matrix E, respectively. The function 'D' finds the sums of the values falling within the donut window for the entry (i,j) of the matrix of interest (represented here as 'A') with chosen parameters p and w (2):

$$D_A(i,j) = \sum_{x=i-w}^{i+w}\sum_{y=j-w}^{j+w} A_{xy} - \sum_{x=i-p}^{i+p}\sum_{y=j-p}^{j+p} A_{xy} - \sum_{x=i-w}^{i-p-1} A_{xj} - \sum_{x=i+p+1}^{i+w} A_{xj} - \sum_{y=j-w}^{j-p-1} A_{iy} - \sum_{y=j+p+1}^{j+w} A_{iy} \tag{2}$$

The 'Lower Left' correction was applied according to (3):

$$E_{ij}^{ll} = \frac{LL_F(i,j)}{LL_E(i,j)} \times E_{ij} \tag{3}$$

where the LL function for a matrix A is defined as in (4) :

$$LL_A(i,j) = \sum_{x=i-w}^{i-1}\sum_{y=j-w}^{j-1} A_{xy} - \sum_{x=i-p}^{i-1}\sum_{y=j-p}^{j-1} A_{xy} \tag{4}$$

A schematic of the donut and lower left windows defined by these functions is shown in **Fig. 3B**. Eqn. 1 generated 'Donut background' matrices (see **Fig. 3C**). Eqn. 3 generated 'Lower left background' matrices (see **Fig. 3D**).

The parameters p and w determine the dimensions of the donut/lower left window surrounding each interaction frequency matrix entry as detailed by Aiden and colleagues (Rao et al. 2014). p and w are defined as the number of bins between the pixel/entry of interest to the inner (p) and outer (w) edges of the donut window, respectively. Thus, if the donut window is conceptualized as two squares, one larger containing the second smaller square, p = (width of

small square – 1) / 2, w = (width of large square – 1) / 2 (**Fig. 3B**). By applying guidelines from *Rao et al.* that *p* should have a distance of 20-25 kb, we set *p* equal to 5 bins of size 4 kb. Similarly, we iterated through values of *w*, ranging from the minimum allowed by the formula (*p+2=6*) to 20 and selected *w*=15.

To capture the most stringent local background model represented within the Donut and Lower Left background models, for each matrix entry we calculated the maximum of the two models and entered this into a new 'Donut/LL Max' background matrix (see **Fig. 3E**). If a matrix entry was non-existent ('NaN') in one background model but not both, the available real background value was utilized. Moreover, to avoid propagating expected values in which we had low confidence, we set the corrected expected matrix entry to 'NaN' and excluded the bin-bin interaction from further analysis if greater than 80% of all possible values within the corresponding donut or lower left window were non-existent.

Probabilistic modeling

As previously described (Beagan et al. 2016), we modeled the background-corrected interaction frequencies as a continuous random variable using the logistic distribution. Using the R fitdistr() function, we parametrized the fit independently for each region and replicate, and computed right-tail p-values. Finally, we computed 'background-corrected interaction scores' with the equation:

$$\mathrm{IS}_{i,j} = -10 \times \log_2(p_{i,j})$$

where $p_{i,j}$ is the logistic p-value for a given entry in the background-corrected interaction frequency matrix. Background-corrected interaction score matrices were plotted as heatmaps to visualize 3D chromatin interactions that were enriched above the local interaction background (**Fig. 3F**).

Removal of interactions below distance limit

We identified 20 kb as our lower limit of bin to bin distance at which we could meaningfully identify 3D interactions; distance-corrected interaction p-value and distance-corrected interaction score entries for bins that were less than 20 kb apart were also set to 'NaN' and excluded from further analysis.

Thresholding interaction scores into cell-type specific interaction classifications

Each background-corrected interaction score matrix entry was subjected to a series of thresholds to classify each into a set of classifications based their value in each cell type (**Fig. 3G**, similar to strategy pursued in Beagan et al. 2016). Both replicates of each cell type were required to pass each threshold in order for an interaction (matrix entry) to be classified into a specific class. Refer to (**Supplemental Fig. 8A**) for visualization of the thresholds discussed below. Matrix entries with interaction scores ≤ 3.22 (p-value of 0.8) across all six replicates were classified as 'background' interactions. If an entry had interaction score from each cell type less than 25.99 (p-value of 0.165, referred to as the 'significance threshold'), it was not classified into any interaction class. Otherwise, if both replicates from at least one cell type cleared the significance threshold, that entry could be classified as either (i) constitutive, (ii) present in two cell types but not the third (i.e. Serum+2i, Serum+NPC, NPC+2i), (iii) specific to one cell type (Serum-only, 2i-only, NPC-only). As in Supplemental Fig. 8A, this is simplified by first considering pairwise combinations of the cell type interaction scores; in this step, assuming the significance threshold has been passed in at least one of the cell types, an entry can be classified as either 'present only in cell type A', 'present only in cell type B', or 'present in both'. Interactions were 'present in both' if: (i) both replicates for each cell type had an interaction score greater than or equal to 40 (p-value of 0.0625, referred to as the 'constitutive threshold'), or (ii) if all four replicates under consideration cleared the significance threshold and the differences between all pairs of the four interaction scores were less than 30.2 (referred to as the 'difference threshold'). Otherwise in these two-way comparisons, entries were considered

only in cell type A or B if in the ‘present’ cell type their interactions scores passed the significance threshold and the difference threshold when compared to the other cell type. Finally, the results of these two-way comparisons were stitched together such that matrix entries were parsed as ‘constitutive’ if always classified as ‘present in both’ of the cell types queried, present in two cell types (Serum+2i, Serum+NPC, NPC+2i) if classified as ‘present in both’ when comparing the two named cell types but classified as ‘present only in’ each of these cell types when compared to the third un-named cell type, or cell type specific (Serum-only, 2i-only, NPC-only) if classified as ‘present only in’ the named cell type across both comparisons with the other two cell types. **Fig. 3H** displays the three-way scatterplot for these classes.

Clustering and Cluster Trimming

Similarly classified interactions that were spatially adjacent were grouped into interaction clusters as previously described (Rao et al. 2014). Briefly, for a given classified interaction, if it existed next to an already identified cluster, the interaction was added to that cluster; if not, a new cluster was assigned to that interaction. After iterating through all classified interactions, adjacent clusters of the same classification were merged.

Interaction clustering enabled us to threshold our data based on interaction size in addition to interaction score. For each interaction cluster, the number of individual interaction matrix entries within that cluster and any clusters directly adjacent (of any classification) was tallied. If the individual interaction sum across itself and all directly adjacent clusters was not greater than 2, that cluster was removed as a low confidence cluster. The process of iterating through all clusters was repeated until no clusters were trimmed. The thresholding, clustering, and trimming methods produced our significant interaction cluster calls (**Fig. 3I**).

Empirical false discover rates

Six simulated 5C replicates were generated for each of our three cellular conditions as described in detail previously (Beagan et al. 2016). The 6 simulated background-corrected interaction frequency replicates were then passed through the same processing stages as the real background-corrected interaction frequency replicates (see above). Because the replicates were simulated to be from the same cell type, any interaction that is classified as a dynamic looping category was considered a false positive. Simulations of six biological replicates of the same condition were performed 1000 times and the average number of interactions that were classified for each cell type across the 1000 simulations were reported (**Fig. S8B**). One simulation round of six 5C library simulations of the NPC condition was chosen as representative in **Figs. S8C-D**.

Parsing Cell-Type Specific YY1

YY1 ChIP-seq datasets (NPC = (Mendenhall et al. 2010), ES = (Sigova et al. 2015), ProB = (Medvedovic et al. 2013)) were downsampled together and peak-called with the MACS2 broad-peak caller using a diffuse p-value of 1e-8 and a broad cutoff of 1e-4 (see above). The subsequent broad peaks were parsed into cell type specific occupancy classes using Galaxy. ES serum only YY1 was defined by the presence of YY1 in serum/LIF ES cells and the absence of YY1 in NPCs and ProB cells (subtraction of NPC YY1 and ProB YY1 peaks from ES serum YY1). NPC only and ProB only peaks were parsed similarly. Constitutive YY1 was defined by the presence of YY1 in ES cells in serum, NPCs and ProB cells (intersection of the ES serum YY1 with NPC YY1 and ProB YY1). A two-way class such as ‘NPC and ProB, not ES’ was parsed via the intersection of NPC and ProB peaks and the subtraction of ES peaks.

Parsing ES and NPC Enhancers

ES enhancers were defined as overlap between H3K27ac peaks and H3K4me1 peaks in ES cells in serum/LIF and absence H3K27ac in NPCs. This was calculated via the intersection of

ES serum H3K27ac ($p < 1x10^{-8}$) with ES serum H3K4me1 ($p < 1x10^{-4}$, from (Beagan et al. 2016)) followed by subtraction of low-confidence NPC H3K27ac ($p < 1x10^{-2}$). Similarly, NPC only enhancers were defined by overlap between H3K27ac peaks in NPCs and H3K4me1 peaks in NPCs and absence H3K27ac in ES cells in serum. To ensure exclusion of all genes from enhancer calls, we required that all parsed ES and NPC enhancers were not within 2 kb of a transcription start site (TSS). ES and NPC enhancers are provided in **Tables S14 and S15** respectively.

Gene expression and Gene Annotation

Normalized, log2 gene expression counts were utilized from (Beagan et al. 2016). Genes were required to have a normalized, log2 expression count of at least 4 across both replicates of the cell type in which they were being considered active. Genes for which all pairwise replicate comparisons of ES serum expression with NPC expression displayed at least a 1.8 fold upregulation in ES cells were then intersected with H3K27ac ($p < 1 \times 10^{-8}$ in ES in serum); the resulting annotations were classified as 'ES-specific genes'. Similarly, genes with at least a 1.8 fold upregulation in NPCs compared to ES cells in serum across all replicates were then intersected with NPC H3K27ac ($p < 1 \times 10^{-8}$) and classified as 'NPC-specific genes'. Active genes across both cell types that exhibited than a 1.8 fold difference with respect to each other were intersected with H3K27ac from both cell types and classified as 'Constitutively expressed'. ES-specific, NPC-specific and Constitutive genes are provided as **Tables S16, S17 and S18,** respectively. Genes with normalized, log2 expression counts less than 2.5 across both cell types were classified as 'Inactive'.

Computing the enrichments of genomic annotations within interaction classes

Enrichments of annotations within interaction classes were calculated and visualized as previously described in detail (Beagan et al. 2016).

CTCF Intersection with Consensus Motif and Directionality Enrichment Calculation

The CTCF position weight matrix was selected from the JASPAR core 2014 vertebrates motifs library. The position and orientation of the motif in the mm9 mouse genome were determined with PWM Tools (http://ccg.vital-it.ch/pwmtools/pwmscan.php). We then intersected our called CTCF peaks with this orientation file to assign orientations to each CTCF peak.

First, we parsed CTCF peaks with forward and reverse consensus motif orientations. We then identified the 4 kb bins intersecting with directionally oriented annotations. To take into account our 16 kb 5C smoothing window, we also considered a bin to contain an annotation if an adjacent bin on either side of the bin in question contained the annotation. Next, for each classified interaction, we determined whether the bins at the base of that interaction contained (i) no CTCF, (ii) CTCF on only one side, (iii) conflicting CTCF orientations over a single peak or in a single bin or (iv) unique CTCF orientations within both bins (**Fig. S9A**). We next parsed the interactions with unique CTCF orientations on both sides by which motif orientations actually appeared in the two bins: (i) interactions with the forward motif orientation in its upstream bin and the reverse orientation in its downstream bin were classified as ‘Convergent’; (ii) interactions with the same orientation on both sides, i.e. both forward or both reverse, were classified as ‘Same Direction’ or ‘Tandem’; finally (iii) an interaction was considered ‘Divergent’ if only reverse motif(s) were present on the upstream side of the interaction and forward motif(s) present on the downstream side. This analysis was performed on the ‘constitutive’, ‘2i+Serum’, and ‘NPC-only’ interaction classes. The enrichment above background for each of these orientations in each interaction class was also calculated as described above (see ‘*Computing the enrichments of genomic annotations within interaction classes*’).

Supplemental References

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