Supplemental Material

Supplemental Materials and Methods

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Supplemental Material and Methods

*DNA adenin methyltransferase identification (DamID)*

DamID was performed as in (Jacinto et al. 2015). Briefly, we cloned the heat shock promoter, ecodam tag and RFC.1 region of the pLgw EcoDam-V5-RFC1 vector (a gift from Dr. B. Van Steensel) into a retroviral pMSCV plasmid. ORFs (GFP, LBR-GFP, Nup153, mNup93) were inserted using the Gateway cloning system (Thermofisher). We generated stable cell lines expressing Dam methyltransferase fusion proteins including a Dam-GFP cell line to correct for effects of local chromatin accessibility (Guelen et al. 2008). Genomic DNA was isolated from the different cell lines using the DNeasy Blood & Tissue Kit (Qiagen). 2.5 ug of genomic DNA from the different cell populations expressing the Dam-fusion proteins were digested with DpnI restriction enzyme (NEB) overnight. Upon ligation and amplification with biotin adapters DNA was sonicated in Diagnode tubes in a Bioruptor (Diagenode) to obtain 100-500 bp fragments. Biotynilated material was pulled down using Streptavidin MyOneC1 Dynabeads (Invitrogen). DNA was eluted from the beads by digestion with DpnII enzyme (NEB) and subsequently purified using the MiniElute PCR purification kit (Qiagen). Resulting DNA was processed according to the TruSeq ChIP Sample Prep kit instructions (Illumina) and size
selection of the libraries performed with AMPure XP beads (Beckman Coulter). Libraries were run in a HiSeq2500 sequencing system (Illumina). Reads were aligned to the human genome (hg19, NCBI37) using Bowtie2 (version 2.2.3) (Langmead and Salzberg 2012). Reads that aligned uniquely to a single genomic location and were used for downstream analysis. In addition, only reads aligning at a GATC sequence in the genome were kept for further analysis. Reads aligning to non-GATC locations were much more likely to non-specific noise. Replicates were combined for subsequent analyses in U2OS. Putative DamID-Seq peaks were identified using HOMER in a manner similar to variable-size ChIP-Seq peaks. Initial peak size was set at 2500 bp, and peaks needed 2-fold more normalized reads than GFP DamID-Seq controls. In addition, peaks were required to contain at least 100 normalized reads per peak (per 10 million reads sequenced) to remove low magnitude sites. Normalized bedGraph files were created for DamID by extending reads 1 kb both upstream and downstream to reflect the relative size of DamID enriched regions. Comparisons between ChIP-Seq peaks, DamID-Seq peaks, and other genomics features such as TSS were performed using the ‘mergePeaks’ program in HOMER. De novo motif discovery, META-gene enrichment profiles, and annotation to nearest or overlapping RefSeq genes were carried out using HOMER.

mRNA sequencing (RNA-Seq)

Reads obtained from the sequencing were aligned to the human genome (hg19, NCBI37) using STAR (version 2.2.0.c, default parameters) (Dobin et al. 2013). Only reads that aligned uniquely to a single genomic location were used for downstream analysis (MAPQ > 10). Gene expression values were calculated for annotated RefSeq genes using
HOMER by counting reads found overlapping exons (Heinz et al. 2010). Differentially expressed genes were found from two replicates per condition using EdgeR (Robinson et al. 2010). Gene Ontology functional enrichment analysis was performed using DAVID (Dennis et al. 2003).

**ChIP-Seq, DNase-Seq, super-enhancer identification**

The following primary antibodies were used for ChIP: CTCF (Active Motif), H3K4me3 (Abcam), H3K27Ac (Abcam), H3K9me2 (Abcam). DNA libraries were generated using the Illumina Tru-Seq ChIP Sample Prep kit (Illumina) or the Kapa Hyper Prep Kit for Illumina Platforms (Kapa biosystems). Libraries were sequenced in a HiSeq 2500 system (Illumina). Replicate samples were generated except for H3k9me2 due to the sequencing depth required. ChIP-Seq and DNase-Seq peaks were identified using HOMER using an FDR of 0.1% and fold-enrichment over input of at least 4-fold (Heinz et al. 2010). Normalized bedGraph files were created for each experiment using HOMER and visualized using the UCSC Genome Browser (Kent et al. 2002).

Super-enhancers were identified with HOMER (Hah et al. 2015), which uses a similar strategy to Rose (Whyte et al. 2013) to stitch together nearby enhancers into super enhancer regions. H3K27ac ChIP-seq and input data for each cell type was used to identify initial peaks, and peaks within 25 kb were merged into putative super enhancer regions. Peaks were ranked by their total H3K27ac minus input read totals, and a cutoff for super-enhancer calling was established where the slope of the normalized signal and rank curve became greater than 1. The specific HOMER parameters used for findPeaks are as follows: “-style super -L 0 –minDist 25000”. Since gene promoters are usually
found within the context of super-enhancers, regions containing H3K4me3 or annotated TSS were not excluded from the analysis.

*Peak Overlap, Feature Enrichment, and Motif Analysis*

Comparisons between ChIP-Seq peaks, DamID-Seq peaks, and other genomics features such as TSS were performed using the ‘mergePeaks’ program in HOMER. Enrichment calculations are carried out by considering the total overlap in bp between two groups of features, such as ChIP-Seq or DamID-Seq peaks, and by considering the total coverage of each set of peaks and the total size of the measureable genome, which was conservatively set to 2 billion bp. Enrichment ratios were calculated assuming both sets of peaks are independent and randomly distributed across the genome. Significance p-values were calculated using the Fisher Exact Test by dividing the genome into bins according to the average feature (peak) size. Histograms displaying feature enrichment relative to the TSS or LAD boundaries were calculated using HOMER’s annotatePeaks.pl program, which compiles peak counts or normalized read counts relative to a set of common features (i.e. TSS). De novo motif discovery was carried out using HOMER’s findMotifsGenome.pl function, which uses a discriminative motif discovery algorithm to identify position specific scoring matrices that are specifically found in DamID-Seq or ChIP-Seq peaks relative to 50,000 randomly selected genomic regions (Heinz et al. 2010). The program also carefully selects genomic background regions with similar GC-content to avoid finding discriminative motifs that simply identify GC-rich sequencing commonly found at promoters.
Supplemental References

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Supplemental Figure Legends

Figure S1. INM and NPC components interactions with the human genome.
(A) Box histogram showing the size (Mb) of LADs identified using replicate LBR DamID experiments. Median size is shown as a line inside the box. (B) Distribution of Nup93 binding sites relative to Nup153 interaction sites (p-value of the enrichment P<1e-10). (C-F) Occupancy of genes (C), DNaseI hypersensitive sites (D), H3K4me3 (E) and CTCF (F) relative to LAD boundaries. (G) Distribution of LBR-LADs (green), Nup153 (gray) and Nup93 (orange) interaction sites relative to transcription start sites. (H) Distribution of Nup93 binding sites relative to annotated genes (interactions at promoters (+/-1Kb of TSS) are shown in dark grey, intragenic interactions (exon and intron) in pale red, intergenic ones in red and with other features in light grey). (I) Distribution of features relative to Nup93 binding sites (H3K4me3 in gray, H3K27Ac in red, DHSs in purple, H3K9me2 in green). (J) Top de novo DNA motif identified within Nup153 binding sites.

**Figure S2. Nup153 and Nup93 interact with SEs in U2OS cells.**

(A-D) Examples of Nup153 and Nup93 interactions with SE regions. Nup153 and Nup93 interactions are shown as black bars. SEs are shown as red bars. H3K27Ac ChIP-Seq profiles and annotated genes show the genomic and epigenetic landscape. (E) Fraction of SEs associated with Nup93. (F) Venn diagram showing the number of U2OS SEs that interact with Nup153, Nup93, both or none. (G) Selected categories of gene ontology analyses performed with Nup153-bound SE-associated genes. (H) Example of Nup153-bound SE-associated gene. Nup153 interactions, black bars. SE, red bar. H3K27Ac ChIP-Seq profiles and annotated genes are shown.
Figure S3. Nup153 interacts with SEs in IMR90 cells.

(A-D) Examples of Nup153 associated SEs in IMR90 cells. Nup153 binding sites are shown as black bars, SEs in red. H3K27Ac ChIP-Seq profiles and UCSC genes are shown. Normalized RNA-Seq profiles in (C) and (D) illustrate expression of the genes in the indicated conditions. (E) Selected categories of gene ontology analyses performed with Nup153-bound SE-associated genes in IMR90 cells. (F) Venn diagram showing the overlap between U2OS and IMR90 SEs. IMR90-specific (442), U2OS-specific (177) and overlapping SEs are indicated. (G) Bar graph indicating the fraction of cell type-specific SEs (U2OS and IMR90-specific SEs) that overlap with Nup153 interaction sites identified in U2OS (gray) and IMR90 (red) cells.

Figure S4. Localization of Nup-associated SEs in U2OS cells.

(A) Representative IF DNA-FISH images in U2OS cells (Lamin A is shown in green, BACs in red, DNA in blue). (B) Quantification of the distribution of FISH signals by the three-zone scoring assay described in Figure 3. Control active regions (Cont), regions within LADs (Lad) and Nup-associated SEs (Nup-SE, interaction with Nup153 or both Nup93 and Nup153 (Nup153/93) is indicated) (n>50 for each region). (C) Distribution of U2OS (blue) and IMR90 (red) SEs relative to LADs. Calculated using LBR-associated domains in U2OS cells.

Figure S5. Intact nuclear compartmentalization in Nup153 knockdown cells.
(A and B) Control and Nup153 depleted U2OS cells were transiently transfected with GFP-NES (nuclear export signal) (A) or GFP-NLS (nuclear localization signal) (B) and stained for Nup153 (red) and DAPI to detect the DNA (blue).

**Figure S6. NPC components Nup153 and Nup93 regulate the transcriptional activity of cell identity genes.**

(A) Comparison of gene expression profiles in control and Nup153 knockdown U2OS cells. Differentially expressed genes are shown in red (FDR<0.05 and >1.5 fold). (B) Expected (gray) versus observed (red) association of Nup153 with genes ranked according to their changes in expression after Nup153 knockdown. (C) U2OS SE-associated genes ranked according to their transcriptional changes upon Nup93 knockdown. Deregulated genes are highlighted in red. (D and E) Examples of genes likely driven by SEs and regulated by Nup153 and Nup93. Nup-genome interactions are represented as black bars, SEs as red bars, H3K27Ac, H3K4me3 ChIP-Seq and RNA-Seq profiles of control, Nup153 and Nup93 siRNA treated cells are shown. (F) To control for the high expression levels of SE driven genes we selected the same number of non-SE genes and with the same expression levels. This selection was repeated 1000 times. The fraction of genes regulated by Nup153 in each permutation was plotted as a distribution (gray) relative to the fraction of regulated SE-associated genes (red line). We calculated the p-value of significance empirically by counting how many of our permutations contained a higher fraction of regulated genes (6/1000 in U2OS, 0/1000 in IMR90).
Figure S7. Model of NE-genome interactions and Nup-mediated regulation of cell identity genes.

LBR (magenta) and nuclear lamina components (red) interact with large, transcriptionally silent, chromosome domains at the NE (grey) while NPCs (orange) associate mainly with transcriptionally permissive regions. NPC-genome interactions are enriched at SEs and their associated cell identity genes in different cell types (cell-identity genes “A”, pink; cell identity genes “B”, green).

Table S1. List of BACs used in this study.
The name used through the paper, BAC code and source are indicated (CHORY: Children’s Hospital Oakland Research Institute).

Table S2. List of Nup153 and Nup93 target genes and differentially expressed genes upon Nup-KD in U2OS cells.

Table S3. List of Nup153 target genes in IMR90 cells and differentially expressed genes upon Nup153-KD.
Nup93 bound 104/345

Nup153
Nup93
SE

H3K27Ac

Gene annot

A

All SEs (U2OS)

E

Nup93 bound 104/345

Nup153
Nup93
SE

H3K27Ac

Gene annot

C

B

Nup-associated-SEs in U2OS

F

Regulation of cell proliferation (p=4e-5)
Osteoblast differentiation (p=1e-4)
Ossification (p=5e-4)
Bone development (p=7e-4)

G

Nup153 bound SE-associated genes (GO-U2OS)

Nup153
SE

H3K27Ac

Gene annot

H

Ibarra287417_FigS2
**Vasculature development** (p=1e-5)

**Regulation of cell proliferation** (p=3e-5)

**Regulation of transcription** (p=1e-4)

**Cell migration** (p=1e-4)

**Cell motion** (p=1e-4)

**Nup153 bound SE-associated genes**

| IMR90 specific SEs | U2OS specific SEs |
|--------------------|--------------------|
| 154                | 168                |
| 442                | 177                |

**Common SEs**

**siControl**

**siNup153**

**RNA-Seq**

**H3K27Ac**

**Gene annot**

**G**

**Fraction of cell type-specific SEs bound by Nup153 (%)**

- **U2OS SE**
- **IMR90 SE**

**Immunoprecipitation**

**Nup153 bound SE-associated genes**

- **Vasculation development**
- **Regulation of cell proliferation**
- **Regulation of transcription**
- **Cell migration**
- **Cell motion**

**Gene annot**

**H3K27Ac**

**Gene annot**

- **LTBP2**
- **NPC2**
- **ISCA2**
- **TCF7**
- **TAF13**
- **DYM**
Fraction of loci

### A

Cont 1  Cont 7  Cont 8  Lad 1  Lad 5  Nup-SE 13  Nup-SE 16

### B

Zone 1  Zone 2  Zone 3

### C

Relative distance (Kb)

LADs

U2OS SEs

IMR90 SEs

SE(bp)/LAD boundary
| Name     | BAC            | Source   |
|----------|----------------|----------|
| Control 1| CTD-3060L19    | Invitrogen|
| Control 2| RP11-120C19    | CHORY    |
| Control 3| RP11-963F11    | CHORY    |
| Control 4| RP11-154B14    | CHORY    |
| Control 5| RP11-29E3      | Invitrogen|
| Control 6| CTD-2648C13    | Invitrogen|
| Control 7| RP11-977B10    | CHORY    |
| Control 8| RP11-258D17    | CHORY    |
| Nup-SE 1 | RP11-426F8     | Invitrogen|
| Nup-SE 2 | RP11-1017A18   | CHORY    |
| Nup-SE 3 | RP11-300D14    | CHORY    |
| Nup-SE 4 | RP11-143B16    | CHORY    |
| Nup-SE 5 | RP11-51H10     | CHORY    |
| Nup-SE 6 | RP11-813D19    | CHORY    |
| Nup-SE 7 | RP11-590D16    | CHORY    |
| Nup-SE 8 | RP11-957B17    | Invitrogen|
| Nup-SE 9 | RP11-352P15    | CHORY    |
| Nup-SE 10| RP11-68O15     | CHORY    |
| Nup-SE 11| RP11-980C20    | CHORY    |
| Nup-SE 12| RP11-179L16    | Invitrogen|
| Nup-SE 13| RP11-1025F23   | CHORY    |
| Nup-SE 14| RP11-844G12    | Invitrogen|
| Nup-SE 15| CTD-3090L15    | Invitrogen|
| Nup-SE 16| RP11-834F19    | Invitrogen|
| Nup-SE 17| RP11-472D15    | CHORY    |
| Nup-SE 18| CTD-2205H22    | Invitrogen|
| Nup-SE 19| RP11-213E22    | Invitrogen|
| Lad1     | RP11-732I13    | CHORY    |
| Lad2     | RP11-946F10    | Invitrogen|
| Lad3     | RP11-458N24    | Invitrogen|
| Lad4     | RP11-927N10    | Invitrogen|
| Lad5     | RP11-268D11    | CHORY    |