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Supplemental Information

MadID, a Versatile Approach to Map Protein-DNA Interactions, Highlights Telomere-Nuclear Envelope Contact Sites in Human Cells

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Figure S1 - related to Figure 1

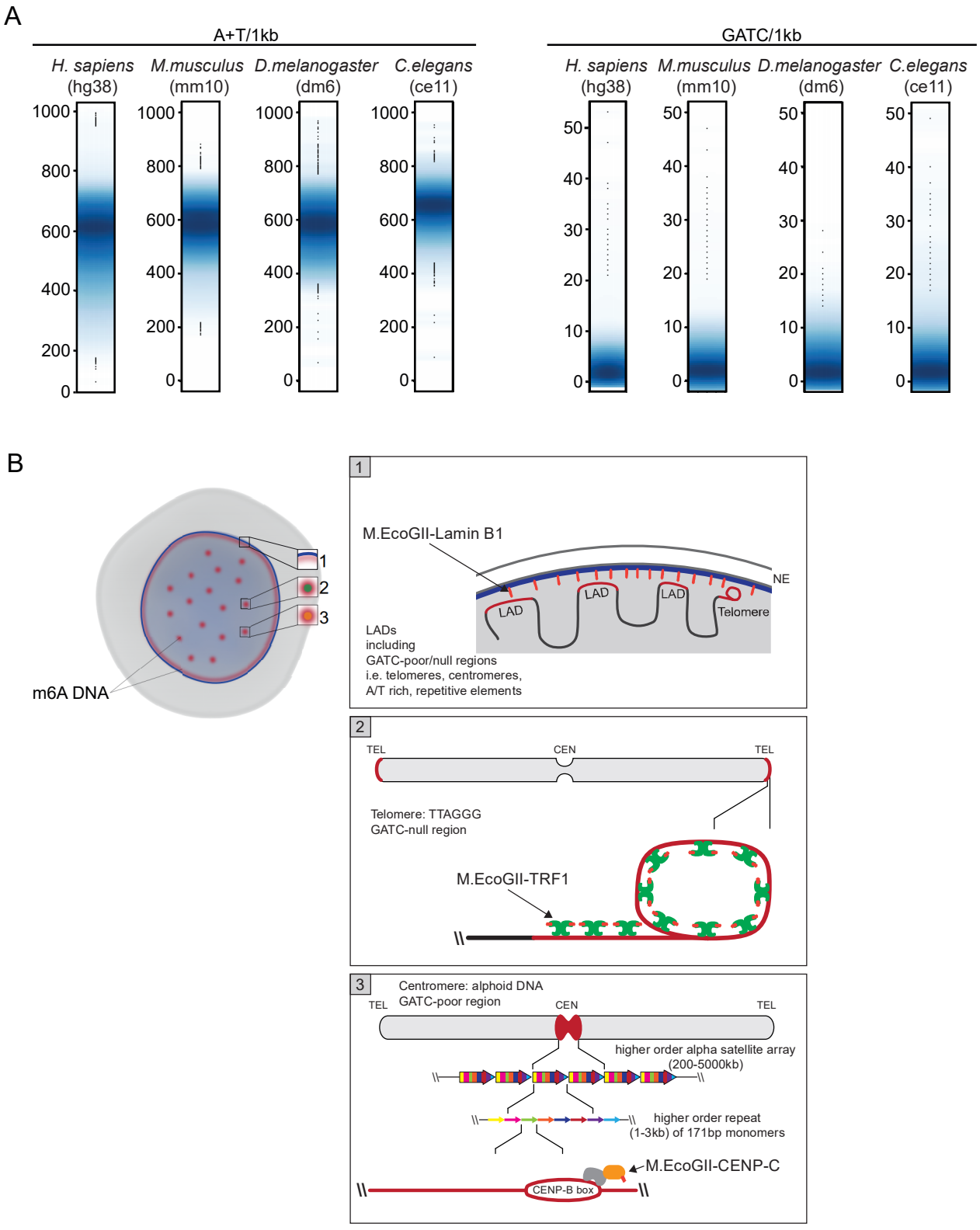


Figure S1 – related to Figure 1

(A) Smooth scatter graphs of A+T nucleotide (left) and GATC motif count (right) per 1kb genome segment for Homo sapiens (hg38), Mus musculus (mm10), Drosophila melanogaster (dm6) and Caenorhabditis elegans (ce11). (B) Graphical illustration of M.EcoGII targeted to 1. The nuclear lamina; 2. Telomeres; 3. Centromeres.

Figure S2 - related to Figure 2

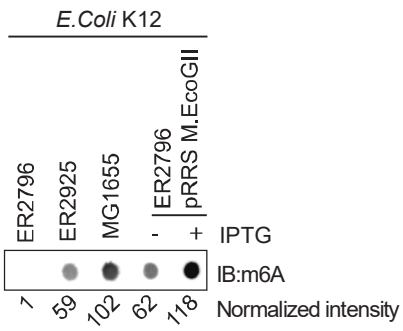
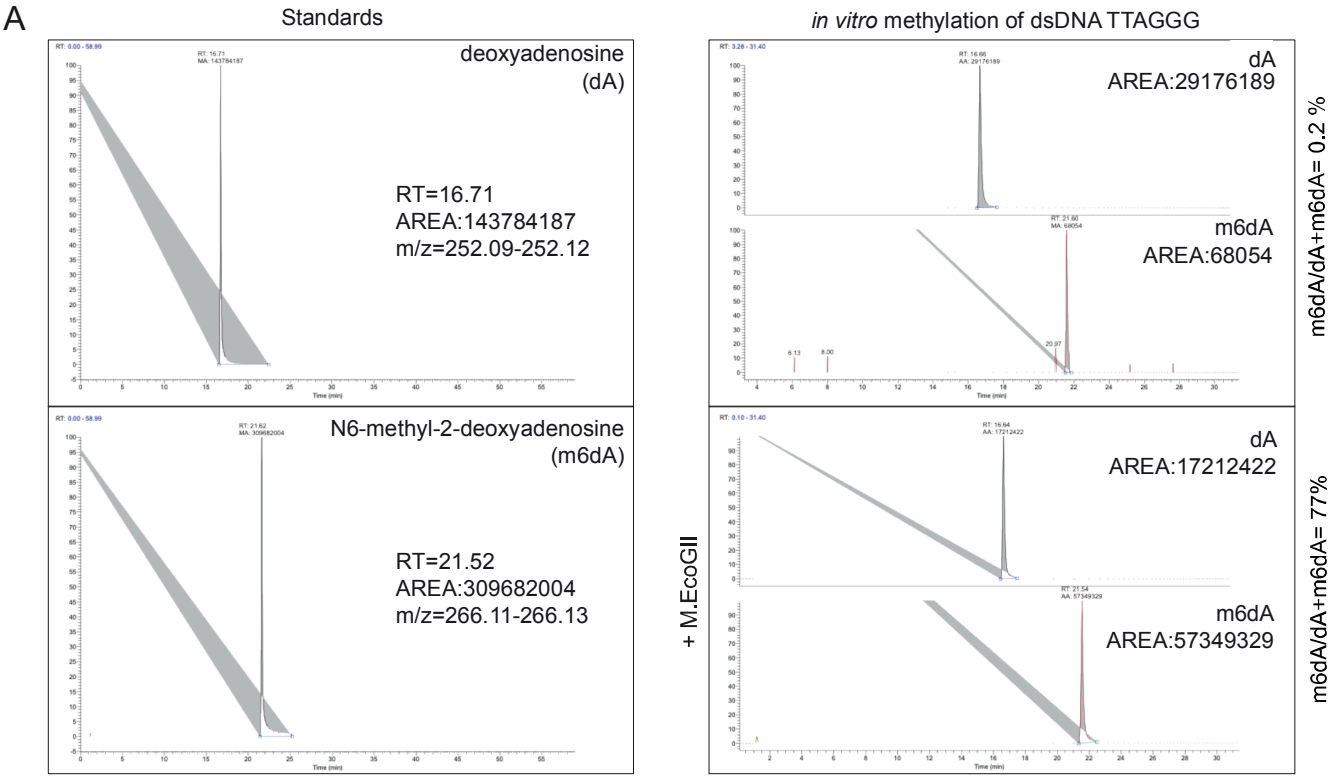
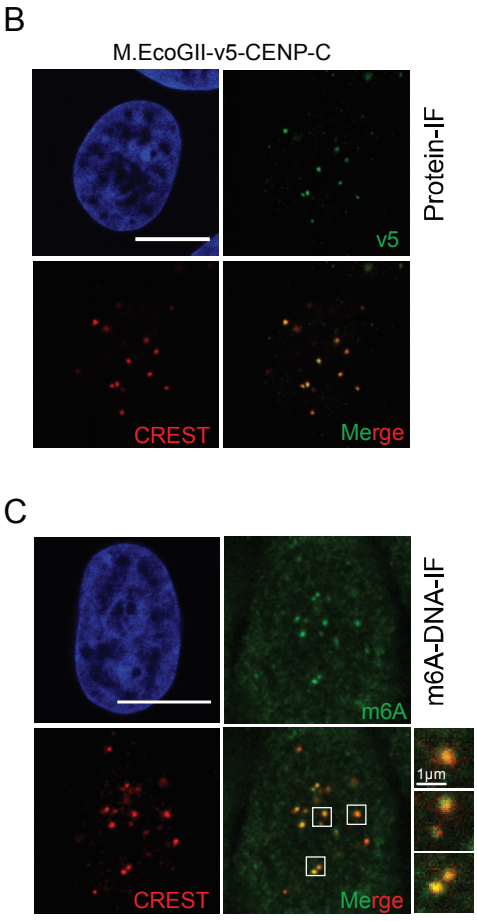


Figure S2 – related to Figure 2

Dot blot of genomic DNA probed with a m6A antibody. Genomic DNA was extracted from E.Coli K-12 ER2796, ER2925, MG1655 strains, which differs in endogenous levels of m6A methylation, and from induced (+IPTG) or not (-IPTG) E.Coli K-12 ER2796 strain carrying pRRS M.EcoGII inducible plasmid strain. Normalized intensity to DNA from the unmethylated strain is shown.

Figure S3 - related to Figure 3





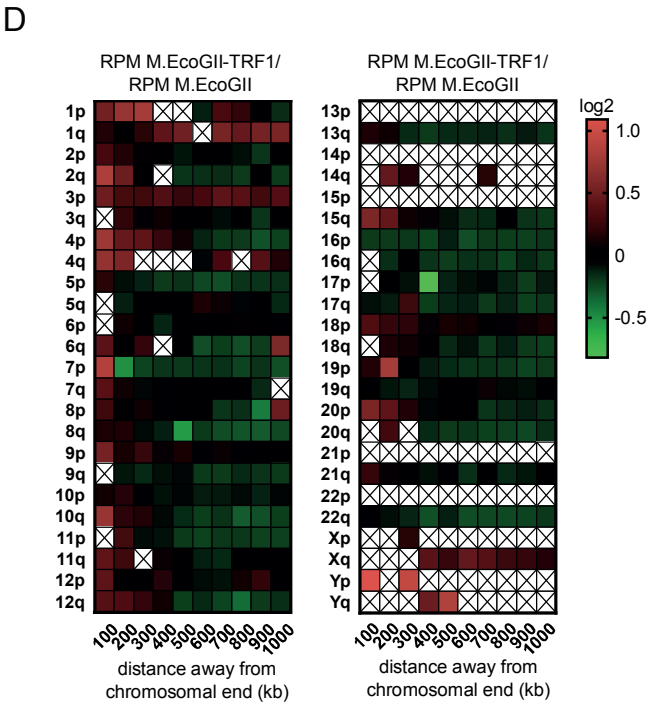


Figure S3 – related to Figure 3

(A) Liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS) analysis. Deoxyadenosine (dA) and N6-methyl-2-deoxyadenosine (m6dA) standards are shown on the left panels. Results obtained from dsDNA corresponding to 800bp of TTAGGG repeats before (TOP) or after (BOTTOM) in vitro methylation with recombinant M.EcoGII is shown on the right panels. The values of peaks area are shown. (B-C) DNA immunostaining of HeLa1.2.11 cells transduced with the indicated vectors. m6A or v5 (green), CREST/TRF2 (red) DNA (blue) and merge. Scale bar, 10 µm. 3 enlarged areas of m6A and CREST/TRF2 staining are shown. Scale bar, 1 µm. (D) Heatmap of the number of reads per million obtained at individual chromosome ends in HeLa1.2.11 cells expressing M-TRF1. The log2 M.EcoGII-TRF1/M.EcoGII ratio is shown. A box with a cross represents a bin without associated DNA sequence, therefore excluded from analysis.

Figure S4 - related to Figure 4

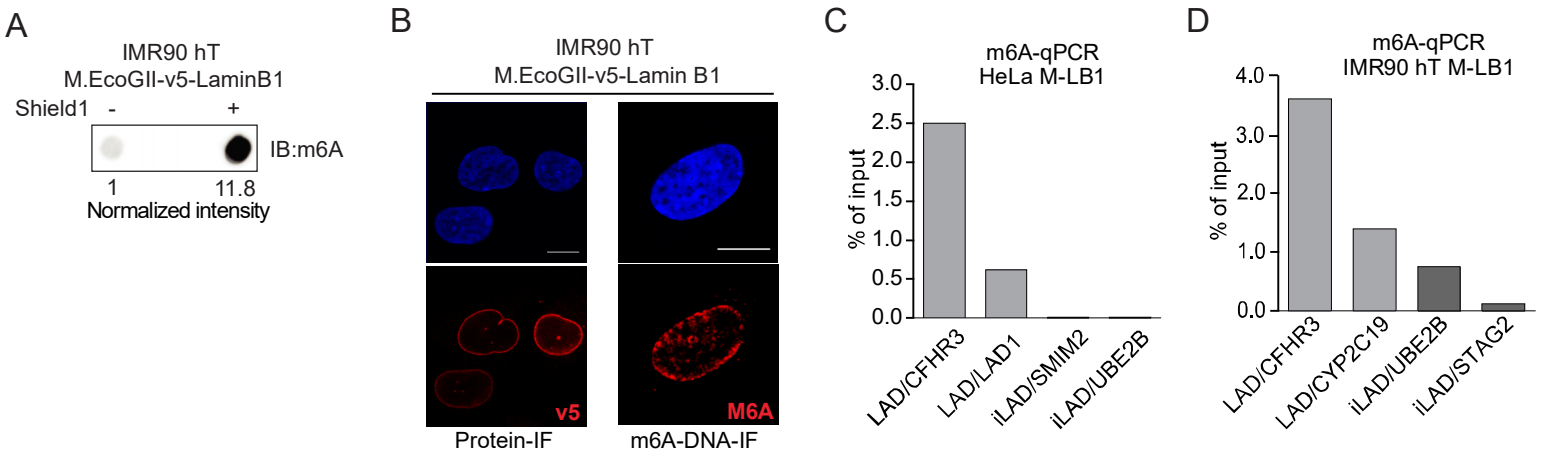


Figure S4 – related to Figure 4

(A) Representative dot blot of genomic DNA from IMR90 hTERT cells induced (+) or not (-) to express M.EcoGII-v5-Lamin B1 (M-LB1). The membrane was probed with a m6A antibody. (B) Exemple of immunofluorescence staining of IMR90 hTERT cells expressing M.EcoGII-v5-Lamin B1. Left: V5-tag (red) and DNA (blue) is shown. Right: m6A (red) DNA (blue). Scale bar, 10 µm. (C) m6A-qPCR analysis of HeLa1.2.11 induced to express M.EcoGII-v5-LaminB1. Enrichment over the input for CFHR3 gene and LAD1 region as a part of LADs (Lamina associated domains), and for SMIM2 and UBE2B genes as a part of iLADs (inter-LADs) is shown. (D) m6A-qPCR analysis in IMR90 hTERT induced to express M.EcoGII-v5-LaminB1. Enrichment over the input for CFHR3 and CYP2C19 genes as a part of LADs (Lamina associated domains), and for UBE2B and STAG2 genes as a part of iLADs (inter Lamina associated domains) is shown.

Figure S5 - related to Figure 5

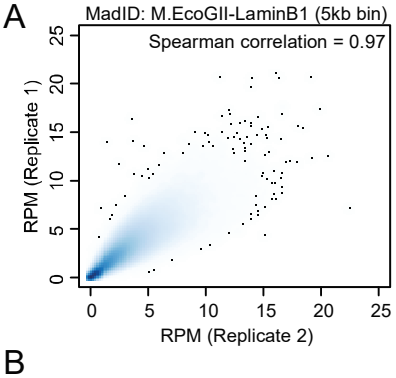
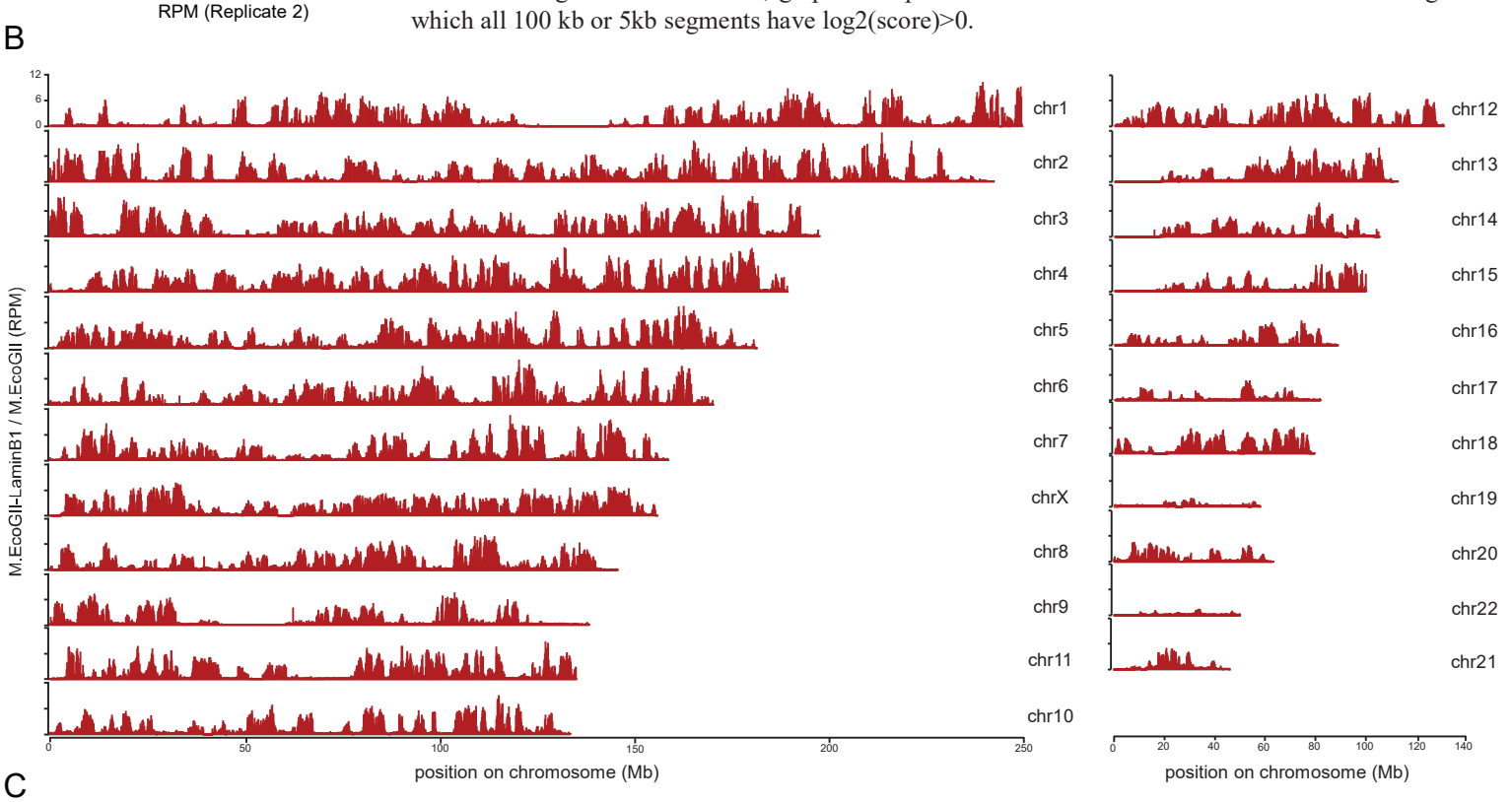
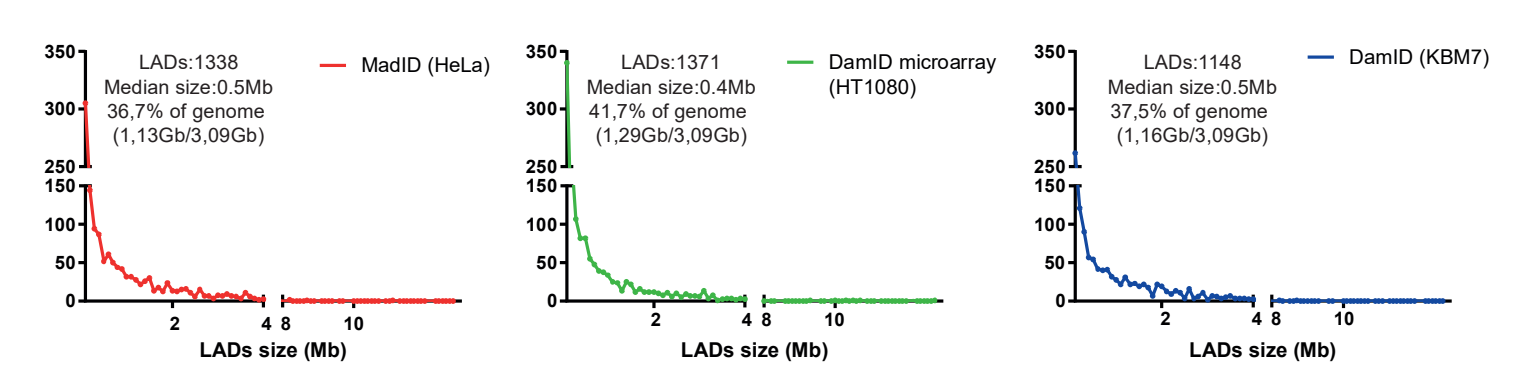


Figure S5 – related to Figure 5

(A) Correlation between sequencing data obtained from two independent experiments of MadID. (B) Nuclear lamina contact maps (red track) for all chromosomes (hg38) identified by MadID (accession E-MTAB-6888). RPM stands for reads per million. (C) Left, frequency distribution (y-axis) of the length of identified LADs (x-axis) using MadID (red line). Center, frequency distribution (y-axis) of the length of identified LADs (x-axis) using conventional DamID based on microarray (green line). Right, frequency distribution (y-axis) of the length of identified LADs using DamID-seq from 118 cells (blue line). (D) Comparison nuclear lamina contact map for a 4.5Mb region of chr1 (hg38) with MadID from HeLa 1.2.11 cells (top profile, accession E-MTAB-6888) and with single-cell DamID-seq of KBM7 cells using 100kb or 5kb binning. Below the tracks, graphical representation of identified LADs as continuous regions in which all 100 kb or 5kb segments have log2(score)>0.





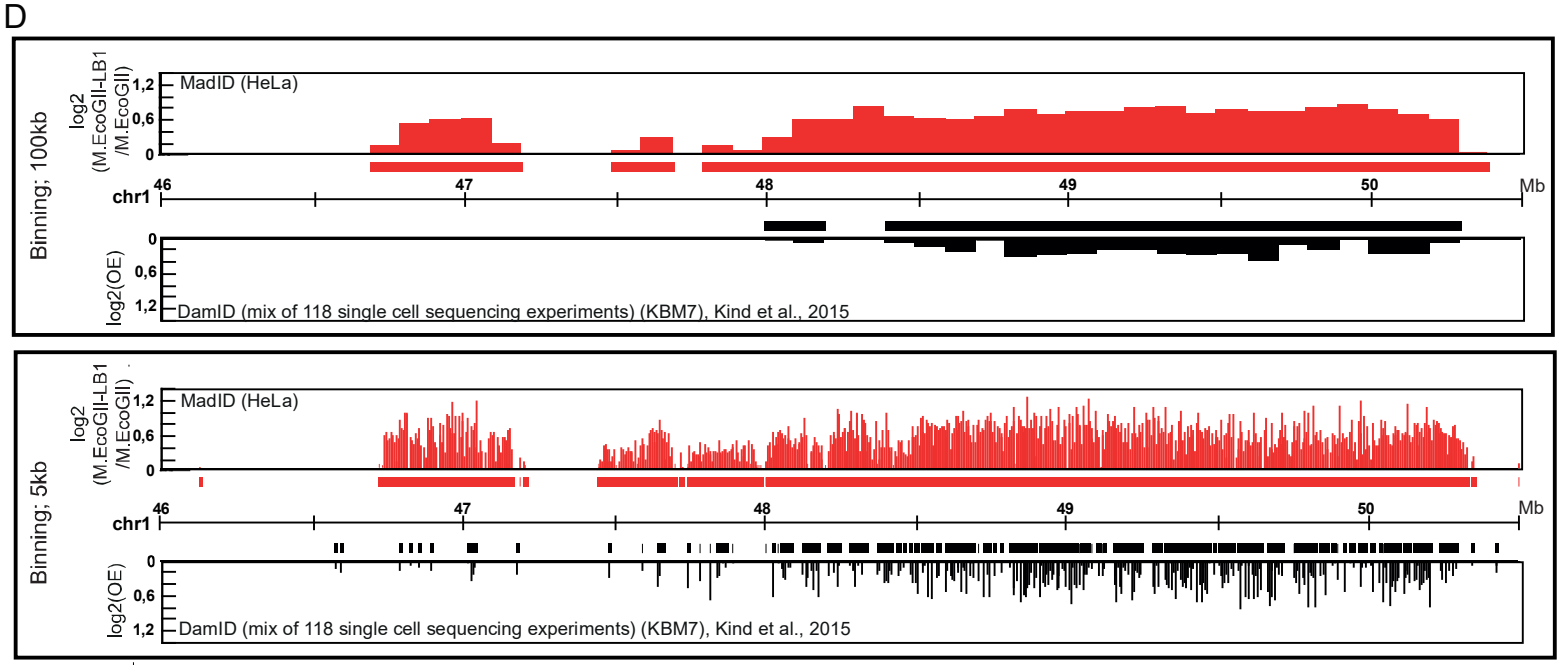


Figure S6 - related to Figure 7

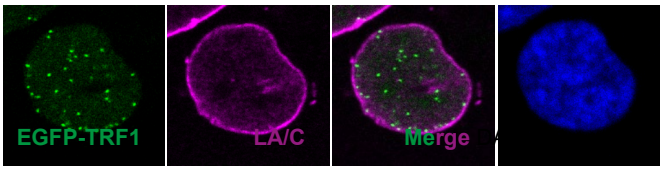


Figure S6 – related to Figure 7
Example of immunostaining of HeLa1.2.11 cells synchronized in early G1 expressing EGFP-TRF1. EGFP (green), Lamin A/C (purple), DNA (blue) and merge is shown.