

**Mapping Nucleolus-associated Chromatin Interactions Using
Nucleolus Hi-C Reveals Pattern of Heterochromatin Interactions**

SUPPLEMENTARY INFORMATION

Supplementary Figures and Legends

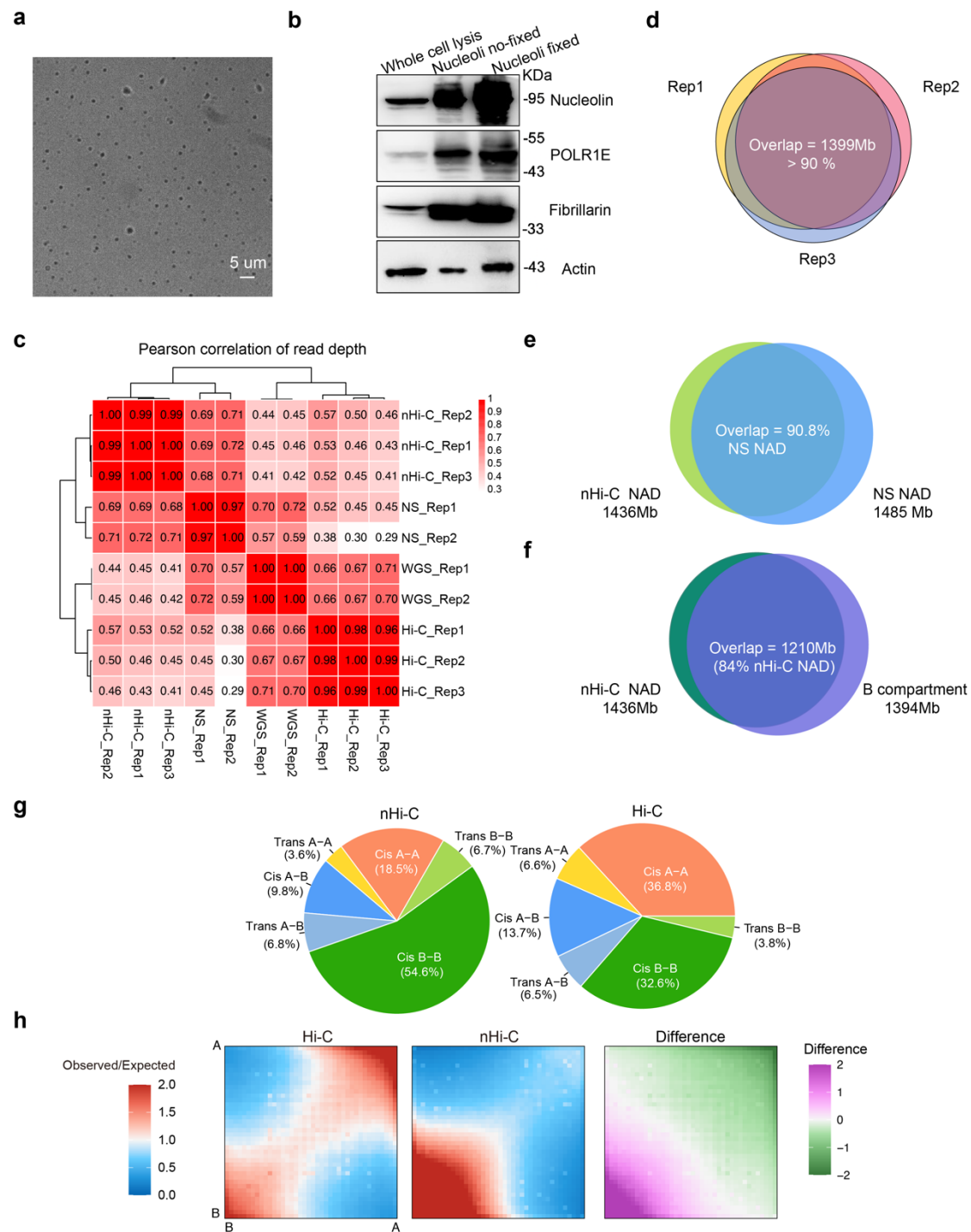


Figure S1. Quality assessment of nHi-C experiments and data in HeLa cells.

a Isolated nucleoli under microscopy (n = 3 biologically independent samples).
b Western blotting of nucleolar proteins from whole cells and isolated nucleoli (n = 3 per group). Uncropped gels are provided in the Source Data file. **c**

Correlation heatmap between sequencing data. NS: nucleolus sequencing. ‘_Rep’ stands for different biological replicates. **d** Overlap of nHi-C NADs in different replicates. **e** Overlap between nHi-C NADs and NS-seq NADs. **f** Overlap between nHi-C NADs and B compartments. **g** Percentage of *cis/trans* A-A, A-B, and B-B compartment interactions captured by nHi-C and *in situ* Hi-C in HeLa cells. **h** Saddle plot of interactions captured by nHi-C and *in situ* Hi-C.

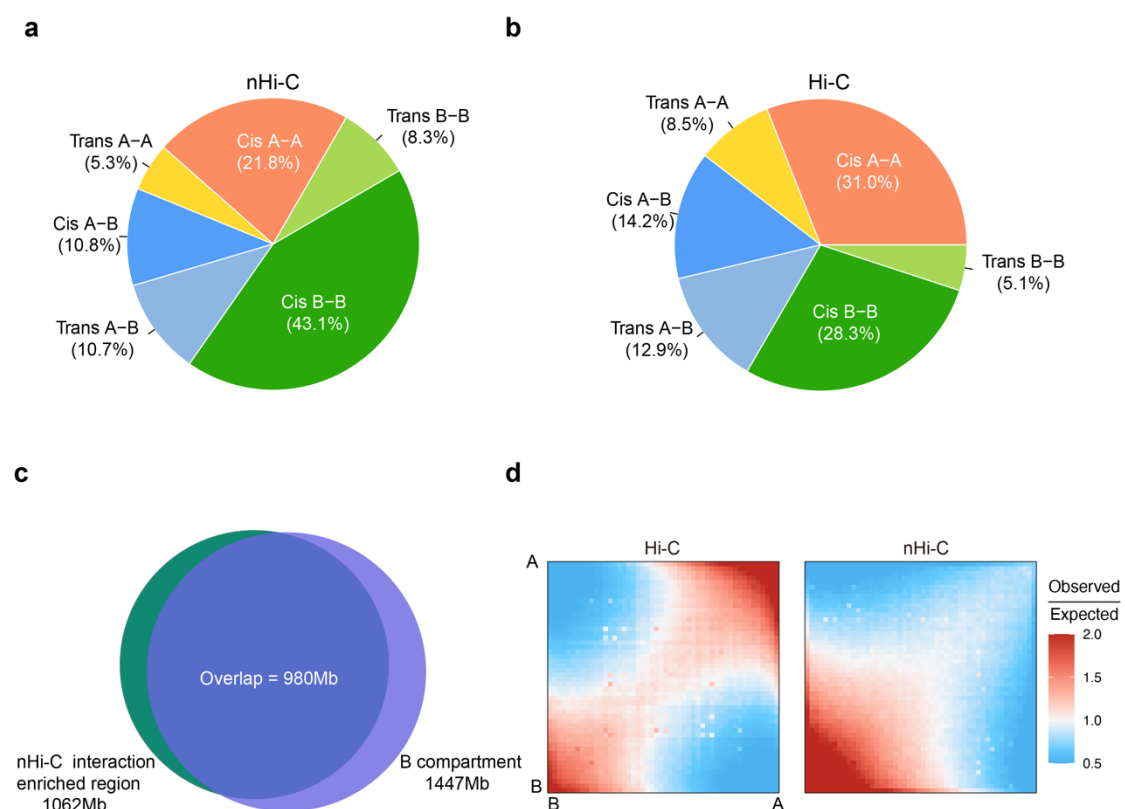


Figure S2. Quality assessment of nHi-C experiments and data in U2OS cells.

a-b Percentage of *cis/trans* A-A, A-B, and B-B compartment interactions captured by nHi-C and *in situ* Hi-C in U2OS cells. **c** Overlap between nHi-C

interaction-enriched regions and B compartments. **d** Saddle plot of interactions captured by nHi-C and *in situ* Hi-C.

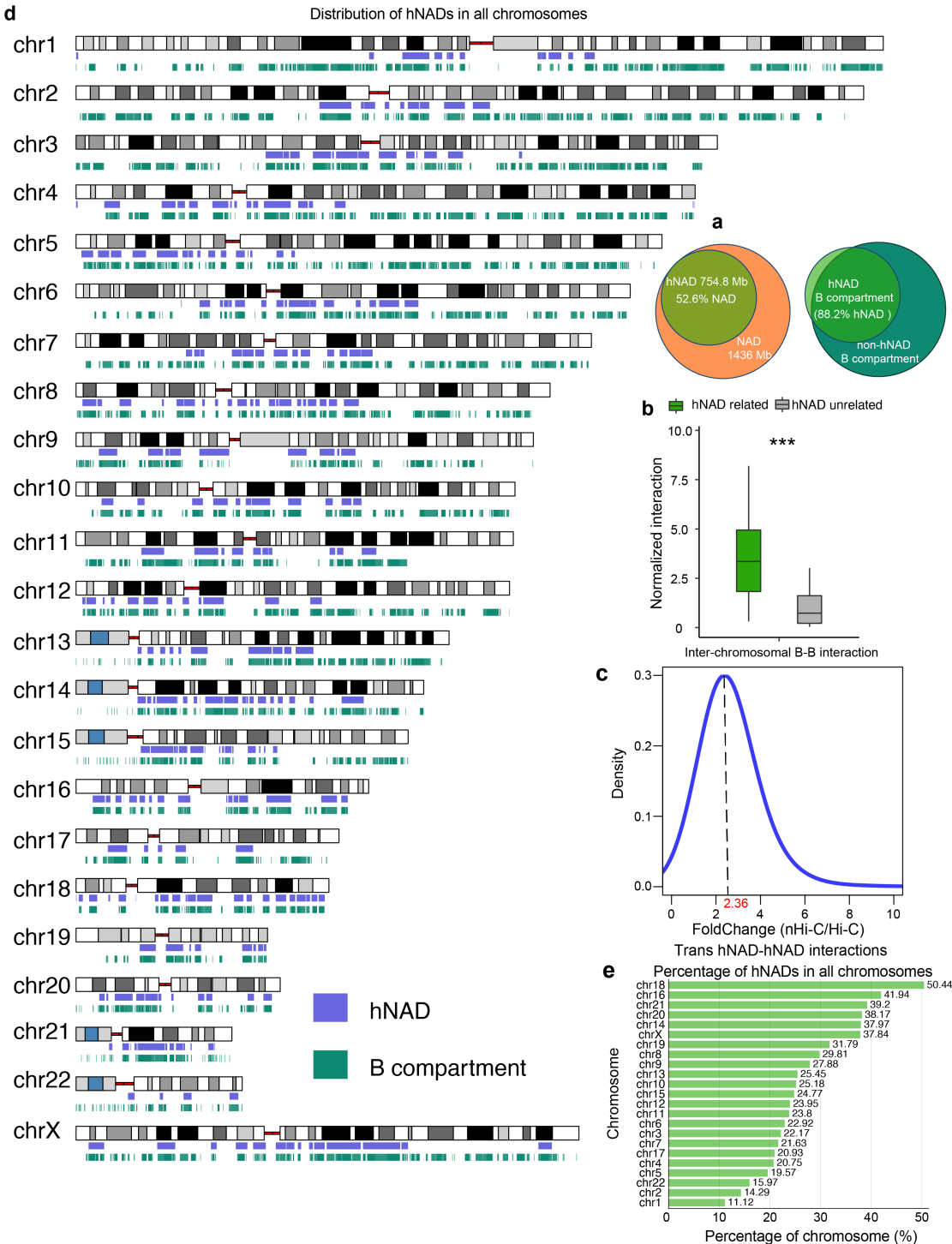


Figure S3. Genomic distribution of hNADs.

a hNADs account for 52.6% of all NADs, and 88.2% of hNADs overlap with B compartments. **b** Comparison of hNAD-related and unrelated inter-

chromosomal B-B interactions. In box-plots, center line stands for median; box limits are 25th and 75th percentiles; whiskers are min to max. $n = 23$ chromosomes. Statistically significant differences are indicated, and were calculated with two-sided Wilcoxon test. $***p < 0.001$. **c** Density distribution of the fold change nHi-C/Hi-C in *trans* hNAD-hNAD interactions. **d-e** Distribution of hNADs in all chromosomes.

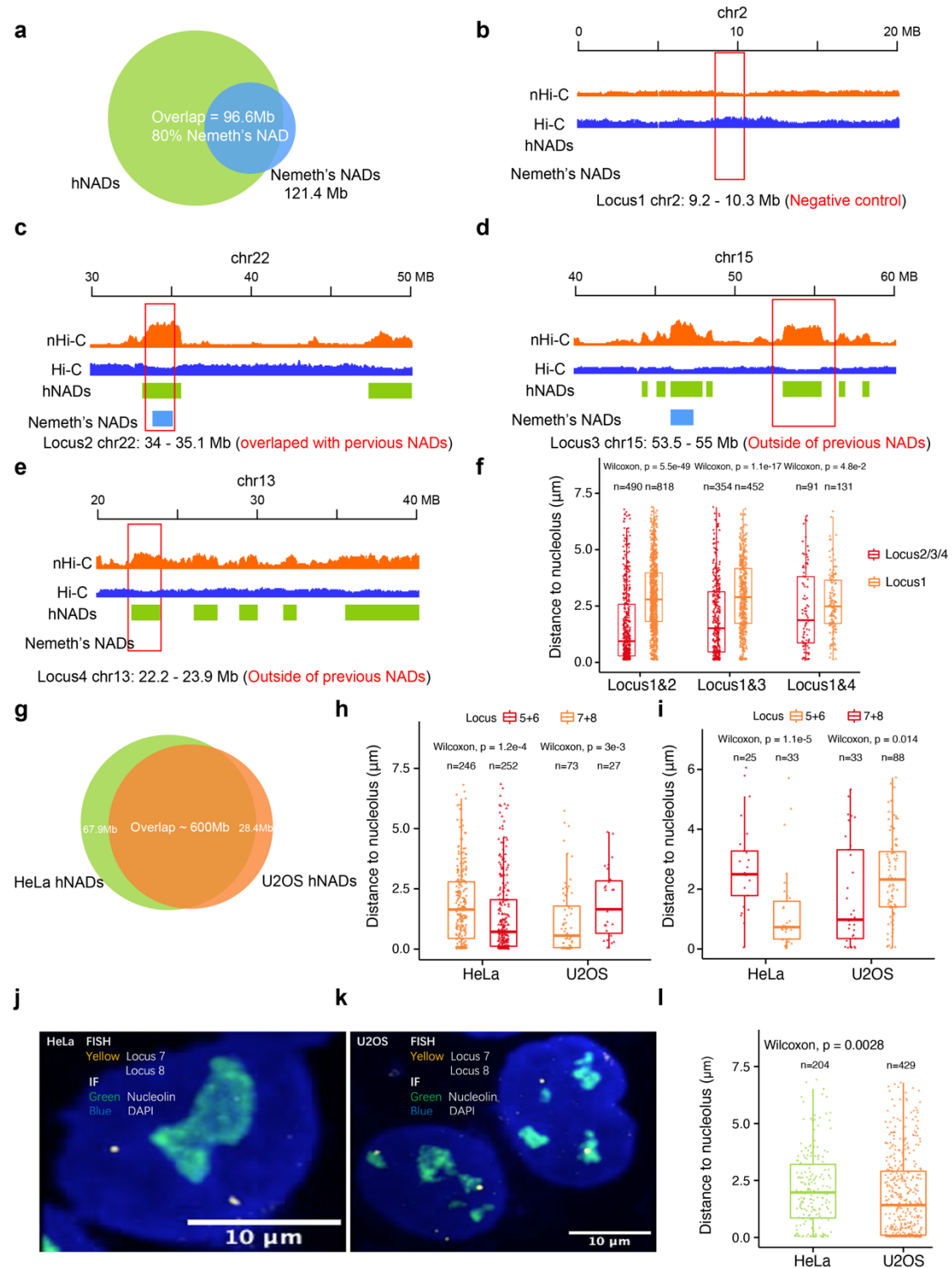


Figure S4. Chosen hNADs for DNA-FISH validation.

a Overlap between hNADs and NADs identified by Nemeth et al. **b-e** Track plot of genome regions chosen to perform DNA-FISH. **f** Distance between hNADs/negative control Locus 1-4 and nucleolus in HeLa cells. **g** Overlap

between hNADs in HeLa cells and U2OS cells. **h-i** Distance between U2OS-specific hNADs Locus 7 and 8/HeLa-specific hNADs Locus 5 and 6 and nucleolus in HeLa and U2OS cells. **j-k** Representative images of U2OS-specific hNADs' Oligopaint FISH probes Locus 7 and 8 in HeLa and U2OS cells (n = 5 biologically independent samples). **l** Distance between U2OS-specific hNADs Locus 7 and 8 and nucleolus in HeLa and U2OS cells. In all box-plots (f, h-i, l), center line stands for median; box limits are 25th and 75th percentiles; whiskers are min to max. The number of 'n' indicates the total number of cells imaged in independent experiments. Statistically significant differences are indicated, and were calculated with two-sided Wilcoxon test.

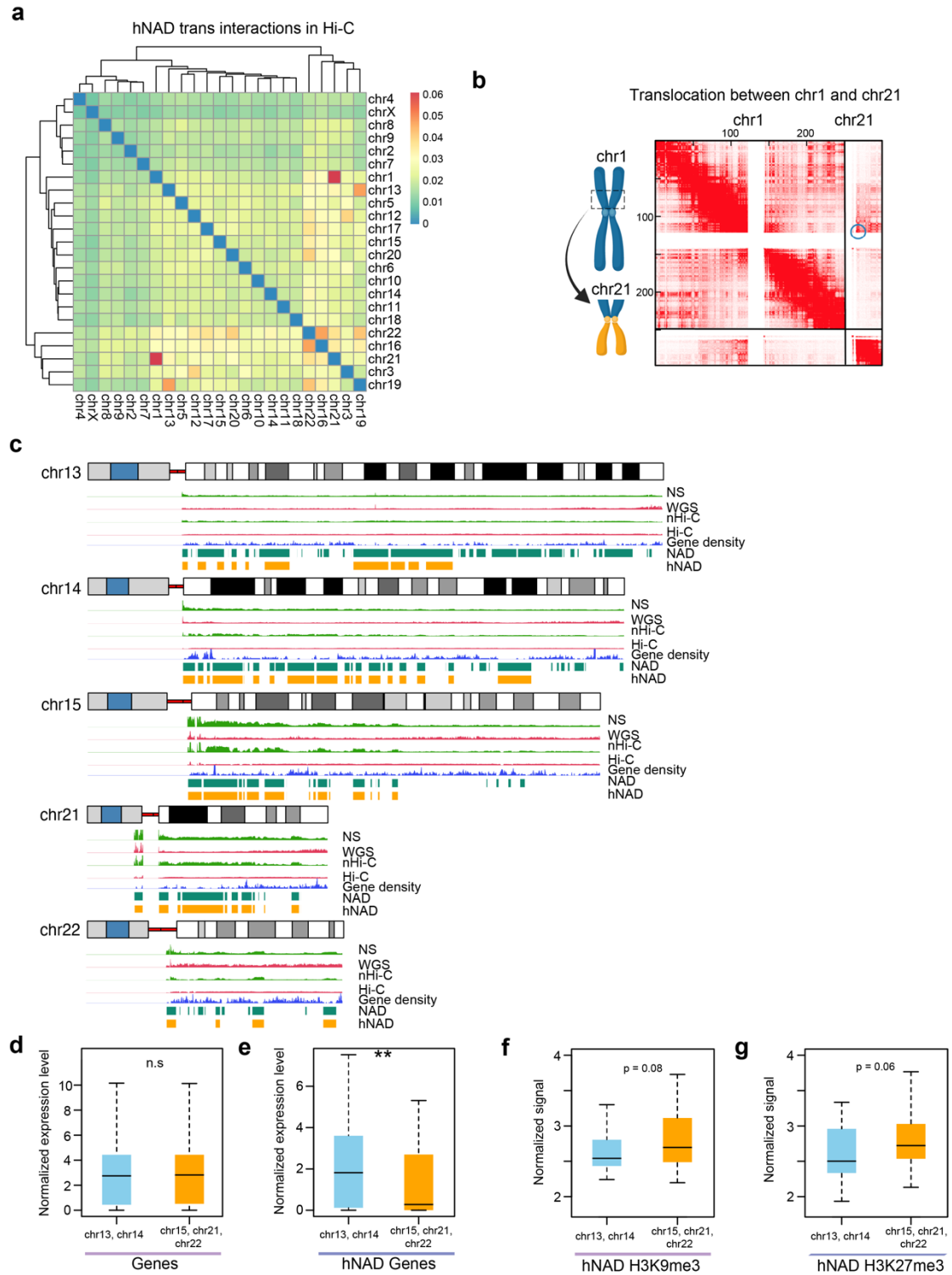


Figure S5. Interactions among the five NOR-bearing chromosomes.

a Chromosome clustering results of hNAD *trans* interactions captured by *in situ* Hi-C. **b** Interaction heatmap of chromosomes 1 and 21. **c** Coverage of nucleolus-seq, WGS, nHi-C, and Hi-C in NOR-bearing chromosomes. **d** Gene

expression levels in NOR-bearing chromosomes. $n = 1263$ genes on chr13/chr14, $n = 1768$ genes on chr15/chr21/chr22. **e** Expression levels of hNAD genes in NOR-bearing chromosomes. $n = 246$ hNAD genes on chr13/chr14, $n = 312$ hNAD genes on chr15/chr21/chr22. **f-g** H3K9me3 (f) and H3K27me3 (g) signals at hNADs located on NOR-bearing chromosomes. $n = 28$ hNADs in chr13/chr14, $n = 31$ hNADs on chr15/chr21/chr22. In all box-plots (d-g), center line stands for median; box limits are 25th and 75th percentiles; whiskers are min to max. Statistically significant differences are indicated, and were calculated with two-sided Wilcoxon test. $**p < 0.01$.

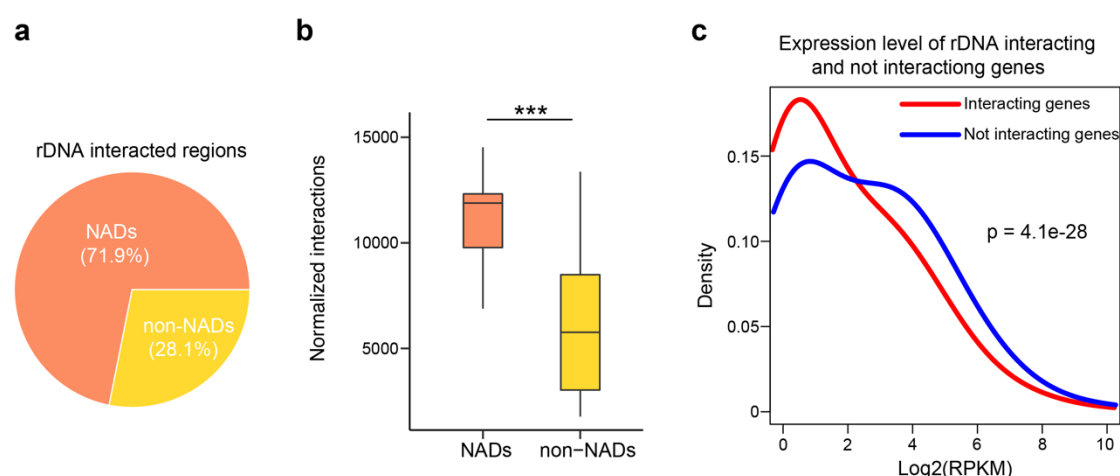


Figure S6. rDNA prefers to form interactions with NADs.

a Percentage of rDNA-interacting regions located in NAD and non-NAD regions.

b rDNA-related interactions are significantly enriched in NAD regions. In box-plots, center line stands for median; box limits are 25th and 75th percentiles; whiskers are min to max. $n = 23$ chromosomes. Statistically significant differences are indicated, and were calculated with two-sided Wilcoxon test.

$***p < 0.001$. **c** rDNA-interacting genes have significantly lower expression

levels than other genes. Statistically significant differences are indicated, and were calculated with two-sided Wilcoxon test.

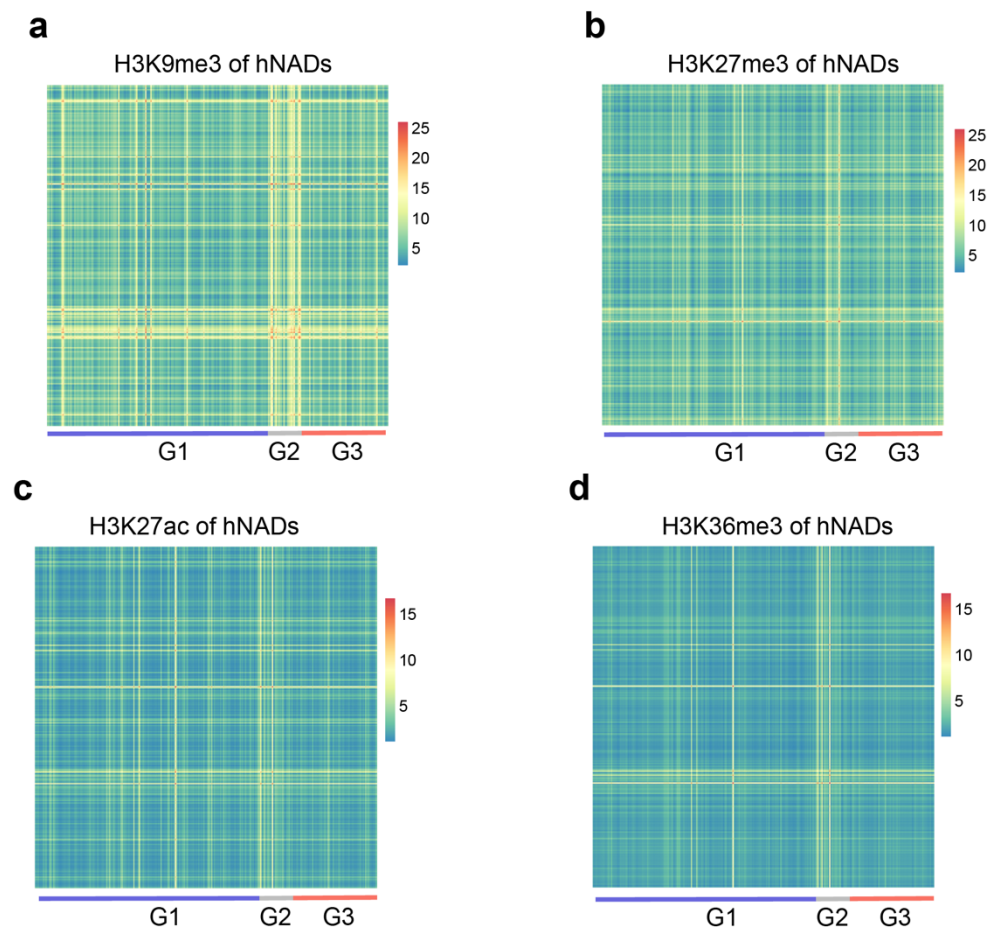


Figure S7. Epigenetic modifications of three hNAD subgroups.

a-d Histone modification levels in the G1, G2, and G3 hNAD regions. a H3K9me3, b H3K27me3, c H3K27ac, d H3K36me3.

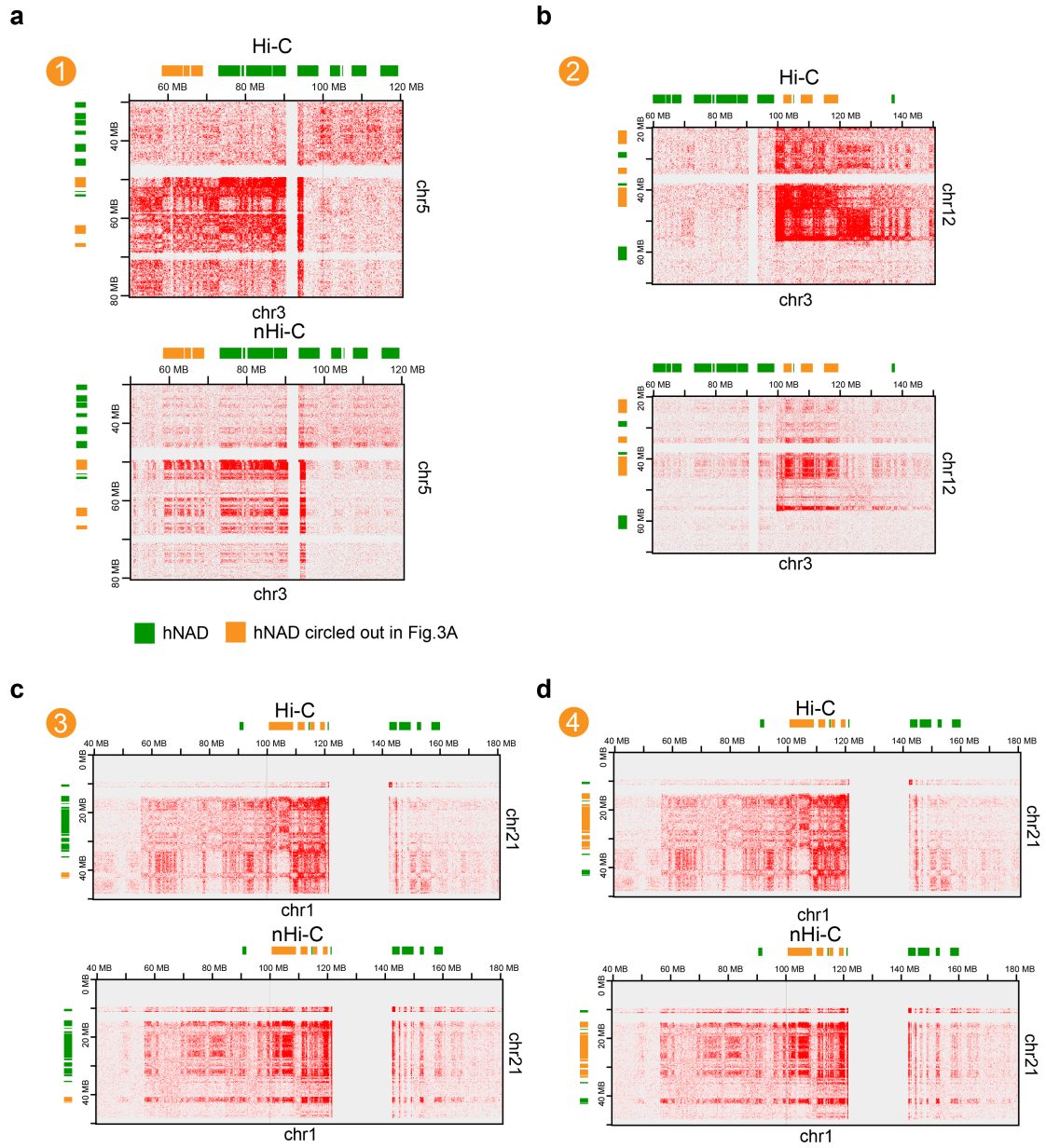


Figure S8. hNAD-related chromosome translocations.

a-d Labeled hotspots in Fig. 3A form butterfly style *trans* interaction heatmaps and are all identified as translocations in both Hi-C and nHi-C.

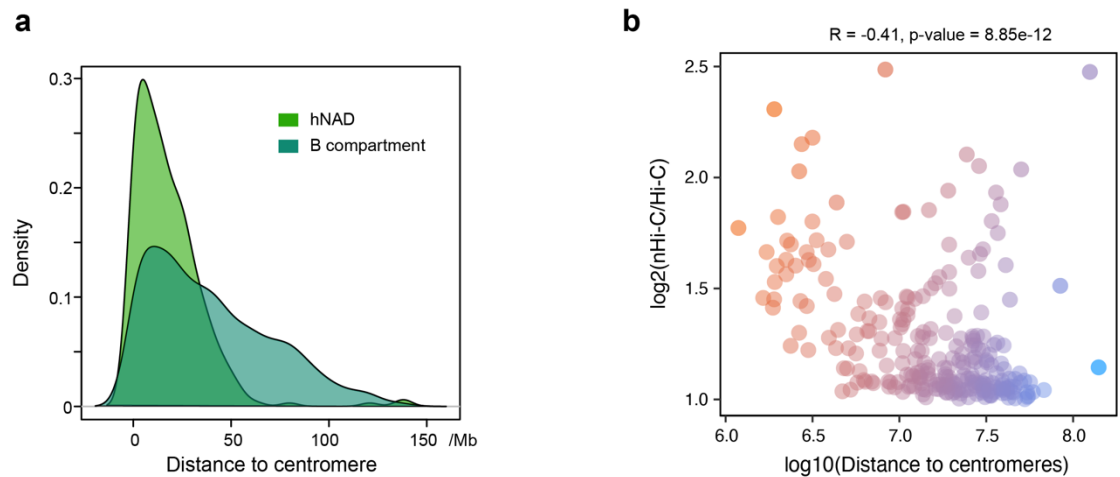


Figure S9. hNADs show positive connections with centromeres.

a The distance of hNAD and B compartment regions from centromeres. **b** Scatter plot of the distance between hNADs and centromeres x axis and the hNAD signal ratio y axis. Statistically significant differences are indicated, and were calculated with two-sided Pearson correlation test.

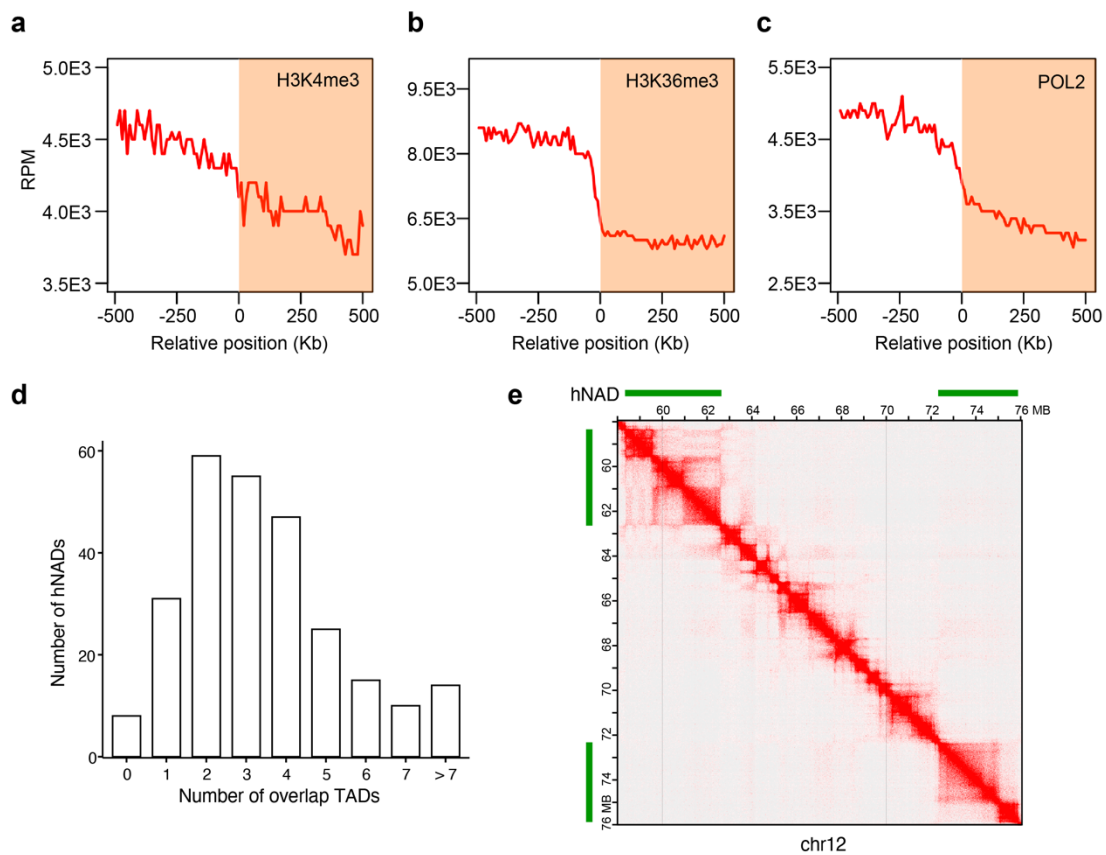


Figure S10. Active histone modifications and RNA polymerase II binding signal at hNAD boundaries.

a H3K4me3, **b** H3K36me3, **c** RNA polymerase II. Orange region indicates hNAD regions. **d** Distribution of hNADs overlapping with TADs. **e** An example of overlap between hNADs and TADs.

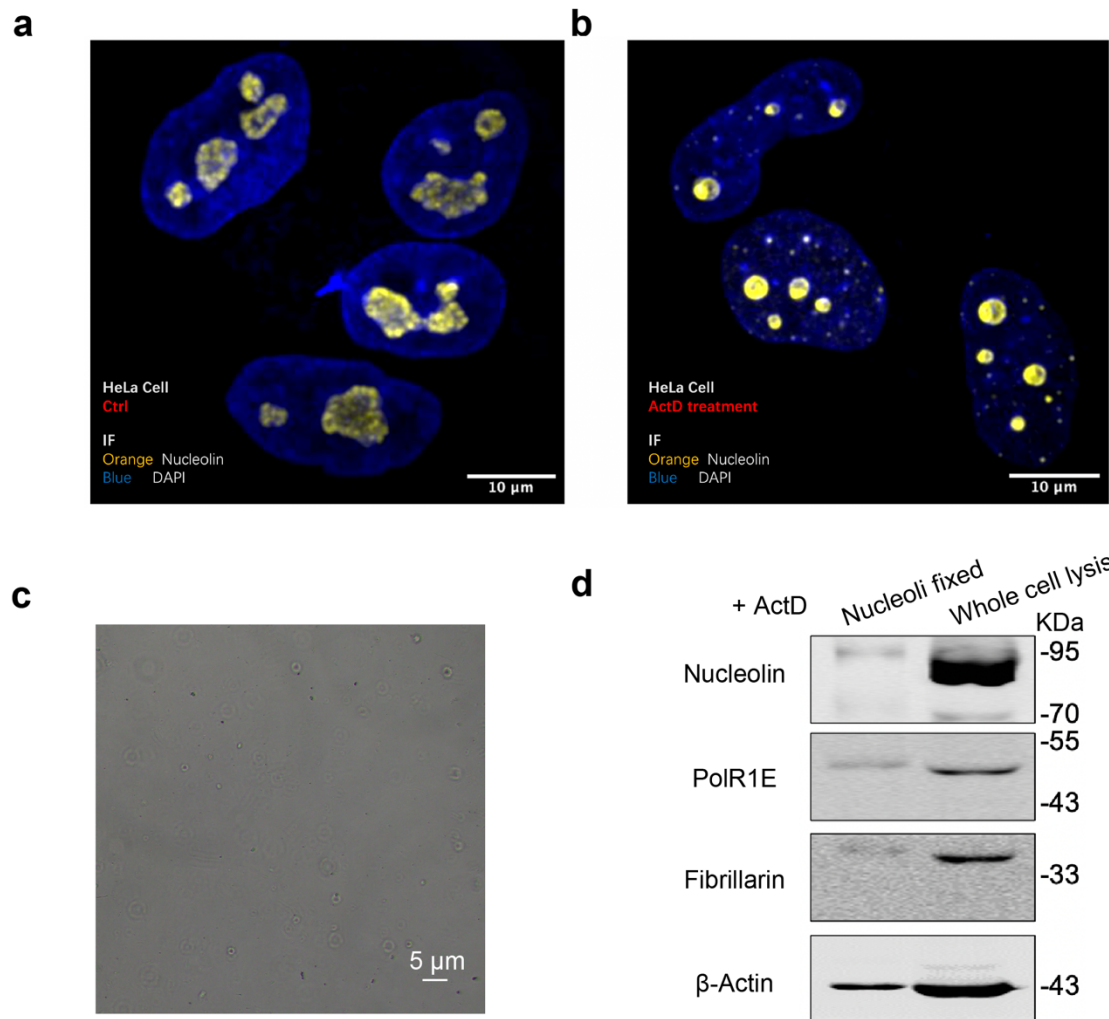


Figure S11. Nucleolus isolation after ActD treatment.

a-b Immunostaining results of nucleolin in ActD non-treat (a) and ActD treated HeLa cells (b) (n = 3 biologically independent samples). **c** Isolated nucleoli after ActD treatment under microscopy (n = 3 biologically independent samples). **d** Western blotting of nucleolar proteins from isolated nucleoli after ActD

treatment (n = 2 per group). Uncropped gels are provided in the Source Data file.

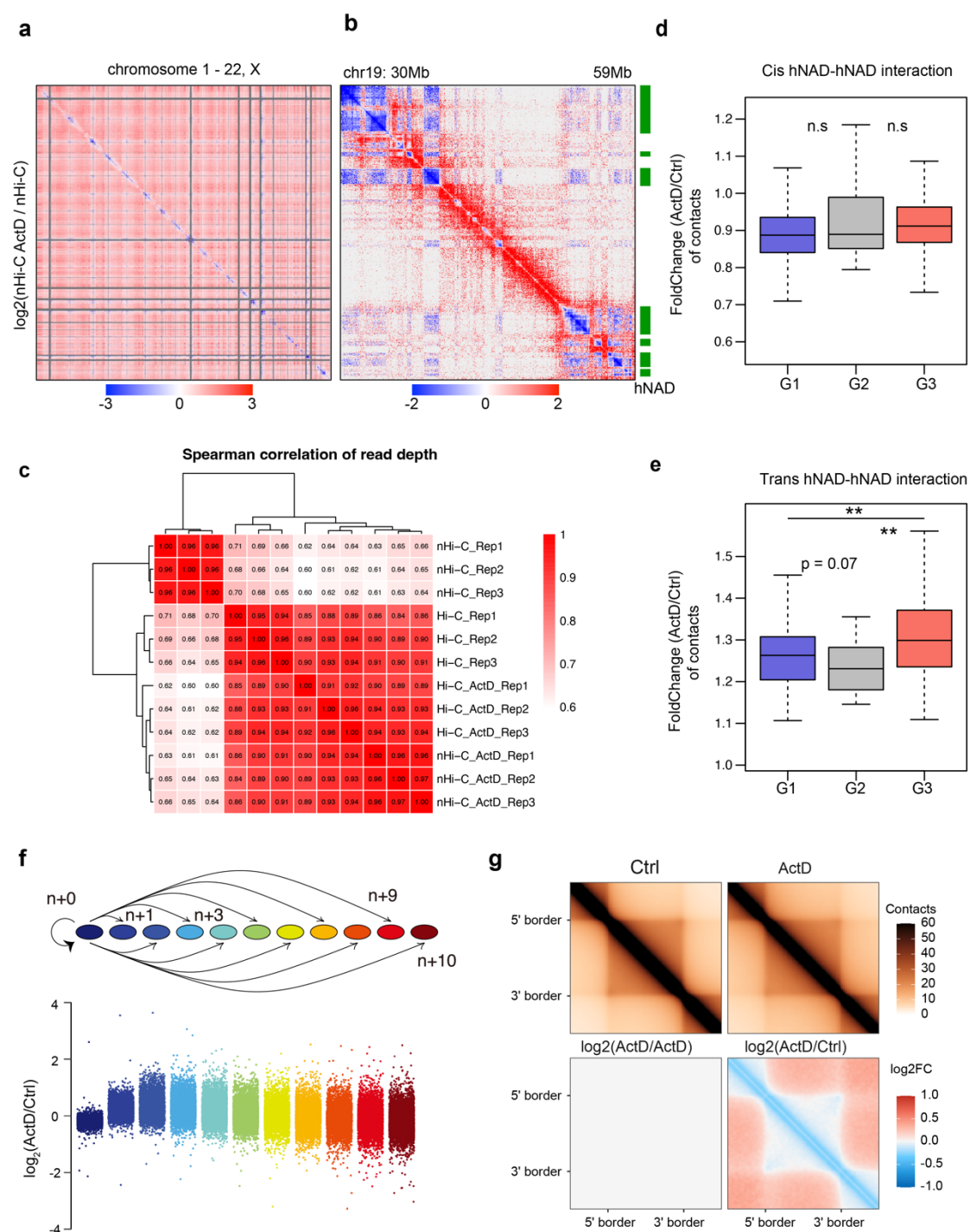


Figure S12. ActD treatment induces genome reorganization in HeLa cells.

a Comparison $\log_2(\text{nHi-C}/\text{Hi-C})$ heatmap of all chromosomes. **b** An example of interaction changes at hNADs. **c** Correlation of read depth in Hi-C and nHi-C

data. ‘_Rep’ stands for different biological replicates. **d-e** *Cis* (d) and *trans* (e) hNAD-hNAD interaction changes at G1, G2, and G3 hNADs. In box-plots, center line stands for median; box limits are 25th and 75th percentiles; whiskers are min to max. n = 177 (G1), n = 22 (G2), n = 65 (G3). Statistically significant differences are indicated, and were calculated with two-sided Wilcoxon test. **p < 0.01. **f** Changes in intra- and inter-TAD interactions after ActD treatment. **g** ATA plot of intra- and inter-TAD interaction changes.