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Supplemental information

Simultaneous mapping of 3D structure and nascent RNAs argues against nuclear compartments that preclude transcription

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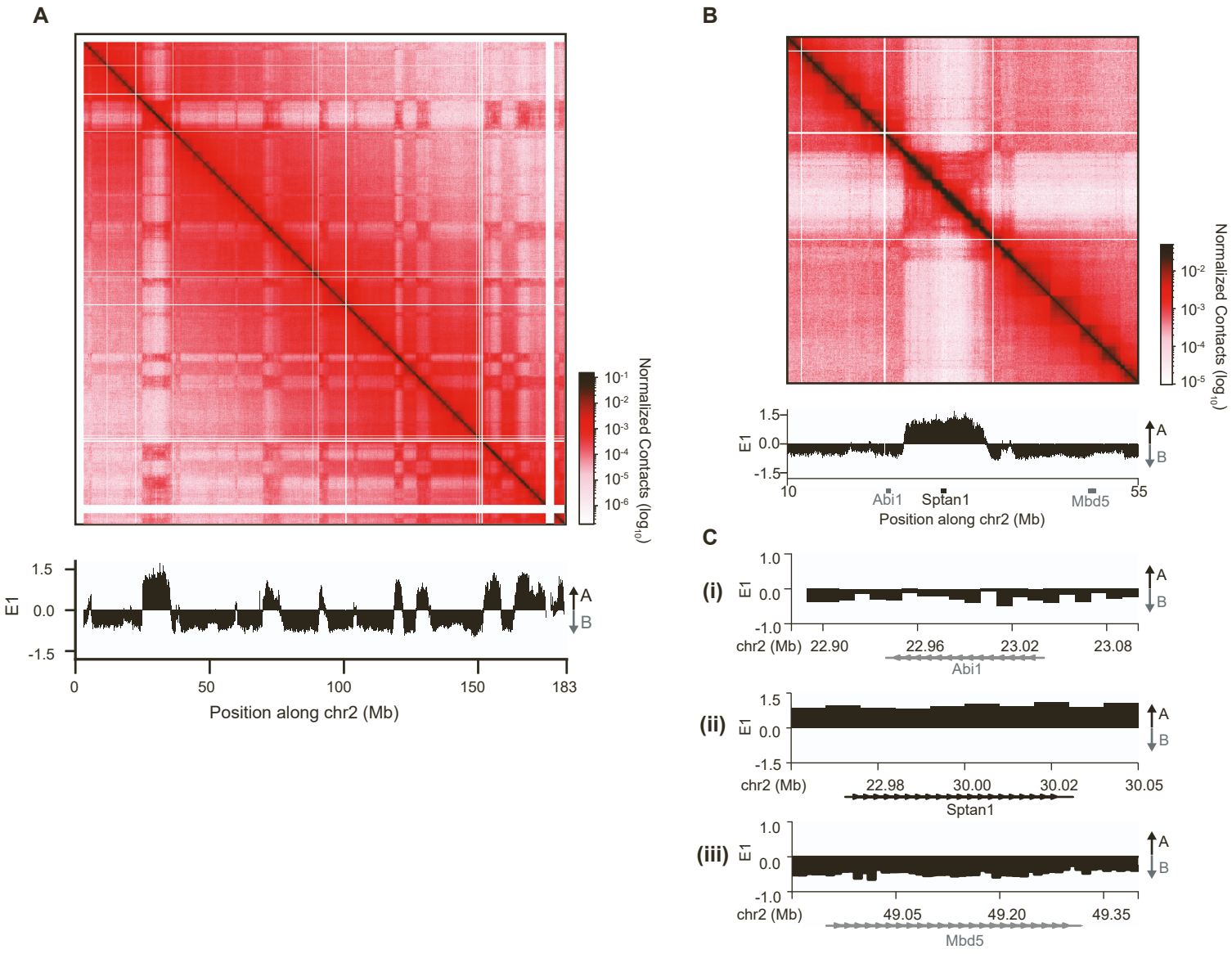
Supplemental Figure 1. Eigenvector 1 (E1) and A/B compartment assignments calculated from RD-SPRITE clusters, corresponding to Figure 2 and Figure 3.



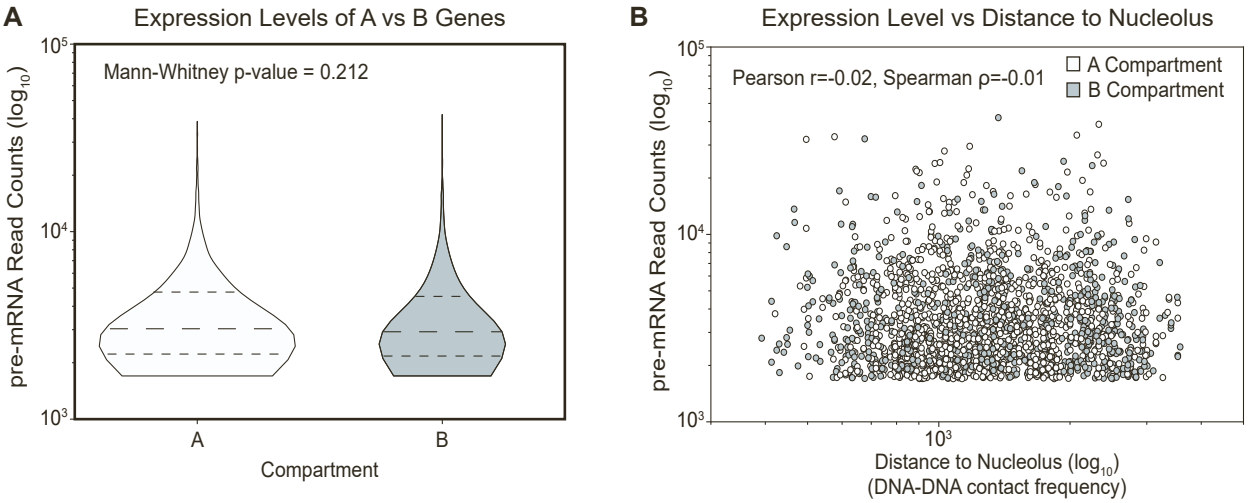
Supplemental Figure 1: Eigenvector 1 (E1) and A/B compartment assignments calculated from RD-SPRITE clusters, corresponding to Figure 2 and Figure 3.

(A) Weighted DNA-DNA contact matrix at 100kb resolution (top) and eigenvector 1 (E1) at 10kb resolution (bottom) along chromosome 2. Both the contact matrix and E1 were calculated from RD-SPRITE clusters containing 2-1000 DNA reads (see **Methods**).

(B) Zoom-in of the 100kb DNA-DNA contact matrix and E1 from (A) near the front of chromosome 2. The positions of one of the A (black) and B (gray) compartment gene triplicates measured by RNA-FISH in Figure 3 (Abi1, Sptan1 and Mbd5) are annotated.

(C) Zoom-in of E1 at 10kb resolution near the gene annotations of the three RNA FISH-measured genes in (B). (i) Abi1 - a B compartment gene, (ii) Sptan1 - an A compartment gene, and (iii) Mbd5 – a B compartment gene. The compartments for each gene were assigned based on the sign of E1 (A compartment > 0; B compartment < 0).

Supplemental Figure 2. Expression level profiles of top 2000 expressed introns, corresponding to Figure 3 and Figure 4.

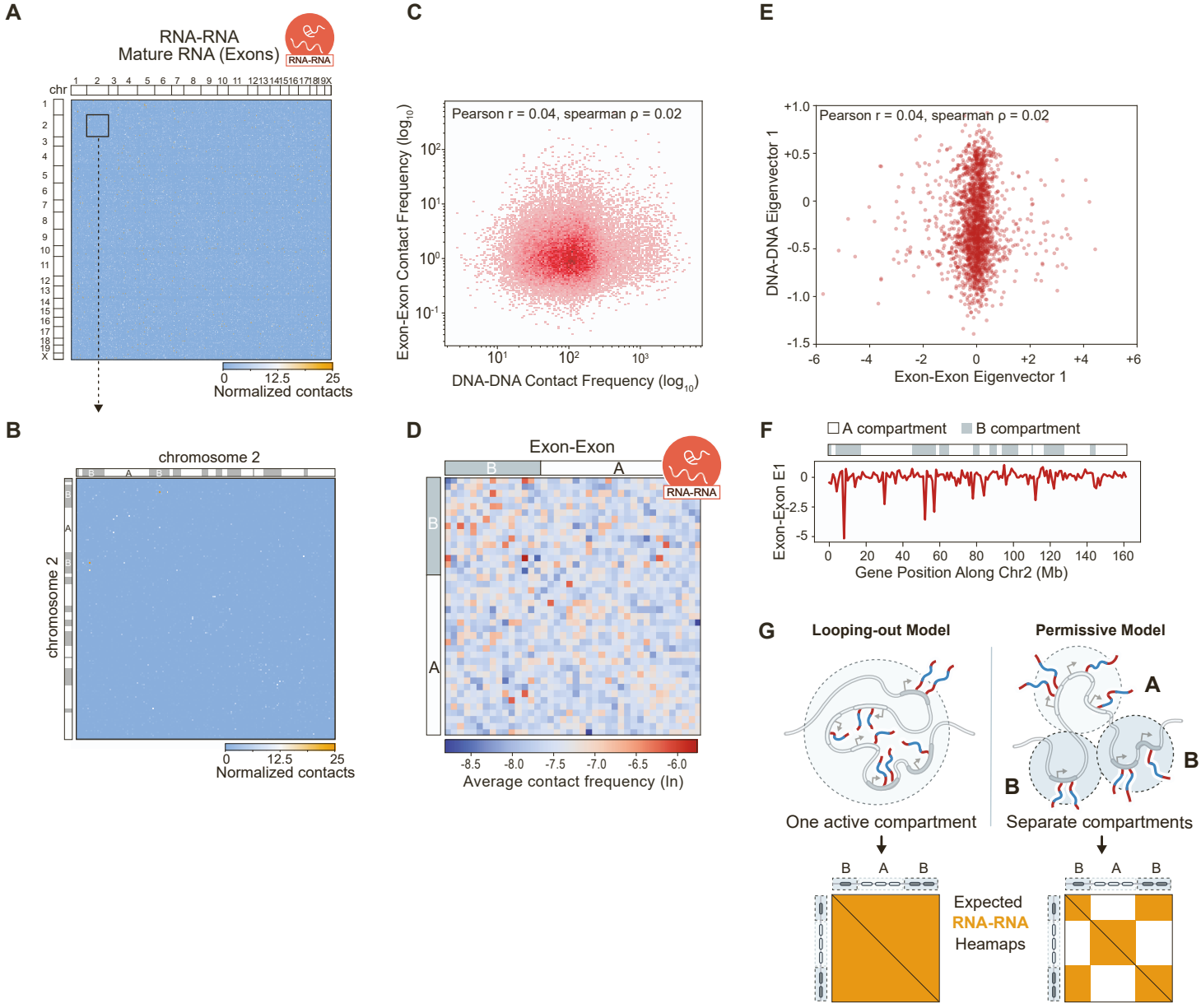


Supplemental Figure 2: Expression level profiles of top 2000 expressed introns, corresponding to Figure 3 and Figure 4.

(A) Violin plot of read counts of selected, top 2000 expressed introns grouped by A/B compartment assignment of the individual genes. Median and quartiles are shown with dotted lines. The bottom of each violin was set to the lowest read count. Mann-Whitney p-value (top) shows no significant difference in the distributions of read counts between A and B compartment genes.

(B) Scatterplot of read counts versus genomic loci distance to nucleolus for top 2000 expressed introns. Distance to nucleolus was calculated based on DNA-DNA contact frequency of the DNA region containing the gene locus and nucleolar hub regions (see **Methods**). Neither Pearson nor spearman statistics show a correlation between counts and distance.

Supplemental Figure 3. RNA-RNA interaction matrix of mature mRNAs (exons), corresponding to Figure 3.



Supplemental Figure 3: RNA-RNA interaction matrix of mature mRNAs (exons), corresponding to Figure 3.

(A) Mature mRNA (exon) gene-level RNA-RNA contact matrix for the exons of genes corresponding to the top 2000 expressed introns, analogous to **Figure 3A**. Genes are sorted based on their genomic position. Chromosomes are annotated along the top and left axes.

(B) Zoom-in of mature mRNA (exon) RNA-RNA contact matrix for chromosome 2.

(C) Correlation of genome-wide, intra-chromosome contact frequencies for gene-level DNA-DNA (x-axis) and mature mRNA (exon) RNA-RNA (y-axis) contact matrices.

(D) Saddle plots generated from mature mRNA (exon) RNA-RNA contact matrix, analogous to **Figure 3F**. Plot shows the average interactions between groups of genes ordered by their compartment signals calculated from a 10 kb-binned DNA-DNA matrix. A/B indicator bar along the axes indicate the compartment assignments of the genes.

(E) Correlation of E1 calculated from gene-level DNA-DNA (y-axis) and mature mRNA (exon) RNA-RNA (x-axis) contact matrices.

(F) E1 calculated from a mature mRNA (exon) RNA-RNA contact matrix along chromosome 2. A/B indicator bar along the top shows compartment assignments based on the sign of E1 generated from a 10 kb-binned DNA-DNA heatmap.

(G) Schematic of the “looping out” model and the corresponding predicted RNA-RNA matrix. If transcription only occurs in the A compartment, nascent transcripts of both A and B compartment genes would interact within a single “active compartment” and there would be no observable compartmentalized structure in an RNA-RNA contact matrix.

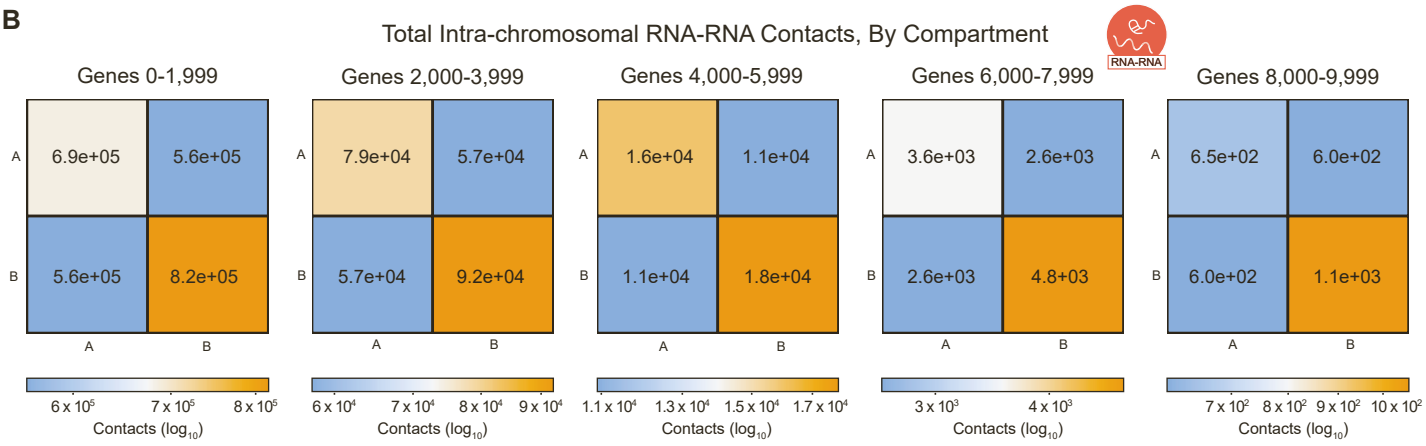
Supplemental Figure 4. RNA-RNA contacts for genes of varied expression levels, corresponding to Figure 3.

A

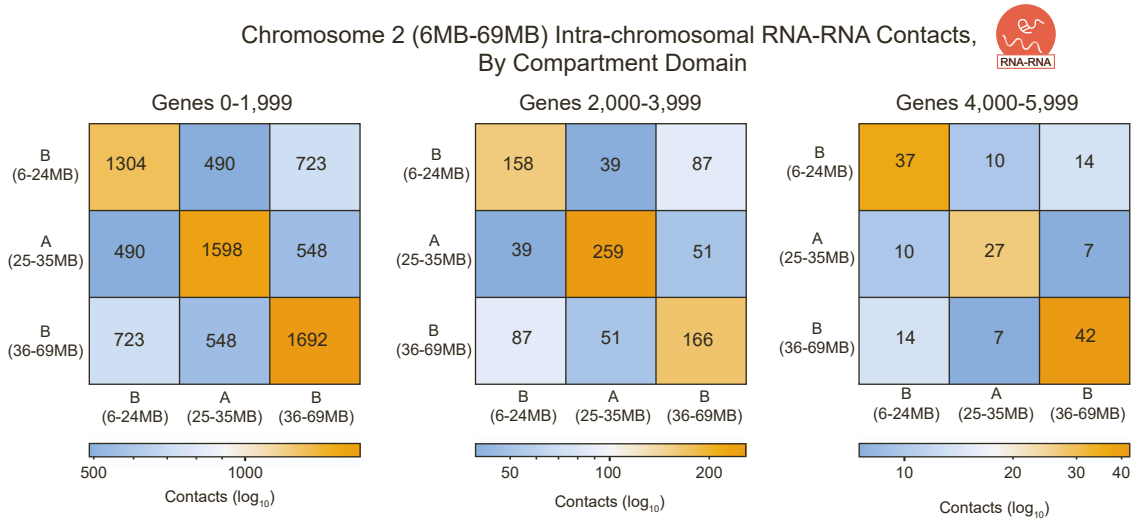
Gene Expression Groups Summary Statistics

Expression Group	Total Expression (Num. Reads)	Maximum Expression (Num. Reads)	Minimum Expression (Num. Reads)	Number of A Compartment Genes	Number of B Compartment Genes
0-1,999	8,421,550	41,983	1731	1214	786
2,000-3,999	2,271,638	1,730	726	1164	836
4,000-5,999	1,001,154	725	338	1220	780
6,000-7,999	468,427	338	151	1274	726
8,000-9,999	200,253	151	61	1264	736

B



C



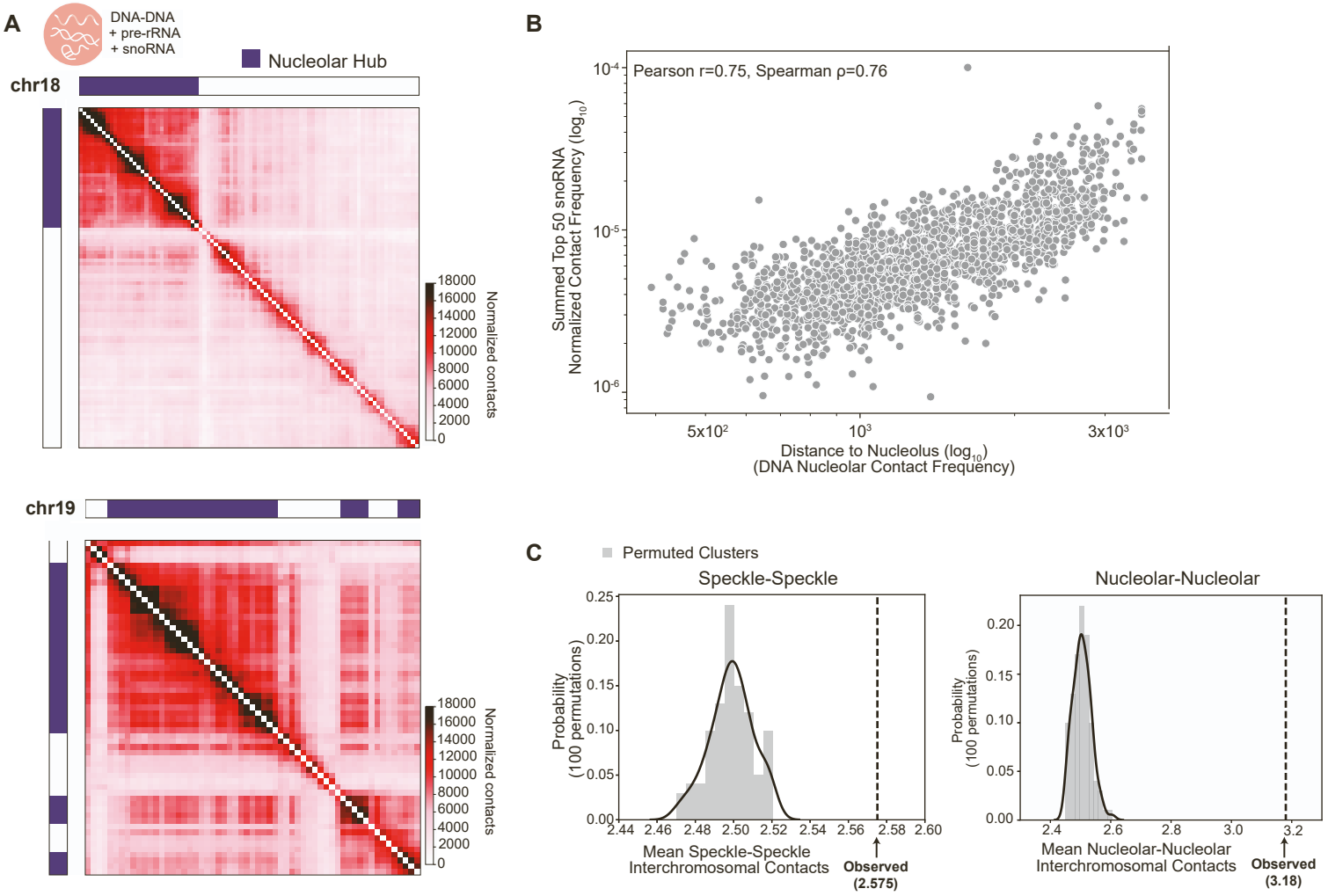
Supplemental Figure 4: RNA-RNA contacts for genes of varied expression levels, corresponding to Figure 3.

(A) Summary statistics for five gene expression groups spanning the top 10,000 expressed pre-mRNA in RD-SPRITE. Each group contains 2,000 genes.

(B) Genome-wide, intrachromosomal RNA-RNA contact matrices for pre-mRNAs within the five gene expression groups, collapsed by A/B compartment assignments of the individual genes.

(C) RNA-RNA contact matrices of pre-mRNAs at the start of chromosome 2 for top three gene expression groups, collapsed by compartment domain assignments of the individual genes.

Supplemental Figure 5. Nucleolar Hub definition and interactions, corresponding to Figure 4.



Supplemental Figure 5: Nucleolar Hub definition and interactions, corresponding to Figure 4.

(A) DNA-DNA contact matrix of snoRNA or pre-rRNA containing clusters for chromosome 18 (top) and chromosome 19 (bottom). Nucleolar hub regions (purple) were defined by clustering of the interchromosomal contacts of the genome-wide DNA-DNA contact matrix generated from clusters containing snoRNA or 45S pre-rRNAs (see **Methods**). Hub regions are annotated with purple bars along the top and left axes.

(B) Scatterplot of nucleolar hub distance versus snoRNA contacts for top 2000 expressed introns. SnoRNA contacts correspond to the total, normalized contact frequency between each pre-mRNA and the top 50 snoRNAs, shown in **Figure 4D**. Pearson and spearman correlation coefficients (top) show positive correlation between distance and contact frequency.

(C) Expected distribution of mean interchromosomal RNA-RNA contacts between speckle hub genes (left) and nucleolar hub genes (right), used for significance testing of **Figure 4H**. RNA reads were randomly permuted among SPRITE clusters 100 times and interchromosomal RNA-RNA contacts were recalculated for each randomization (see **Methods**). Observed interchromosomal contact frequencies from **Figure 4H** are shown as dotted lines and are located at considerably larger values than the expected distributions (permuted).