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Supplemental Information

Condensin II Counteracts Cohesin and RNA Polymerase II in the Establishment of 3D Chromatin Organization

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SUPPLEMENTAL INFORMATION

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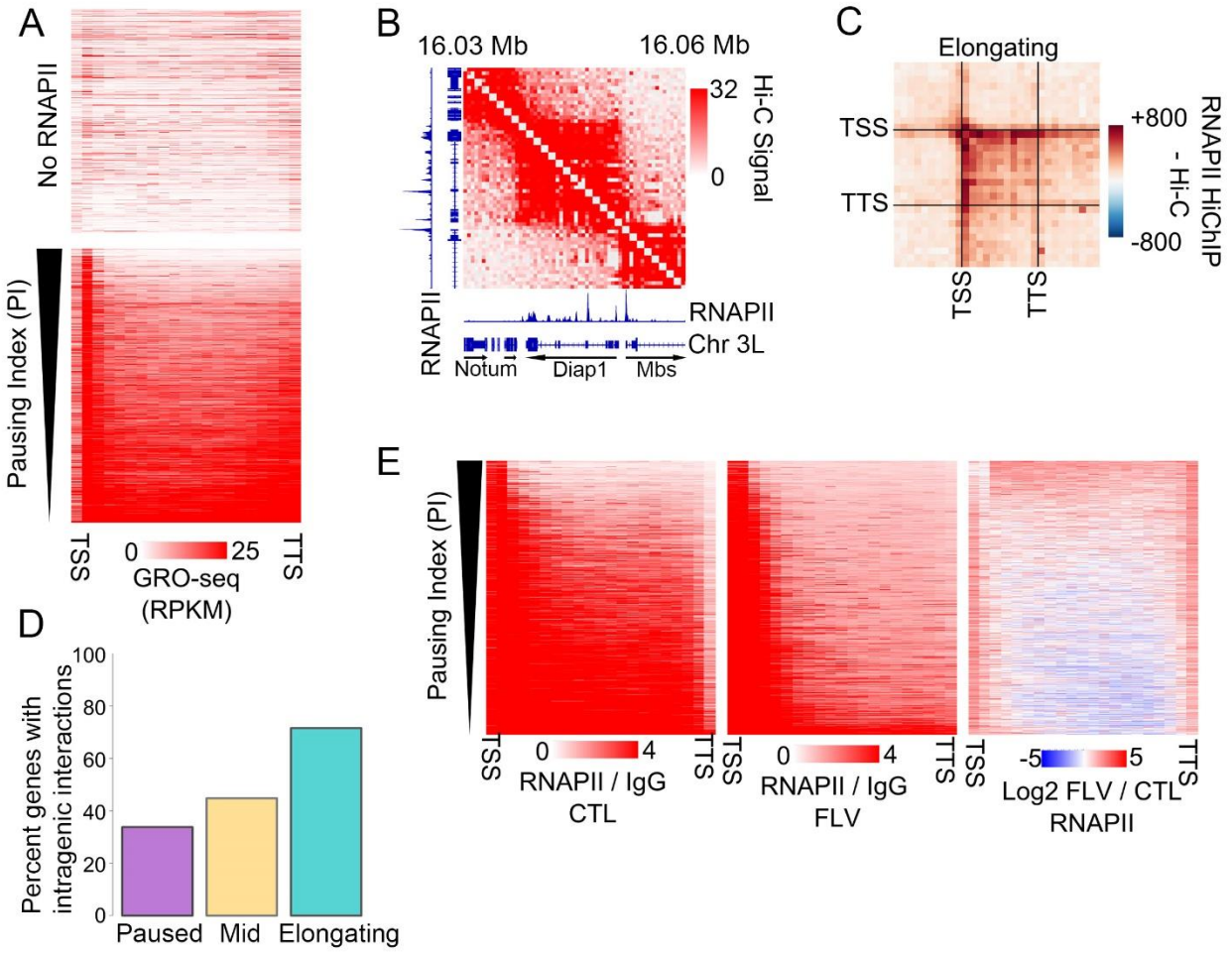

Figure S1 Related to Figure 1

(A) Heatmap of GRO-seq signal across scaled genes ranked by pausing index. Top heatmap represent the category determined to have no RNAPII at TSSs.

(B) Hi-C signal at the elongating gene *Diap1* showing intragenic signal. RNAPII ChIP-seq signal is also shown.

(C) Metaplot showing the difference between RNAPII HiChIP and Hi-C signals in elongating genes. Interactions within scaled genes as well as with one gene length to the left and to the right are shown.

(D) Percentage of genes with significant interaction determined from RNAPII HiChIP where both anchors lie within the same gene.

(E) Heatmap of RNAPII signal in the control (left), after flavopiridol treatment (middle), and the difference (right) across scaled genes ranked by pausing index.

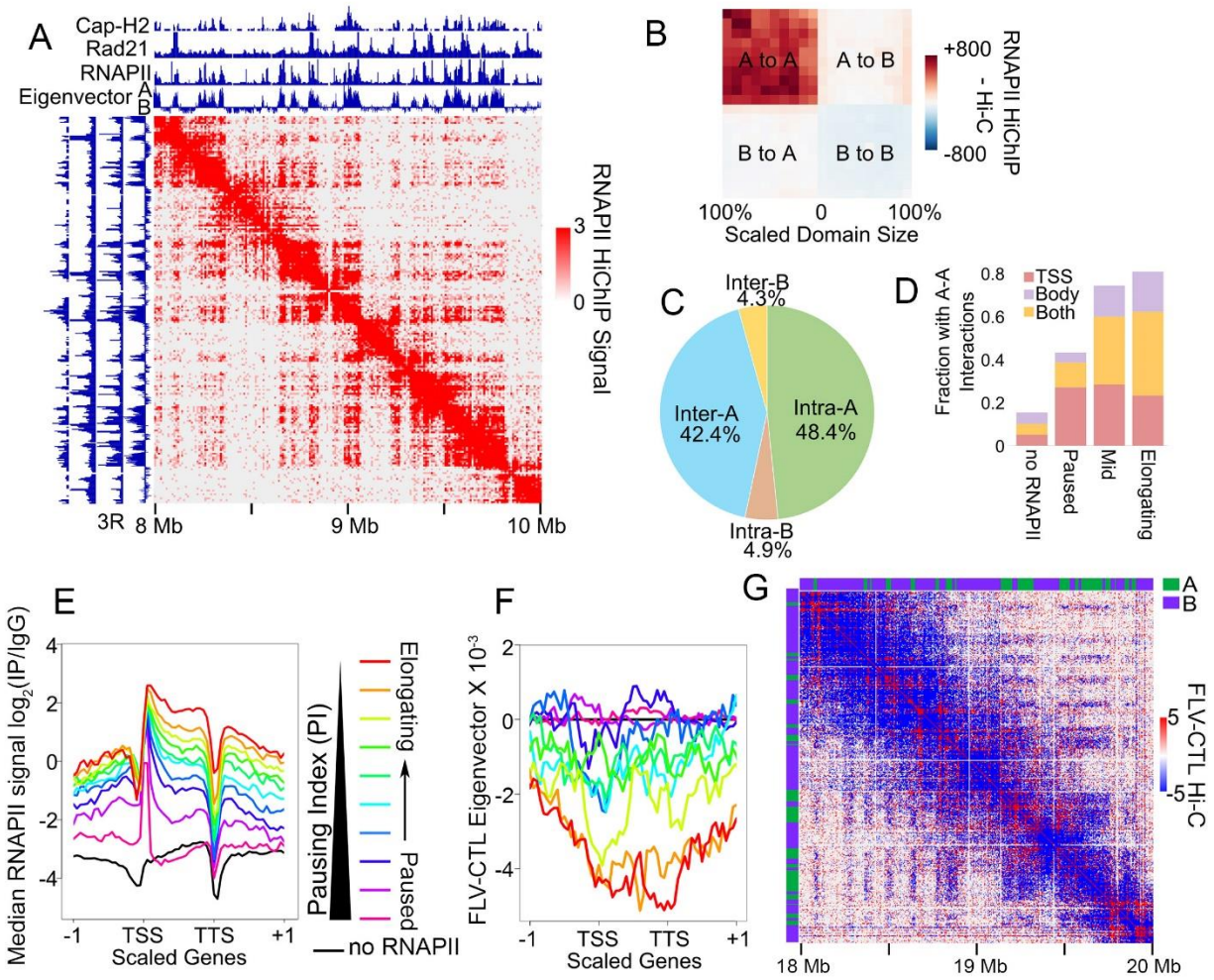

Figure S2 Related to Figure 2

(A) RNAPII HiChIP signal showing that most interaction signal localizes to A compartmental domains designated by positive eigenvector shown above. Cap-H2, Rad21, and RNAPII ChIP-seq data are shown.

(B) Metaplot showing the difference between RNAPII HiChIP and Hi-C signals for compartmental interactions.

(C) Distribution of RNAPII HiChIP significant interactions. Intra-A or intra-B indicates that both anchors lie within a single compartmental domain (domain near the diagonal). Inter-A or inter-B indicates that anchors cross multiple compartmental domains (compartmental signal away from the diagonal).

(D) Fraction of RNAPII HiChIP significant inter-A (A-A) interactions where both anchors occur at TSSs of different genes (peach, TSS), both occur in the bodies of different genes (purple, Body), or one anchor occurs at a TSS while the other occurs in the body of a different gene (yellow, Both).

(E) Profiles of RNAPII ChIP-seq signal across scaled genes and one gene length upstream and downstream. Gene categories as in Figure 2F.

(F) Profiles showing the difference in the compartmental eigenvector from Hi-C after flavopiridol treatment (FLV) compared to the control (CTL) across scaled genes and one gene length upstream and downstream. Gene categories as in Figure 2F.

(G) Differential Hi-C heatmap showing the reduction of signal after flavopiridol (FLV) compared to the control (CTL). The location of A (green) and B (purple) compartmental domains is shown.

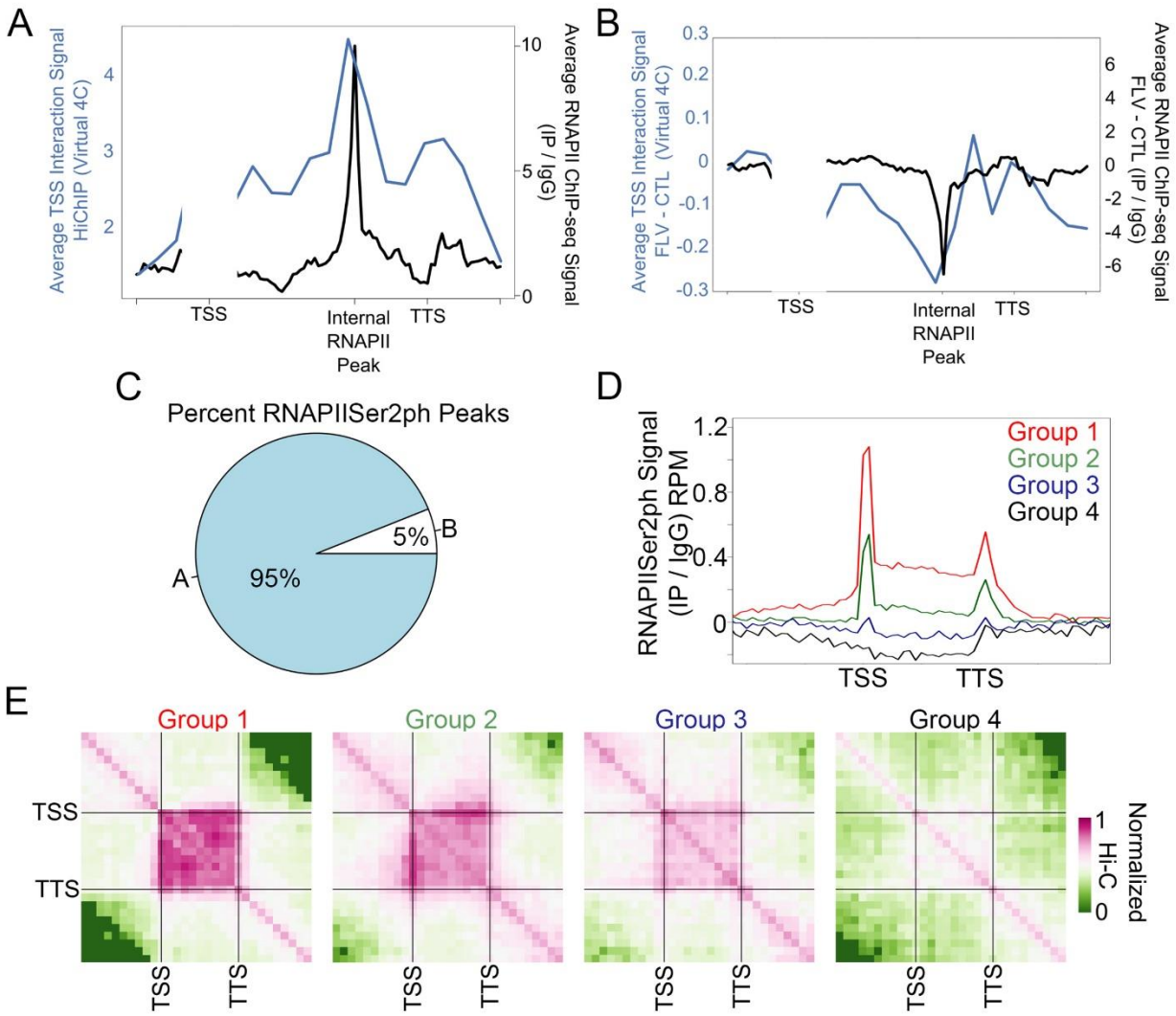

Figure S3 Related to Figure 3

(A) Average distance-normalized RNAPII HiChIP signal between the TSS and gene body (blue), and RNAPII ChIP-seq signal (black), across the gene portion between the TSS, internal RNAPII peak, and the TTS.

(B) Average difference in flavopiridol treated cells compared to control cells in distance-normalized HiC signal between the TSS and gene body (blue), and in RNAPII ChIP-seq signal (black) across the gene portion between the TSS, internal RNAPII peak, and the TTS.

(C) Relative percent of RNAPIISer2ph ChIP-seq peaks in mouse sperm that overlap A and B compartments.

(D) Average RNAPIISer2ph ChIP-seq signal in mouse sperm along genes. Groups 1-4 represent genes categorized from high to low levels of RNAPIISer2ph.

(E) Metaplot of distance-normalized median Hi-C signal in mouse sperm within genes categorized by levels of RNAPIISer2ph. Interactions within scaled genes as well as that same scaled distance upstream and downstream of the gene are shown.

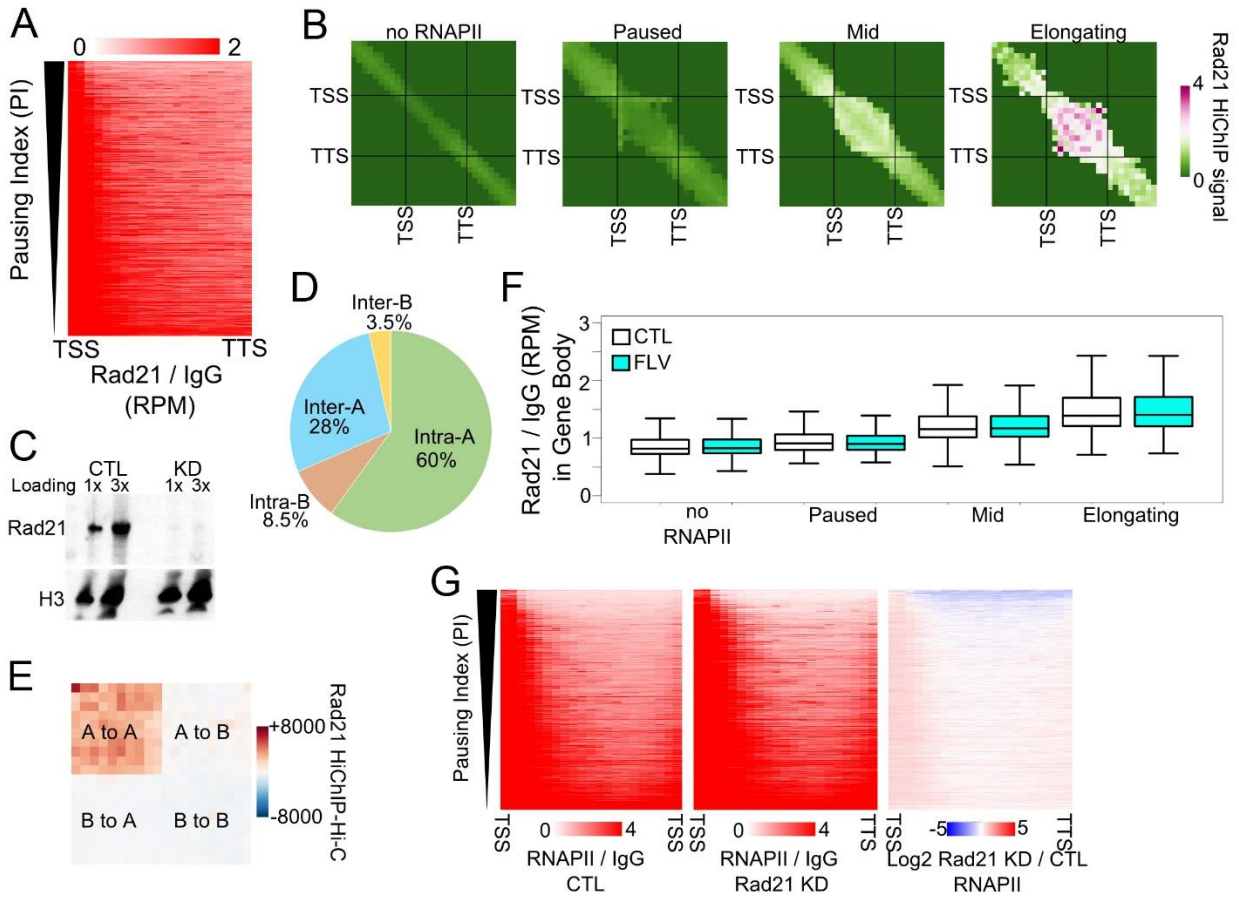

Figure S4 Related to Figure 4

(A) Heatmap of Rad21 ChIP-seq signal across scaled genes ranked by pausing index.

(B) Metaplot of median Rad21 HiChIP signal within genes categorized by the pausing index. Interactions within scaled genes as well as one gene length to the left and to the right are shown.

(C) Western blot of Rad21 in control and after Rad21 knockdown. H3 is shown as a loading control. 1x and 3x indicate relative amount of loaded extract.

(D) Distribution of significant Rad21 HiChIP interactions. Intra-A or intra-B indicates that both anchors lie within a single compartmental domain (domain located near the diagonal of the Hi-C heatmap). Inter-A or inter-B indicates that anchors cross multiple compartmental domains (compartmental signal away from the diagonal of the Hi-C map).

(E) Metaplot showing the difference between Rad21 HiChIP and Hi-C signals for compartmental interactions.

(F) Rad21 ChIP-seq signal in the control (CTL, white) and after flavopiridol treatment (FLV, blue) in the bodies of genes categorized by the pausing index. To exclude TSS signal, only the portion of the gene body between +500 bp and the TTS was considered.

(G) Heatmap of RNAPII signal in the control (left), after Rad21 KD (middle), and the difference (right) across scaled genes ranked by pausing index.

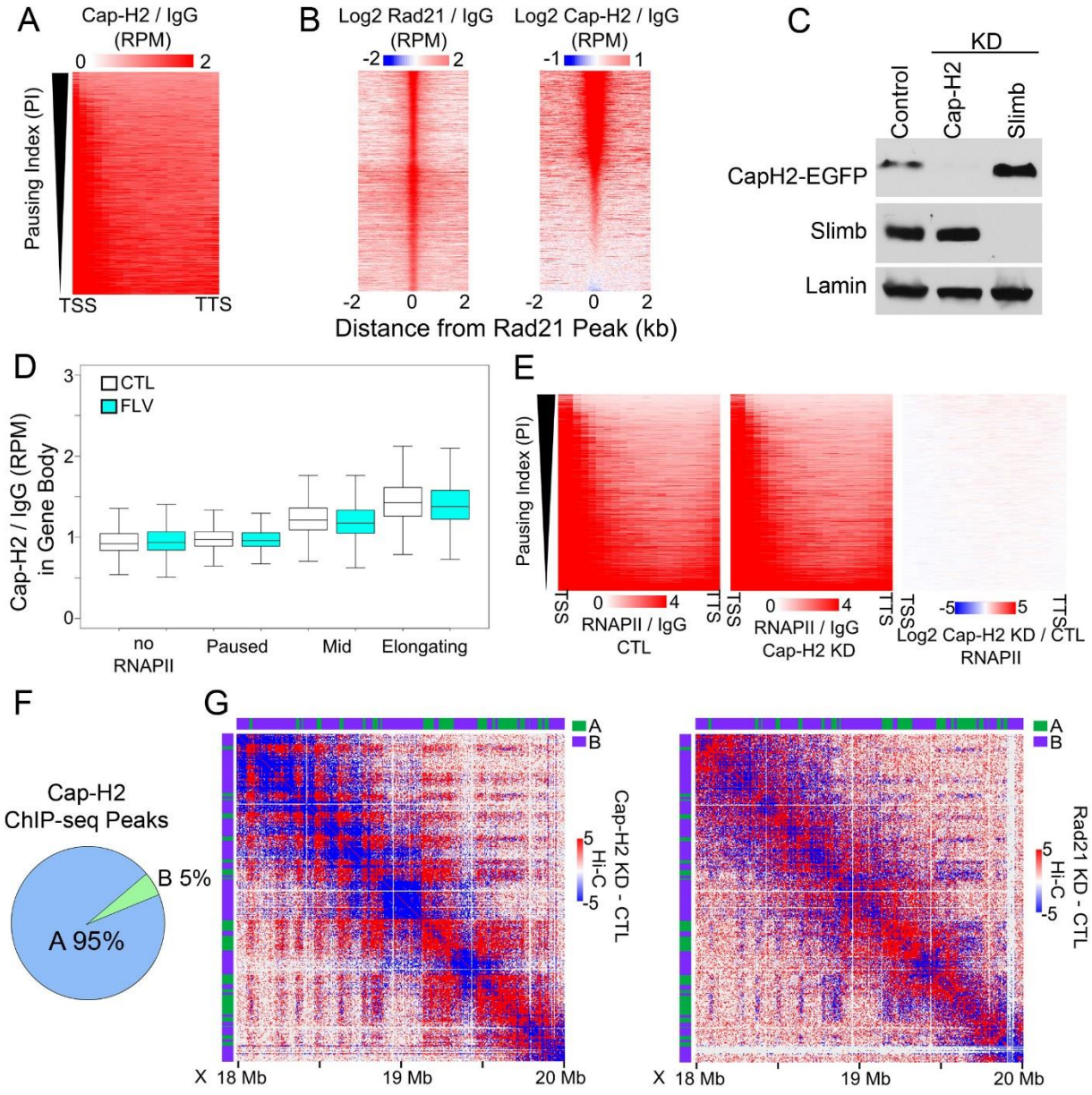

Figure S5 Related to Figure 5

(A) Heatmap of Cap-H2 ChIP-seq signal across scaled genes ranked by pausing index.

(B) Heatmap of Rad21 (left) or Cap-H2 (right) ChIP-seq signal in a 2 kb window on either side of Rad21 peaks.

(C) Western blot of Cap-H2-EGFP and Slimb after knockdown of Cap-H2 or Slimb compared to the control. Lamin is shown as a loading control.

(D) Cap-H2 ChIP-seq signal in the control (CTL, white) and after flavopiridol treatment (FLV, blue) in the bodies of genes categorized by the pausing index. To exclude TSS signal, only the portion of the gene body between +500 bp and the TTS was considered.

(E) Heatmap of RNAPII signal in the control (left), after Cap-H2 KD (middle), and the difference (right) across scaled genes ranked by pausing index.

(F) Distribution of Cap-H2 peaks in A (blue) or B (green) compartmental domains.

(G) Differential Hi-C heatmap showing the reduction of signal after Cap-H2 KD (left) or Rad21 KD (right) compared to the control (CTL). Location of A (green) and B (purple) compartmental domains is shown.

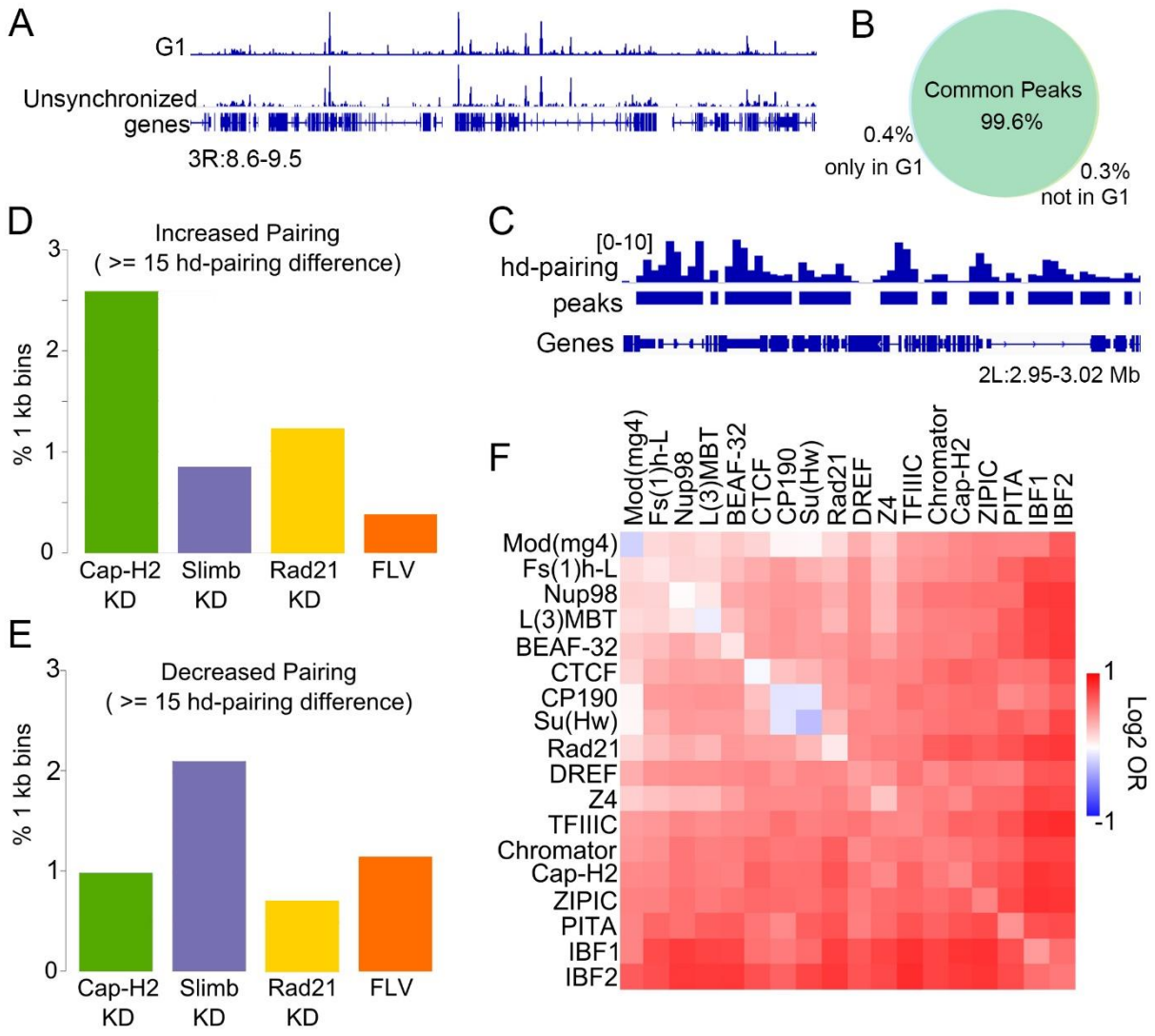

Figure S6 Related to Figure 7

(A) hd-pairing signal track for S2 cells in G1 (top) or unsynchronized (bottom).

(B) Overlap of hd-pairing peaks in G1 and unsynchronized cells.

(C) Example locus showing the hd-pairing profile (top track) and peaks called by MACS (middle).

(D) Percentage of bins with large increases in hd-pairing in cells knocked down for various genes compared to the control.

(E) Percentage of bins with large decreases in hd-pairing in cells knocked down for various genes compared to the control.

(F) Enrichment of insulator proteins on hd-pairing peaks compared to random ATAC-seq peaks determined by the odds ratio (Log2 OR). Sites for architectural proteins alone (diagonal) or in combination with each other are shown.

Table S1 Related to Figure 1. Mapping statistics of HiChIP libraries after alignment to the dm6 *Drosophila* genome

Experiment description	RNAPII HiChIP	RNAPII ChIA-PET (Rowley et al. 2017)
Sequenced Read Pairs	59,568,990	33,708,212
Normal Paired	46,214,591 (77.58%)	21,050,572 (62.45%)
Chimeric Paired	71,249 (0.12%)	3,821,972 (11.34%)
Chimeric Ambiguous	23,723 (0.04%)	7,570,272 (22.46%)
Unmapped	13,259,427 (22.26%)	1,265,396 (3.75%)
Ligation Motif Present	24,664,806 (41.41%)	88,205 (0.26%)
Alignable (Normal+Chimeric Paired)	46,285,840 (77.70%)	24,872,544 (73.79%)
Unique Reads	8,045,481 (13.51%)	14,769,195 (43.81%)
PCR Duplicates	38,239,528 (64.19%)	10,098,920 (29.96%)
Optical Duplicates	831 (0.00%)	4,429 (0.01%)
Library Complexity Estimate	8,071,577	21,598,913
Intra-fragment Reads	541,163 (0.91% / 6.73%)	7,539,437 (22.37% / 51.05%)
Below MAPQ Threshold	2,094,713 (3.52% / 26.04%)	1,431,119 (4.25% / 9.69%)
Hi-C Contacts	5,409,605 (9.08% / 67.24%)	5,798,639 (17.20% / 39.26%)
Ligation Motif Present	2,230,087 (3.74% / 27.72%)	35,778 (0.11% / 0.24%)
3' Bias (Long Range)	76% - 24%	51% - 49%
Pair Type %(L-I-O-R)	25% - 25% - 25% - 25%	25% - 25% - 25% - 25%
Inter-chromosomal	376,897 (0.63% / 4.68%)	281,654 (0.84% / 1.91%)
Intra-chromosomal	5,032,708 (8.45% / 62.55%)	5,516,985 (16.37% / 37.35%)
Short Range (<20Kb)	2,989,418 (5.02% / 37.16%)	4,893,337 (14.52% / 33.13%)
Long Range (>20Kb)	2,043,278 (3.43% / 25.40%)	623,626 (1.85% / 4.22%)
Combined Hi-C Contacts		11,208,244

Table S2 Related to Figure 1 and Figure 2. Mapping statistics of Hi-C libraries after alignment to the dm6 *Drosophila* genome of flavopiridol treated Kc167 cells

Experiment description	FLV Hi-C
Sequenced Read Pairs	303,966,432
Normal Paired	208,276,132 (68.52%)
Chimeric Paired	6,674 (0.00%)
Chimeric Ambiguous	5,810 (0.00%)
Unmapped	95,677,816 (31.48%)
Ligation Motif Present	190,054,447 (62.52%)
Alignable (Normal+Chimeric Paired)	208,282,806 (68.52%)
Unique Reads	150,117,038 (49.39%)
PCR Duplicates	58,161,687 (19.13%)
Optical Duplicates	4,081 (0.00%)
Library Complexity Estimate	299,674,204
Intra-fragment Reads	3,909,669 (1.29% / 2.60%)
Below MAPQ Threshold	47,829,337 (15.74% / 31.86%)
Hi-C Contacts	98,378,032 (32.36% / 65.53%)
Ligation Motif Present	50,827,276 (16.72% / 33.86%)
3' Bias (Long Range)	88% - 12%
Pair Type %(L-I-O-R)	25% - 25% - 25% - 25%
Inter-chromosomal	6,905,351 (2.27% / 4.60%)
Intra-chromosomal	91,472,681 (30.09% / 60.93%)
Short Range (<20Kb)	47,580,993 (15.65% / 31.70%)
Long Range (>20Kb)	43,889,726 (14.44% / 29.24%)
Published Data Hi-C Contacts:	34887399
Combined Data Hi-C contacts:	133265431

Table S3 Related to Figure 4. Mapping statistics of HiChIP libraries after alignment to the dm6 *Drosophila* genome

Experiment description	Rad21 HiChIP
Sequenced Read Pairs	21,551,557
Normal Paired	17,303,732 (80.29%)
Chimeric Paired	156 (0.00%)
Chimeric Ambiguous	310 (0.00%)
Unmapped	4,247,359 (19.71%)
Ligation Motif Present	8,746,408 (40.58%)
Alignable (Normal+Chimeric Paired)	17,303,888 (80.29%)
Unique Reads	5,192,745 (24.09%)
PCR Duplicates	12,110,880 (56.19%)
Optical Duplicates	263 (0.00%)
Library Complexity Estimate	5,414,357
Intra-fragment Reads	352,905 (1.64% / 6.80%)
Below MAPQ Threshold	1,340,006 (6.22% / 25.81%)
Hi-C Contacts	3,499,834 (16.24% / 67.40%)
Ligation Motif Present	1,565,627 (7.26% / 30.15%)
3' Bias (Long Range)	82% - 18%
Pair Type %(L-I-O-R)	25% - 25% - 25% - 25%
Inter-chromosomal	95,257 (0.44% / 1.83%)
Intra-chromosomal	3,404,577 (15.80% / 65.56%)
Short Range (<20Kb)	2,760,432 (12.81% / 53.16%)
Long Range (>20Kb)	644,141 (2.99% / 12.40%)

Table S4 Related to Figure 5. Mapping statistics of Hi-C libraries in Slimb KD Kc cells after alignment to the dm6 *Drosophila* genome

Experiment description	Slimb KD Hi-C Rep1	Slimb KD Hi-C Rep2
Sequenced Read Pairs	198,433,468	222,741,284
Normal Paired	146,300,446 (73.73%)	162,798,128 (73.09%)
Chimeric Paired	11,954 (0.01%)	13,757 (0.01%)
Chimeric Ambiguous	6,919 (0.00%)	7,956 (0.00%)
Unmapped	52,114,149 (26.26%)	59,921,443 (26.90%)
Ligation Motif Present	96,122,134 (48.44%)	119,995,791 (53.87%)
Alignable (Normal+Chimeric Paired)	146,312,400 (73.73%)	162,811,885 (73.09%)
Unique Reads	59,393,336 (29.93%)	129,631,529 (58.20%)
PCR Duplicates	86,892,695 (43.79%)	33,178,874 (14.90%)
Optical Duplicates	26,369 (0.01%)	1,482 (0.00%)
Library Complexity Estimate	66,909,011	343,113,579
Intra-fragment Reads	7,662,865 (3.86% / 12.90%)	11,186,278 (5.02% / 8.63%)
Below MAPQ Threshold	17,000,368 (8.57% / 28.62%)	45,431,846 (20.40% / 35.05%)
Hi-C Contacts	34,730,103 (17.50% / 58.47%)	73,013,405 (32.78% / 56.32%)
Ligation Motif Present	16,951,041 (8.54% / 28.54%)	35,110,804 (15.76% / 27.09%)
3' Bias (Long Range)	87% - 13%	88% - 12%
Pair Type %(L-I-O-R)	25% - 25% - 25% - 25%	25% - 25% - 25% - 25%
Inter-chromosomal	6,513,642 (3.28% / 10.97%)	5,921,397 (2.66% / 4.57%)
Intra-chromosomal	28,216,461 (14.22% / 47.51%)	67,092,008 (30.12% / 51.76%)
Short Range (<20Kb)	15,361,535 (7.74% / 25.86%)	31,658,552 (14.21% / 24.42%)
Long Range (>20Kb)	12,854,350 (6.48% / 21.64%)	35,430,979 (15.91% / 27.33%)
Combined Hi-C Contacts		107743508