Supplemental Table 1. Peripheral localization of the early and late replicating regions analyzed in the initial FISH analysis. The BAC probe names are given, their chromosomal locations, average replication timing value (data from replicationdomain.org) and the percentage of alleles localizing to the nuclear periphery.

Supplemental Table 2. Localization of select early and late replicating regions with three repressive nuclear subcompartments. The association of 4 late and 2 early replicating regions with peripheral heterochromatin (PH), perinucleolar heterochromatin (PNH) and pericentromeric heterochromatin (PCH) was assessed simultaneously by multicolor 3D ImmunoFISH. Values are the percentages of alleles associating with the given subcompartment. The total repression is the cumulative value of the allele's association with any of the three subcompartments (many alleles contact more than one subcompartment at a time, the total localization to repressive subcompartments is thus not a simple addition of the association with the individual subcompartments).

Supplemental Table 3. Localization of select early and late replicating regions with the three repressive nuclear subcompartments on two small non NOR-containing chromosomes. Presentation of data is the same as for Supplemental Table 2.

Supplemental Table 4. Repressive nuclear subcompartments exhibit redundancy. The localization of four different late replicating loci on three different chromosomes vis-à-vis the PNH, PCH and PH subcompartments as well as their association to all three subcompartments collectively (total repression) was assessed prior to and after two hours of actinomycin D treatment. Values are the percentages of alleles associating with the given subcompartment. The 'PNH only' column lists what percentage of the test regions associate with only PNH and not another repressive subcompartment at the same time. Localization analysis of regions exhibiting significant PNH association prior to actinomycin D treatment **(A)** and control regions that do not exhibit significant association with PNH to begin with **(B)**.

Supplemental Table 5. List of all BAC probes and their chromosomal locations used in this study.

Supplemental Figue 1. The size of the replication domain affects nuclear localization. Detail of the replication timing profile of chromosome 21 in GM06990 lymphoblastoid cells (adapted from replicationdomain.org, visualized on http://genome.ucsc.edu; E = early, L= late replicating regions). The blue (=early) and red (=late) bars above the track indicate the positions of BAC FISH probes used in the analysis. Percentages given above reflect the association of these regions with the nuclear periphery. The two probes within the early replication domains shaded green exhibit a higher association with the nuclear periphery than those in the purple shaded domains, most likely due to the small size of the early domains and since they are nestled in between larger late replicating domains.

Supplemental Figue 2. Genome association with the nuclear lamina in human lymphoblastoid cells by DamID. Maps of LaminB1 interactions with all human chromosomes (orange = enrichment, blue = depletion), above their respective replication timing profiles (RT, E = early, L = late). The LADs track in the center shows contiguous segments of prominent lamina association.

Supplemental Figue 3. The LaminB1 association of the genome closely follows the replication timing map with few exceptions on large chromosomes. Details of the DamID profiles and replication timing tracks for segments on chromosomes 3 and 1. Shaded areas illustrate discrepancies between the two tracks; they are often smaller late replicating domains within larger segments of the chromosome that does not exhibit significant LaminB1 association.

Supplemental Figue 4. Genome association with the nuclear lamina in human lymphoblastoid cells compared to human fibroblasts. DamID maps of LaminB1 interactions with chromosome 3 in the lymphoblastoid cells GM06990 (top track) and the fibroblast Tig3 cell line (bottom track, data from Guelen et al.). The LAD tracks in between depict all LADs in the GM06990 cells, followed by those unique to this tissue, LADs present in both cell types, LADs unique to the fibroblast background, and finally all LADs in the Tig3 fibroblasts. The detail of a 45-50MB segment of the chromosome reveals the similarities and differences between the LADs of the two tissues.

Supplemental Figue 5. Multicolor ImmunoFISH to determine the association of genomic loci with three repressive nuclear subcompartments simultaneously. The two rows represent two slices through one GM06990 cell nucleus depicting both alleles of one genomic locus: Detection of the lamina (LaminB1 antibody), nucleoli (nucleolin antibody), pericentromeric heterochromatin (human pancentromeric DNA probe, PCH) and a genomic locus (BAC DNA probe). The last panels show all channels merged – lamina (grey), nucleolin (blue), PCH (red) and BAC DNA (green) channels. The example shown also illustrates how the three repressive subcompartments can be adjacent to each other and even overlap. Moreover, one of the two alleles (bottom row) localizes to both PCH and PNH.

Supplemental Figue 6. Association of early and late replicating loci on two small non NOR-containing chromosomes (Chr19, 20) with the three repressive nuclear subcompartments. The localization of several loci was determined simultaneously vis-à-vis the nuclear periphery (PH = peripheral heterochromatin), pericentromeric heterochromatin (PCH) and perinucleolar heterochromatin (PNH) by multicolor ImmunoFISH. Association of the loci to any of the three repressive subcompartments collectively (black) is significantly higher than association only with the PH subcompartment (blue). BAC probe identities: Chr19 A=RP11-451E20, B=RP11-206D24, C=RP11-69B20, Chr20 A=RP11-273F19, B=RP11-359H4, C=RP11-1008E7, D=RP11-709F1.

Supplemental Figue 7. Actinomycin D treatment of GM06990 cells does not abrogate RNA polymerase II activity. Cells were pulse labeled with EU prior and following actinomycin D treatment. The EU incorporation was visualized by the Click-It reaction (Life Technologies). Left panel shows GM06990 cells prior to any actinomycin D treatment, prominent rRNA synthesis is visible within the nucleoli and mRNA synthesis in the remaining nucleoplasm. Right panel shows GM06990 cells following actinomycin D treatment; while rRNA synthesis has been abrogated by RNA polymerase I inhibition, mRNA synthesis by RNA polymerase II continues within the remainder of the nucleoplasm.

Supplemental Figue 8. Actinomycin D treatment of GM06990 cells does not substantially alter expression levels of annotated open reading frames (ORFs) in test regions. qPCR on nuclear RNA isolated prior to and following 2hrs of actinomycin D treatment. Expression levels were assayed of all ORFs located in the regions tested in the localization analysis, shown are only results and primer pairs for ORFs yielding reliably detectable qPCR signal. Exon/intron primer pairs were selected to assay specifically for nascent transcripts, exon/exon primer pairs to control for genomic DNA contamination. The graph shows Ct values of averages of three independent PCR reactions performed in triplicate (+/- SD), all normalized with GAPDH, which remained invariant with treatment. We aimed to ensure that the actinomycin D treatment would not lead to aberrant increased expression that might affect the localization of the genomic segment. No increase in expression was observed, and only in one case a slight decrease (PITNB).

Supplemental Table 1

	Chr	Position	RT value	% Peripheral
RP11-119L4	3	2.74	-0.21	93.7
RP11-708D1	3	14.75	0.85	24.3
RP11-17C24	3	21.58	-0.86	94.3
RP11-107O1	3	189.07	1.02	62.2
RP11-492I23	4	3.43	1.07	20.9
RP11-362J17	4	20.83	-0.82	76.9
RP11-213G21	4	38.59	1.09	32.9
RP11-423P14	4	66.18	-0.65	87.7
RP11-69O1	4	141.47	-0.26	70.3
RP11-228F3	4	185.72	1.18	37.3
RP11-68M15	14	23.56	1.15	9.5
RP11-91J1	14	46.29	-0.80	54.6
RP11-79B13	14	68.75	0.77	7.1
RP11-88N20	14	87.63	-0.98	73.0
RP11-42M14	17	50.70	-0.98	47.3
RP11-142B17	17	56.75	1.14	7.6
RP11-243E20	17	68.62	-0.75	59.8
RP11-800K7	21	24.59	-0.88	53.4
RP11-705P12	21	26.80	0.70	28.9
CTD-2207L7	21	28.75	-1.07	54.5
RP11-385G3	21	30.43	0.99	29.9
RP11-315H16	21	31.81	-0.95	47.3
CTD-2059E17	21	34.73	0.97	13.0
RP11-105O24	21	38.87	0.84	8.8
RP11-771K8	21	42.20	-0.92	29.1
RP11-22M5	22	22.32	1.14	2.9
RP11-79G21	22	28.25	-0.26	15.6
RP11-49M22	22	45.17	0.41	3.9
RP11-66M5	22	48.36	-0.59	14.6

Supplemental Table 2

	BAC	Chr	RT	PH	PNH	PCH	Repressive
Late	RP11-42M14	17	-0.98	48.60	33.21	43.90	87.80
	CTD-2207L7	21	-1.07	48.05	63.02	45.01	96.84
	RP11-315H16	21	-0.95	48.44	63.63	44.52	96.07
	RP11-79G21	22	-0.26	24.14	45.61	31.03	73.06
Early	RP11-142B17	17	1.14	10.91	7.62	5.30	23.40
	CTD-2059E17	21	0.97	20.51	34.89	13.50	50.00

Supplemental Table 3

	BAC	Chr	RT	PH	PNH	PCH	Repressive
Late	RP11-206D24	19	-0.52	13.84	27.66	41.30	67.40
	RP11-69B20	19	-0.80	19.66	31.08	46.70	73.90
	RP11-1008E7	20	-0.97	53.54	19.48	32.26	78.49
	RP11-709F1	20	-1.08	45.10	36.85	58.88	93.66
Early	RP11-451E20	19	1.13	8.74	17.92	11.03	32.40
	RP11-273F19	20	0.97	30.07	13.54	18.79	55.76
	RP11-359H4	20	1.20	15.43	9.34	19.05	40.48

Supplemental Table 4

A

		PH	PCH	PNH	PNH only	total Repression
Chr20	Ctrl	44.94	56.96	36.08	15.19	91.77
(RP11-709F1)	Actino	55.33	58.38	11.68	2.54	87.31
Chr21 A	Ctrl	47.80	41.51	68.55	22.64	94.97
(RP11-315H16)	Actino	59.77	45.98	18.97	2.87	87.93
Chr21 B	Ctrl	48.70	43.04	66.52	21.74	96.52
(CTD-2207L7)	Actino	59.45	41.55	9.14	2.74	82.96
Chr22	Ctrl	26.94	31.00	49.08	26.81	73.06
(RP11-79G21)	Actino	36.73	34.91	13.45	3.09	64.00

B

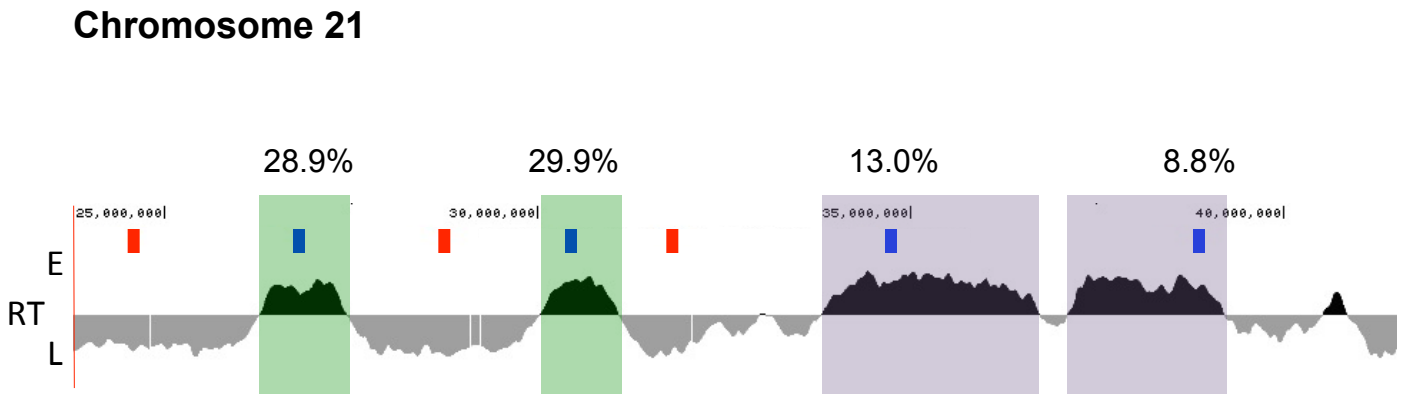
		PH	PCH	PNH	PNH only	total Repression
Chr3 A	Ctrl	41.69	6.09	8.67	4.45	50.82
(RP11-708D1)	Actino	40.17	6.07	4.60	2.51	45.82
Chr3 B	Ctrl	95.30	10.07	6.04	2.01	99.33
(RP11-17C24)	Actino	96.15	5.77	0.00	0.00	96.15
Chr17	Ctrl	24.60	12.70	18.25	10.32	43.65
(RP11-142B17)	Actino	23.77	11.48	3.28	1.64	32.79

Supplemental Table 5

List of all BACs used in this study

BAC	Chr	Pos. start	Pos. end
RP11-119L4	chr3	2653216	2820466
RP11-708D1	chr3	14676541	14828209
RP11-17C24	chr3	21501635	21662360
RP11-107O1	chr3	188990099	189149481
RP11-492I23	chr4	3334580	3525884
RP11-362J17	chr4	20738801	20920843
RP11-213G21	chr4	38499262	38681142
RP11-423P14	chr4	66083619	66283525
RP11-69O1	chr4	141384114	141554828
RP11-228F3	chr4	185635303	185803183
RP11-68M15	chr14	23476852	23652426
RP11-91J1	chr14	46188441	46392714
RP11-79B13	chr14	68674505	68835127
RP11-88N20	chr14	87552921	87703166
RP11-142B17	chr17	56666964	56840758
RP11-90L11	chr17	68470215	68632969
RP11-243E20	chr17	68539717	68697062
RP11-206D24	ch19	9267502	9437626
RP11-451E20	ch19	16397567	16563349
RP11-69B20	ch19	53715397	53864483
RP11-273F19	chr20	5583318	5745127
RP11-1008E7	chr20	8566634	8756158
RP11-359H4	chr20	33931910	34099003
RP11-709F1	chr20	59252029	59427394
RP11-800K7	chr21	24505210	24680695
RP11-705P12	chr21	26716253	26892398
CTD-2207L7	chr21	28687795	28814324
RP11-385G3	chr21	30357341	30510899
RP11-315H16	chr21	31712164	31908166
CTD-2059E17	chr21	34648731	34807381
RP11-105O24	chr21	38795458	38951057
RP11-771K8	chr21	42116785	42286115
RP11-22M5	chr22	22239869	22394983
RP11-79G21	chr22	28169393	28327386
RP11-49M22	chr22	45090621	45258577
RP11-66M5	chr22	48279484	48441934

Supplemental Figure 1



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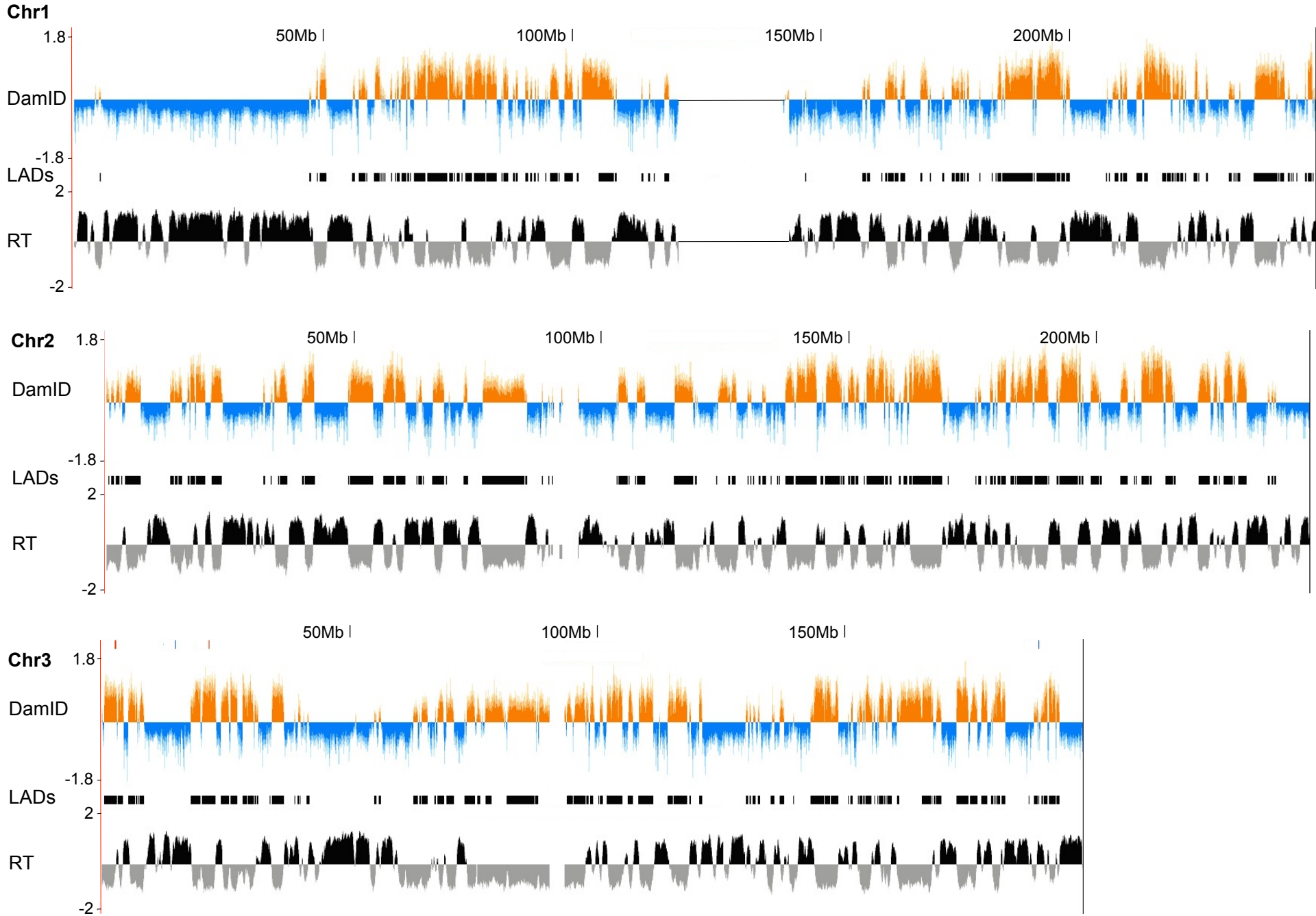

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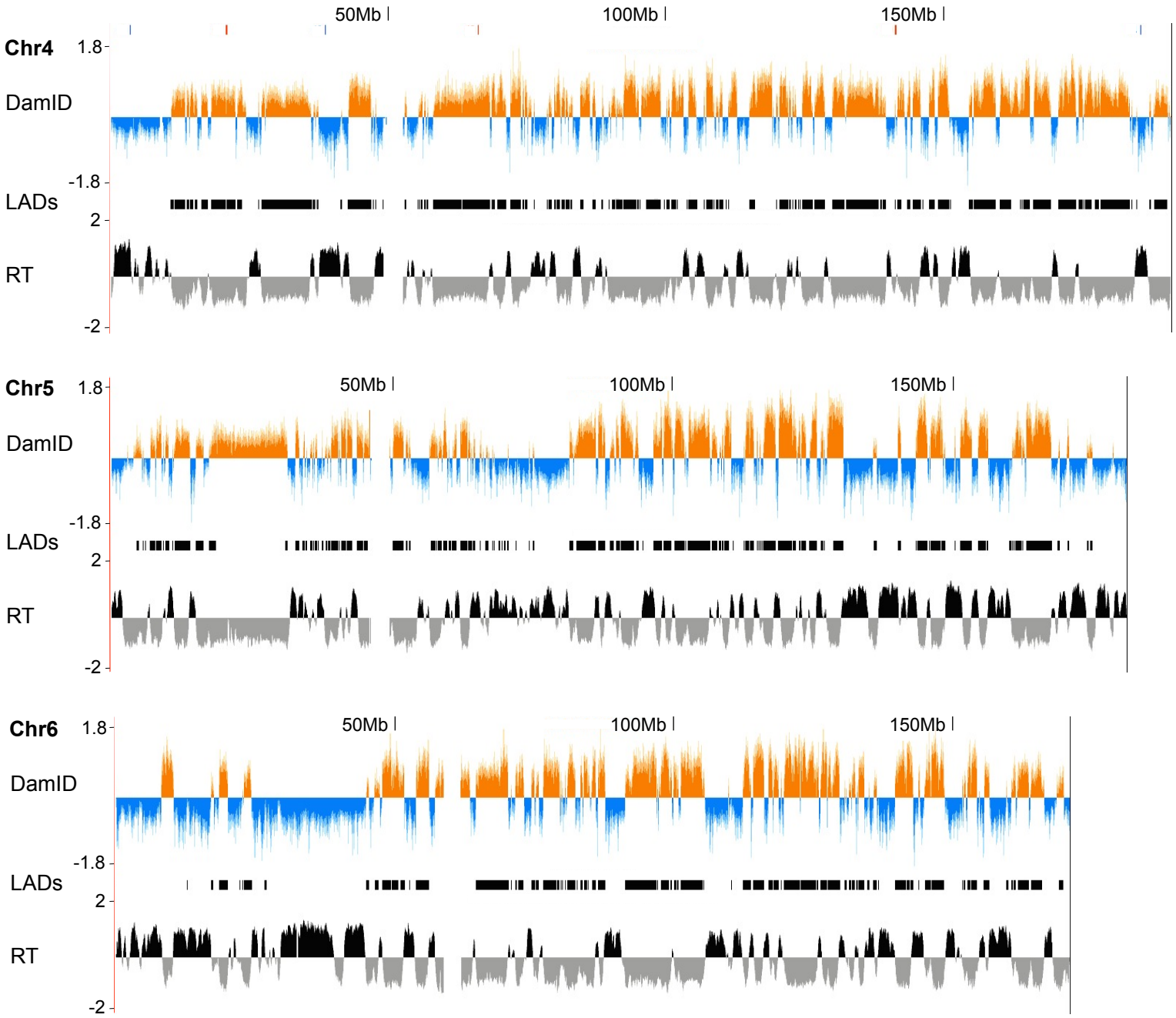

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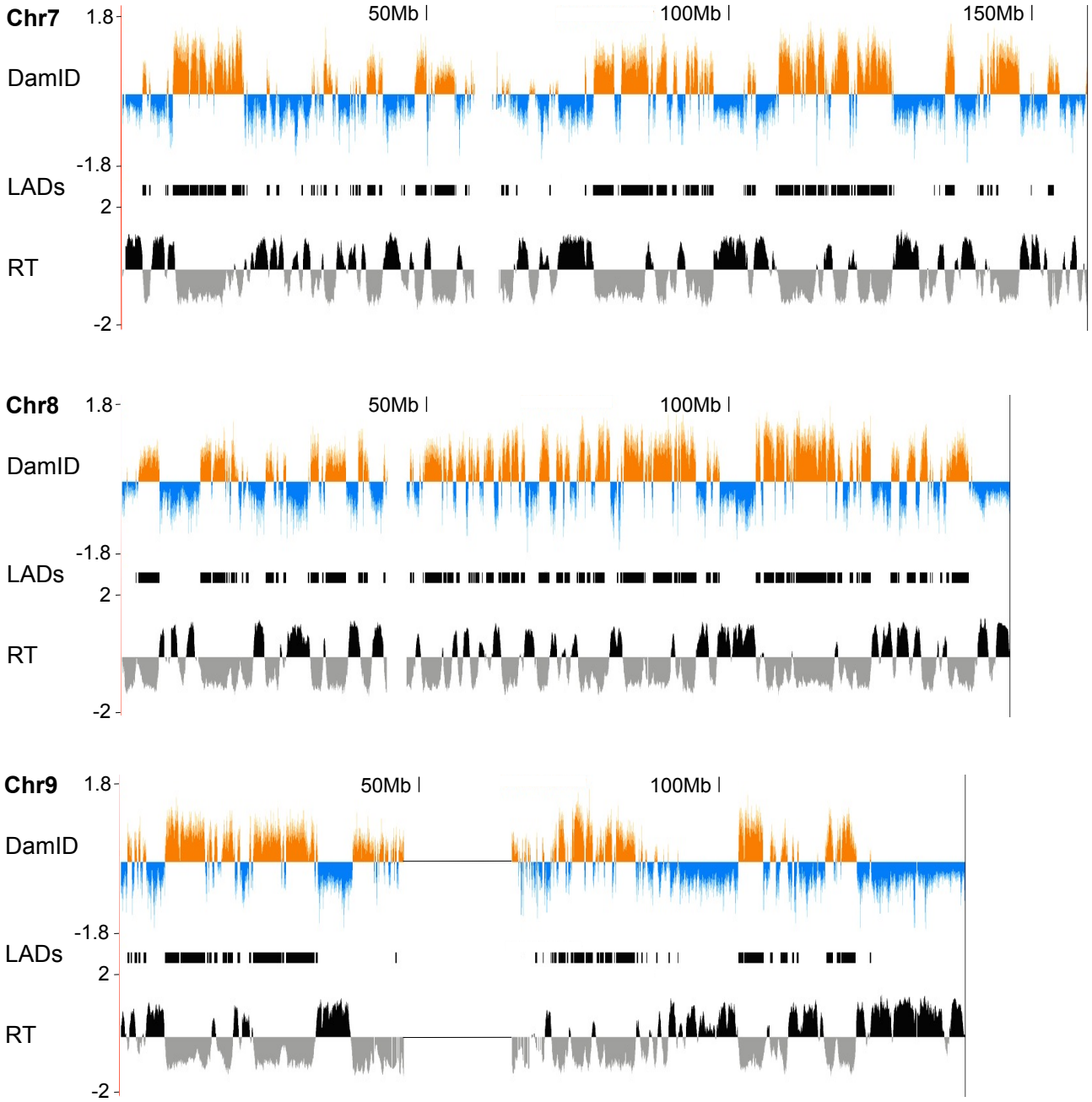

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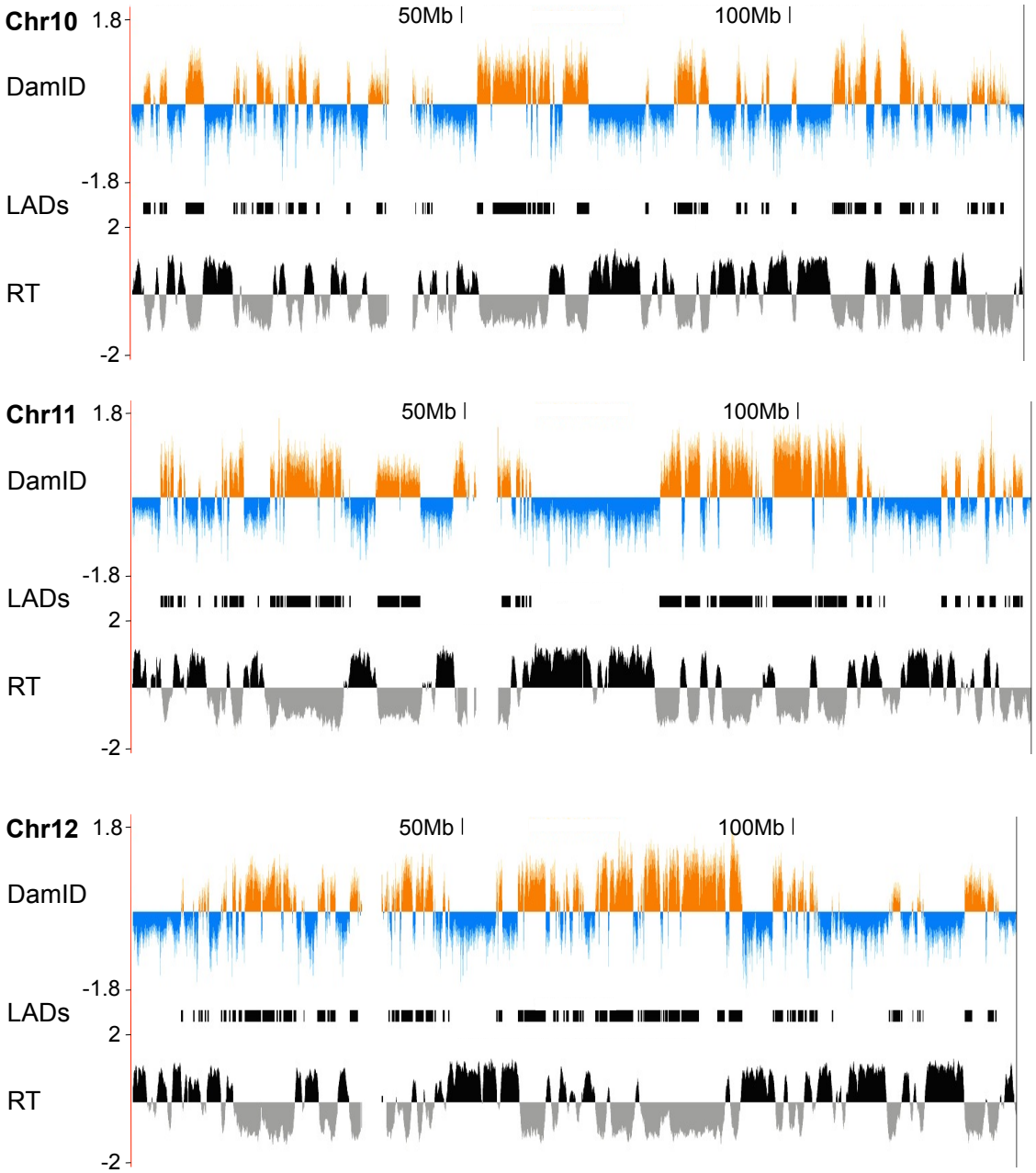

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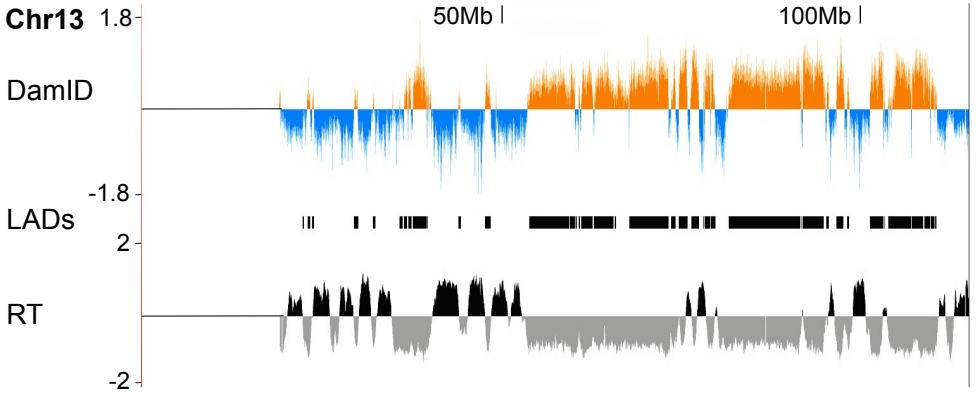

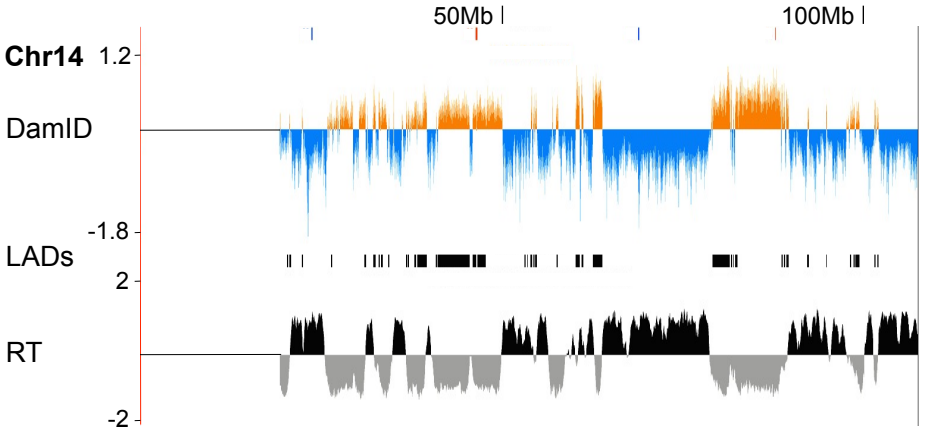

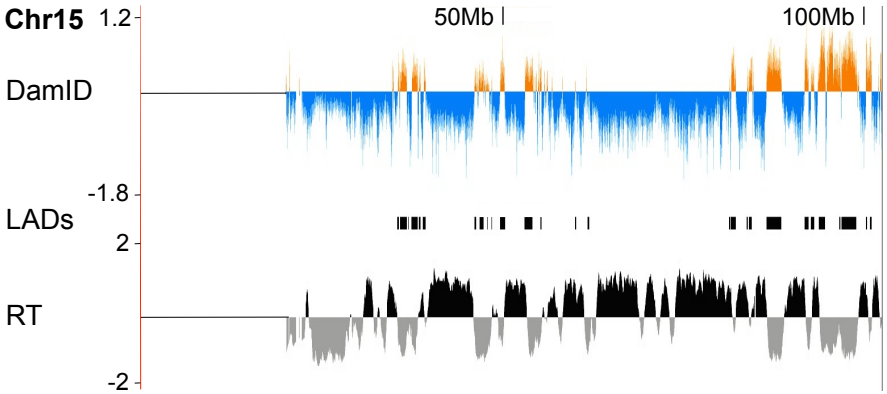

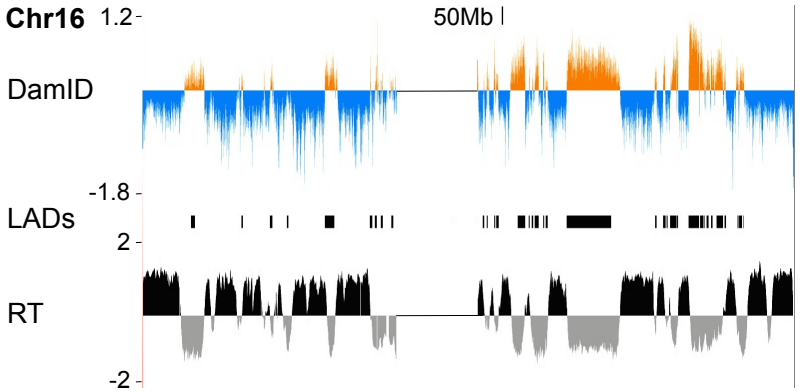

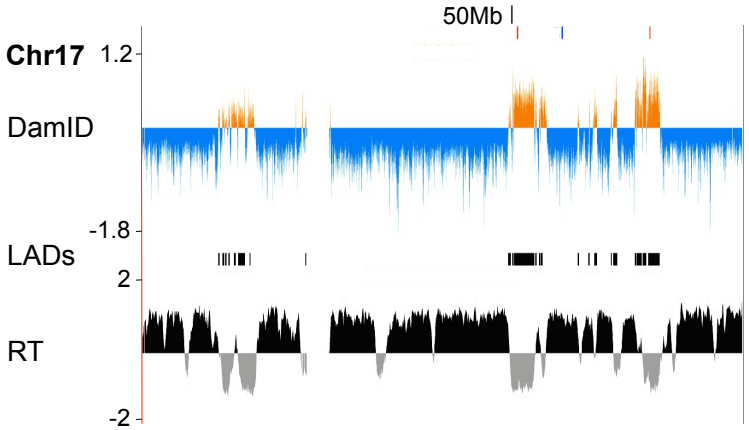

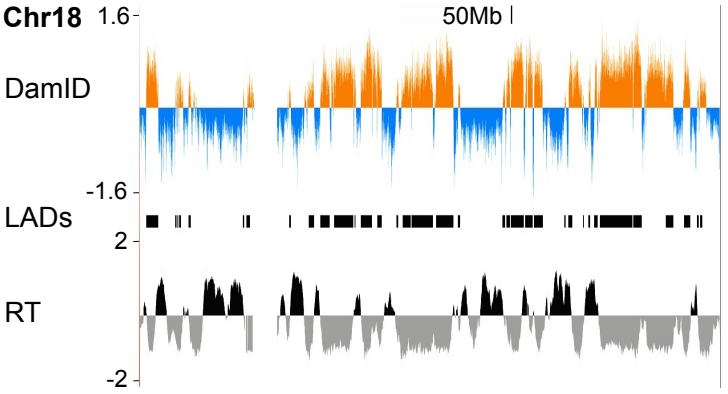

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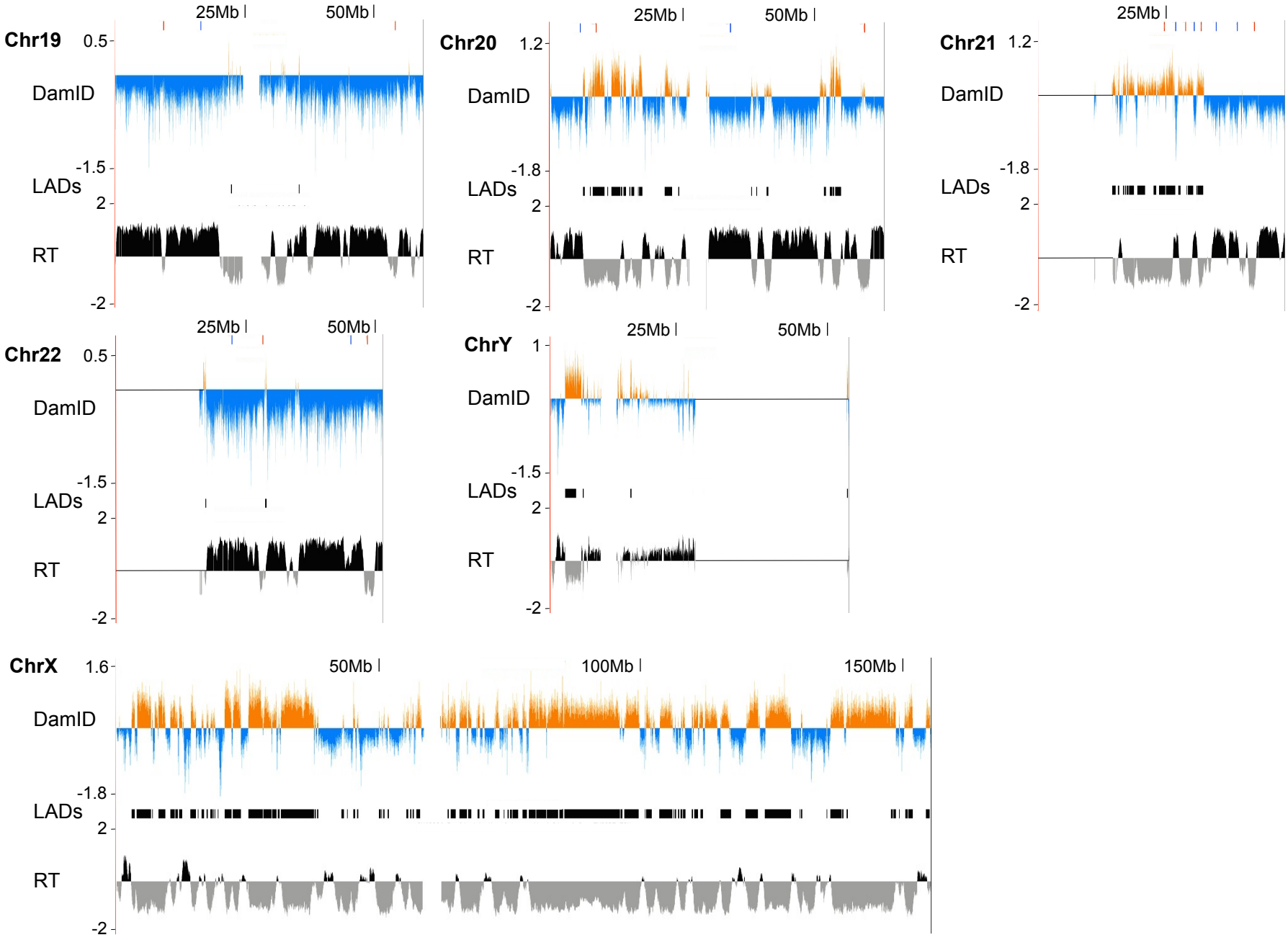

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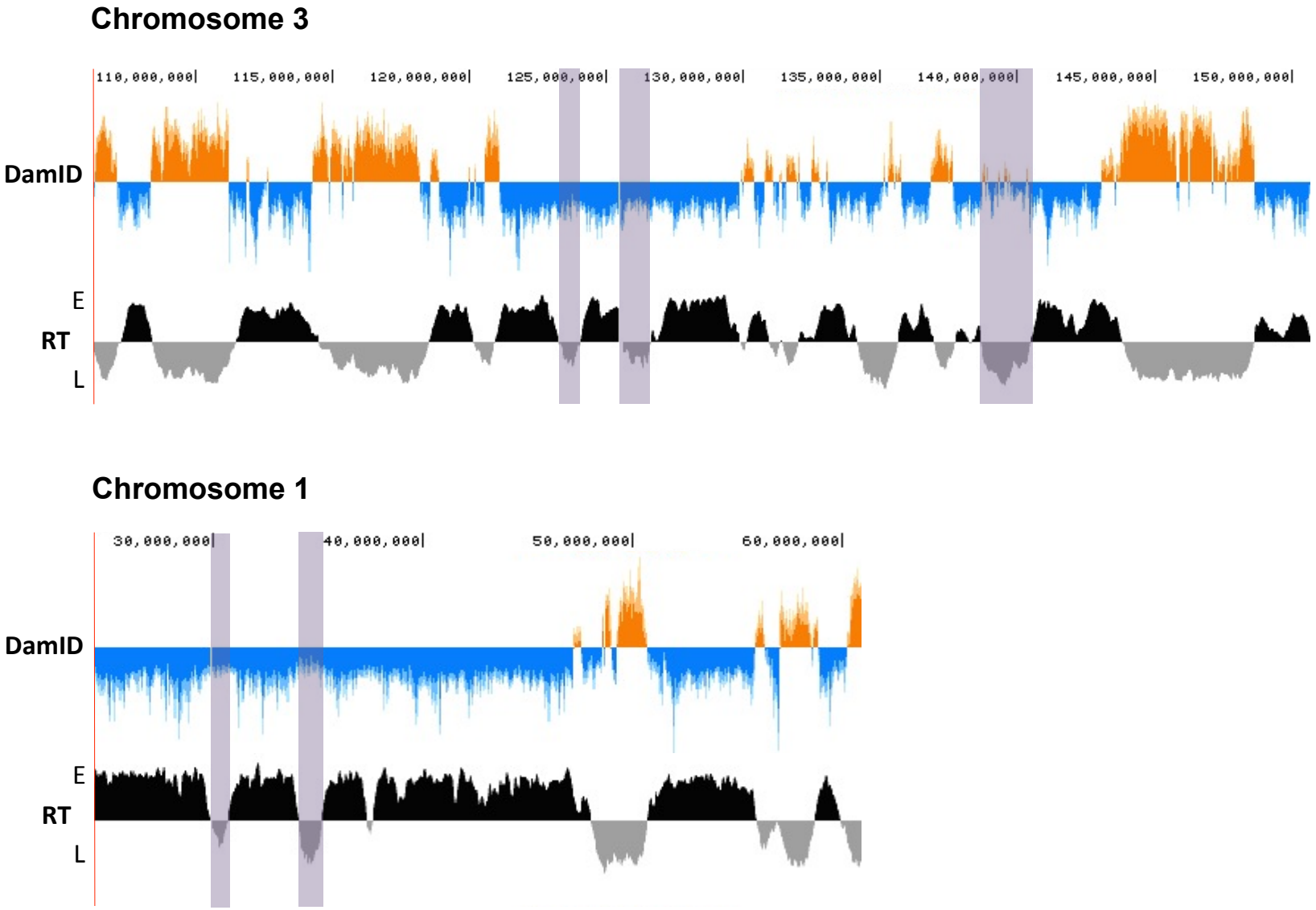
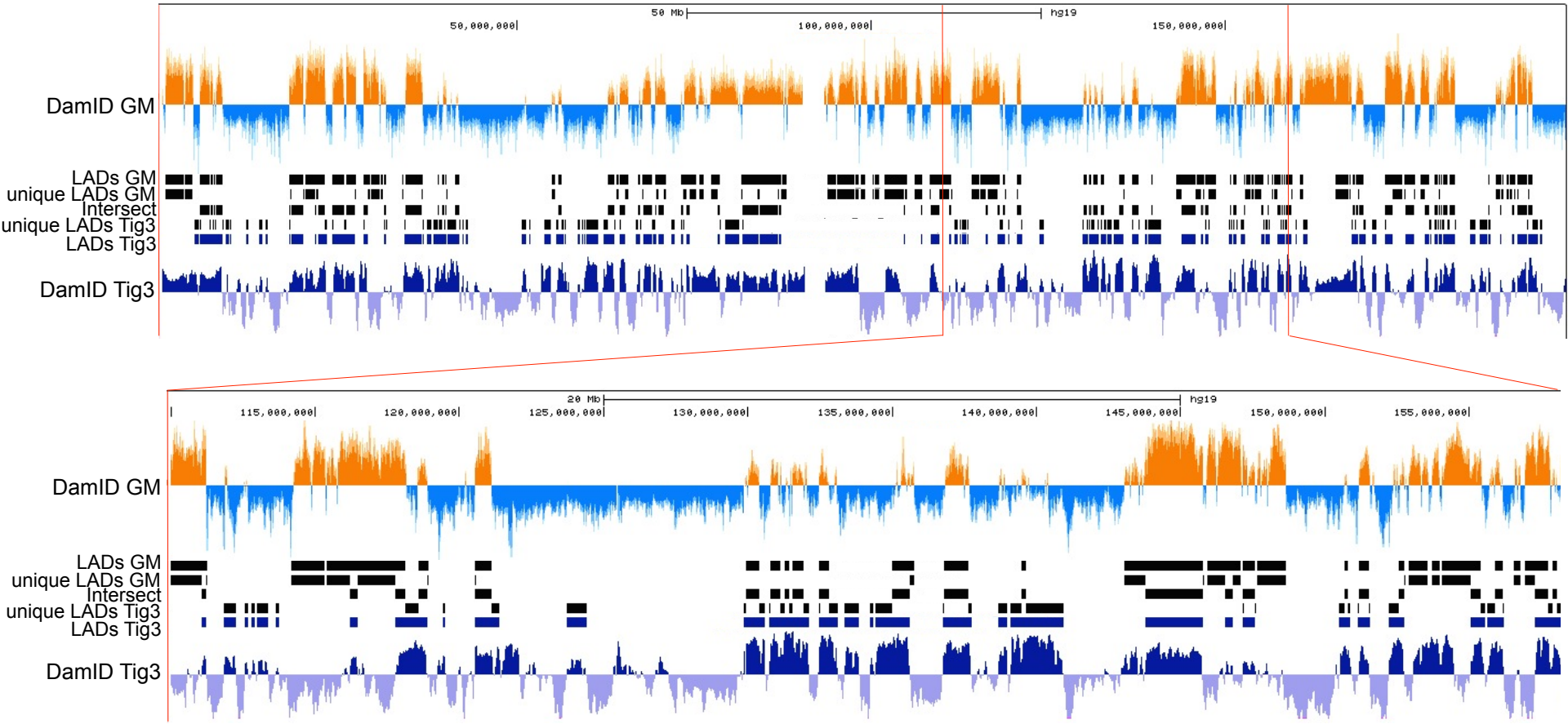
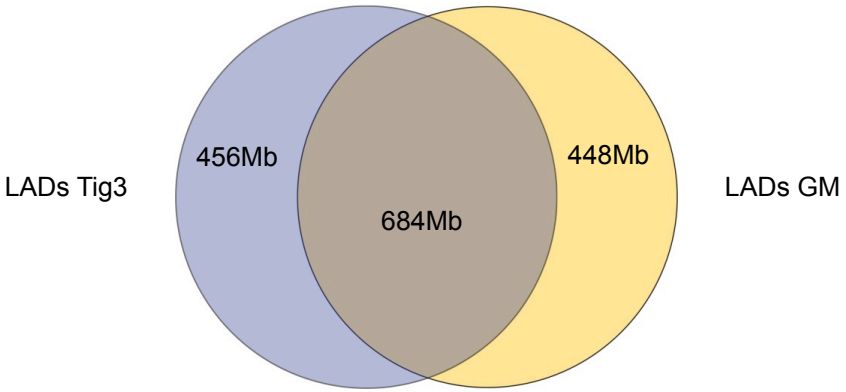


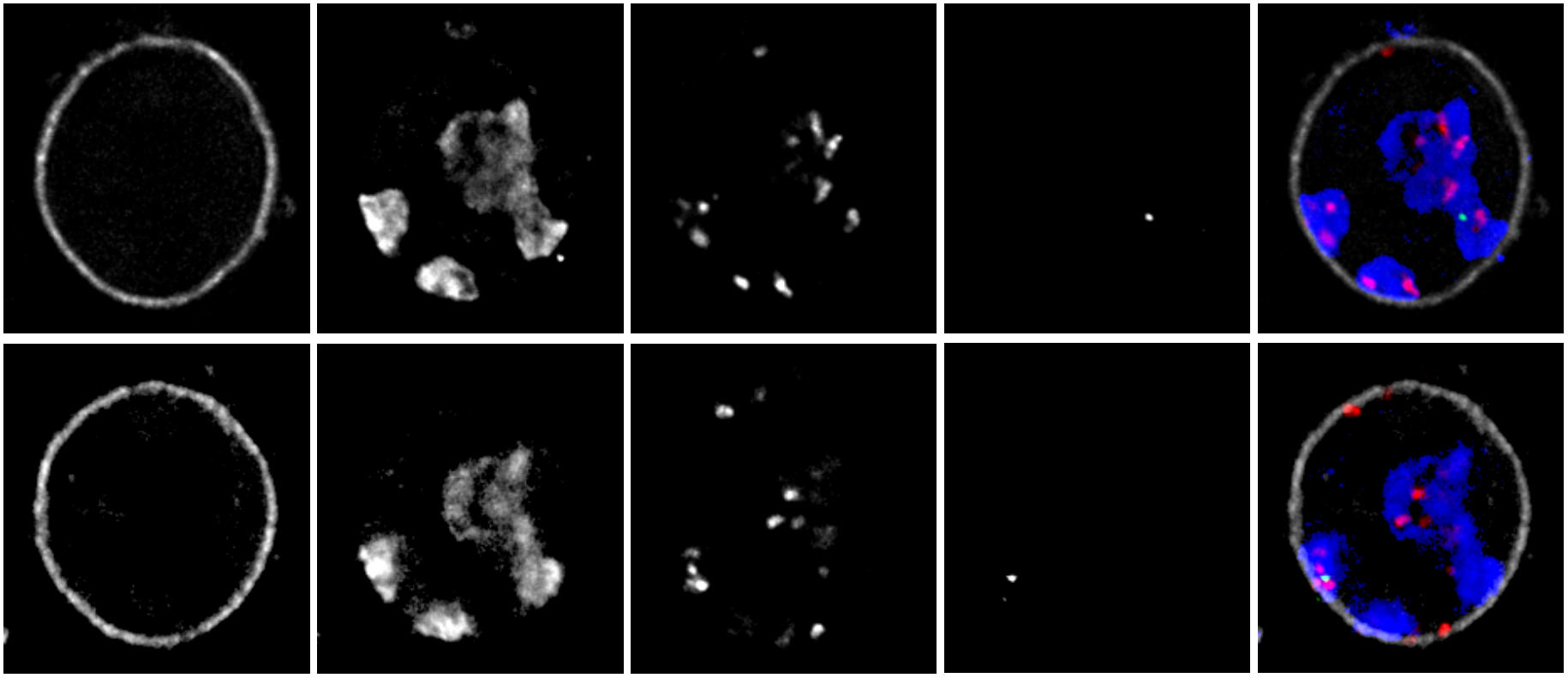
Supplemental Figure 4

Chromosome 3



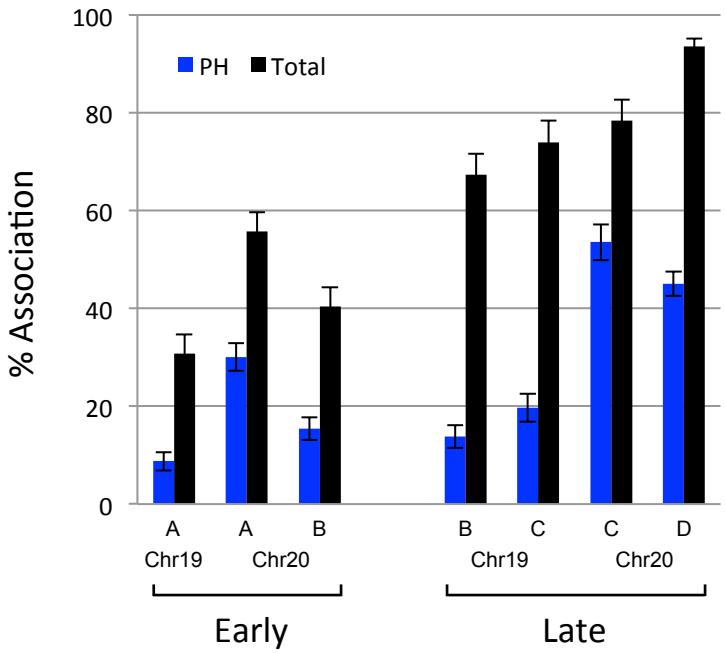


Supplemental Figure 5

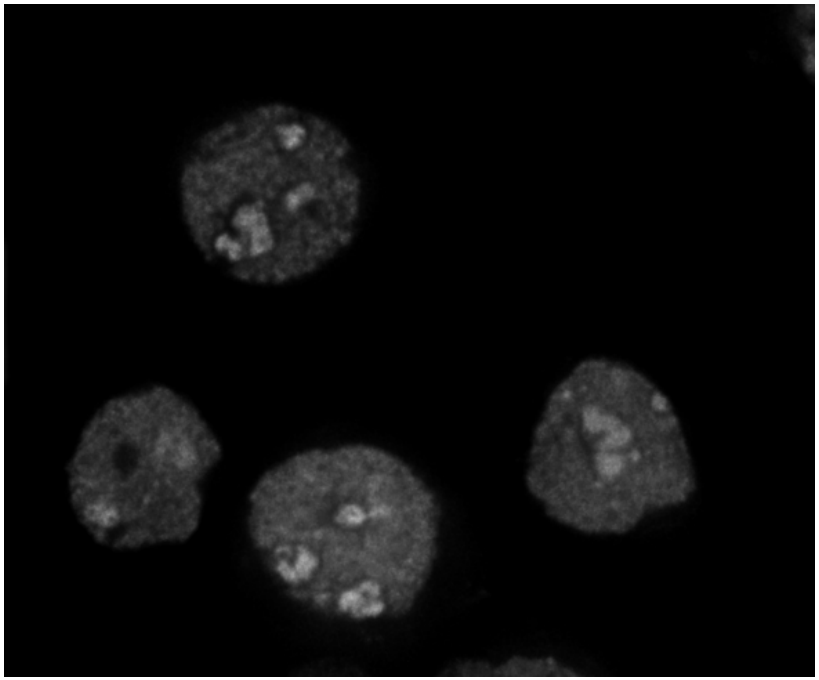


Lamina | Nucleolin | PCH | BAC | Lamina/Nucleolin/ PCH/BAC

Supplemental Figure 6

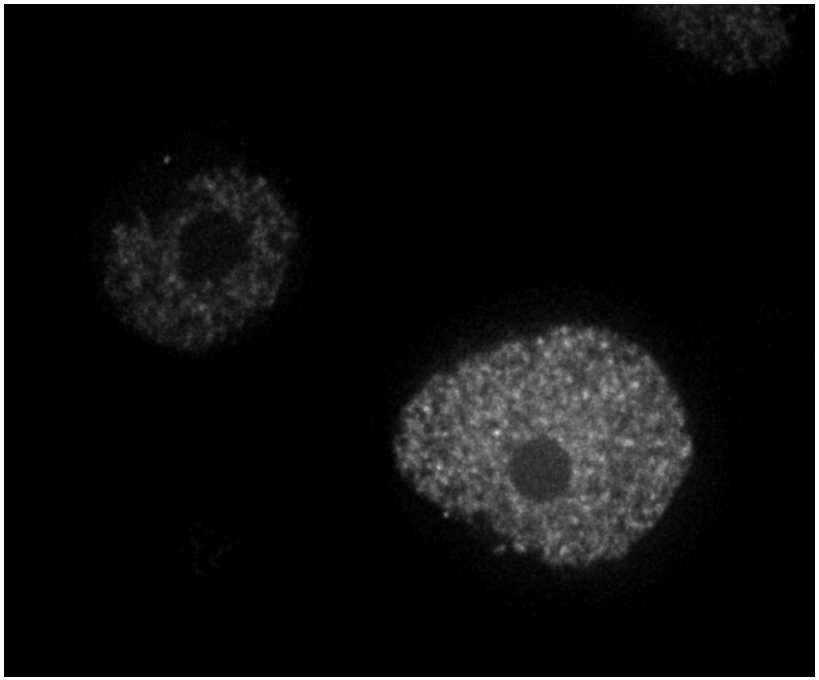


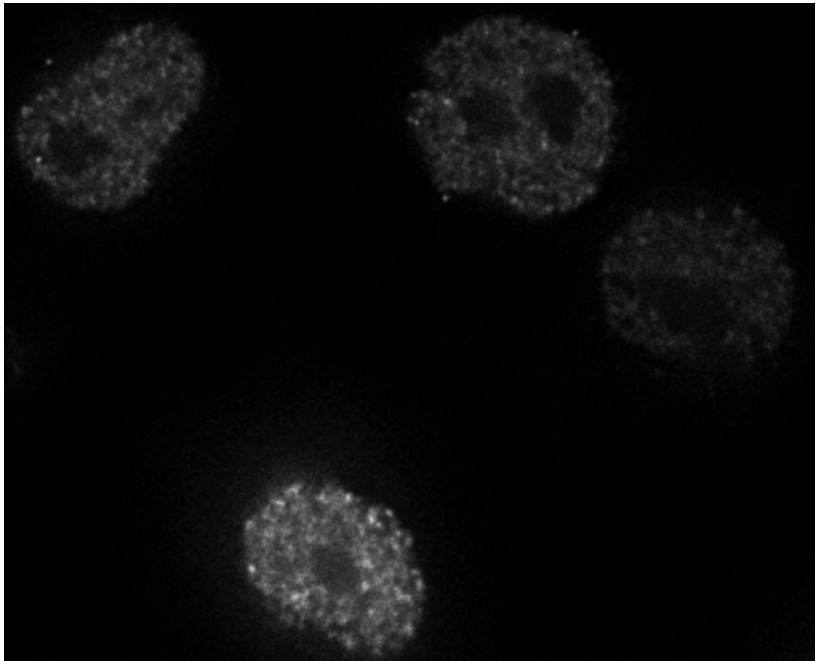
Supplemental Figure 7



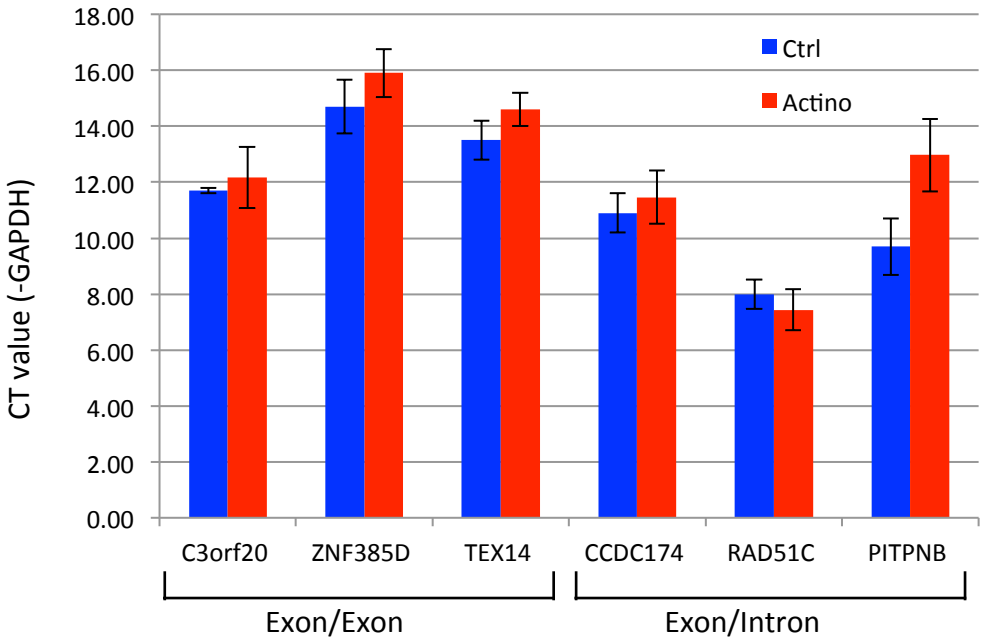
untreated

200ng/ml actinomycinD





Supplemental Figure 8



Primers used for RT

C3orf20 F GGAGAAGCTGCAGGAGTTGT C3orf20 R CTGCAGTGGGAGGGTAATGT	Chr3 BAC RP11-119L4 @ 14.75Mb
CCDC174 F TTGCCAAACTTCGACAAAAA CCDC174 R TTGGTGTCCTTCCTCTCCTG	Chr3 BAC RP11-119L4 @ 14.75Mb
Tex14 F AGCCTTCTCACAGCATCACA Tex14 R CAGTGCCTCCTCCATCACTT	Chr17 BAC RP11-142B17 @ 56.75Mb
ZNF385D F AAGACAGAGCTGCTGGGAAG ZNF385D R GCTGCAGCTGCTAGAGGATT	Chr3 BAC RP11-17C24 @ 21.58Mb
RAD51C F GGTTCCAGACTGCTGAGGAA RAD51C R TTATGAAGCCCTGGGTATGC	Chr17 BAC RP11-142B17 @ 56.75Mb
PITPNB F GTCCCCAGATGTGTGCCTAT PITPNB R TGTCTTCCATCGTGAGATCG	Chr22 BAC RP11-79G21 @ 28.25Mb

Supplemental Methods

EU incorporation

5-Ethynyl uridine (EU) for incorporation into RNA was added to a final concentration of 1mM to untreated GM06990 cells and one hour following the addition of actinomycin D. After a one hour incubation the cells were harvested and deposited onto polylysine coated slides and fixed. The EU was labeled with Alexa-594 using the Click-iT® reaction according to manufacturer's instructions (Life Technologies; C10330).

Nuclear RNA isolation and RT-PCR

GM nuclei were isolated by cell lysis in sucrose buffer and the detergent NP-40 on ice. Following centrifugation at 1,500g the nuclei pellet was washed in sucrose buffer without detergent and centrifuged again. Nuclear RNA was isolated using the TRIzol LS Reagent according to manufacturer's instructions (Life Technologies; 10296-028) and reverse transcription was performed on 100ng of DNase treated recovered RNA using random primers and SuperScript II (Life Technologies; 18064-014). qPCR was performed on an ABI 7900HT Real Time PCR machine and analyzed with SDS 2.4 software (Applied Biosystems/Life Technology) with SYBR Green incorporation to detect PCR products. The qPCR reactions were performed three times in triplicate, the CT values for the GAPDH control (which was invariant with actinomycin D treatment) were subtracted from those of the test ORFs.