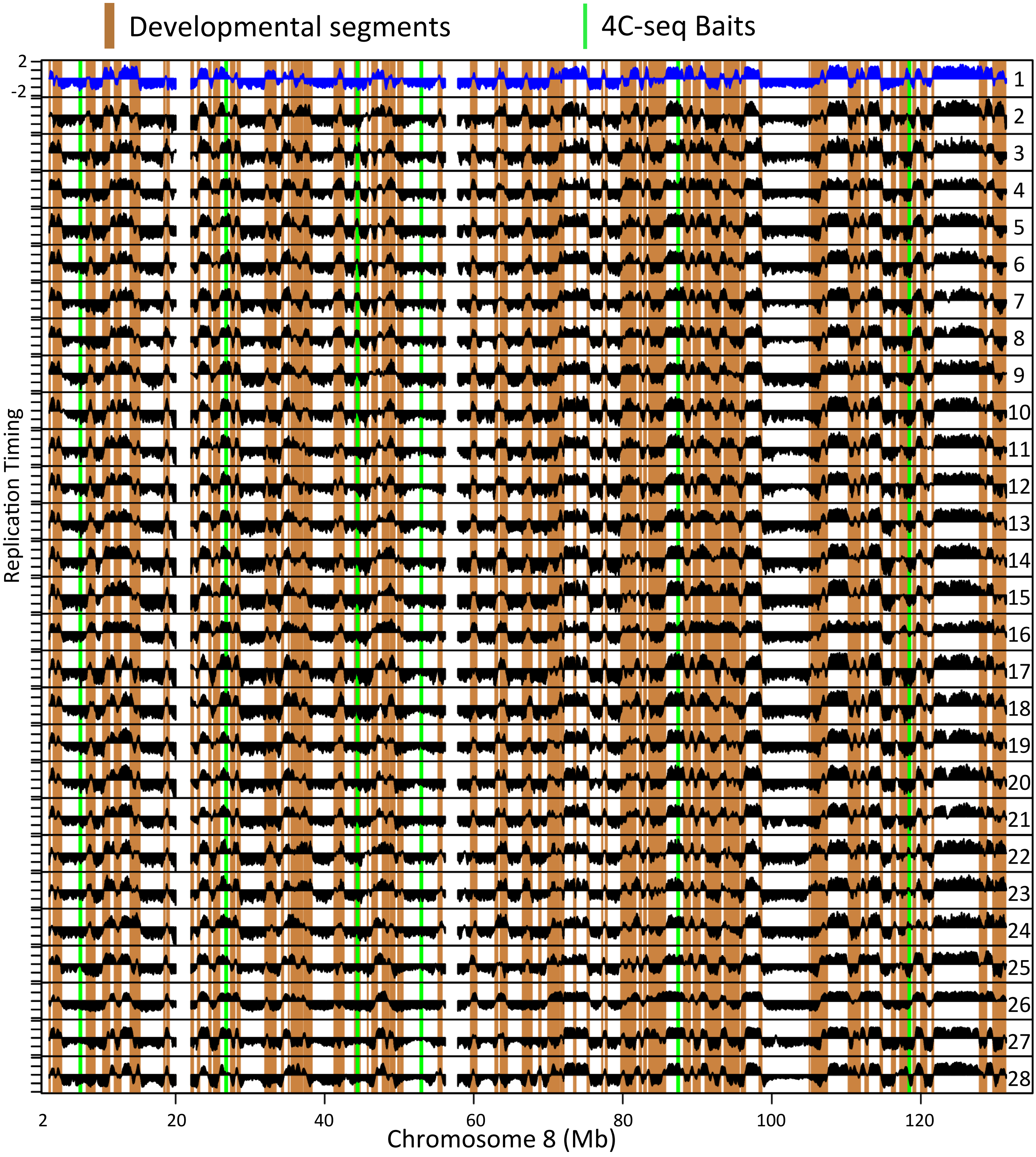
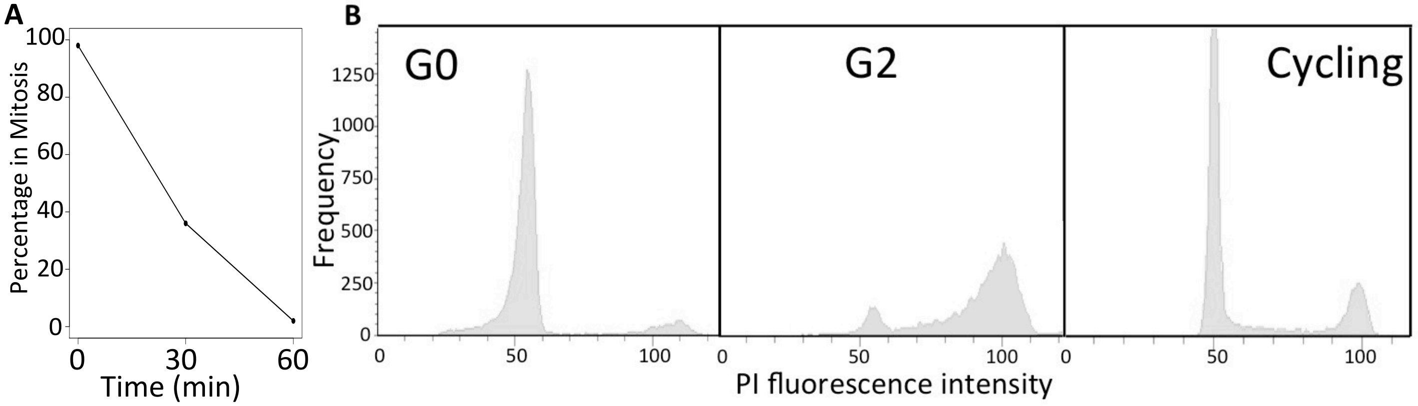
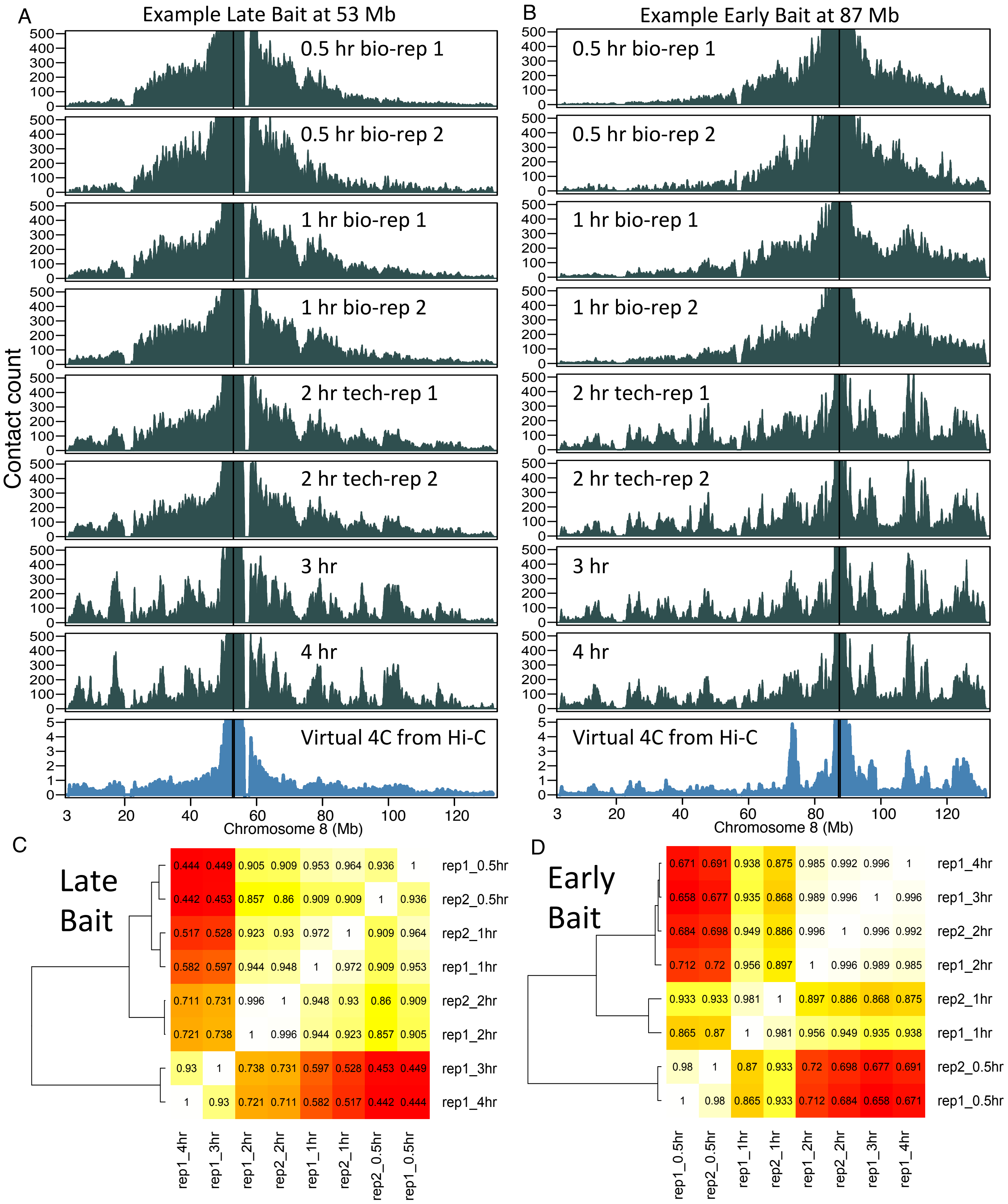


Supplementary Figure 1: Choosing baits from constitutive and developmental regions. Windowed replication timing profiles (40 kb) of 28 different mouse cell lines (replicationdomain.org) were compared to classify the genome into constitutive or developmental segments. Plot of Chr 8 is shown here with developmental regions shaded brown. Green lines indicate positions of baits. C127 replication timing profile is shown in blue.



Supplementary Figure 2: Cell cycle synchronization

A) Plot showing percentage mitotic cells vs time after release from nocodazole block. **B)** FACS profiles showing synchrony at G2 and G0 compared to cycling C127 cells.

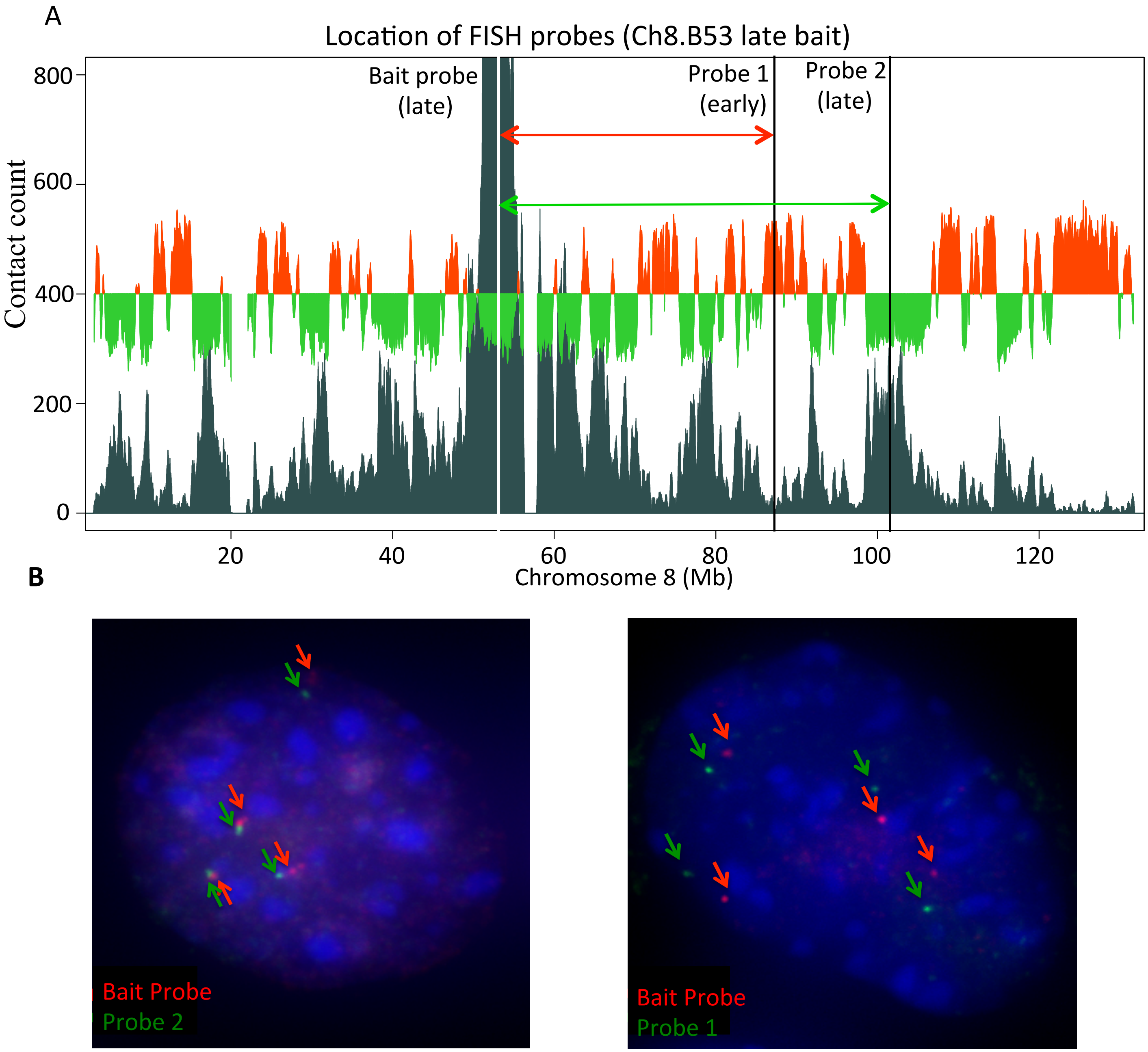


	rep1_4hr	rep1_3hr	rep1_2hr	rep2_2hr	rep1_1hr	rep2_1hr	rep2_0.5hr	rep1_0.5hr
rep1_0.5hr	0.444	0.449	0.905	0.909	0.953	0.964	0.936	1
rep2_0.5hr	0.442	0.453	0.857	0.86	0.909	0.909	1	0.936
rep2_1hr	0.517	0.528	0.923	0.93	0.972	1	0.909	0.964
rep1_1hr	0.582	0.597	0.944	0.948	1	0.972	0.909	0.953
rep2_2hr	0.711	0.731	0.996	1	0.948	0.93	0.86	0.909
rep1_2hr	0.721	0.738	1	0.996	0.944	0.923	0.857	0.905
rep1_3hr	0.93	1	0.738	0.731	0.597	0.528	0.453	0.449
rep1_4hr	1	0.93	0.721	0.711	0.582	0.517	0.442	0.444

	rep1_0.5hr	rep2_0.5hr	rep1_1hr	rep2_1hr	rep1_2hr	rep2_2hr	rep1_3hr	rep1_4hr
rep1_4hr	0.671	0.691	0.938	0.875	0.985	0.992	0.996	1
rep1_3hr	0.658	0.677	0.935	0.868	0.989	0.996	1	0.996
rep2_2hr	0.684	0.698	0.949	0.886	0.996	1	0.996	0.992
rep1_2hr	0.712	0.72	0.956	0.897	1	0.996	0.989	0.985
rep2_1hr	0.933	0.933	0.981	1	0.897	0.886	0.868	0.875
rep1_1hr	0.865	0.87	1	0.981	0.956	0.949	0.935	0.938
rep2_0.5hr	0.98	1	0.87	0.933	0.72	0.698	0.677	0.691
rep1_0.5hr	1	0.98	0.865	0.933	0.712	0.684	0.658	0.671

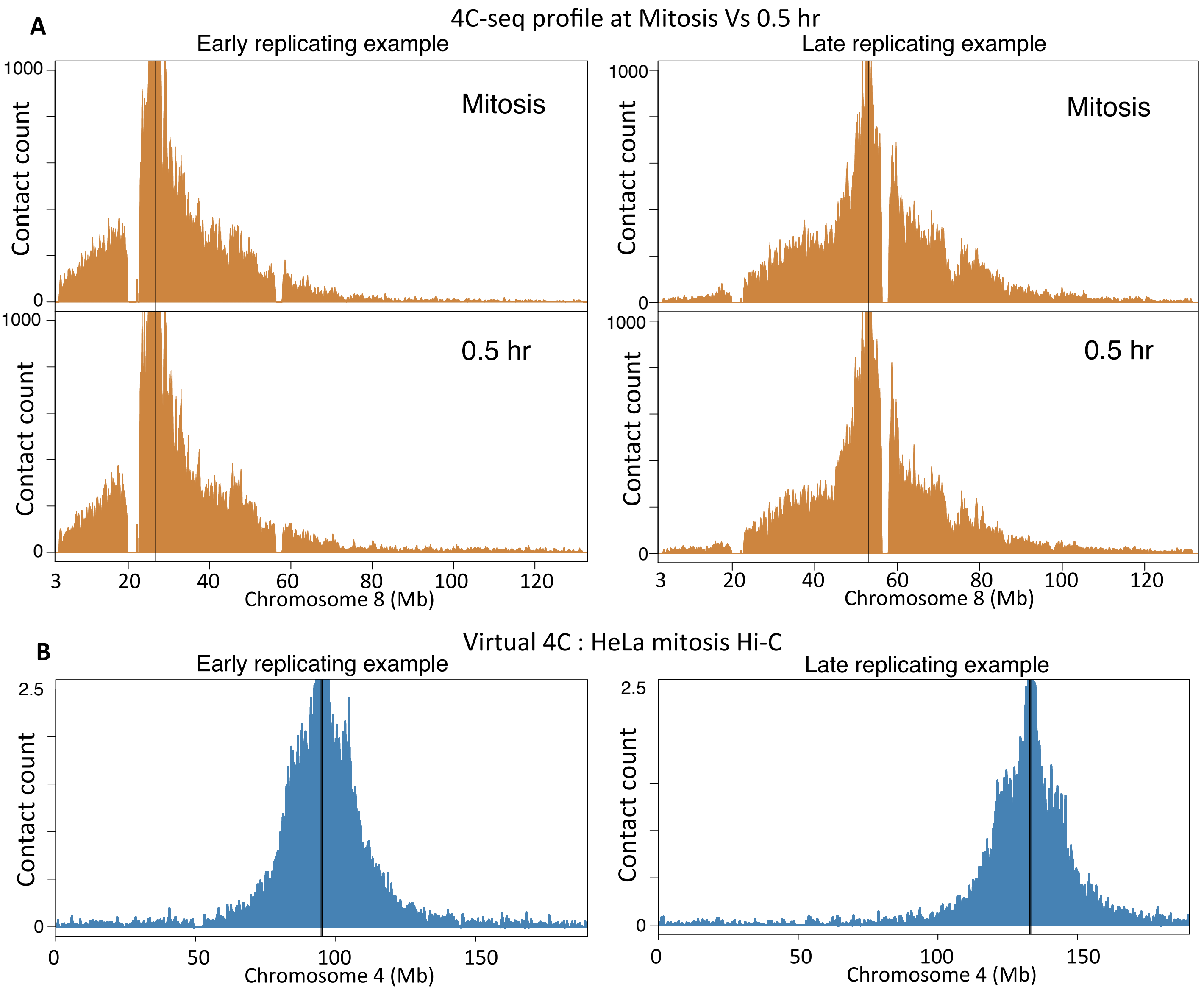
Supplementary Figure 3: Reproducibility of 4C-seq profiles.

A, B) Comparison between replicates, adjacent time-points and virtual 4C generated from mouse ESC Hi-C data for a late replicating (left) and early replicating (right) region. Biological replicates at 0.5, 1 hr and technical replicate at 2 hr are highly reproducible. 4C-seq profiles between 3 hr and 4 hr also show almost identical profiles indicating the reproducibility of our 4C-seq. Bottom panels (blue plots) show virtual 4C derived from Hi-C from mouse ESCs also shows high similarity to our 4C profile for C127, except that existing Hi-C datasets have lower sequencing depth compared to 4C for a given locus. **C, D)** Heat-map and clustering based on Pearson correlation values of plots shown in A and B. 4C datasets cluster according to replicates and time points for both the early and late example shown here.



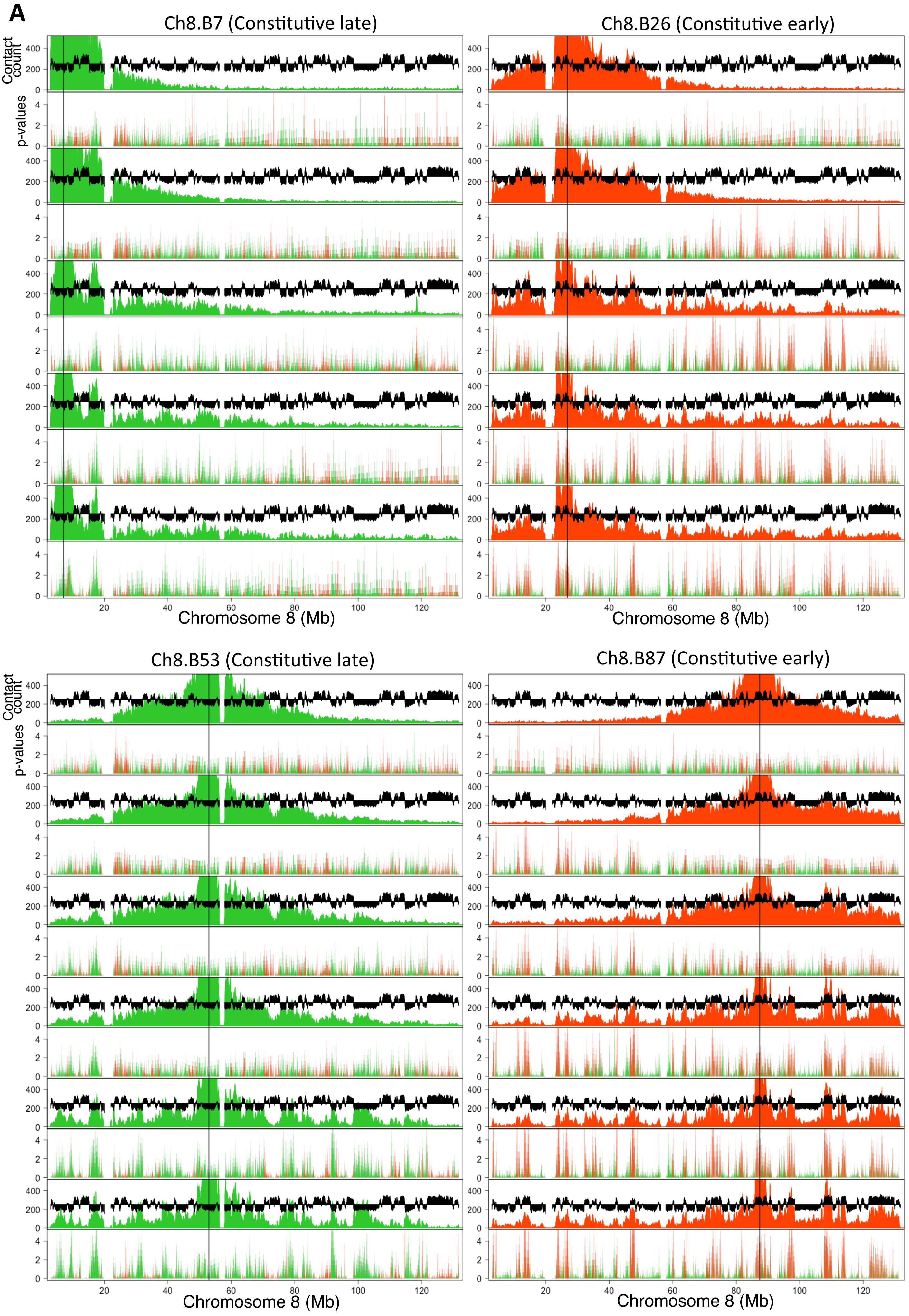
Supplementary Figure 4: 3D FISH validation of 4C-seq

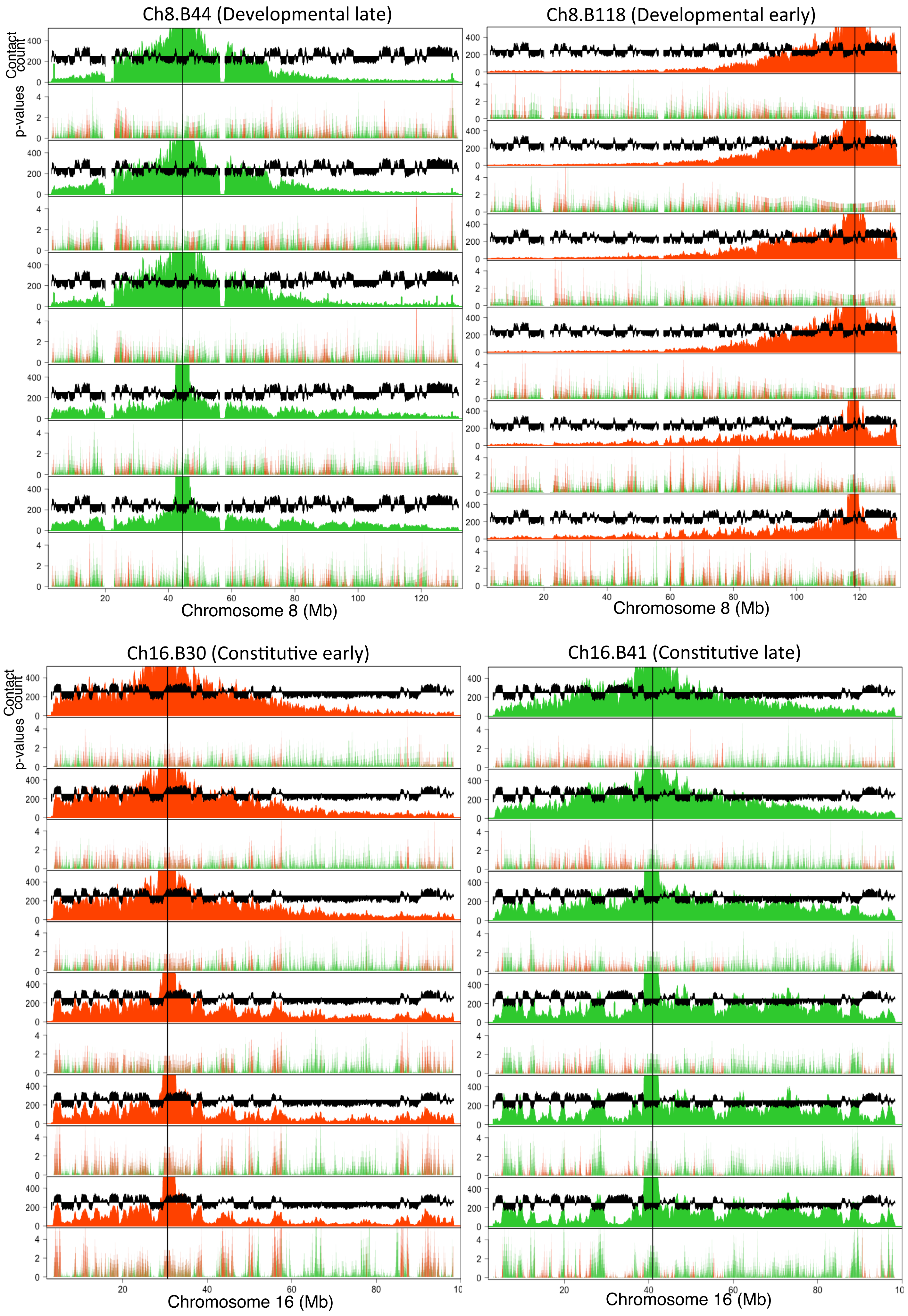
A) Location of FISH probes at a region with low 4C contact count (probe 1) or high 4C contact count (probe 2) on chromosome 8. **B**) Exemplary FISH images, from data shown in Figure 1A showing agreement of 3D distance with 4C data. Even though probe 2 is further away from the bait than probe 1 on the linear chromosome, probe 2 is separated by a lesser distance from the bait in 3D in the nucleus. C127 cells are tetraploid and therefore shows 4 FISH signals.

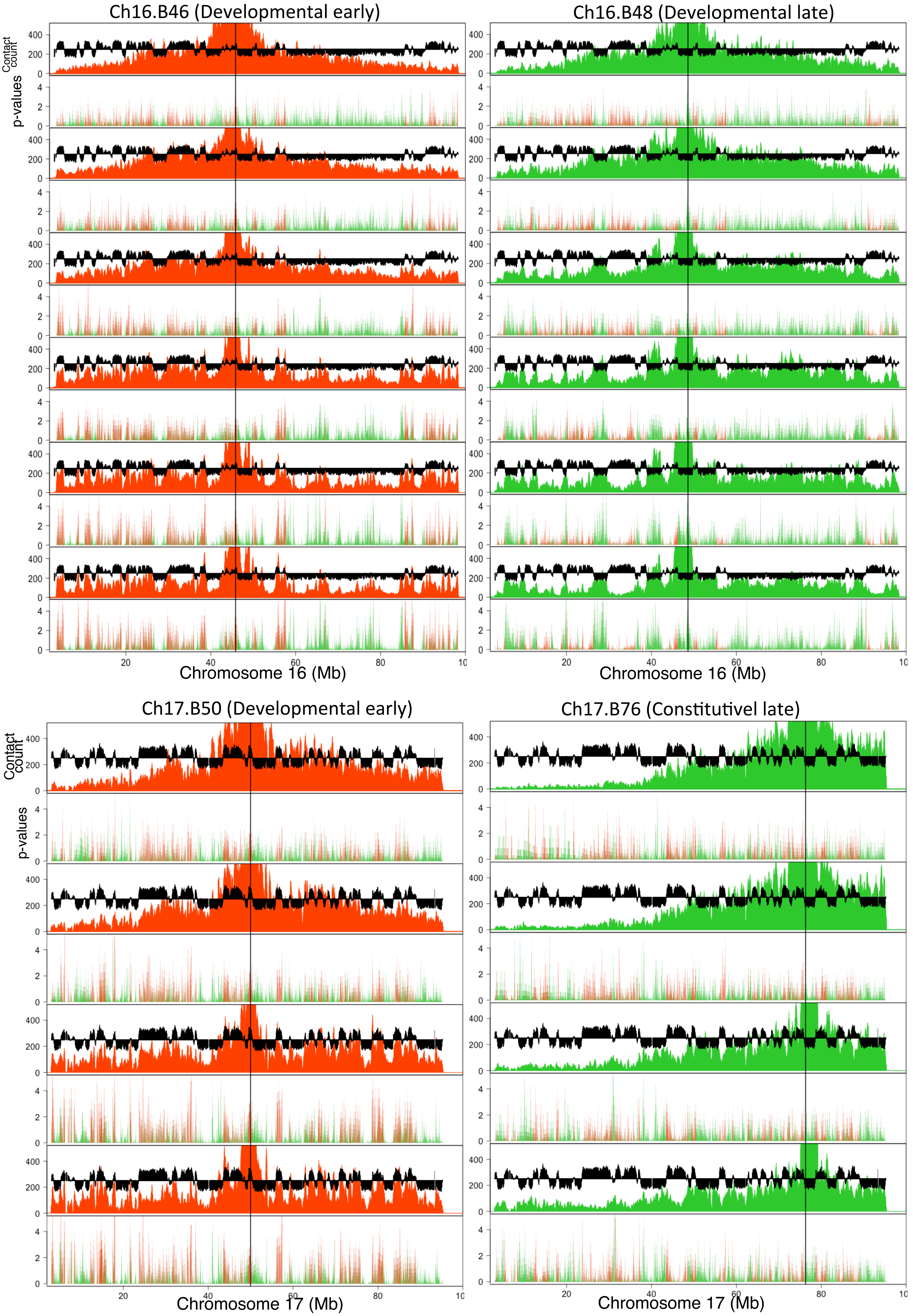


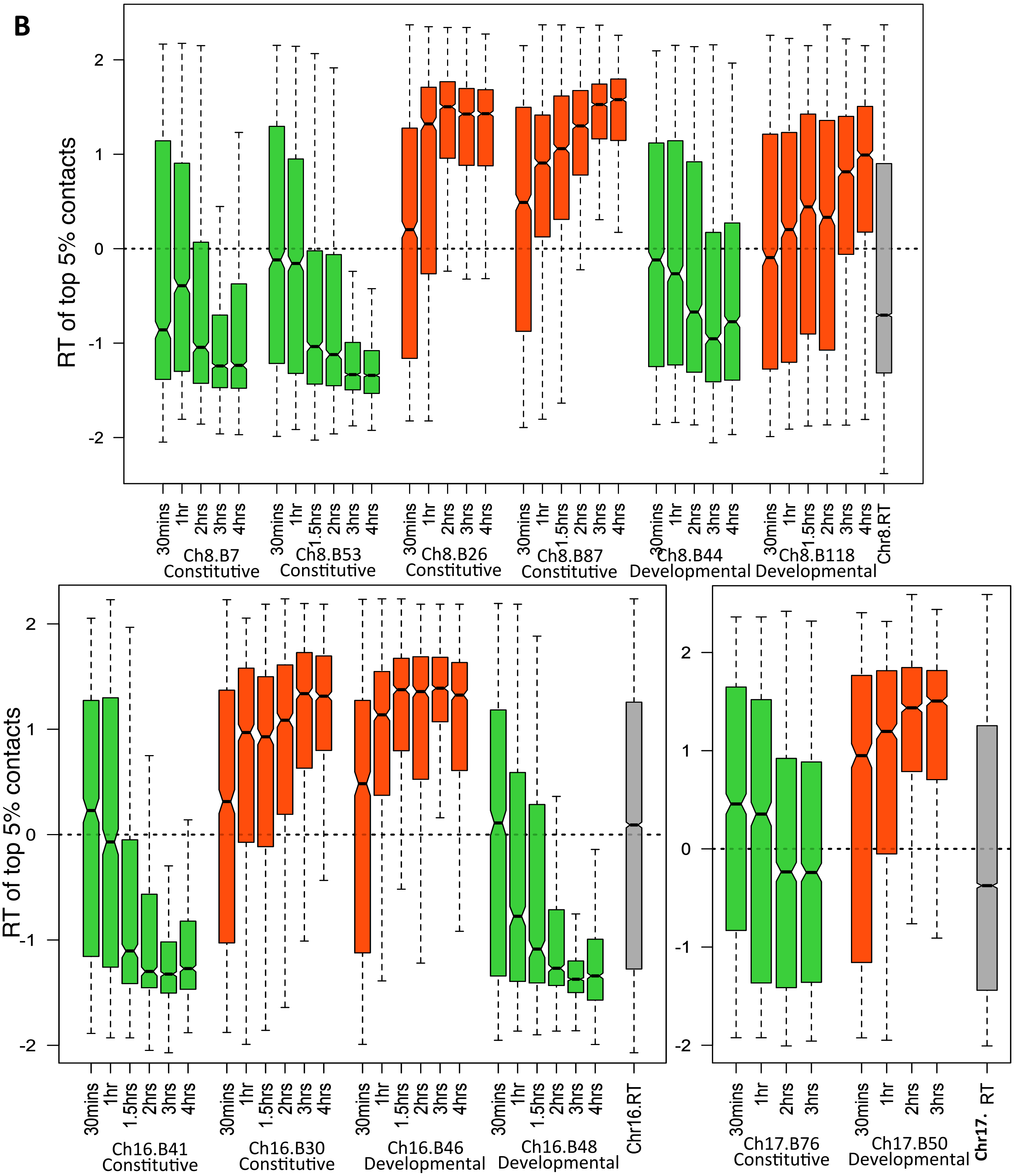
Supplementary Figure 5: Comparison of 4C-seq at mitosis, 0.5 hr post-mitosis in C127 cells and Hi-C at mitosis in HeLa cells.

A) Comparison of 4C contact counts between mitosis and 0.5 hr post-mitosis for baits in an early replicating region and late replicating region in C127 cells showing lack of compartmentalization. **B)** Virtual 4C for an early and late replicating region generated from Hi-C in HeLa cells synchronized in mitosis showing a similar lack of compartmentalization.

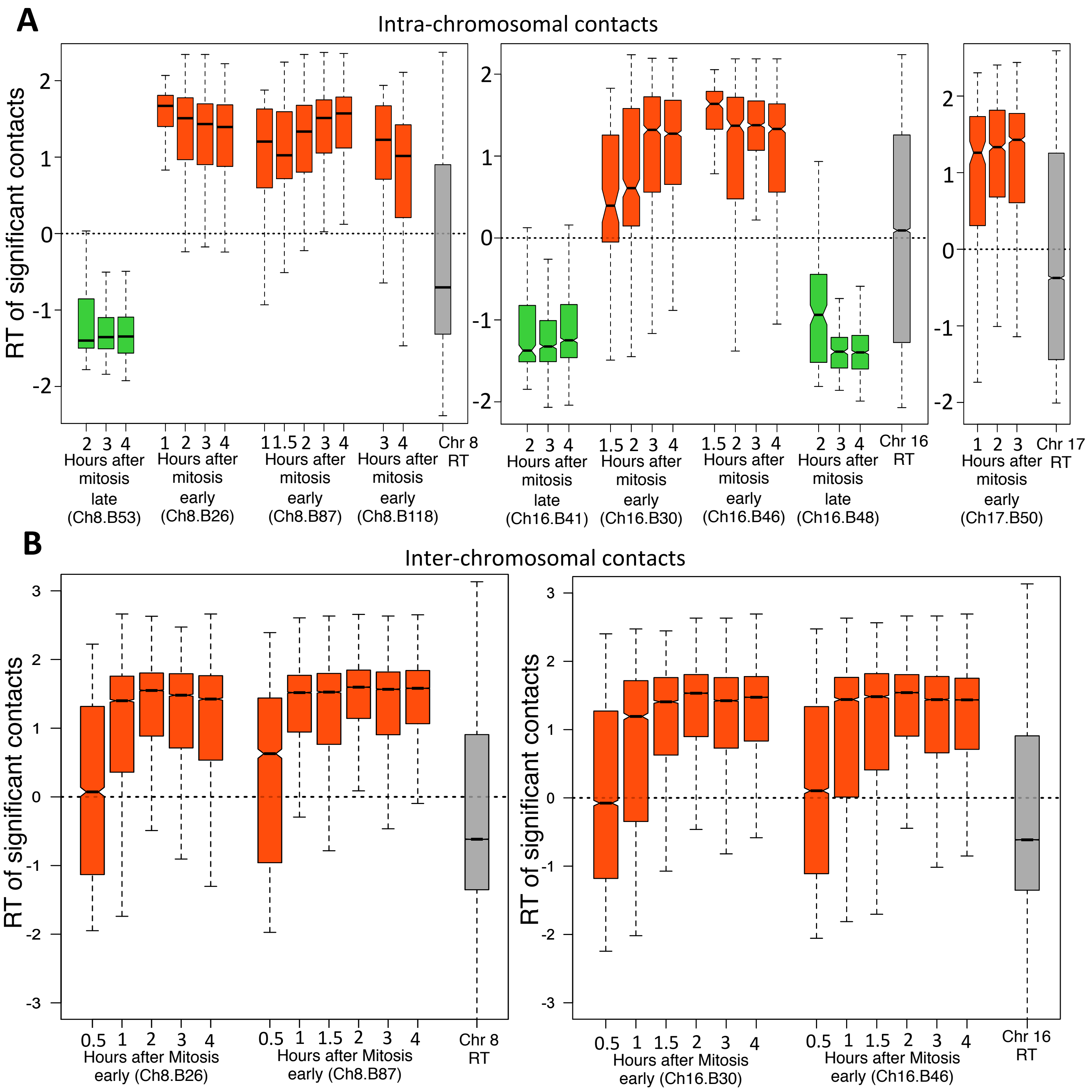

A
Ch8.B7 (Constitutive late)
Ch8.B26 (Constitutive early)
Ch8.B53 (Constitutive late)
Ch8.B87 (Constitutive early)
Contact count
p-values
400
200
0
4
2
0
20
40
60
80
100
120
Chromosome 8 (Mb)


Ch8.B44 (Developmental late)
Ch8.B118 (Developmental early)
Contact count
p-values
400
200
0
4
2
0
20
40
60
80
100
120
Chromosome 8 (Mb)
Ch16.B30 (Constitutive early)
Ch16.B41 (Constitutive late)
Chromosome 16 (Mb)
10

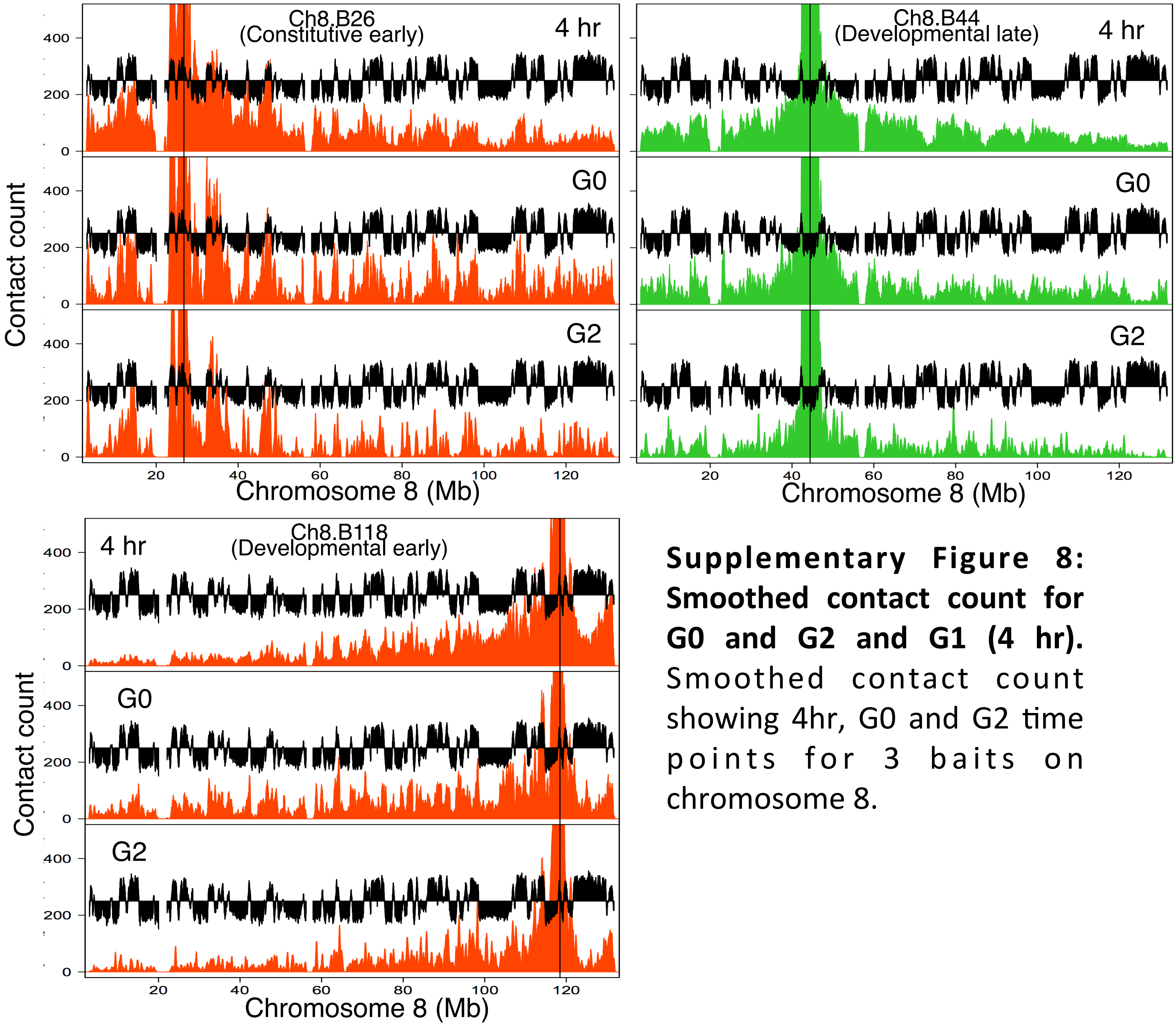

Ch16.B46 (Developmental early)
Ch16.B48 (Developmental late)
Contact count
p-values
400
200
0
4
2
0
20
40
60
80
Chromosome 16 (Mb)
Ch17.B50 (Developmental early)
Ch17.B76 (Constitutivel late)
Chromosome 17 (Mb)



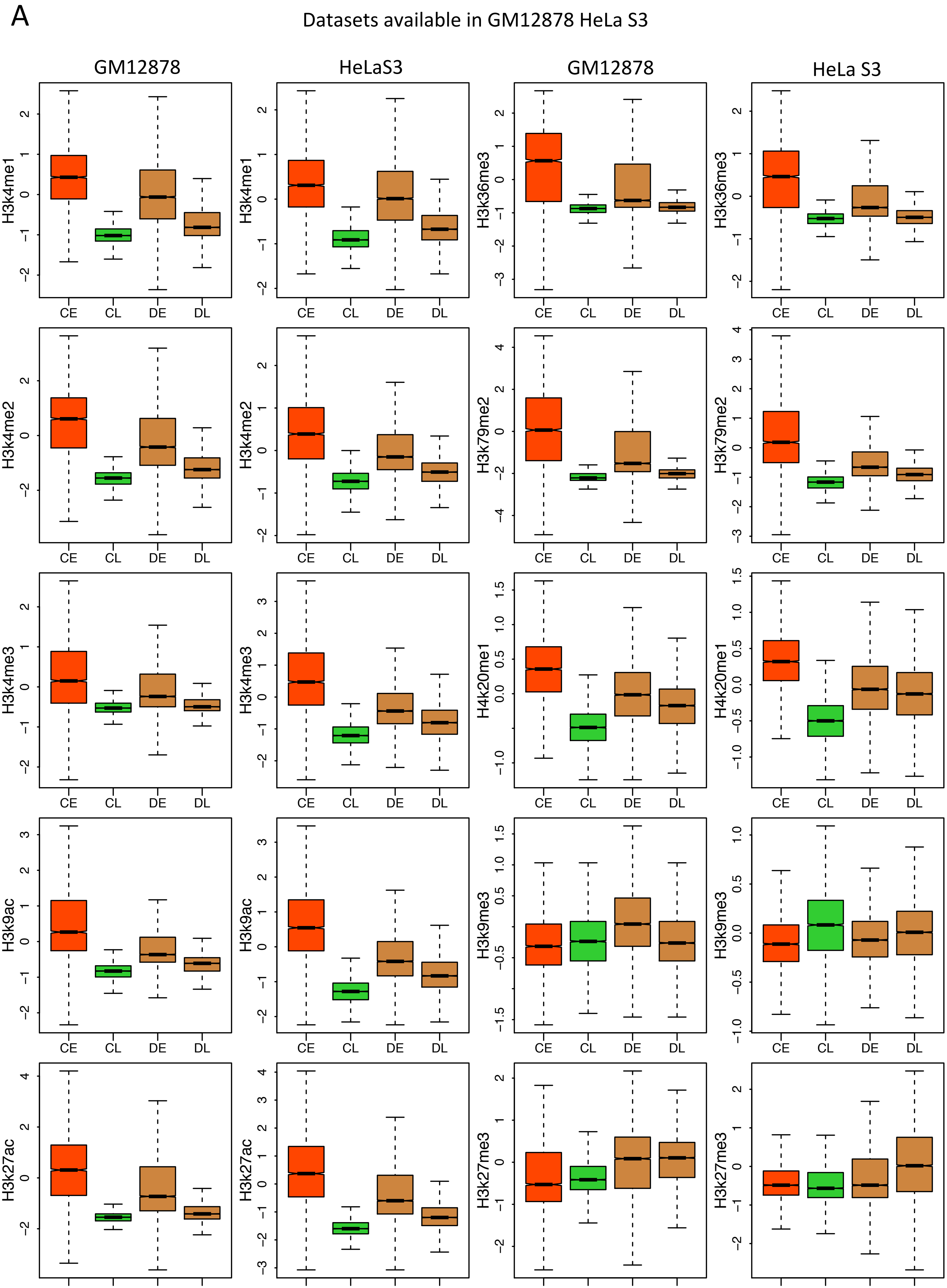
Supplementary Figure 6: Smoothed contact count and p-values for all 12 baits. A) Smoothed contact count and –log10(p-values) are shown for all 12 baits across all time-points analyzed. For contact count plots, early and late baits are shown by red and green colors respectively. For p-value plots, red and green indicates interaction with early and late replicating loci respectively **B)** Distribution of RT values for the top 5 percentile of contacts for all baits. Positive values of RT indicate early replication and negative values of RT indicate late replication. For reference, the RT distribution of all HindIII loci for the respective chromosome is shown in grey.



Supplementary Figure 7: Replication timing of significant contacts. A) Replication timing of the FDR corrected significant intra-chromosomal contacts (FDR 0.1) for bait/time-points with at least 100 significant contacts. There were less than 100 significant post-TDP contacts for Ch8.B7, Ch8.B44 and Ch17.B76 **B)** Replication timing of the FDR corrected significant inter-chromosomal contacts (FDR 0.1). Significance calculation is described in the methods section. Baits that had low number of post-TDP significant contacts (<10000 genome wide) are not shown.



Supplementary Figure 8: Smoothed contact count for G0 and G2 and G1 (4 hr). Smoothed contact count showing 4hr, G0 and G2 time points for 3 baits on chromosome 8.


A
Datasets available in GM12878 HeLa S3
GM12878
HeLaS3
GM12878
HeLa S3
H3k4me1
H3k4me1
H3k36me3
H3k36me3
H3k4me2
H3k4me2
H3k79me2
H3k79me2
H3k4me3
H3k4me3
H4k20me1
H4k20me1
H3k9ac
H3k9ac
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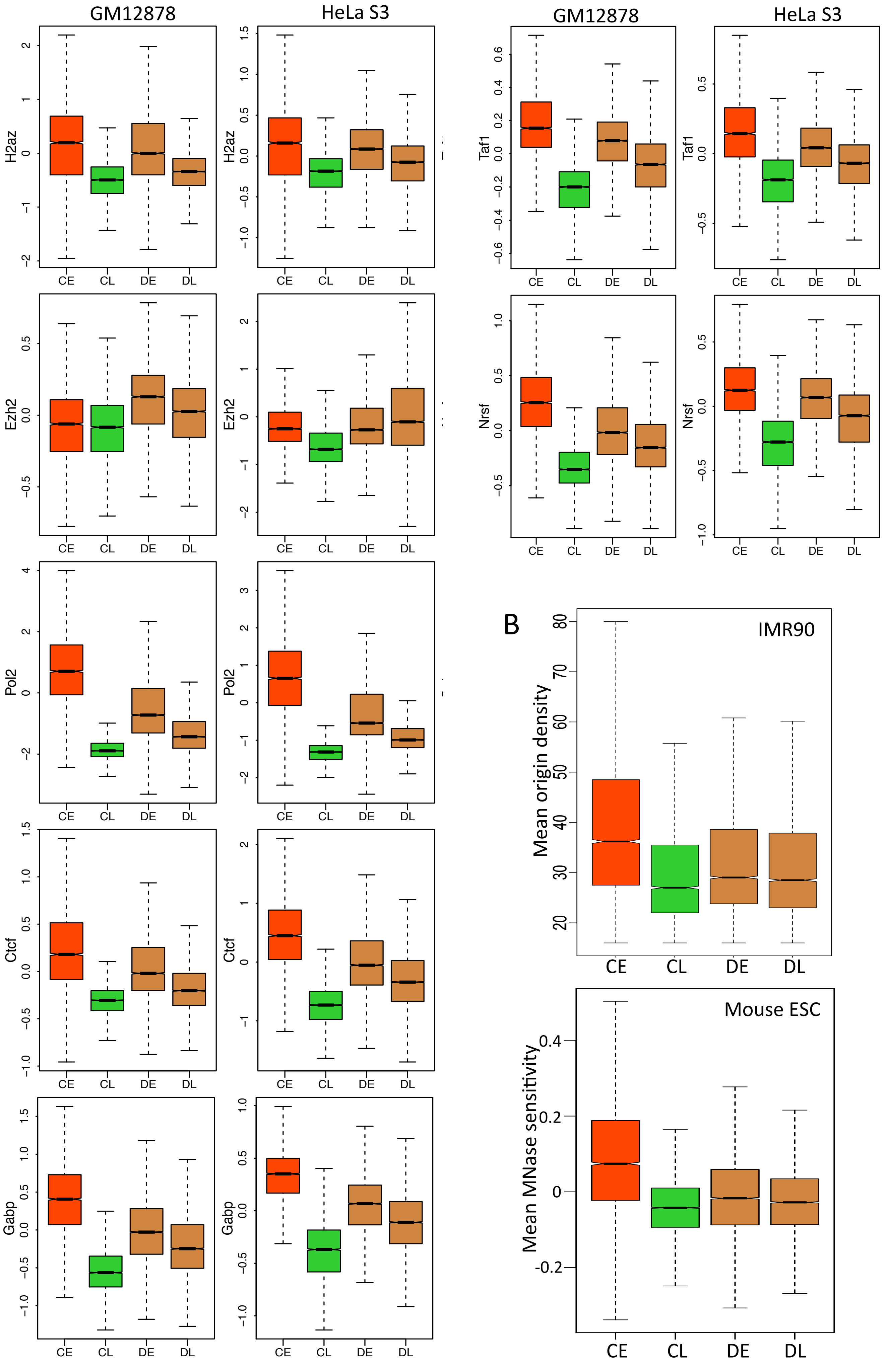
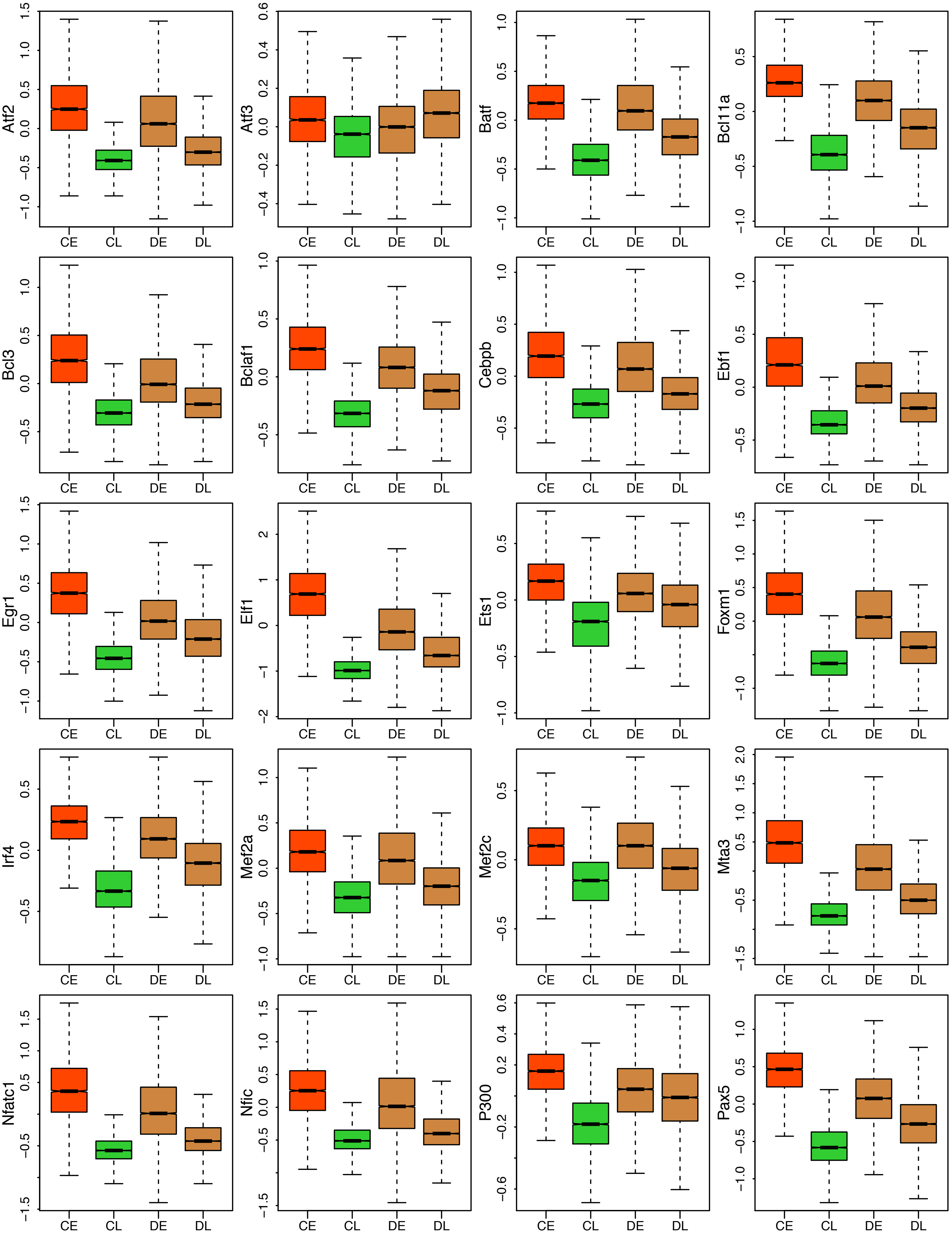
H3k27me3
CE
CL
DE
DL

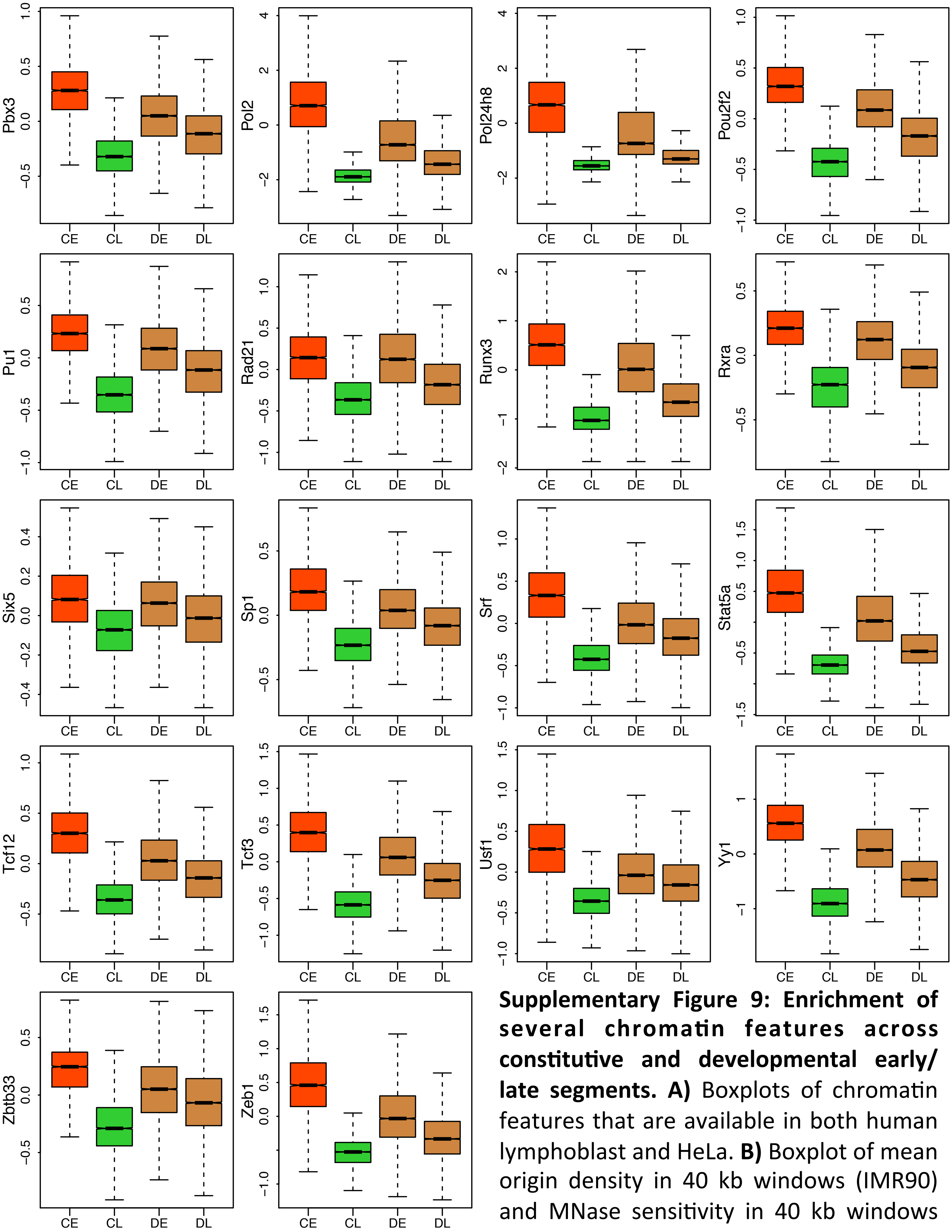

GM12878
HeLa S3
GM12878
HeLa S3
H2az
H2az
Taf1
Taf1
Ezh2
Ezh2
Nrsf
Nrsf
Pol2
Pol2
Ctcf
Ctcf
Gabp
Gabp
CE
CL
DE
DL
B
IMR90
Mean origin density
20
30
40
50
60
70
80
Mouse ESC
Mean MNase sensitivity
-0.2
0
0.2
0.4

C

Datasets available only in GM12878



Datasets available only in GM12878



Supplementary Figure 9: Enrichment of several chromatin features across constitutive and developmental early/late segments. A) Boxplots of chromatin features that are available in both human lymphoblast and HeLa. **B)** Boxplot of mean origin density in 40 kb windows (IMR90) and MNase sensitivity in 40 kb windows (mouse ESC). **C)** Boxplots of chromatin features that are available only in human lymphoblast.

Bait Name/ category	CHR	Position (mm9)	Sequence
Ch8.B7 Constitutive late replicating	Chr8	7223950	F:5’AATGATACGGCGACCACCGAACACTCTTTCCCTACACGACGCTCTTCCGATCTTATTAGGACAGACAGAAGCTT R:5’CAAGCAGAAGACGGCATACGAAGAAGAAAGCAATCACTCAC
Ch8.B53 Constitutive late replicating	Chr8	52961959	F:5’AATGATACGGCGACCACCGAACACTCTTTCCCTACACGACGCTCTTCCGATCTAAACATGTTTGGCCAAGCTT R:5’CAAGCAGAAGACGGCATACGATGTCCAGTGAAGATTTGCTCTT
Ch8.B26 Constitutive early replicating	Chr8	26774313	F:5’AATGATACGGCGACCACCGAACACTCTTTCCCTACACGACGCTCTTCCGATCTTTGAGTTTGACGCCAAGCTT R:5’CAAGCAGAAGACGGCATACGACTTTTCGCCCAGAGTAAACG
Ch8.B87 Constitutive early replicating	Chr8	87434064	F:5’AATGATACGGCGACCACCGAACACTCTTTCCCTACACGACGCTCTTCCGATCTGGGCGGAGATGGAAGCTT R:5’CAAGCAGAAGACGGCATACGATTGGCAAAGCTCTGGAAGGT
Ch8.B44 Developmental (late replicating in C127)	Chr8	44417335	F:5’AATGATACGGCGACCACCGAACACTCTTTCCCTACACGACGCTCTTCCGATCTGGTCATCCCTTTAGAAGCTT R:5’CAAGCAGAAGACGGCATACGATAAGTTCAATTCCCAGCAAC
Ch8.B118 Developmental (early replicating in C127)	Chr8	118459309	F:5’AATGATACGGCGACCACCGAACACTCTTTCCCTACACGACGCTCTTCCGATCTCCTCATACAGCTGGAAGCTT R:5’CAAGCAGAAGACGGCATACGAATGGCTAAACCCCACTCTCT
Ch17.B76 Constitutive late replicating	Chr17	76305544	F:5’AATGATACGGCGACCACCGAACACTCTTTCCCTACACGACGCTCTTCCGATCTGGTCATCCCTTTAGAAGCTT R:5’CAAGCAGAAGACGGCATACGATAAGTTCAATTCCCAGCAAC
Ch17.B50 Developmental (early replicating in C127)	Chr17	49968658	F:5’AATGATACGGCGACCACCGAACACTCTTTCCCTACACGACGCTCTTCCGATCTCCTCATACAGCTGGAAGCTT R:5’CAAGCAGAAGACGGCATACGAATGGCTAAACCCCACTCTCT
Ch16.B30 Constitutive early replicating	Chr16	30595243	F:5’AATGATACGGCGACCACCGAACACTCTTTCCCTACACGACGCTCTTCCGATCTCGGAAACTCAGCGGTAAAGC R:5”CAAGCAGAAGACGGCATACGATTAGGCCCTGAATGTTGGTC
Ch16.B41 Constitutive late replicating	Chr16	40919975	F:5’AATGATACGGCGACCACCGAACACTCTTTCCCTACACGACGCTCTTCCGATCTAAGGCACAGGTAGCCAAGC R:5’CAAGCAGAAGACGGCATACGAAGGCTCCTATCAGCAAGCAC
Ch16.B46 Constitutive early replicating	Chr16	45838128	F:5’AATGATACGGCGACCACCGAACACTCTTTCCCTACACGACGCTCTTCCGATCTGCTACCTAACTGGGCAAAGC R:5’CAAGCAGAAGACGGCATACGATGTCCTGCTTCCTGTGTGAG
Ch16.B48 Developmental (late replicating in C127)	Chr16	48602104	F:5’AATGATACGGCGACCACCGAACACTCTTTCCCTACACGACGCTCTTCCGATCTGGCTGTAGCTTAGGGAAGC R:5’CAAGCAGAAGACGGCATACGAGGGGTCTCATAAGCTGTGGA

Supplementary Table 1: 4C-seq forward and reverse primers used for Illumina sequencing.

Sample	Mapped reads	Sample	Mapped reads	Sample	Mapped reads	Sample	Mapped reads
Ch17.B76.PM.3hrs.bio.replicate1	1525018	Ch8.B26.G0.bio.replicate2	3422887	Ch8.B26.PM.4hrs.bio.replicate1	2179299	Ch16.B41.PM.3hrs.bio.replicate1	283213
Ch17.B76.PM.30mins.bio.replicate1	6722592	Ch8.B44.G0.bio.replicate1	3060249	Ch8.B118.G0.bio.replicate2	943564	Ch17.B50.PM.3hrs.bio.replicate1	1506835
Ch8.B118.PM.4hrs.bio.replicate1	3444712	Ch16.B30.PM.30mins.bio.replicate1	5556148	Ch8.B44.PM.3hrs.bio.replicate1	1876253	Ch17.B76.PM.1hr.bio.replicate1	1412223
Ch8.B44.PM.2hrs.tech.replicate2	2878582	Ch16.B48.PM.1.5hrs.bio.replicate1	3946062	Ch8.B118.PM.1hr.bio.replicate2	719854	Ch16.B48.PM.1hr.bio.replicate1	1381420
Ch8.B44.PM.2hrs.tech.replicate1	1361387	Ch17.B50.PM.2hrs.bio.replicate1	3840074	Ch8.B118.PM.1hr.bio.replicate1	266006	Ch16.B30.PM.2hrs.bio.replicate2	1138984
Ch16.B46.PM.2hrs.bio.replicate1	5780630	Ch8.B118.PM.1.5hrs.bio.replicate1	3665663	Ch16.B46.PM.3hrs.bio.replicate1	7831923	Ch8.B44.PM.4hrs.bio.replicate1	1061114
Ch16.B46.PM.2hrs.bio.replicate2	1218419	Ch16.B41.PM.4hrs.bio.replicate1	2266020	Ch8.B53.PM.1hr.bio.replicate2	4868171	Ch16.B30.PM.2hrs.bio.replicate1	1265421
Ch8.B44.PM.30mins.bio.replicate2	3626764	Ch8.B26.PM.2hrs.tech.replicate2	531754	Ch8.B53.PM.1hr.bio.replicate1	7215800	Ch8.B118.PM.2hrs.tech.replicate1	1432325
Ch8.B7.PM.3hrs.bio.replicate1	2007587	Ch8.B26.PM.2hrs.tech.replicate1	1255171	Ch8.B7.G2.bio.replicate1	4535650	Ch8.B118.PM.2hrs.tech.replicate2	1460428
Ch8.B44.PM.30mins.bio.replicate1	1921831	Ch16.B46.PM.30mins.bio.replicate1	1697496	Ch8.B87.PM.4hrs.bio.replicate1	6219399	Ch8.B53.PM.1.5hrs.bio.replicate1	1310109
Ch16.B30.PM.4hrs.bio.replicate1	2615498	Ch8.B118.PM.30mins.bio.replicate1	8826711	Ch8.B118.G2.bio.replicate1	5921514	Ch16.B41.PM.1hr.bio.replicate1	1181384
Ch8.B7.M.0hr.bio.replicate1	383908	Ch8.B118.PM.30mins.bio.replicate2	4032833	Ch8.B7.G0.bio.replicate2	6614698	Ch8.B87.PM.2hrs.tech.replicate2	914363
Ch8.B7.PM.2hrs.tech.replicate1	1371401	Ch16.B41.PM.2hrs.bio.replicate1	6373010	Ch8.B26.G0.bio.replicate1	5097919	Ch8.B87.PM.2hrs.tech.replicate1	1598251
Ch8.B7.PM.2hrs.tech.replicate2	4912157	Ch16.B48.PM.30mins.bio.replicate1	2992780	Ch17.B50.PM.1hr.bio.replicate1	2733464	Ch8.B53.G2.bio.replicate1	2749821
Ch16.B46.PM.1.5hrs.bio.replicate1	4627368	Ch16.B41.PM.2hrs.bio.replicate2	6987700	Ch8.B53.PM.4hrs.bio.replicate1	2163015	Ch16.B30.PM.3hrs.bio.replicate1	2515052
Ch8.B53.PM.2hrs.tech.replicate2	1370094	Ch8.B87.PM.1.5hrs.bio.replicate1	3470068	Ch16.B46.PM.1hr.bio.replicate1	1073125	Ch8.B7.PM.4hrs.bio.replicate1	2771825
Ch8.B53.M.0hr.bio.replicate1	3246749	Ch8.B53.PM.30mins.bio.replicate2	4198215	Ch8.B118.PM.3hrs.bio.replicate1	244438	Ch8.B44.PM.1hr.bio.replicate2	3183404
Ch8.B53.PM.2hrs.tech.replicate1	5293681	Ch8.B53.PM.30mins.bio.replicate1	6110678	Ch16.B46.PM.4hrs.bio.replicate1	278667	Ch8.B44.PM.1hr.bio.replicate1	2813671
Ch16.B48.PM.2hrs.bio.replicate2	1260841	Ch8.B7.PM.1hr.bio.replicate1	2425567	Ch8.B7.PM.30mins.bio.replicate1	186019	Ch8.B44.G0.bio.replicate2	2027030
Ch16.B48.PM.2hrs.bio.replicate1	2691084	Ch8.B87.PM.1hr.bio.replicate1	803137	Ch8.B7.PM.30mins.bio.replicate2	182669	Ch16.B30.PM.1.5hrs.bio.replicate1	3281061
Ch8.B26.G2.bio.replicate1	678688	Ch8.B87.PM.1hr.bio.replicate2	468921	Ch8.B53.G0.bio.replicate2	203016	Ch8.B87.PM.3hrs.bio.replicate1	3301246
Ch8.B53.PM.3hrs.bio.replicate1	2502399	Ch8.B7.PM.1hr.bio.replicate2	3770333	Ch8.B87.PM.30mins.bio.replicate2	289399	Ch16.B30.PM.1hr.bio.replicate1	2820728
Ch8.B118.G0.bio.replicate1	2385700	Ch17.B76.PM.2hrs.bio.replicate1	3644303	Ch8.B87.G0.bio.replicate1	223239	Ch16.B41.PM.1.5hrs.bio.replicate1	3081112
Ch8.B26.PM.3hrs.bio.replicate1	809466	Ch8.B26.PM.30mins.bio.replicate2	1448846	Ch8.B87.PM.30mins.bio.replicate1	273020	Ch16.B48.PM.4hrs.bio.replicate1	2898355
Ch16.B41.PM.30mins.bio.replicate1	1540182	Ch8.B26.PM.30mins.bio.replicate1	3860921	Ch8.B26.M.0hr.bio.replicate1	268806	Ch8.B53.G0.bio.replicate1	2765889
Ch8.B87.G2.bio.replicate1	7268391	Ch8.B44.G2.bio.replicate1	2977454	Ch8.B87.G0.bio.replicate2	191295	Ch8.B26.PM.1hr.bio.replicate2	2241038
Ch16.B48.PM.3hrs.bio.replicate1	3752253	Ch17.B50.PM.30mins.bio.replicate1	1420425	Ch8.B7.G0.bio.replicate1	185000	Ch8.B26.PM.1hr.bio.replicate1	2637584
						Ch8.B44.M.0hr.bio.replicate1	3024047
						Sum	291093963

Supplementary Table 2: Number of mapped reads for all bait and time point combinations.

Dataset	Figure	Reference	GEO Accession
Hi-C data	Fig 5B, 5C, 5D	Rao SSP, Huntley MH, Durand NC, Stamenova EK, Bochkov ID, Robinson JT, Sanborn AL, Machol I, Omer AD, Lander ES, et al. 2014. A 3D Map of the Human Genome at Kilobase Resolution Reveals Principles of Chromatin Looping. *Cell* **159**: 1665–1680.	GSE63525
Human fibroblast (Tig3) LaminB damID	Fig 6A, 6B	Guelen, L., Pagie, L., Brasset, E., Meuleman, W., Faza, M., Talhout, W., … van Steensel, B. (2008). Domain organization of human chromosomes revealed by mapping of nuclear lamina interactions. Nature, 453(7197), 948–951	GSE8854
Mouse astrocyte LaminB damID	Fig 6A, 6B	Peric-Hupkes, D., Meuleman, W., Pagie, L., Bruggeman, S., Solovei, I., Brugman, W., … van Steensel, B. (2010). Molecular maps of the reorganization of genome-nuclear lamina interactions during differentiation. *Molecular Cell*, *38*(4), 603–613.	GSE36132
IMR90 origin mapping	Fig 6A, Supplementary Fig 9	Besnard, E., Babled, A., Lapasset, L., Milhavet, O., Parrinello, H., Dantec, C., … Lemaitre, J.-M. (2012). Unraveling cell type-specific and reprogrammable human replication origin signatures associated with G-quadruplex consensus motifs. *Nature Structural & Molecular Biology*, *19*(8), 837–844.	GSE37757
Segway/ChromHMM segmentation labels for HeLa and Human Lymphoblast (GM12878)	Fig 6D	Hoffman, M. M., Ernst, J., Wilder, S. P., Kundaje, A., Harris, R. S., Libbrecht, M., … Noble, W. S. (2013). Integrative annotation of chromatin elements from ENCODE data. *Nucleic Acids Research*, *41*(2), 827–41.	
ChIP-seq	Fig 6A, Supplementary Fig 9	ENCODE	
Replication timing data, MNase sensitivity	Fig 6A, Supplementary Fig 9	www.replicationdomain.org	

Supplementary Table 3: Public datasets analyzed for genome-wide analyses.

Trans-acting Factor	**Constitutive early**	**Constitutive late**	**Developmental early**	**Developmental late**	**No:**
Atf2, Batf, Bcl11a, Bcl3, Bclaf-1, Cebpb, Ctcf, Ebf1, Egr1, Elf1, Ets1, Foxm1, Gabp, Irf4, Mef2a, Mef2c, Mta3, Nfatc1, Nfic, Nrsf, P300, Pax5, Pbx3, Pol2, Pol24h8, Pou2f2, Pu1, Rad21, Runx3, Rxra, Six5, Sp1, Srf, Stat5a, Taf1, Tcf12, Tcf3, Usf1, Yy1, Zbtb33, Zeb1	Enriched	Depleted	Moderately Enriched	Moderately Depleted	42
Atf3, Ezh2	No significant enrichment				2

Chromatin marks	**Constitutive early**	**Constitutive late**	**Developmental early**	**Developmental late**	**No:**
H2az, H3k27ac, H3k4me1, H3k4me2, H3k9ac, H4k20me1	Enriched	Depleted	Moderately Enriched	Moderately Depleted	6
H3k36me3	Enriched	Depleted	Moderately Enriched	Depleted	1
H3k27me3, H3k9me3	No significant enrichment				2
H3k4me3, H3k79me2	Different between Gm12878 and HelaS3				2

Supplementary Table 4: Enrichment of trans-acting factors and histone marks shown in Supplementary Figure 8 across constitutive early/late and developmental early/late segments of the genome.