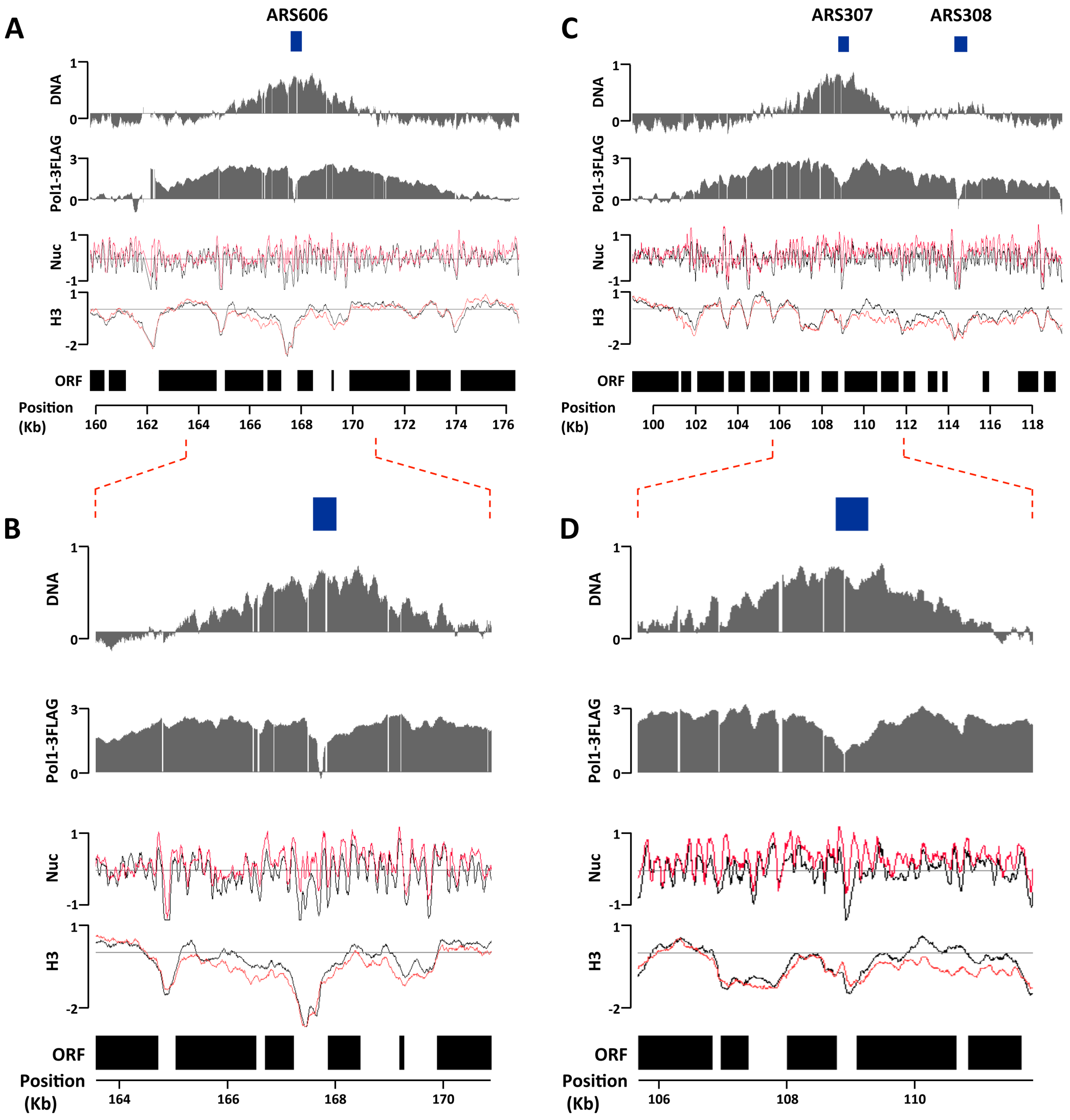
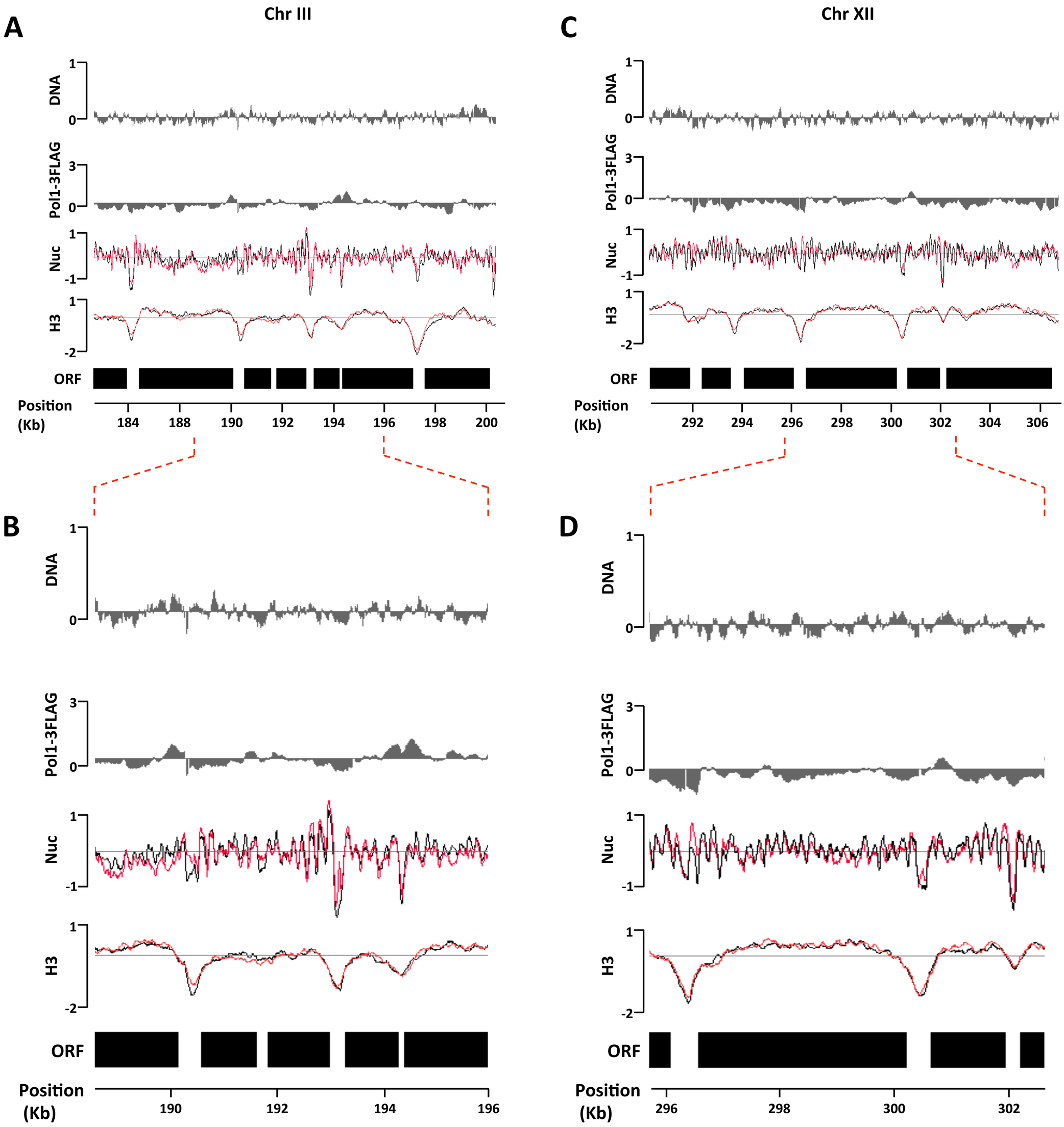
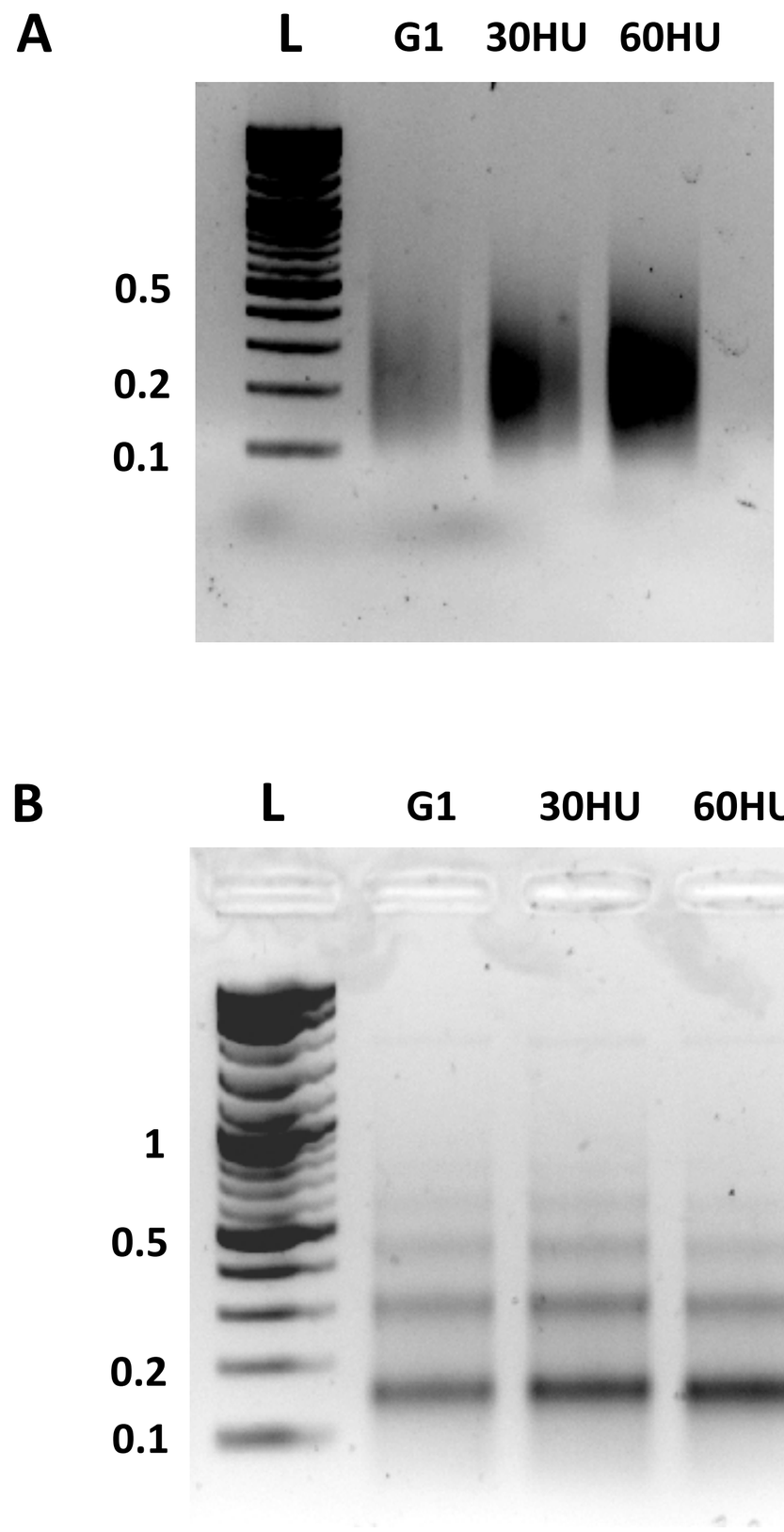


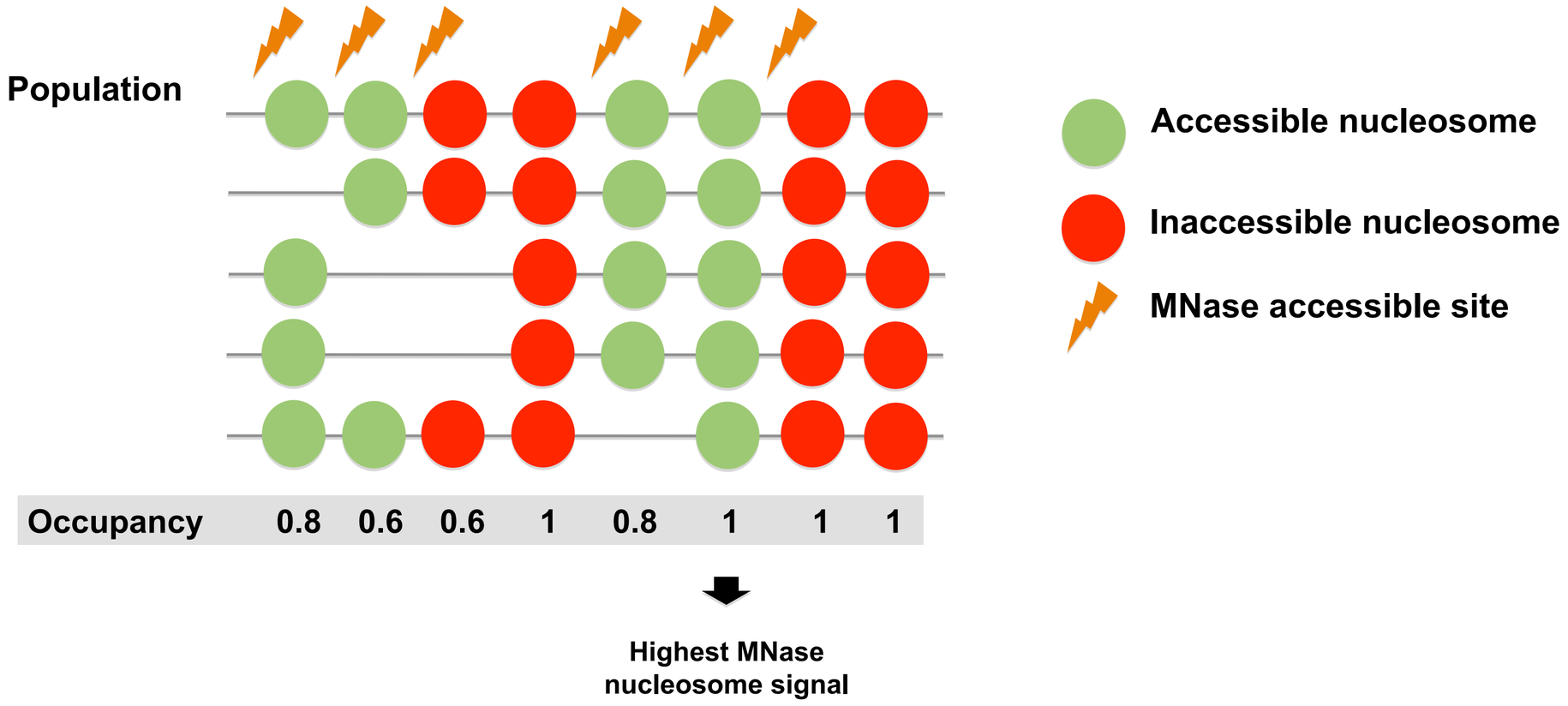
Supplemental Figure S1. Chromatin changes at replicating genomic locations in the presence of replication stress. G1 arrested cells were released into S phase in media containing 200 mM HU. (A) map shows 16 Kb region around early ORI ARS606 on right arm of chromosome VI. DNA profiles (DNA) and Pol 1 ChIP (Pol1-3FLAG) data correspond to 30' post release into S phase. MNase nucleosome signal (Nuc), and histone H3 ChIP (H3) data from G1 arrested cells (black line) and 30' HU cells (red line). (B) magnification of the region around ARS606 approximately spanning 6.5 Kb. (C) same as (A) showing 18 Kb around the early ARS307 around the centromeric region on chromosome III. (D) magnification of the region around ARS307 approximately spanning 6 Kb.



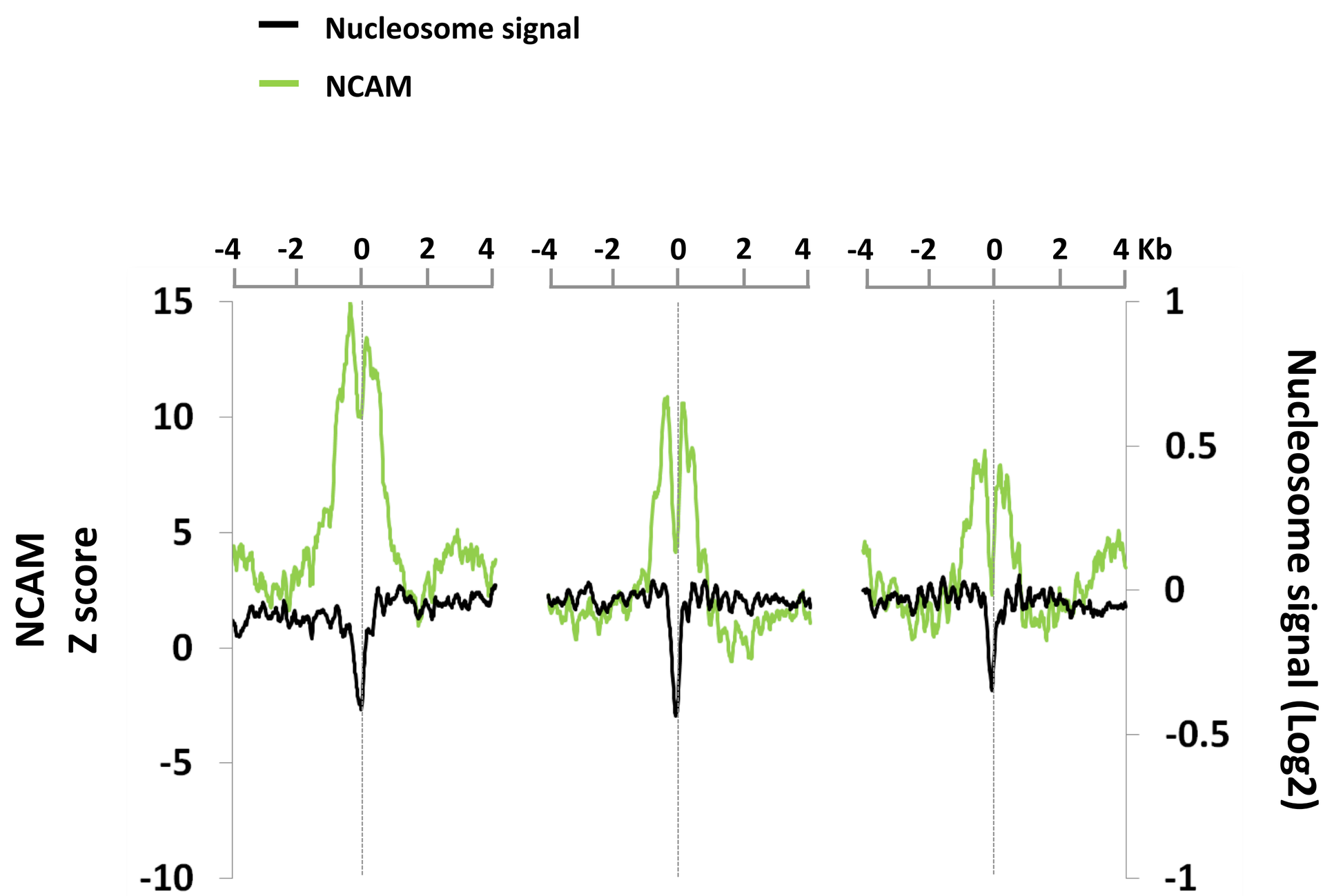
Supplemental Figure S2. Chromatin structure at non-replicating genomic locations in the presence of replication stress. G1 arrested cells were released into S phase in media containing 200 mM HU. (A) map shows 17 Kb of a non-replicating region on the right arm of chromosome III. DNA profiles (DNA) and Pol 1 ChIP (Pol1-3FLAG) data correspond to 60' post release into S phase. MNase nucleosome signal (Nuc), and histone H3 ChIP (H3) data from G1 arrested cells (black line) and 60' S phase cells (red line). (B) magnification of the chromosome III region approximately spanning 7.5 Kb. (C) same as (A) showing a 16 Kb on the right arm of chromosome XII. (D) magnification of the chromosome XII region approximately spanning 6.5 Kb.



Supplemental Figure S3. Sample preparation. (A) Chromatin was extensively sonicated to an average fragment size of 250 bp prior to immunoprecipitation with specific antibodies. (B) Samples were MNase digested and the mononucleosome DNA fraction (arrowhead) was excised from the gel and purified by electroelution. (C) Isolated mononucleosome DNA ready to fragment and label after dephosphorilation of 5' and 3' ends with CIP. Genomic DNA (D) was routinely fragmented with DNaseI to an average fragment size of 150 bp (E). Molecular size marker (L) is expressed in kilobases.

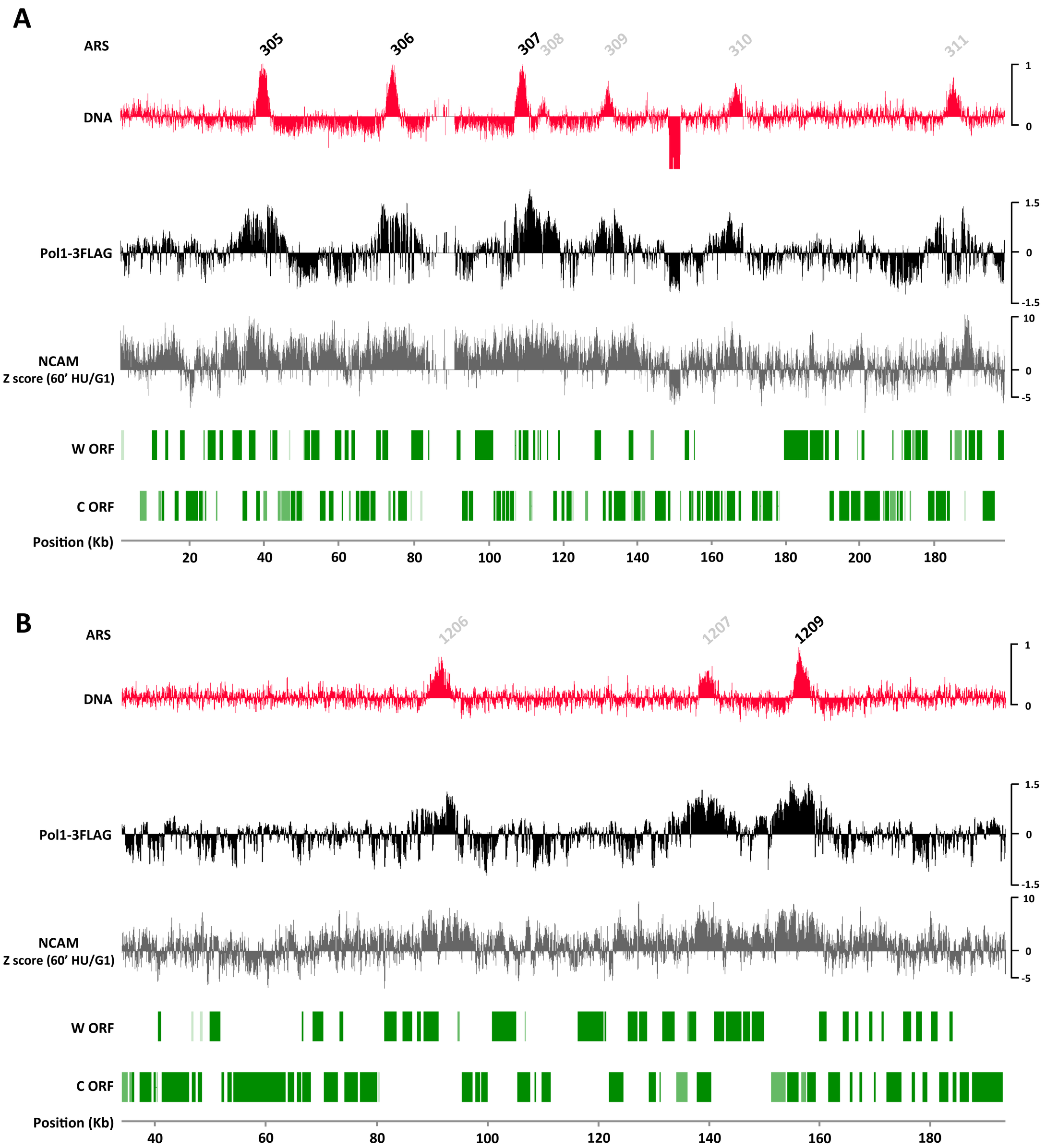


Supplemental Figure S4. MNase nucleosome signal is a function of nucleosome accessibility and occupancy. Diagram represents a given genomic location in 5 different cells within a population. The final amount of DNA recovered from any given nucleosome within the population after non-extensive digestion with MNAse will depend on two factors: first, how many cells have a nucleosome protecting a given DNA sequence (nucleosome occupancy within the population), and second, how accessible the nucleosome is within each of the cells. Therefore, the highest nucleosomal DNA recovery would theoretically correspond to regions 100% occupied by 100% accessible nucleosomes.

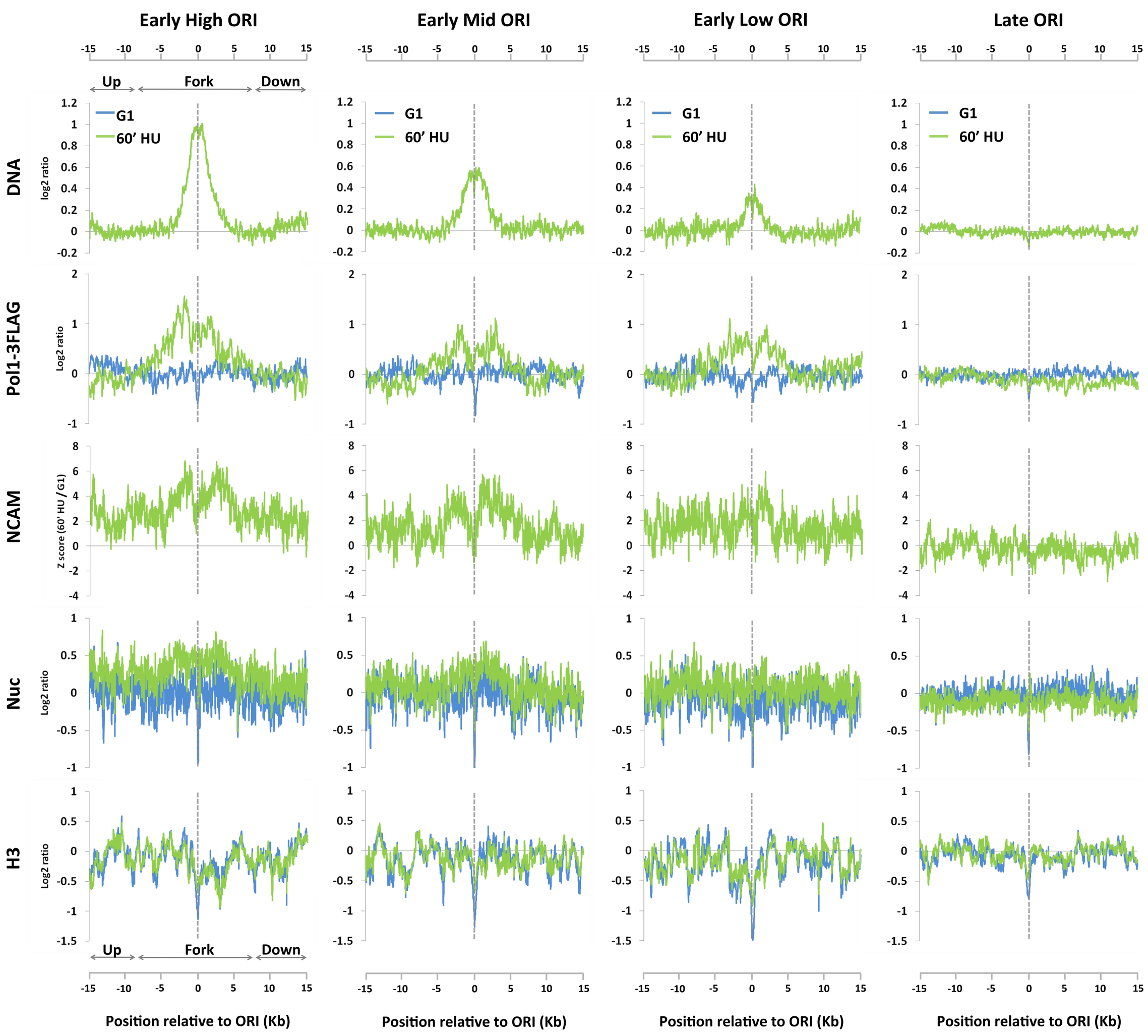


Average Trancription	3.05 ± 1.44 (n=200)	0.7 ± 0.41 (n=400)	-0.26 ± 0.24 (n=170)

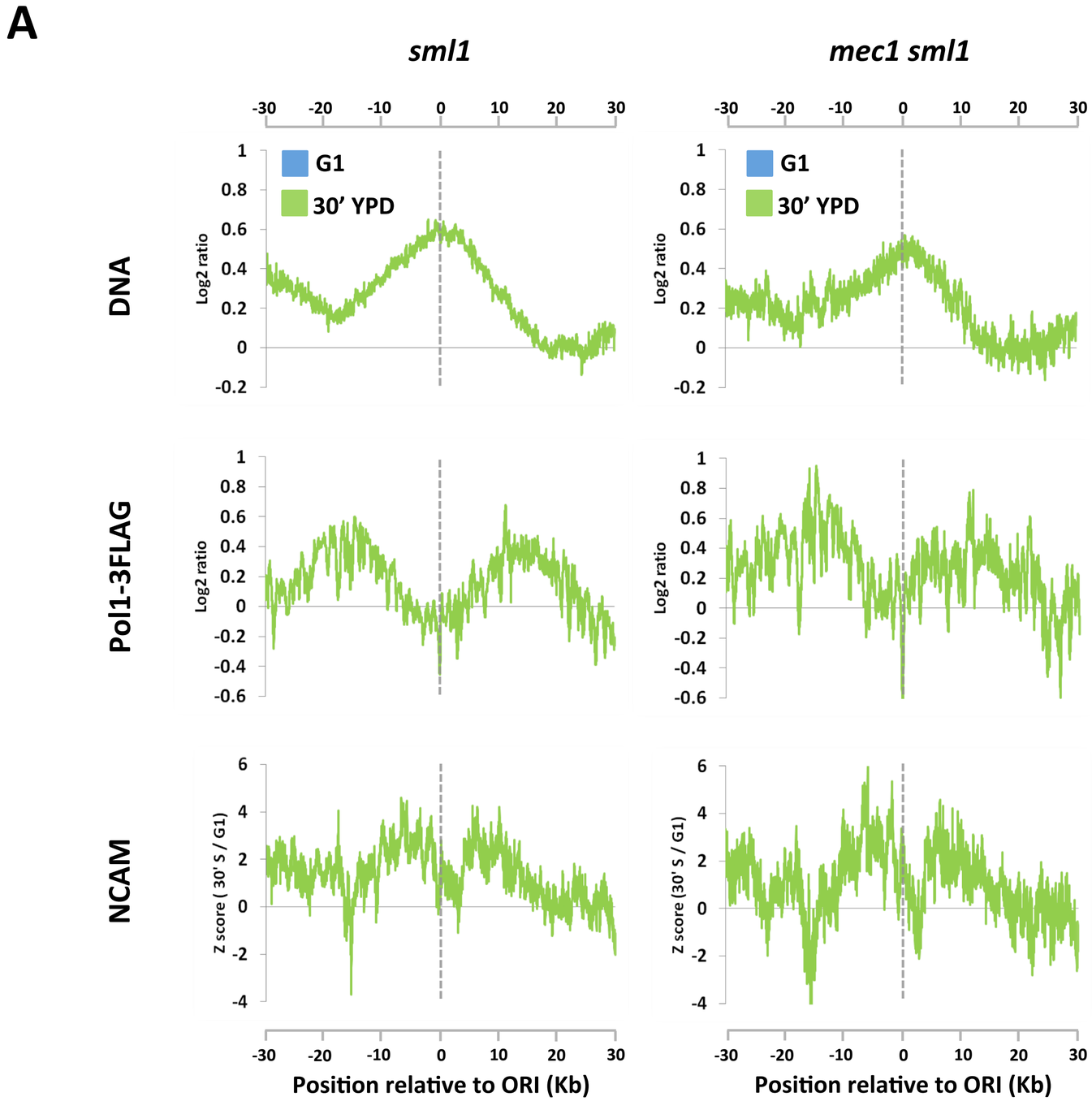
Supplemental Figure S5. Transcription and NCAM correlation. G1 Transcription levels were obtained from 770 genes across chromosomes III, VI and XII. Genes were assigned to three groups based on their transcription levels. Average transcription levels and standard deviation are shown underneath each group. For each group, averaged nucleosome signals (black lines) were aligned at the transcription start site (TSS) (grey dashed lines). The G1 averaged NCAM data (green lines) were also aligned at the TSS for each one of the three groups. The average NCAM was highest in the most highly transcribed group of genes, and lowest in the least transcribed group of genes.

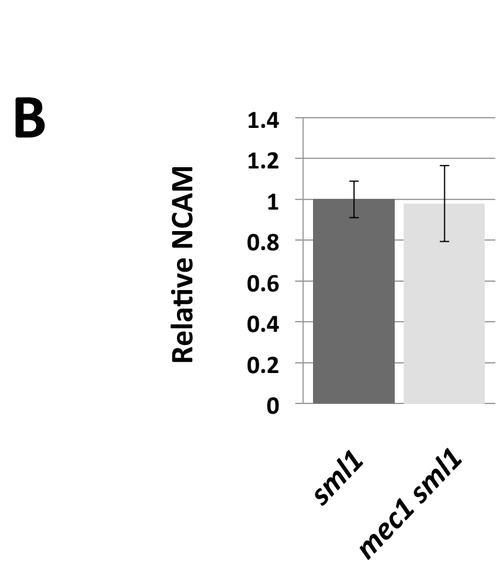


Supplemental Figure S6. Increases in NCAM take place over broad genomic regions around actively replicating ORIs. (A) The screen shot shows a fragment of ~200 Kb of chromosome III starting at its left telomere. The region includes the three efficient ORIs (in black) in chromosome III. Data shown includes DNA profiles (in red), Pol1-3FLAG ChIP (in black) and increases in NCAM (in grey; 60 min HU versus G1) in cells incubated for 60 minutes in 200 mM HU containing media after release from G1 arrest. Regions where NCAM increases in S phase, compared to G1, have positive values. The positions of Watson (W) as well as Crick (C) open reading frames (ORF) are depicted as green boxes. Relative positions are expressed in kilobases (Kb). (B) Same as in (A) for ~160 Kb region in chromosome XII.

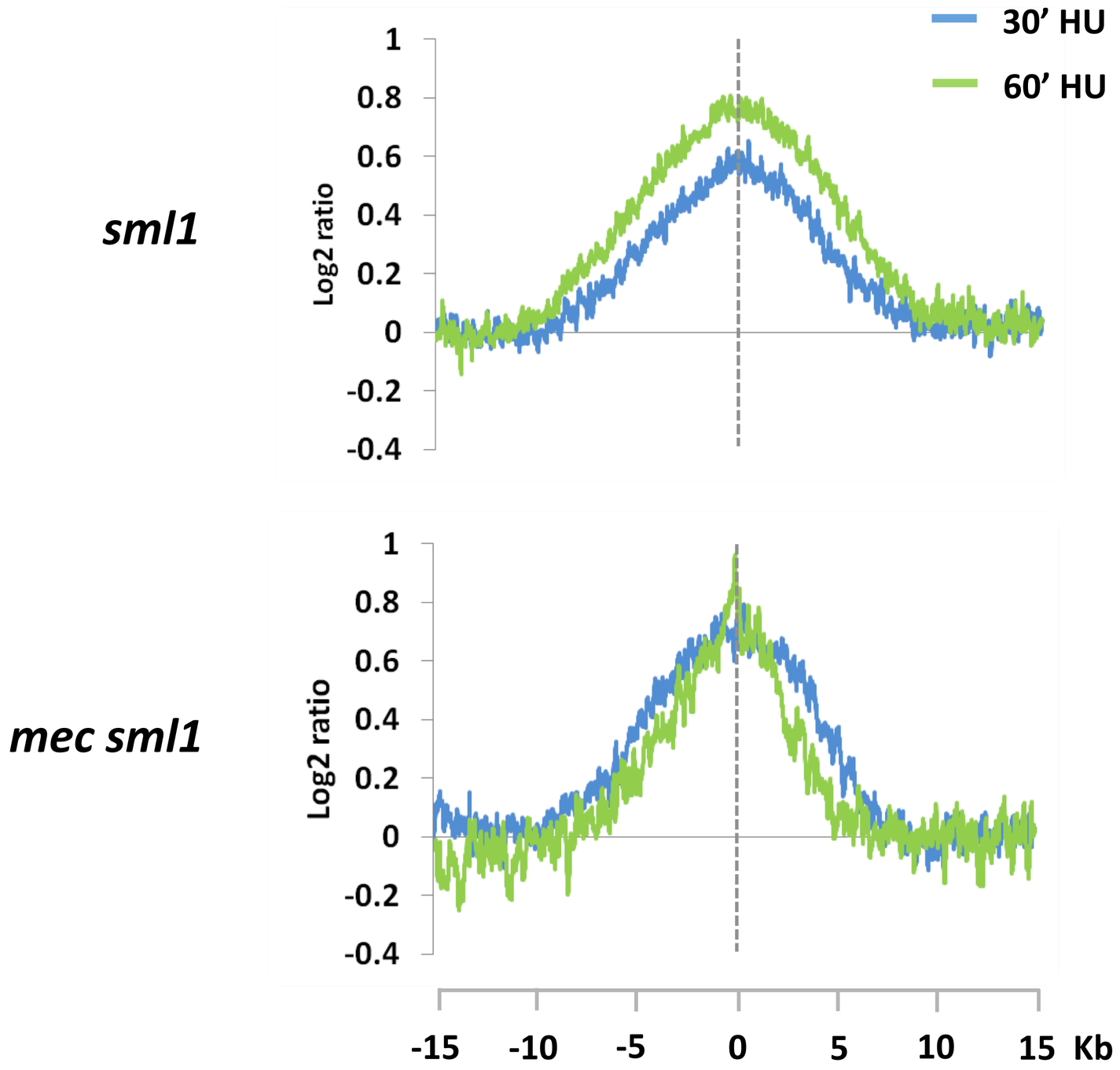


Supplemental Figure S7. Chromatin landscape at HU-stalled replication forks in WT cells. G1 data (in blue) and S phase data (60' in HU, in green) corresponding to DNA profiles (DNA), Pol1 ChIP (Pol1-3FLAG), NCAM difference between S and G1 phases (NCAM), nucleosome mapping (Nuc), and H3 ChIP (H3), averaged at the midpoints of, from left to right columns, early efficient, early mid efficient, early low efficient, and late ORIs. Data sets are aligned at the peak of DNA profiles, which precisely coincide with the previously published ORI midpoints. The Y-axes are on the log2 scale, except for the NCAM panels.

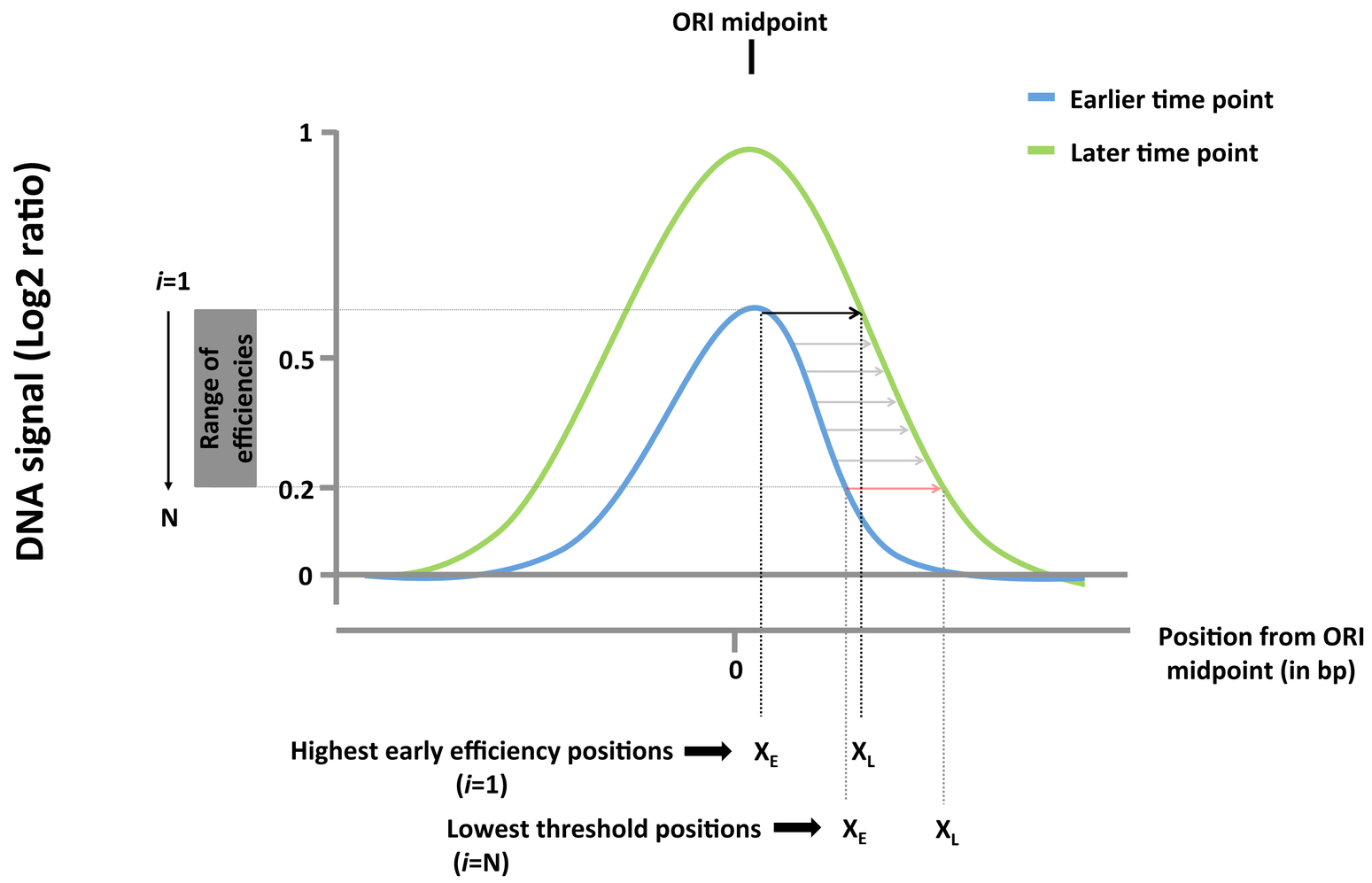




Supplemental Figure S8. No differences in NCAM are detected between *sml1* and *mec1-100 sml1* strains during normal S phase progression. (A) DNA, Pol1-3FLAG and NCAM profiles from cells harvested 30 minutes after alpha factor release into YPD media at room temperature (24°C). (B) Quantification of the relative increase in NCAM data in (A). The average from two independent biological replicates is shown, where the value for sml1 mutant is set to 1. Vertical bars indicate standard error of the mean.



Supplemental Figure S9. DNA degradation occurs in *mec1 sml1* cells in the presence of prolonged replication stress. *sml1* (top) and *mec1 sml1* (bottom) cells were released from G1 arrest into media containing 200 mM HU and harvested at 30 and 60 minutes after release. DNA signal from early efficient ORIs (n=8) was aligned at the ORI midpoints and an average DNA signal was generated for 30 (in blue) and 60 (in green) minutes. In both cell types we detected higher DNA signal at ORI midpoints at 60 minutes compared to 30 minutes, clearly indicating that ORIs continued to fire between the two time points in both strains. However, the width of the DNA signal was reduced from 30 to 60 minutes only in *mec1 sml1* cells, clearly indicating that DNA is being degraded in this strain in the presence of replication stress. This was not the case of *sml1* cells, where the width of the DNA signal increased from 30 to 60 minutes, as we would expect for a strain that is synthesizing DNA and remains stable.

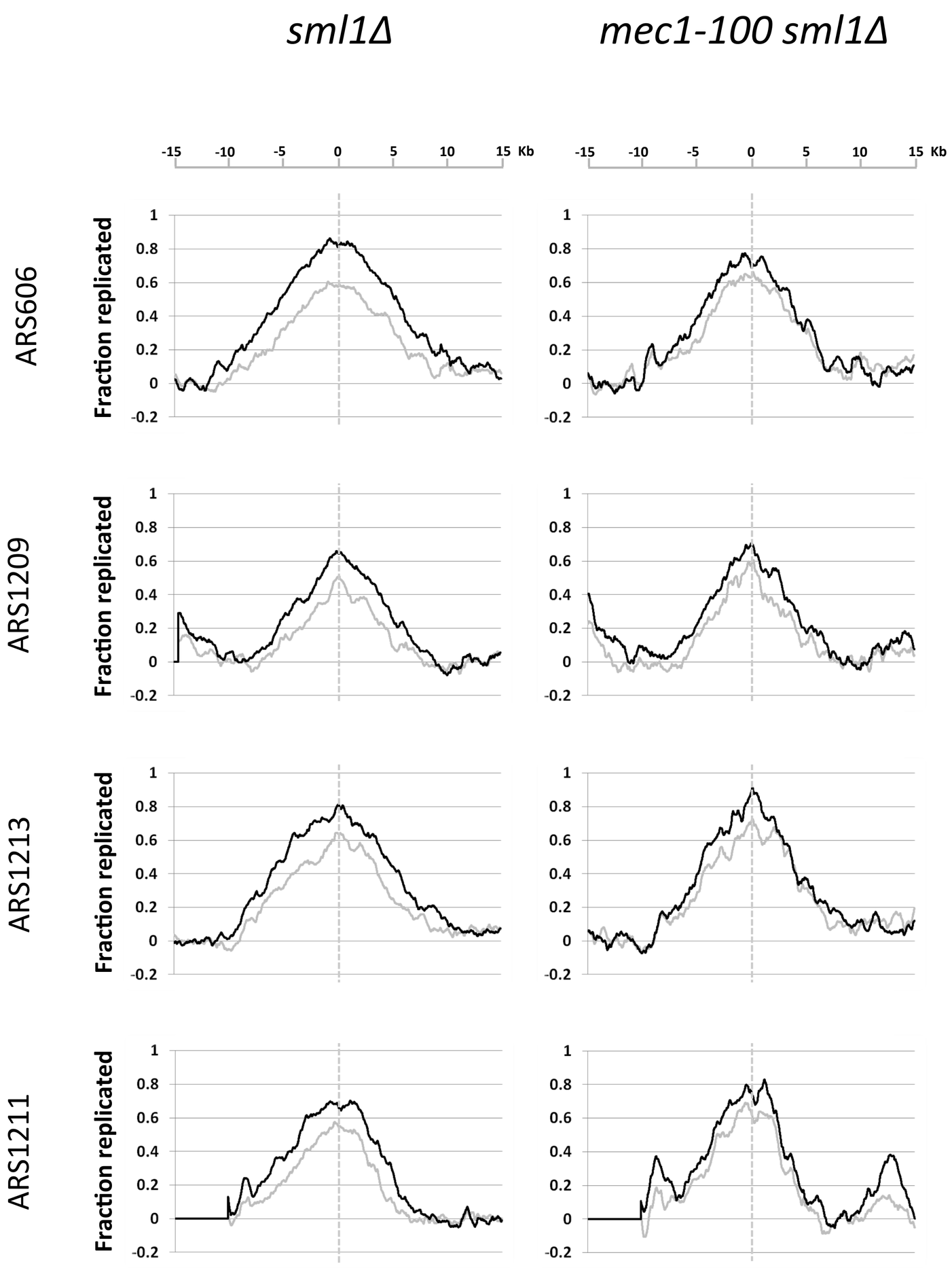


$$\text{Left or right fork rate} = (X_L - X_E)/\,\Delta t$$

(for each efficiency value)

$$\text{Average left or right fork rate} = \frac{\sum_{i=1}^{N} (X_L - X_E)/\,\Delta t}{N}$$

Supplemental Figure S10. Estimation method of replication fork rate. This method can be applied to individual ORIs as well as to averaged signal from multiple ORIs. For each ORI, DNA signals from an early time point (blue distribution) and a later time point (green distribution) are aligned at the ORI midpoint (black box above graph). For an actively replicating ORI, the with of the DNA distribution (arrows) increases with time, as DNA replication proceeds. Thus, fork rate is measured at each independent fork (left and right) as the quotient between the distance in base pairs traveled by the side of the distribution (X_L- X_E) and the elapsed time (Δt), measured at equally efficient data points in both the early and late DNA distributions. This calculation is performed for each data point that falls within a certain range of ORI efficiencies (N, grey box). This range of efficiencies is defined for each ORI (or averaged signal from multiple ORIs) by the highest efficiency of the early time point (upper limit, upper black arrow) and a the lowest threshold (lower limit, lower red arrow). The lowest threshold is defined by the minimal DNA signal that we can confidently detect above the background noise across the different experiments analyzed, which we set at 0.2. The number of points analyzed (N) across experiments was on average 803 (range 538 to 1078).



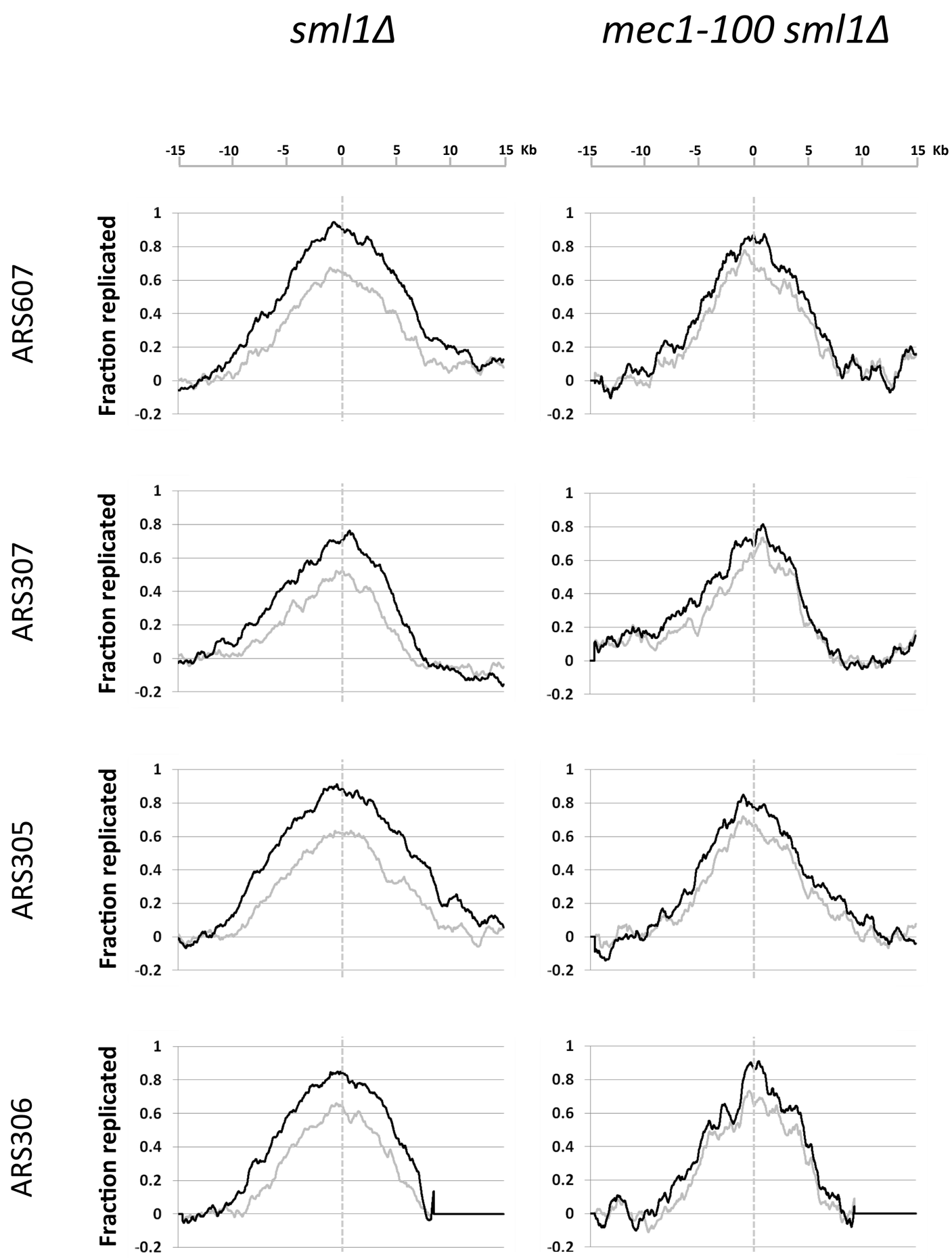
	sml1Δ	*mec1-100 sm1Δ*	P value
Left fork	81±12 (851)	37±16 (885)	<2e-15
Right fork	96±20 (957)	28±15 (758)	<2e-15

	sml1Δ	*mec1-100 sm1Δ*	P value
Left fork	62±17 (666)	35±11 (616)	<2e-15
Right fork	65±17 (563)	45±17 (712)	<2e-15

	sml1Δ	*mec1-100 sm1Δ*	P value
Left fork	68±34 (964)	45±22 (810)	<2e-15
Right fork	68±16 (991)	28±23 (807)	<2e-15

	sml1Δ	*mec1-100 sm1Δ*	P value
Left fork	68±15 (845)	32±14 (680)	<2e-15
Right fork	52±13 (605)	28±18 (538)	<2e-15

Supplemental Figure S11. *mec1-100 sml1* cells show severe defects in fork progression. Each data row corresponds to an early firing ORI. Panels on the right show individual quantification of fork rate (average plus minus standard deviation) for each individual fork (left and right). Numbers in parenthesis indicate the number of data points (N) included in the quantification. P values are given from two-tailed T tests.



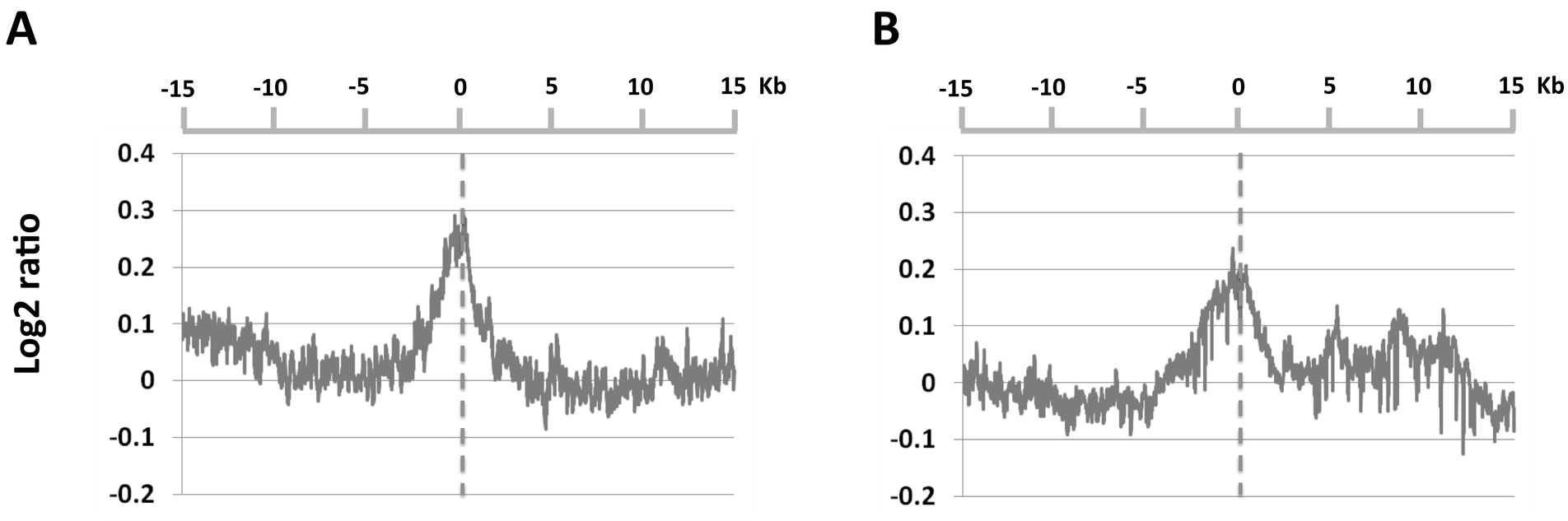
	sml1Δ	*mec1-100 sm1Δ*	P value
Left fork	76±16 (916)	36±19 (765)	<2e-15
Right fork	95±31 (855)	42±24 (870)	<2e-15

	sml1Δ	*mec1-100 sm1Δ*	P value
Left fork	91±16 (855)	61±24 (975)	<2e-15
Right fork	76±26 (666)	24±20 (696)	<2e-15

	sml1Δ	*mec1-100 sm1Δ*	P value
Left fork	99±27 (967)	36±11 (682)	<2e-15
Right fork	106±27 (919)	48±21 (1078)	<2e-15

	sml1Δ	*mec1-100 sm1Δ*	P value
Left fork	84±21 (951)	36±19 (714)	<2e-15
Right fork	76±31 (897)	40±25 (656)	<2e-15

Supplemental Figure S12. *mec1-100 sml1* cells show severe defects in fork progression. Each data row corresponds to an early firing ORI. Panels on the right show individual quantification of fork rate (average plus minus standard deviation) for each individual fork (left and right). Numbers in parenthesis indicate the number of data points (N) included in the quantification. P values are given from two-tailed T tests.



Supplemental Figure S13. DNA profiles from late ORIs (n=20) in *mec1-100 sml1* cells are shown averaged at ORI midpoints (dashed vertical line) by 60 (A) and 40 (B) minutes. Experiments were performed in the presence of 200 mM HU.