Supplemental Material

Local nucleosome dynamics and eviction following a double-strand break are reversible by NHEJ-mediated repair in the absence of DNA replication

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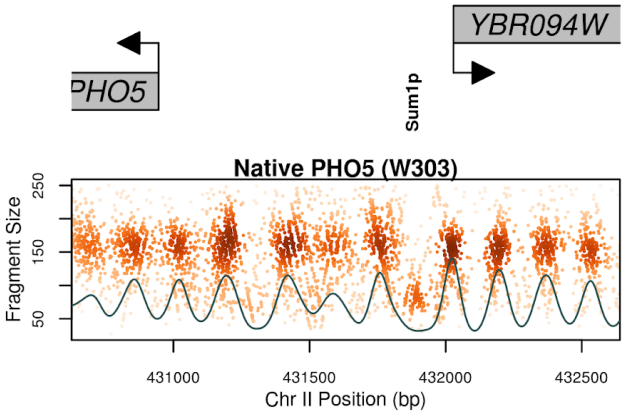
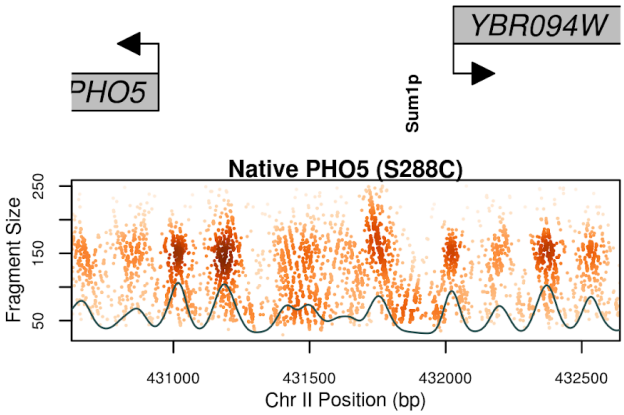
Supplemental Data and Figures

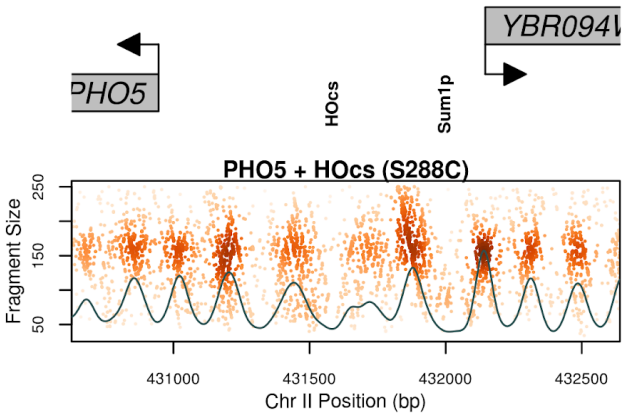
Supplemental Table 1. Oligonucleotide sequences.

Pho5_southern_f	*TTTCACACCCTCCATTGTCA*
Pho5_southern_r	*TTTGGGGCACATAAGGATTC*
DNL4-ko-f	*AATAAAAATCTAGAACTGAAGGAAATAGTA ACGGATTATTTAGGTcagattgtactgagagtgc*
DNL4-ko-r	*GTATTAAATAAACTTCAAAAAATTAAGCCTC CGCAAAACGCACCAccttacgcatctgtgcgg*
YKU70-ko-f	*AGCTATGATTTGTTAAGTGACTCTAAGCCTG ATTTTAAAACGGGAATATTcggcatcagagcaga ttgta*
YKU70-ko-r	*CTACCAAATATTGTATGTAACGTTATAGATA TGAAGGATTTCAATCGTCTctgatgcggtattttctc ct*
MRE11-ko-f	*GCAGACAATTGACGCAAGTTGTACCTGCTCA GATCCGATAAAACTCGACTcggcatcagagcaga ttgta*
MRE11-ko-r	*AAGCCCTTGGTTATAAATAGGATATAATATA ATATAGGGATCAAGTACAActgatgcggtattttct cct*
Pho5-gRNA-f	*GTGTCGCACGCTCTCTTTACGTTTT*
Pho5-gRNA-r	*GTAAAGAGAGCGTGCGACACGATCA*
Pho5_HOcs_CRISPR-Cas9_gBlock	*GACCTGATGTCAGTCCCCACGCTAATAGCG GCGTGTCGCAGATCTAAATAAATTCGTTTTC AATGATTAAAATAGCATAGTCGGGTTTTTCT TTTAGTTTCAGCTTTCCGCAACAGTATAATT TTATAAACCCTGGTTTTGGTTTTGTAGAGTG GTTCGCTCTCTTTACAGGACGCCGGAGACC GGCATTACAAGGA*

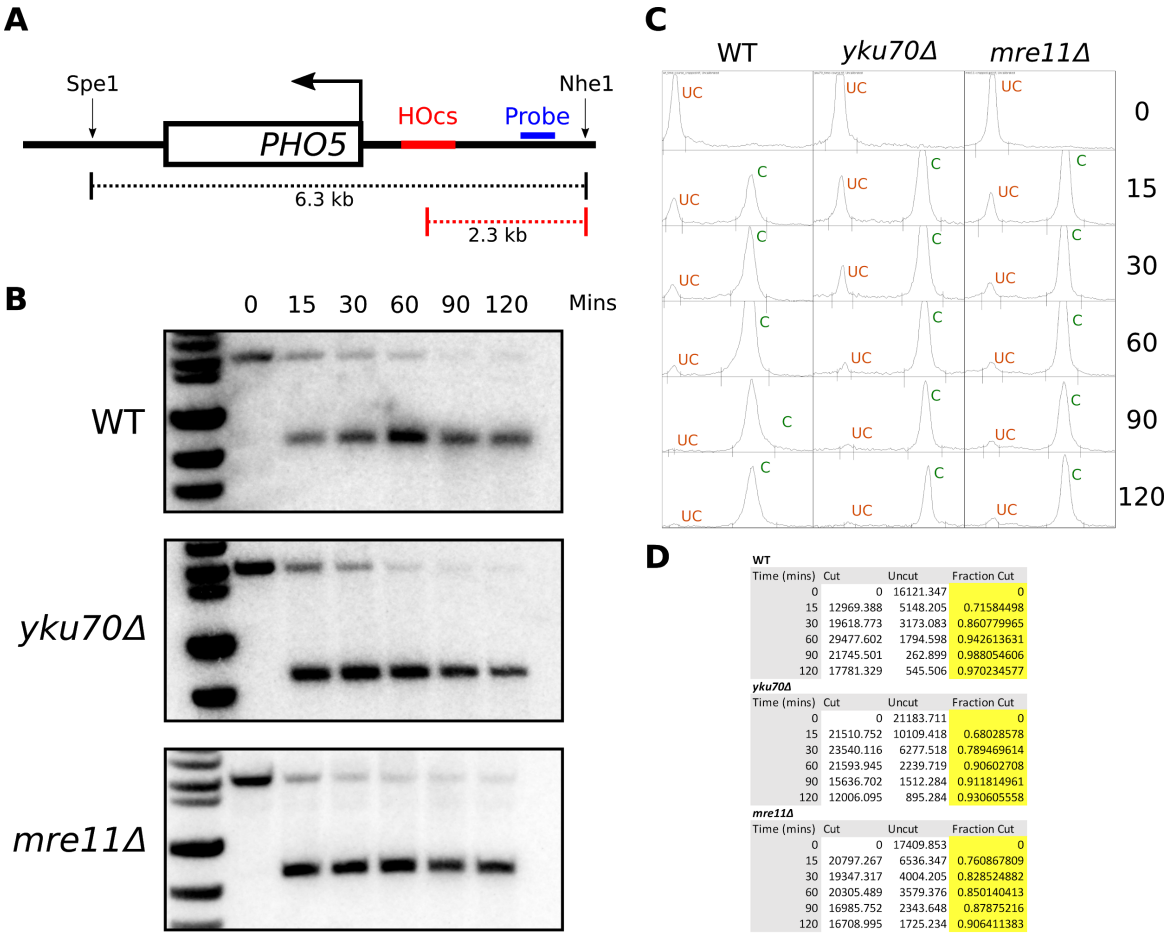
Supplemental Table 2. Summary statistics of sequencing libraries and concordance data for replicate experiments. See `Supplemental_Table_S2.xlsx`.


PHO5
YBR094W
Sum1p
Native PHO5 (W303)
Fragment Size
50
150
250
431000
431500
432000
432500
Chr II Position (bp)

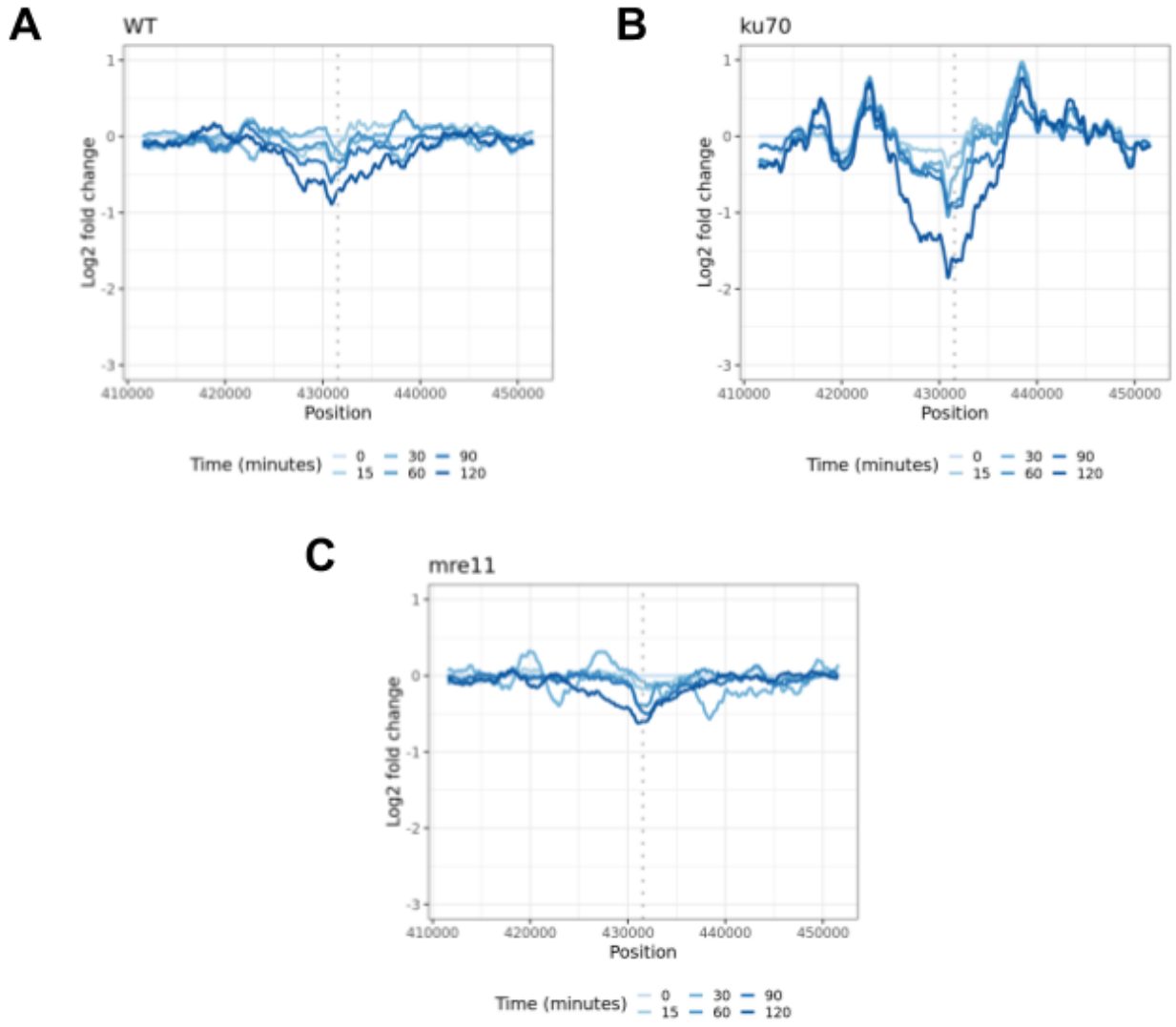

PHO5
YBR094W
Sum1p
Native PHO5 (S288C)
Fragment Size
50
150
250
431000
431500
432000
432500
Chr II Position (bp)


PHO5
HOcs
Sum1p
PHO5 + HOcs (S288C)
Fragment Size
50
150
250
431000
431500
432000
432500
Chr II Position (bp)

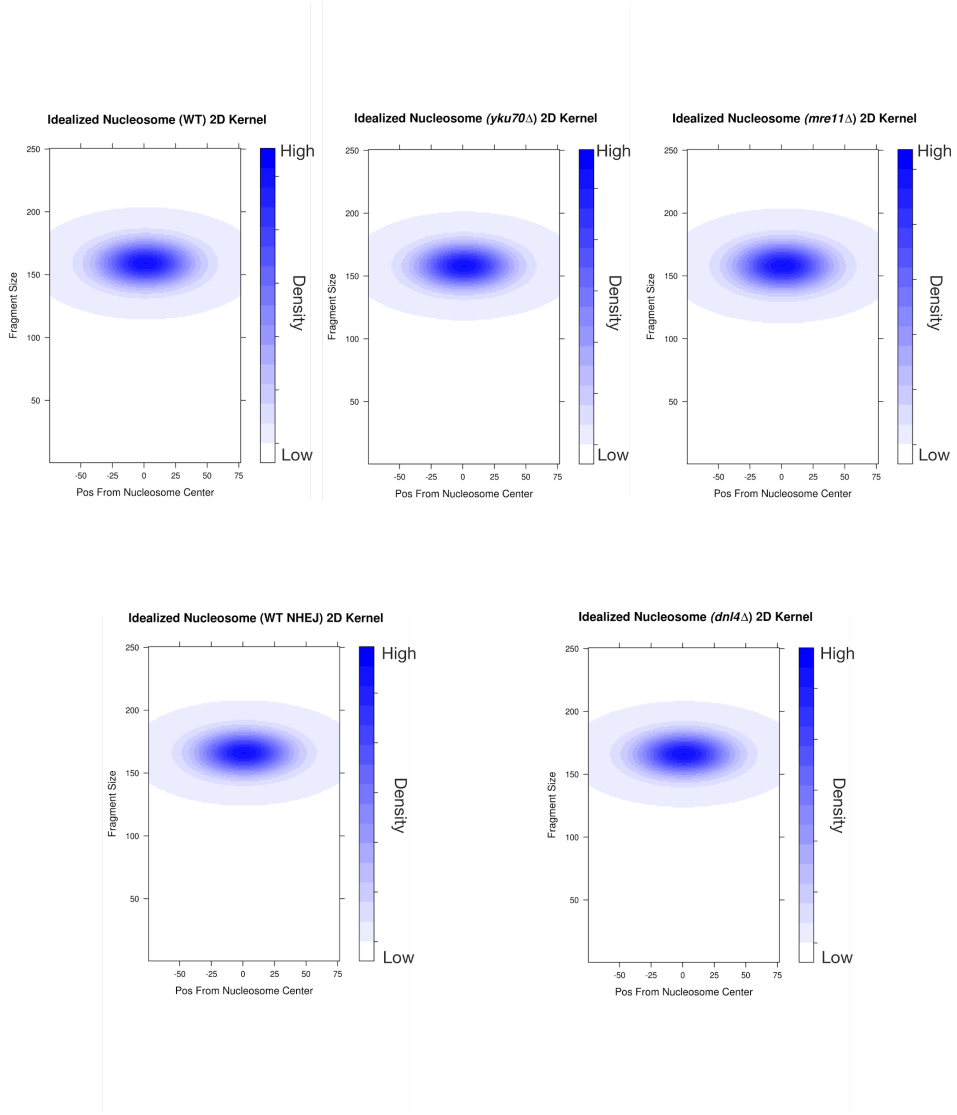
Supplemental Figure 1. Structure of the *PHO5* locus prior to and after the HOcs insertion. Top panel is the native *PHO5* locus from the W303 yeast strain background while the middle panel is the same locus in the JKM139 strain, which is largely derived from S288C. The bottom panel is the S288C strain with the HOcs insertion. The +1 nucleosome of *PHO5* is the 4L nucleosome in **Figure 1A**.



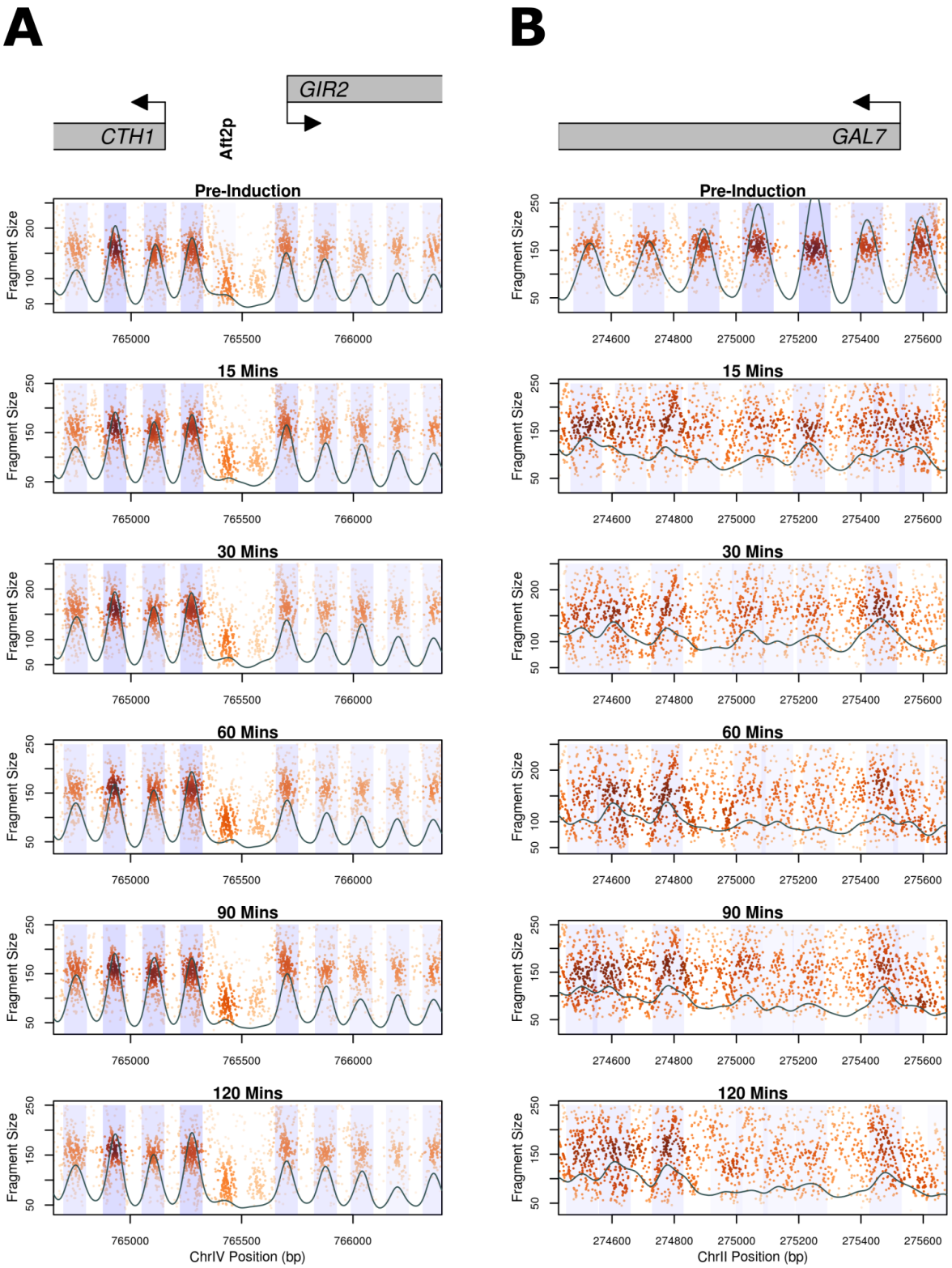
Supplemental Figure 2. Southern Blotting of WT, yku70Δ, and mre11Δ strains. **A.** Schematic of the southern blotting probe and the detected fragment sizes at the *PHO5* locus with the 117 bp HO recognition site. **B.** Southern blots of the 3 strains profiled in this study. **C.** Line profiles of each lane that were analyzed in ImageJ to extrapolate uncut (UC, orange) and cut (C, green) band intensity. **D.** Table of the data obtained from the analysis in **C**, fraction cut is determined by dividing the cut signal by the sum of the uncut and cut signals.



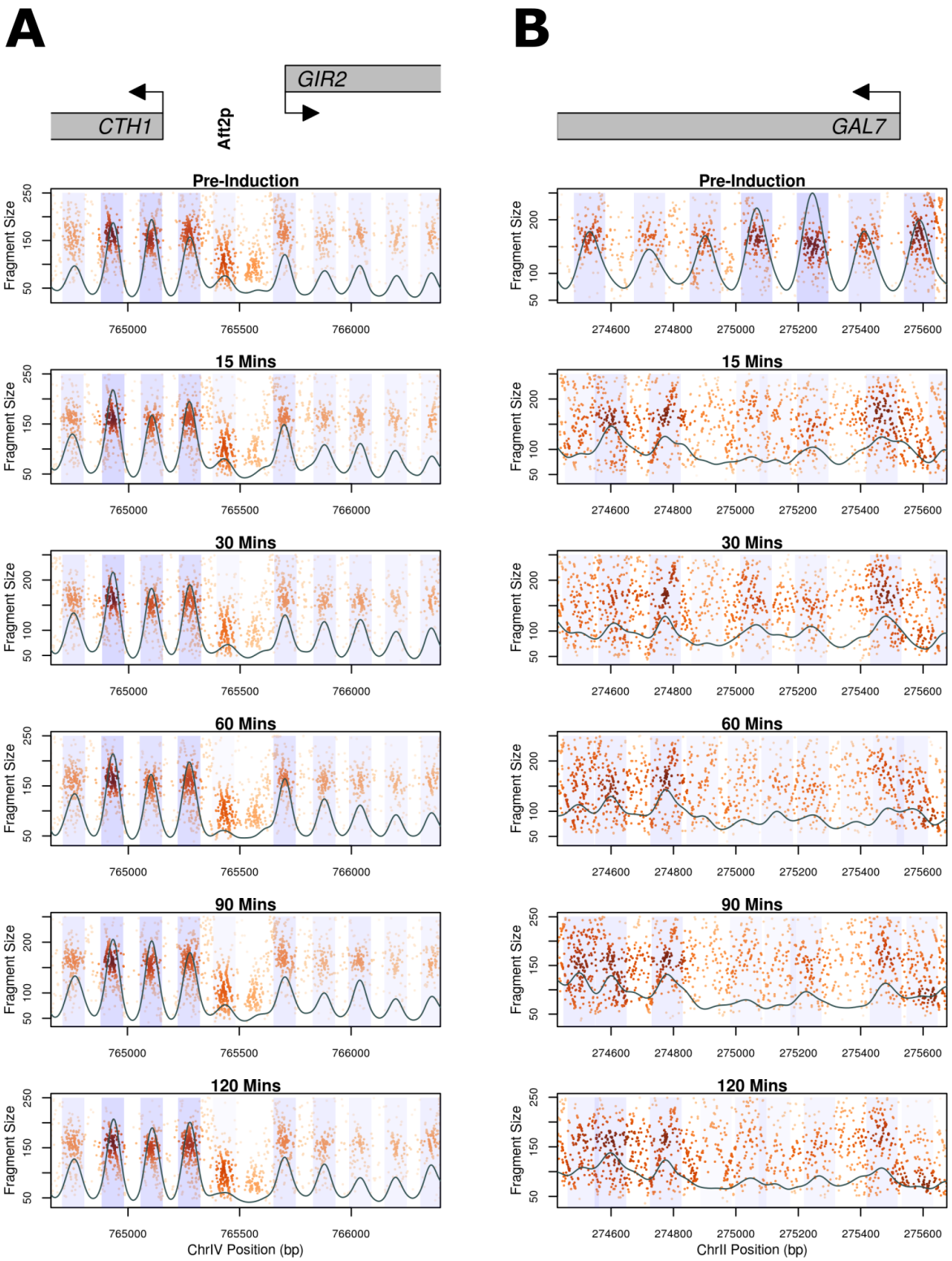
Supplemental Figure 3. DNA resectioning surrounding the HO induced break at the *PHO5* locus. Total DNA was recovered and the relative copy number was assessed for each timepoint by high throughput sequencing. Panels A, B, and C represent WT, *yku70Δ* and *mre11Δ* strains. Greater time post-induction (min) is plotted in darker shades of blue indicated below each plot.



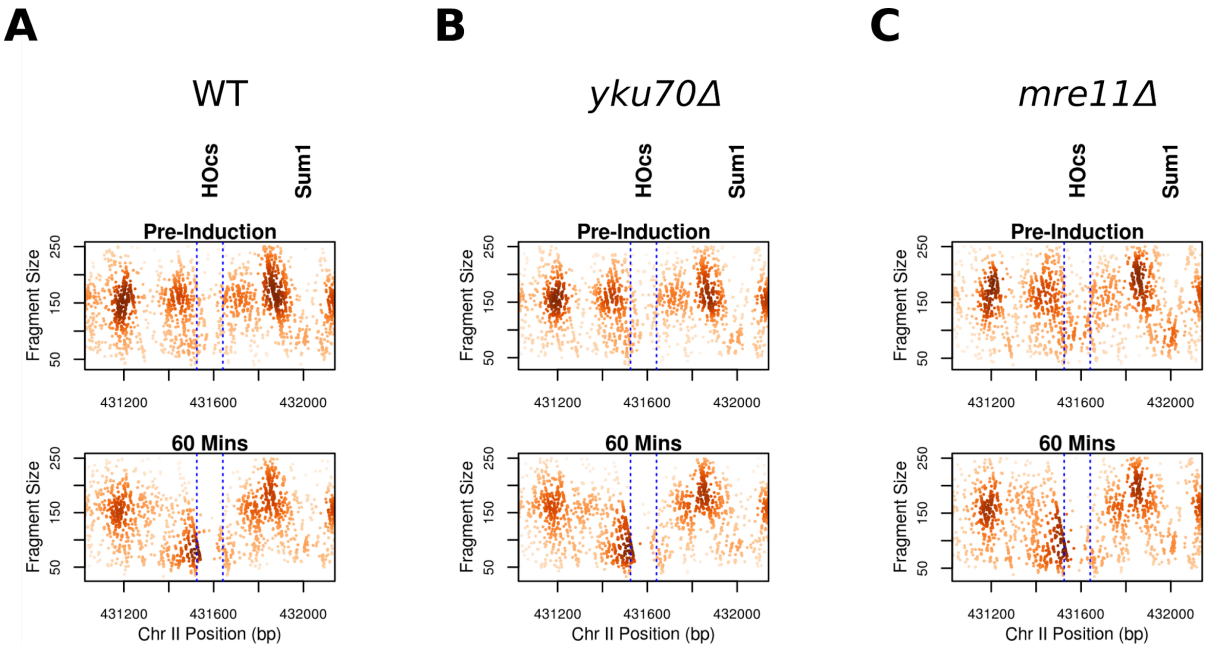
Supplemental Figure 4. Idealized two-dimensional density (2D) kernel of nucleosomes derived from the pre-induction sample for each time-course. The size and fragment distribution parameters for this nucleosome were derived from analyzing the 8,632 nucleosomes chemically mapped (Brogaard et al. 2012) on Chr IV for each experiment which were similar across all experiments.


A
B
CTH1
GIR2
Aft2p
GAL7
Pre-Induction
15 Mins
30 Mins
60 Mins
90 Mins
120 Mins
Fragment Size
ChrIV Position (bp)
ChrII Position (bp)

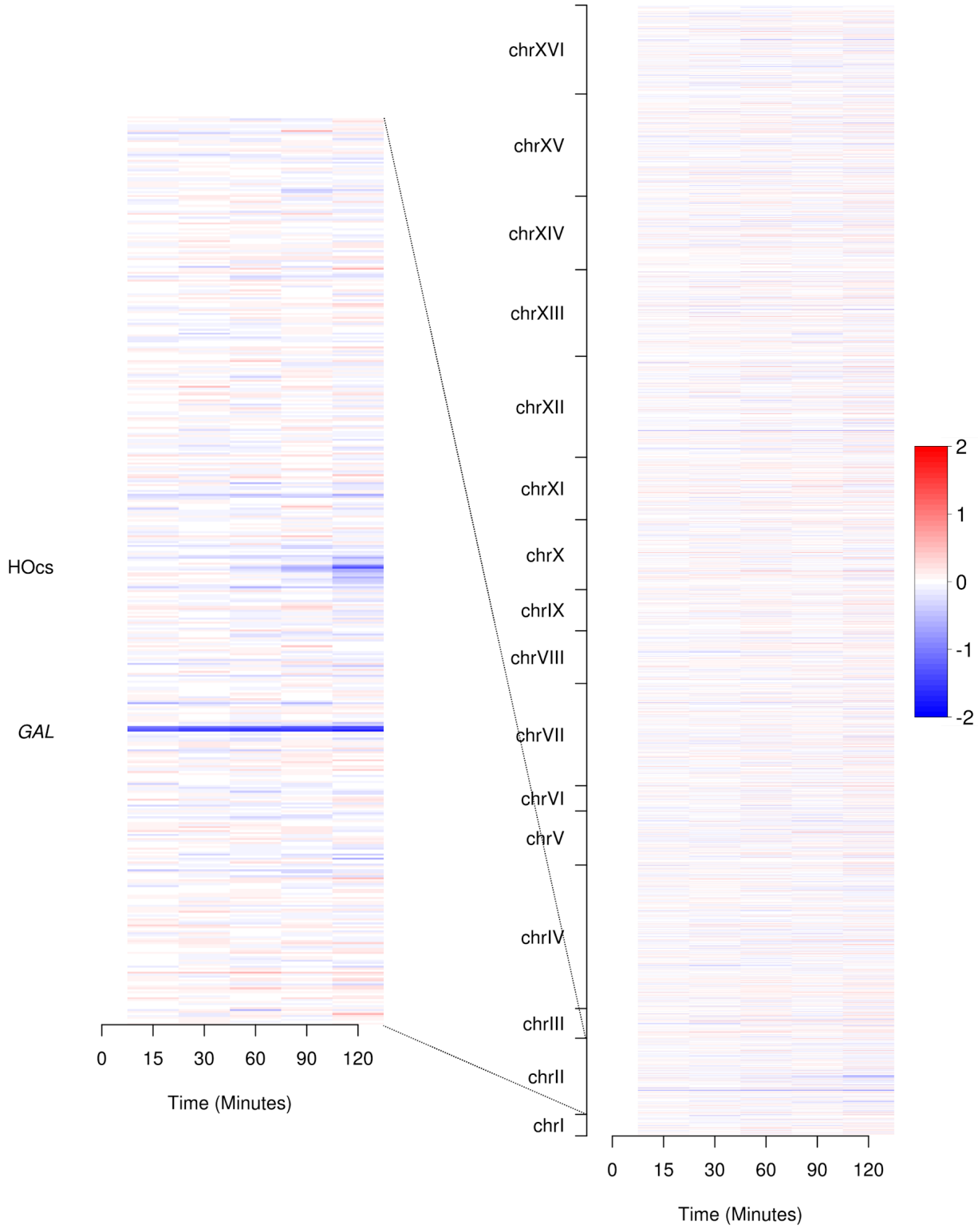
Supplemental Figure 5. *yku70Δ* control loci genome wide chromatin occupancy profiles (GCOPs). **A.** GCOP for the *CTH1* and *GIR2* loci which serve as a control for unaltered chromatin. Gray boxes depict gene bodies, with arrows indicating the direction of transcription. The bold vertical text indicates the Aft2p binding site between these two genes. A two-dimensional cross correlation with an idealized nucleosome (see Methods) is calculated at every base pair and depicted as a continuous black trace. The peaks of this cross-correlation analysis represent the most likely position of a locally detected nucleosome and we have shaded +/- 0.5 standard deviations of nucleosome position from the center of each detected peak. The intensity of the shaded color is proportional to the cross-correlation score of the nucleosome peak. **B.** GCOP for the *GAL7* locus following galactose induction.


A
B
CTH1
Aft2p
GIR2
GAL7
Pre-Induction
15 Mins
30 Mins
60 Mins
90 Mins
120 Mins
Fragment Size
ChrIV Position (bp)
ChrII Position (bp)

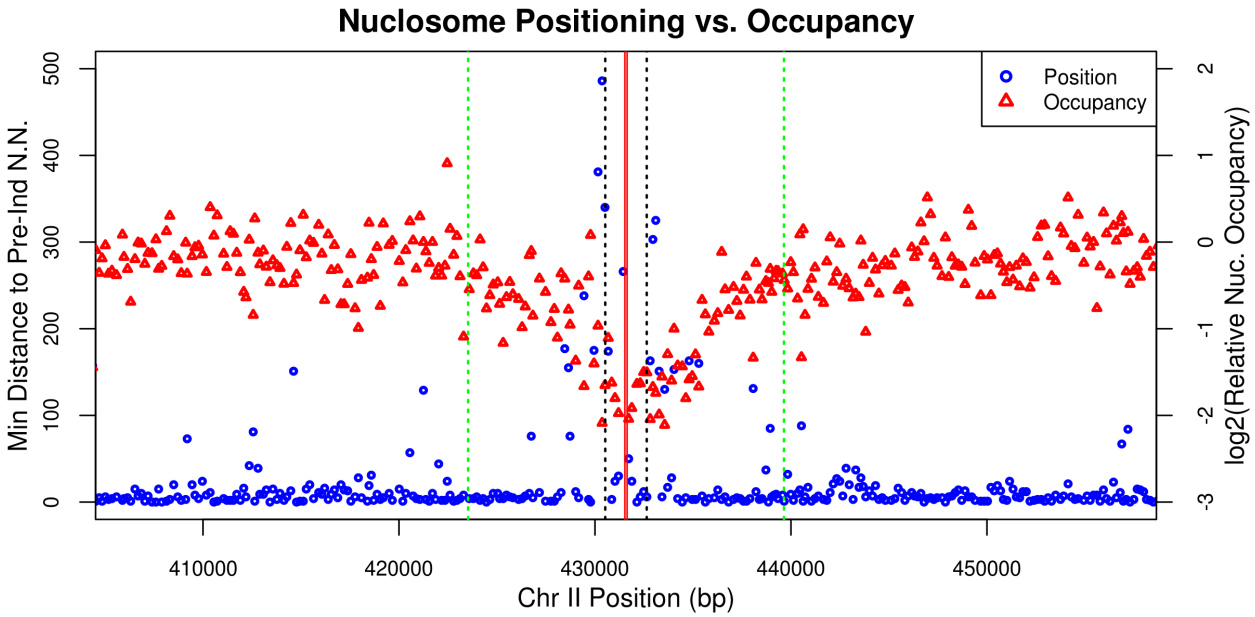
Supplemental Figure 6. *mre11Δ* control loci genome wide chromatin occupancy plots (GCOPs) **A.** GCOP for the *CTH1* and *GIR2* loci which serve as a control for unaltered chromatin. Gray boxes depict gene bodies, with arrows indicating the direction of transcription. The bold vertical text annotates the Aft2p binding site between these two genes. A two-dimensional cross correlation with an idealized nucleosome (see Methods) is calculated at every base pair and depicted as a continuous black trace. The peaks of this cross-correlation analysis represent the most likely position of a locally detected nucleosome and we have shaded +/- 0.5 standard deviations of nucleosome position from the center of each detected peak. The intensity of the shaded color is proportional to the cross-correlation score of the nucleosome peak. **B.** GCOP for the *GAL7* locus following galactose induction.



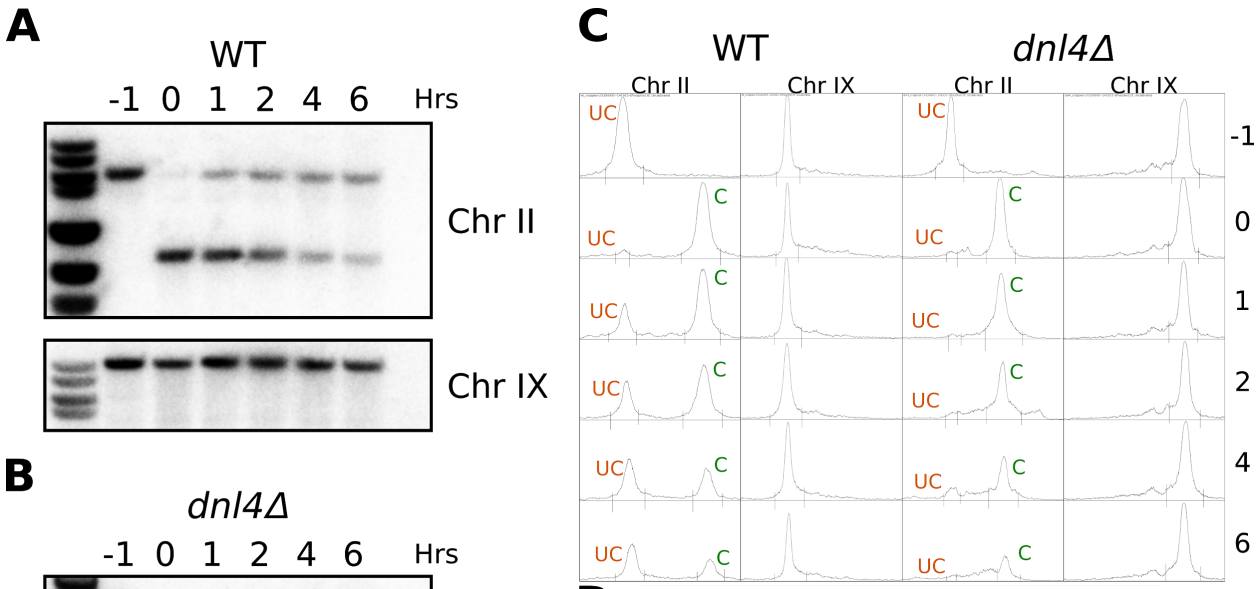
Supplemental Figure 7. Zoomed-in view of genome wide chromatin occupancy plots (GCOPs) of the WT (**A**), *yku70Δ* (**B**), and *mre11Δ* (**C**) strains.


HOcs
GAL
0
15
30
60
90
120
Time (Minutes)
chrXVI
chrXV
chrXIV
chrXIII
chrXII
chrXI
chrX
chrIX
chrVIII
chrVII
chrVI
chrV
chrIV
chrIII
chrII
chrI
0
15
30
60
90
120
Time (Minutes)
2
1
0
-1
-2

Supplemental Figure 8. Genome wide genic nucleosome analysis in wild type cells following a DSB. The 2D cross correlation score for genic nucleosomes in each time point is expressed as a log2-fold change relative to the pre-induction state. The coordinates corresponding to the *GAL* loci and the HO induced DSB are annotated on the zoomed in view of Chr II (left panel).



Supplemental Figure 9. Relative nucleosome position and nucleosomal occupancy ~25 kb from the DSB. We detected 307 nucleosomes in a ~50 kb window around the DSB (arrayed on the x axis according to their position) on Chr II and plotted their relative distance to the nearest neighboring nucleosome (in pre-induction) throughout the time course (blue circles). Separately we calculated the occupancy of each individual nucleosome and plotted the log2-ratio of the 120 min post-induction occupancy to the pre-induction occupancy (red triangles). The dotted green lines denote coordinates 8 kb from the break while the dotted black lines denote 1 kb from the break.



D

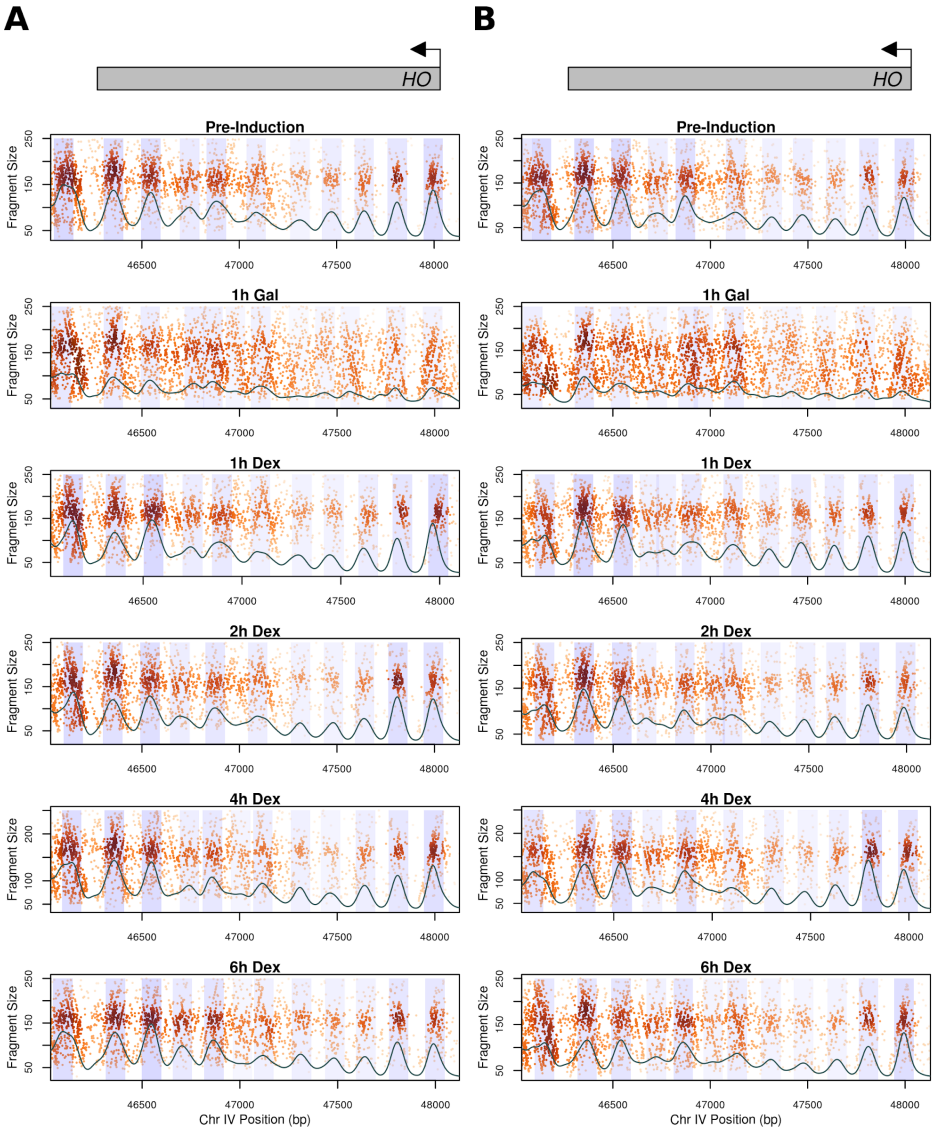
WT

Time	Control	Relative DNA (to -1)	Uncut	Cut	Fraction Uncut
-1	24851.945	1	21926.773	0	1
0	20887.652	0.840483592	1418.113	20224.48	0.076949691
1	26808.087	1.078711827	7177.125	17608.702	0.303438267
2	28813.057	1.15938841	8260.075	14784.652	0.324922896
4	26322.108	1.059156859	9799.267	7342.903	0.421947665
6	18467.409	0.74309713	8280.61	5224.953	0.508208619

dnl4Δ

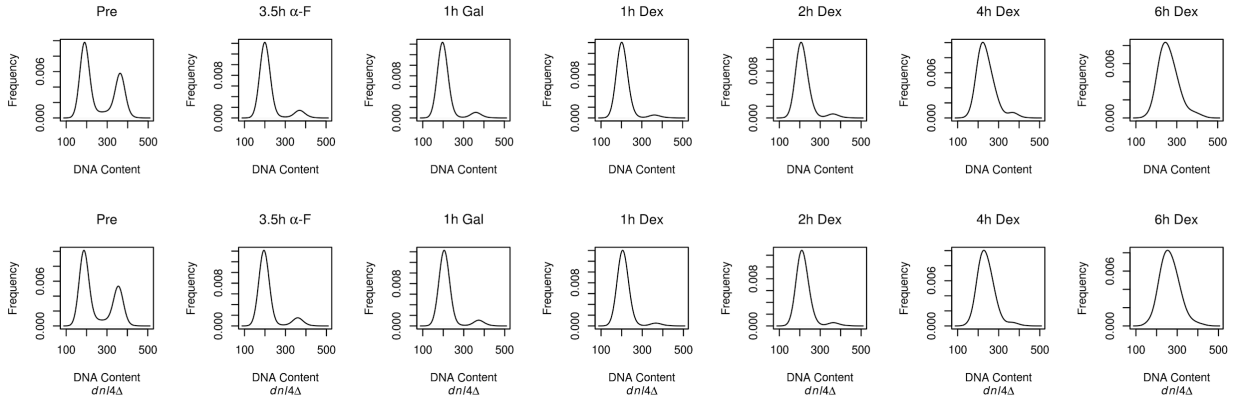
Time	Control	Relative DNA (to -1)	Uncut	Cut	Fraction Uncut
-1	18768.731	1	20591.217	0	1
0	20644.388	1.099935206	1375.941	21886.045	0.060750617
1	17126.267	0.912489342	1029.355	21542.451	0.054784205
2	17247.924	0.91897124	1495.77	18044.673	0.079046179
4	20330.602	1.083216654	3302.619	12515.631	0.148067973
6	18344.066	0.977373803	1086.991	6380.246	0.054011127

Supplemental Figure 10. Southern Blotting of WT and *dnl4Δ* strains. Southern blots of the WT (**A**) and *dnl4Δ* (**B**) strains along with an unrelated control locus from Chr IX. **C.** Line profiles of each lane that were analyzed in ImageJ to extrapolate uncut (UC, orange), cut (C, green) and control (Chr IX) band intensity. **D.** Table of the data obtained from the analysis in **C,** fraction uncut is determined by dividing the uncut signal at each time point by the uncut signal from pre-induction. All of these values are then normalized to the band intensity from Chr IX in the corresponding lane.



Supplemental Figure 11. Genome wide chromatin occupancy plots (GCOPs) of the HO gene body in the WT (**A**) and *dnl4Δ* (**B**) strains over the NHEJ time course. Gray boxes depict gene bodies, with arrows indicating the direction of transcription. A two-dimensional cross correlation with an idealized nucleosome (see Methods) is calculated at every base pair and depicted as a continuous black trace. The peaks of this

cross-correlation analysis represent the most likely position of a locally detected nucleosome and we have shaded +/- 0.5 standard deviations of nucleosome position from the center of each detected peak. The intensity of the shaded color is proportional to the cross-correlation score of the nucleosome peak.



Supplemental Figure 12. FACS profiles of the WT (top) and *dnl4Δ* (bottom) strains throughout the NHEJ time course. DNA content is measured on the horizontal axis while cell count is measured on the vertical axis.