

Supplemental Methods

Sequence Alignment

Sequencing reads were aligned in paired-end mode to the sacCer3/R61 version of the *S. cerevisiae* genome using Bowtie 0.12.7 (Langmead et al. 2009).

Analysis of chromatin organization and structure

Biological replicates were merged for all data analyses. Merged files were then sampled to obtain equal read depth. Each merged experiment represents a minimum of 30 million reads. To characterize chromatin structure in nascent and mature chromatin at gene bodies, nucleosome-size reads between 140 bp and 180 bp in length were used to calculate a density curve of nascent, mature, and bulk chromatin across the length of each gene, with a 30 bp bandwidth Gaussian kernel. The pairwise Pearson correlation between the density curves at each gene was calculated for nascent and bulk, or mature and bulk chromatin.

Similarly, we obtained nucleosome-size reads overlapping each gene and determined the midpoint location of each. We then determined the density distribution of all the read midpoints with a 30 bp bandwidth Gaussian kernel. This output signal was smoothed and the refined predicted values generated a curve where each peak represents a nucleosome dyad and each trough represents linker DNA. We used an autocorrelation function (ACF) to determine the pattern of organization for the first four nucleosomes (+1, +2, +3, and +4) within each gene in nascent and mature chromatin.

This allowed us to directly measure the chromatin structure at all gene locations in the two chromatin states. To do this, midpoint locations of sequencing reads between 140 and 180 bps in length were used to generate density estimates at each gene locus in nascent and mature chromatin. To calculate the ACF value for each gene, we first determined the step corresponding to the nucleosome periodicity across all gene bodies from the bulk chromatin sample. We found that the periodicity lag was 172 bp, which includes the length of linker DNA. We then determined the ACF value corresponding to the lag at 172 for each gene from either the nascent or mature chromatin samples.

Signal normalization of histone post-translational modifications, H2A.Z variant occupancy, and NET-seq

The accession number GSE61888 was used to locate and download ChIP-seq raw histone post translational modification (PTM) and htz1 histone variant data from (Weiner et al. 2015). Raw NET-seq data was obtained using the accession number GSE25107 (Churchman and Weissman 2011). Reads were aligned in single end mode to the sacCer3/R61 version of the *S. cerevisiae* genome using Bowtie 0.12.7 (Langmead et al. 2009).

To determine PTM and H2A.Z occupancy, we calculated all the mapped reads overlapping gene bodies and normalized read counts using RPKM (Reads Per Kilobase of transcript per Million mapped reads). NET-seq data was processed similarly; however, the enrichment of reads was determined for sequences that spanned 100 bp

upstream of the gene start to 300 bp downstream of the gene start. Gene orientation was considered during this calculation.

Individual nucleosome positioning and occupancy scores

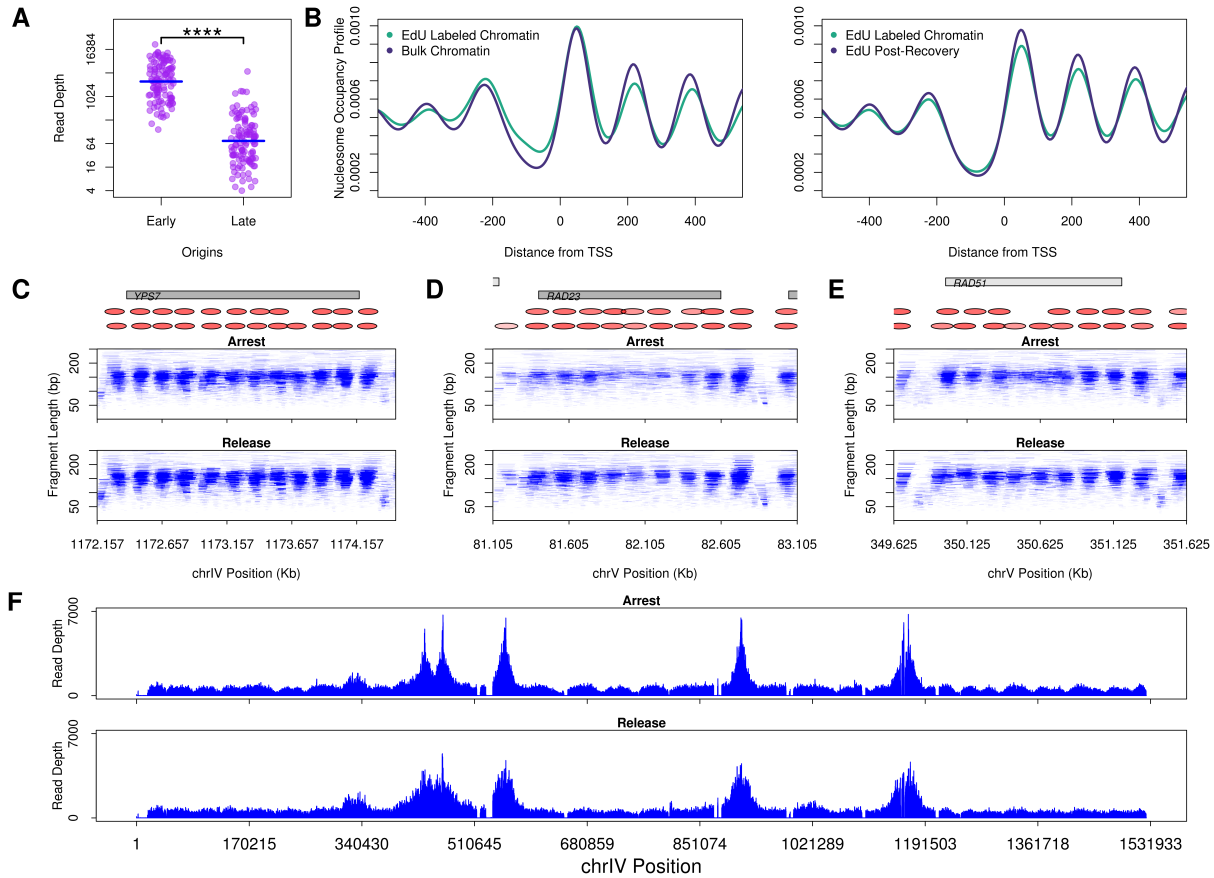
Nucleosome locations were determined from bulk chromatin by obtaining nucleosome-size reads between 140bp and 180 bp in length and calculating a density curve across the length of each chromosome with a 30 bp bandwidth Gaussian kernel. Each chromosome-length density calculation was passed through the *turnpoints* function in the *pastecs* R package to determine all the potential peaks from the density curves. These nucleosome dyad peaks were used to calculate nucleosome coordinates 70 bp upstream and downstream from each peak (140 bp total width). These nucleosome locations were used as reference for subsequent calculations of nascent and mature chromatin structure.

For nascent and mature EdU-labeled chromatin, the nucleosome-size reads from each chromosome were acquired and their midpoint positions determined. Then, the distance of each midpoint to the nearest nucleosome dyad identified from bulk chromatin was calculated. This average was used as the nucleosome positioning score. To ascertain nucleosome occupancy, the total number of normalized sequencing reads mapping within 70 bp upstream and downstream of a nucleosome dyad identified from bulk chromatin was determined.

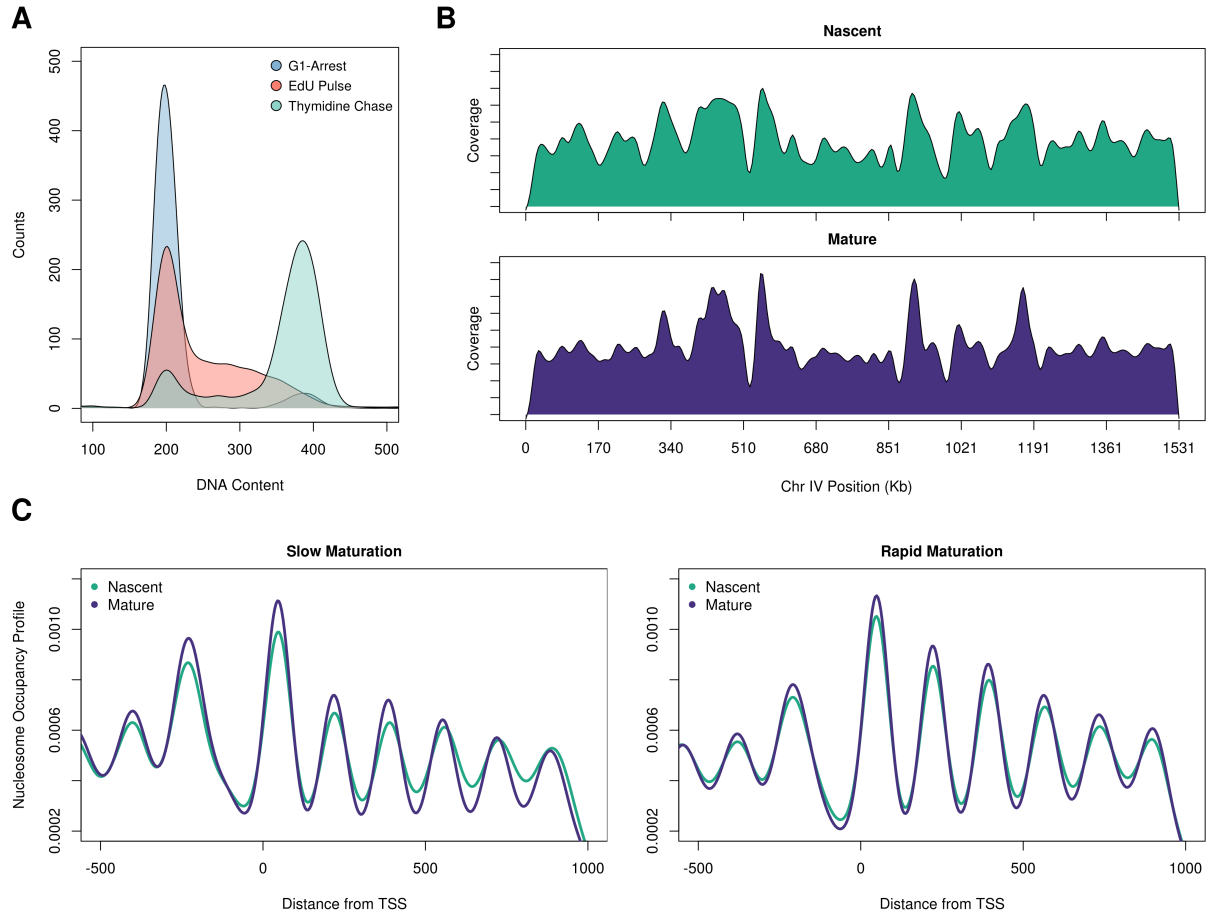
References

- Churchman LS, Weissman JS. 2011. Nascent transcript sequencing visualizes transcription at nucleotide resolution. *Nature* **469**: 368–373.
- Langmead B, Trapnell C, Pop M, Salzberg SL. 2009. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biol* **10**: R25.
- Weiner A, Hsieh T-HS, Appleboim A, Chen HV, Rahat A, Amit I, Rando OJ, Friedman N. 2015. High-resolution chromatin dynamics during a yeast stress response. *Mol Cell* **58**: 371–386.

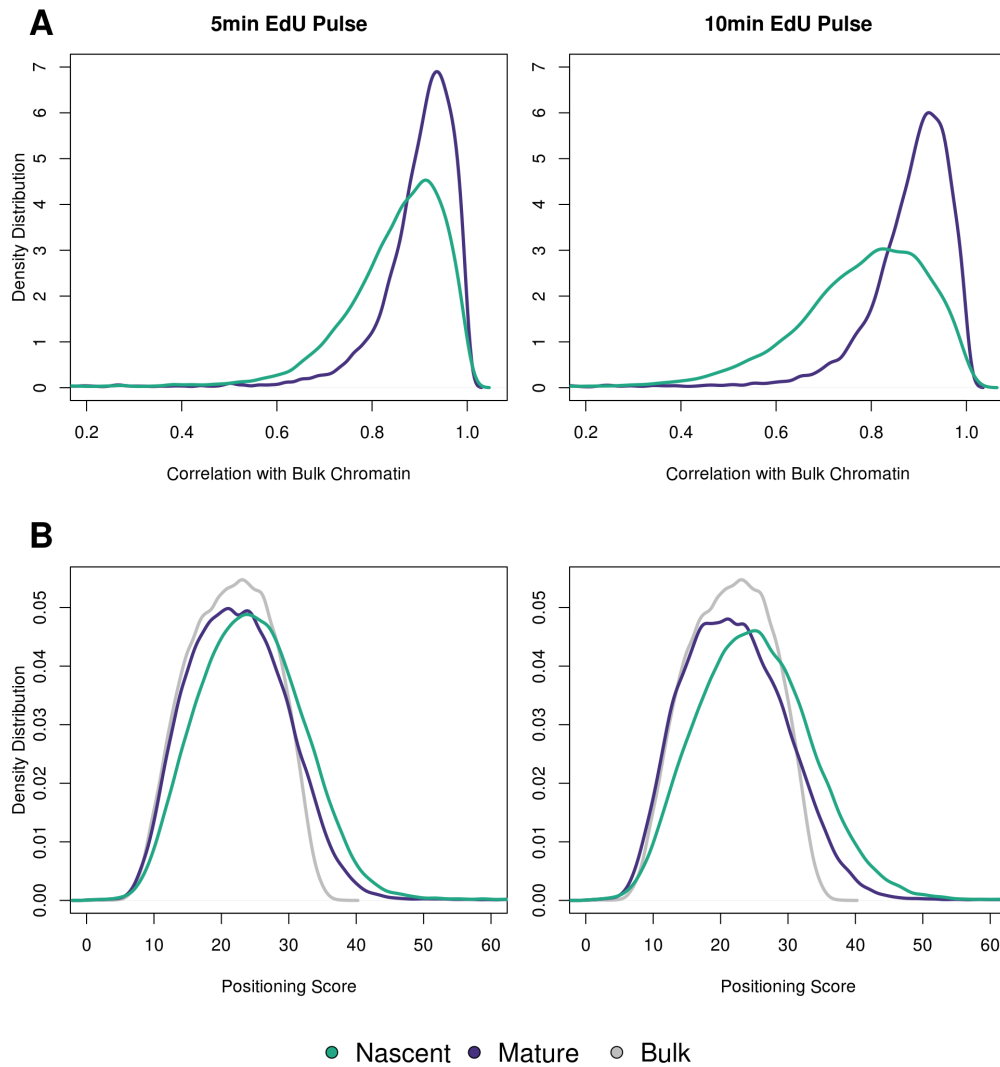
Supplemental Figures



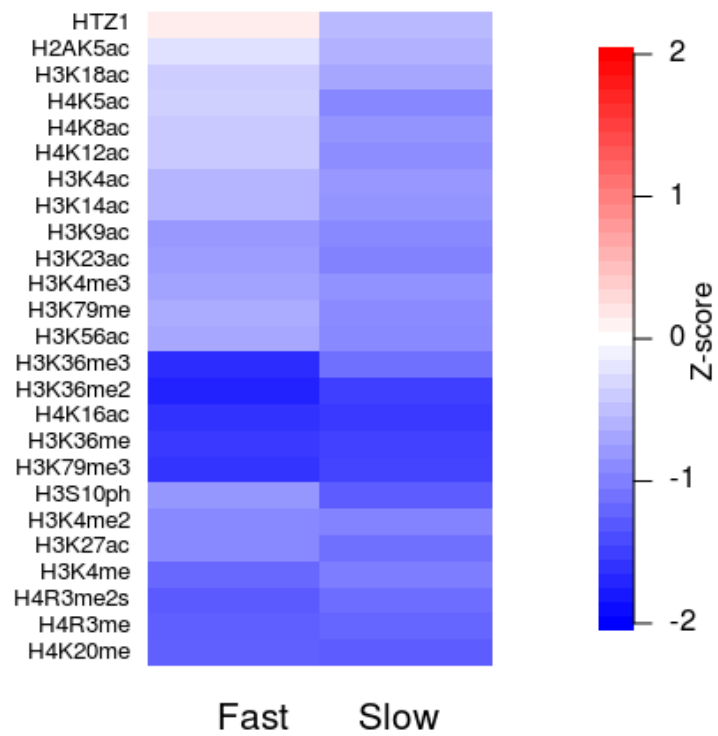
Supplemental Figure S1. *EdU-labeling and recovery of chromatin surrounding early origins of DNA replication.* **A.** Sequencing read depth at 114 early and 114 late replicating origins (Belsky et al. 2015) from the data described in Figure 1A (note the log₂ scale). Mean sequencing coverage of early and late origins is 4163.8 and 207.7 reads respectively. *t-test* **** $p \leq 0.0001$. **B and C.** Nucleosome occupancy profiles for the EdU early origin labeling experiment described in Figure 1 and for a separate experiment where following the HU-arrest, the EdU-labeled cells were allowed to proceed back into the cell cycle. Nucleosome occupancy profiles were calculated from 539 gene promoters located within 3500 bp of an early origin. **C - E.** NCOPs showing EdU labeled chromatin from arrested cells in HU and following a 2 hour release. The gene locations are the same as described in Fig 1B, C and D. **F.** Coverage plots of EdU-labeled chromatin from cells arrested in alpha factor and released into HU for 2 hours (top) and following wash and release from the HU arrest for an additional cell cycle period (bottom).



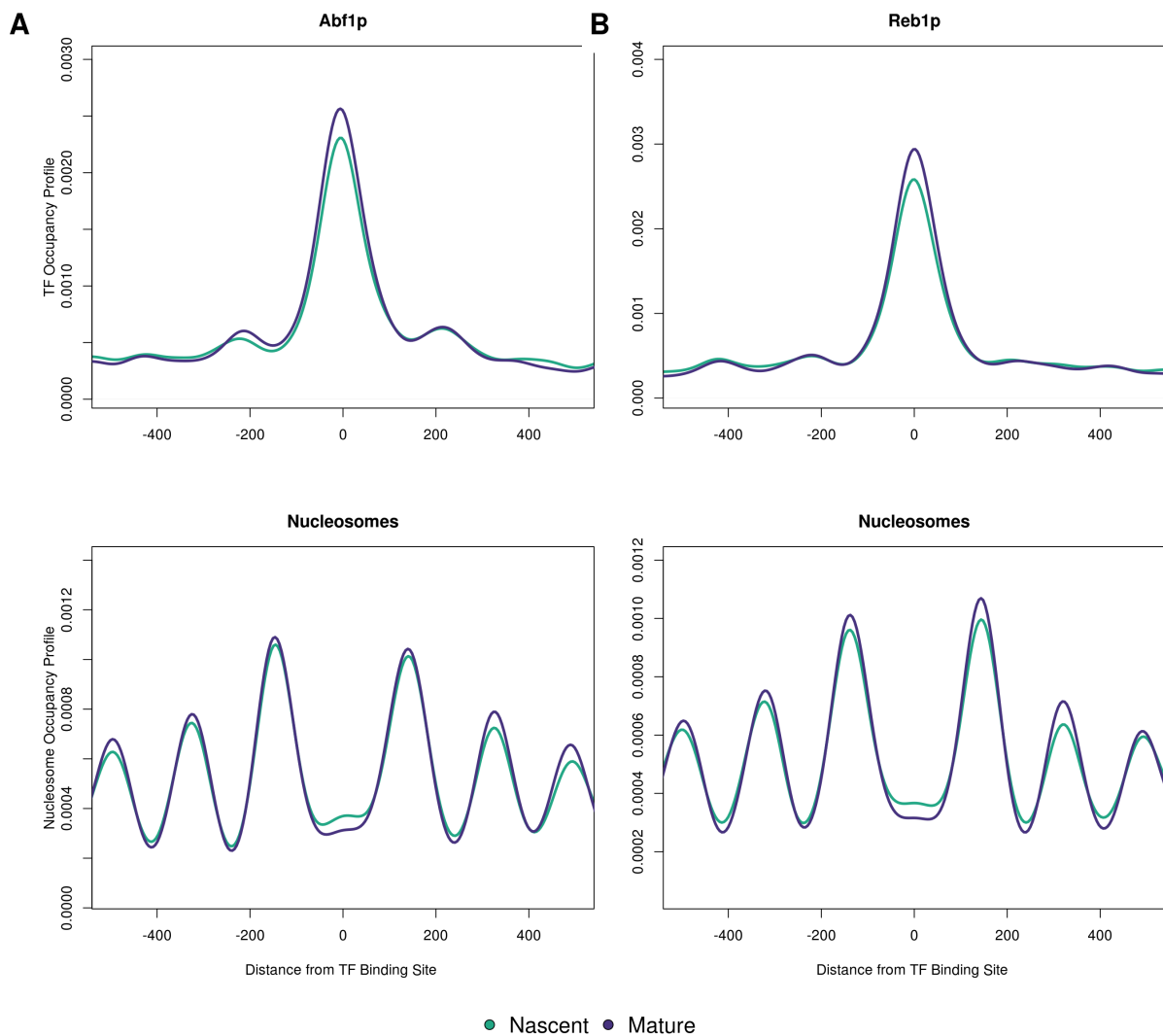
Supplemental Figure S2. *Experimental conditions to capture nascent and mature chromatin.* **A.** A representative flow cytometry plot shows the cell cycle profile of yeast labeled with EdU (red track) and the state of cells following a 30 min thymidine chase (green track). **B.** Coverage plot of nascent and mature chromatin over chromosome IV shows relatively similar levels of EdU incorporation across the chromosome. **C.** Nucleosome occupancy profiles of the slow maturing (first quintile) and fast maturing (fifth quintile) genes.



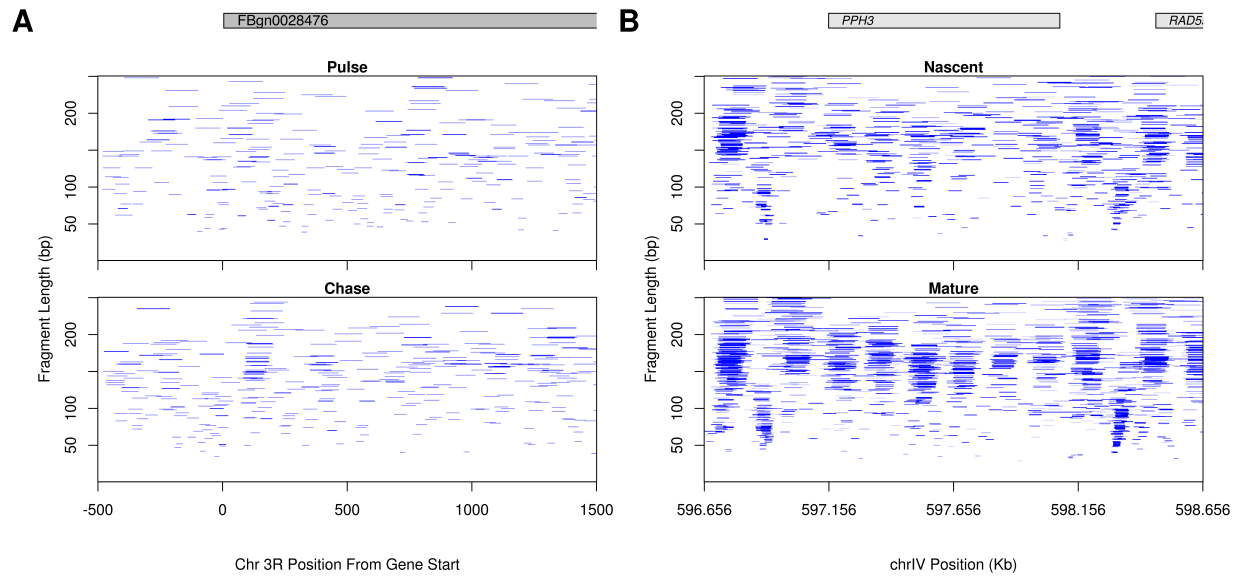
Supplemental Figure S3. *Nucleosome organization for nascent and mature chromatin from a 5 or 10 min EdU pulse.* The efficacy of a 5 or 10 minute EdU pulse was assessed in replicate for each experiment. **A.** Correlation of nascent or mature chromatin with bulk chromatin from either a 5 (left) or 10 (right) min EdU pulse for gene bodies. **B.** Distribution of the nucleosome positioning scores genome-wide for nascent and mature chromatin from the 5 and 10 min EdU labeling pulse (see figure 3A).



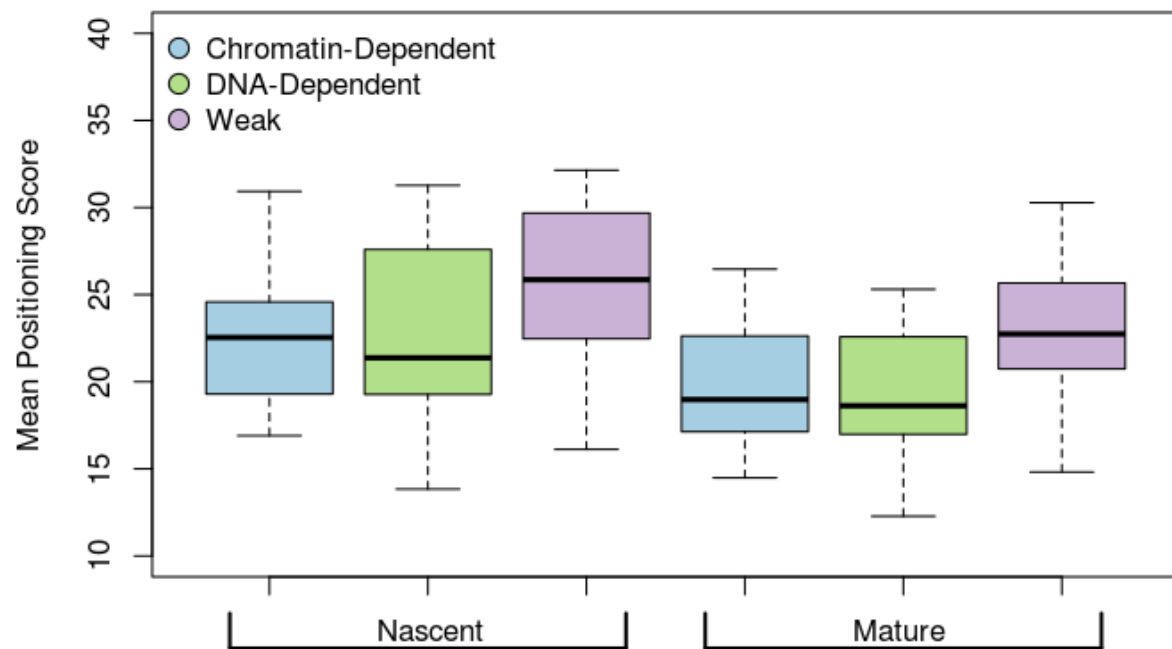
Supplemental Figure S4. Enrichment of histone post-translational modifications and variants at 269 origins with fast or slow chromatin maturation. Heatmap represents the mean Z-scores.



Supplemental Figure S5. *Chromatin profiles at general regulatory factor binding sites. A.* Transcription factor occupancy for 151 Abf1p and **B.** 156 Reb1p binding sites (top two panels) and the corresponding nucleosome occupancy profiles (bottom two panels).



Supplemental Figure S6. Comparison of NCOPs derived from *Drosophila* and *S. cerevisiae*. **A.** *Drosophila* MINCE-Seq data plotted as NCOPs. **B.** *S. cerevisiae* NCOP for a similar sized locus. The increased sequence coverage provides for a detailed locus-specific view of chromatin.



Supplemental Figure S7. Chromatin organization at origins of replication where ORC binding is chromatin-dependent, DNA-dependent or weak.