Supplemental Figure 1. BrdU incorporation is constant across the four S-phase gates. A. FACS profile and replication timing gates for Kc167 cells. Total DNA content (light grey), and BrdU positive cells (dark gray) are plotted as a histograms, along with four gates representing early (red), early-mid (green), late-mid (purple), and late (blue) S-phase fractions. **B.** For both BrdU positive PI and total PI staining cells, the area under the curve was determined within each S-phase gate, and plotted as a ratio.

Supplemental Figure 2. The *Drosophila* genome is partitioned into primarily early and late replicating domains. The pairwise correlations (Spearman's rho) between each S-phase fraction (early (E), early-mid (E-M), late-mid (L-M), and late (L)) are shown for each cell line.

Supplemental Figure 3. Replication timing profiles for Kc167 (black), S2 (gray) and DmBg3 (orange) cells. Replication timing ratios were derived from the four fractions to generate a relative timing value for each genomic position. High values represent early replicating regions and low values represent late replicating regions.

Supplemental Figure 4. Replication timing correlates with the chromatin landscape. A. The relative replication timing values from DmBg3 cells were binned into 10 kb windows and ordered from late (left, blue) to early (right, red). Also plotted are normalized enrichments for gene expression, DNA binding proteins and histone modifications as well as ORC density and promoter density. Histone marks and DNA binding proteins were ordered by row according to their genome wide co-association (determined by k-means clustering of genome-wide correlations, k=2). **B.** Same as **A.** except for Kc167 cells.

Supplemental Figure 5. Segmentation of the genome into early and late replicating domains. A. The S2 genome was segmented into discrete early (red) and late (blue) replicating domains, using a 3-state hidden Markov model representing early, late or indeterminate replication timing. Early and late domains calls are plotted along each chromosome along with the corresponding replication timing profile. **B.** Same as **A.** for DmBg3 cells. **C.** same as **A.** for Kc176 cells.

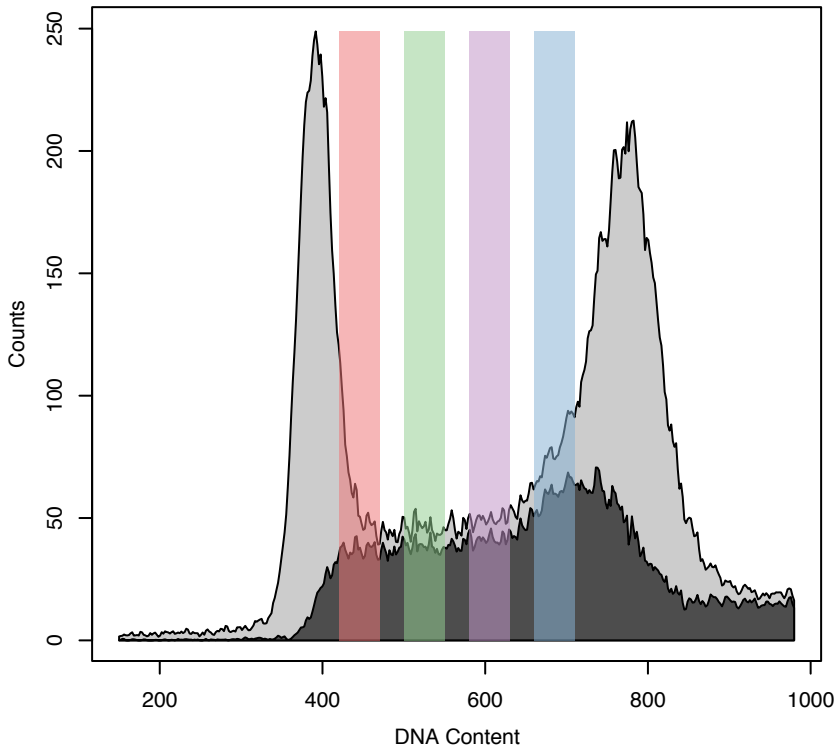
Supplemental Figure 6. Static replication domains for Kc167 cells. The

chromatin landscape for static early (left) and late (right) domains are shown. For each domain (columns), the median score for 15 histone modifications and 2 DNA binding proteins were determined, grouped into three clusters (via K-mean analysis), and plotted along with the replication timing ratio (log2 difference in RPKM between early and late fractions), normalized expression and ORC and TSS density (counts per 10 kb).

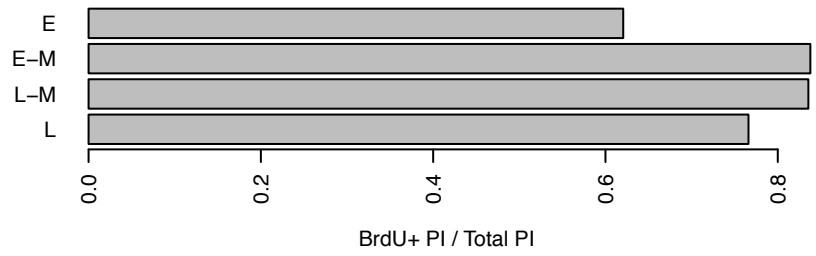
Supplemental Figure 7. ORC ChIP-seq signal is plotted around ORC peaks found in either early replicating domains (red) or late replicating domains (blue) in S2, DmBg3 or Kc167 cells.

Supplemental Figure 8. The fraction of each chromosome that is contained in early (red) or late (blue) domains within each cell line.

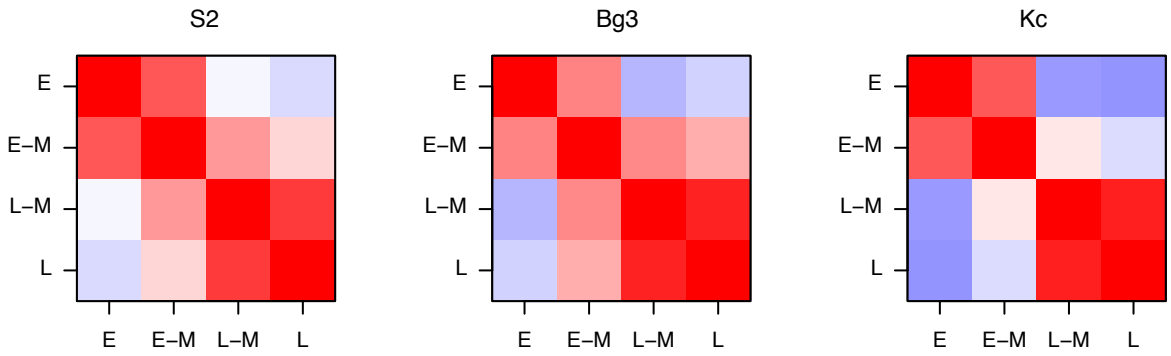
A



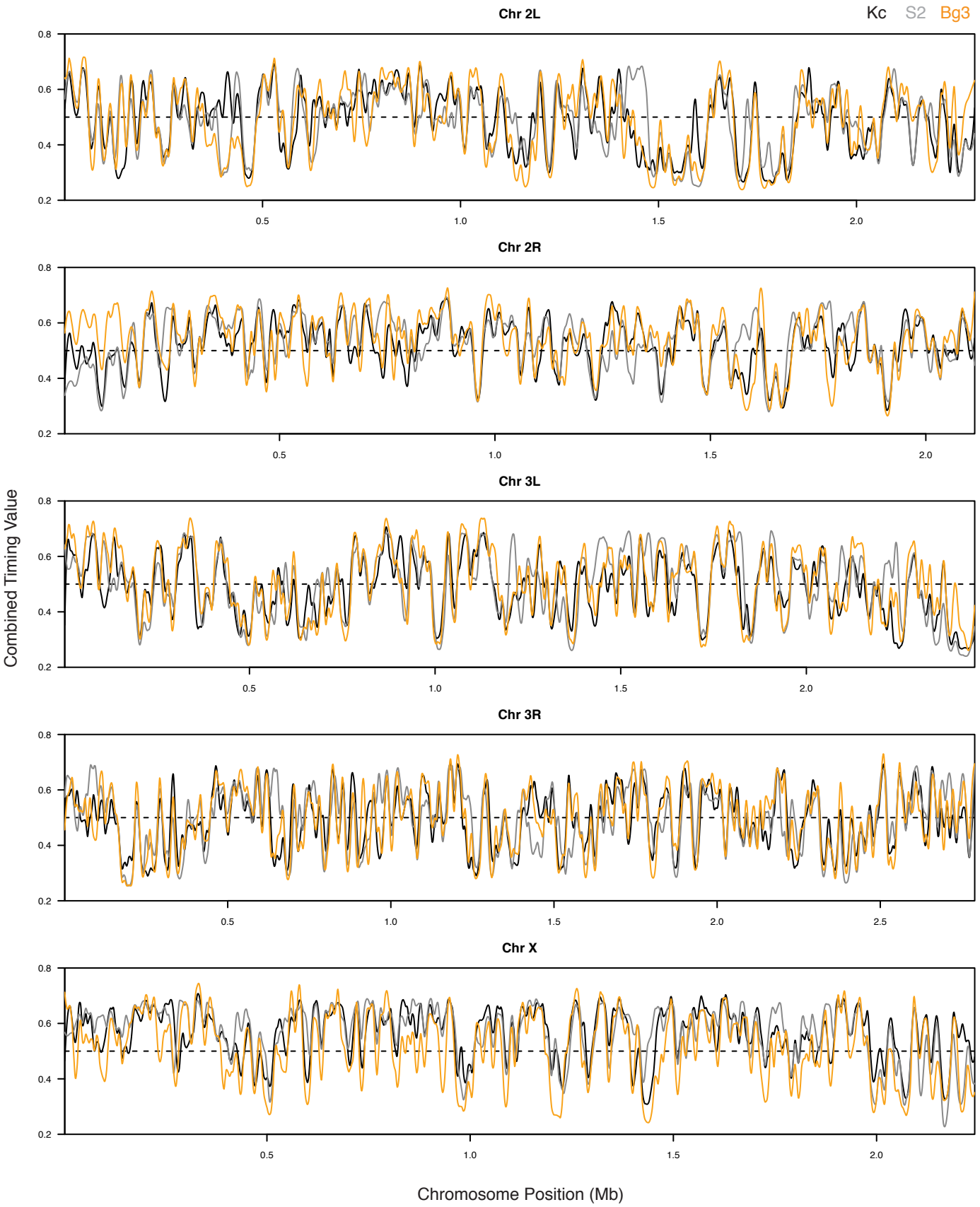
B



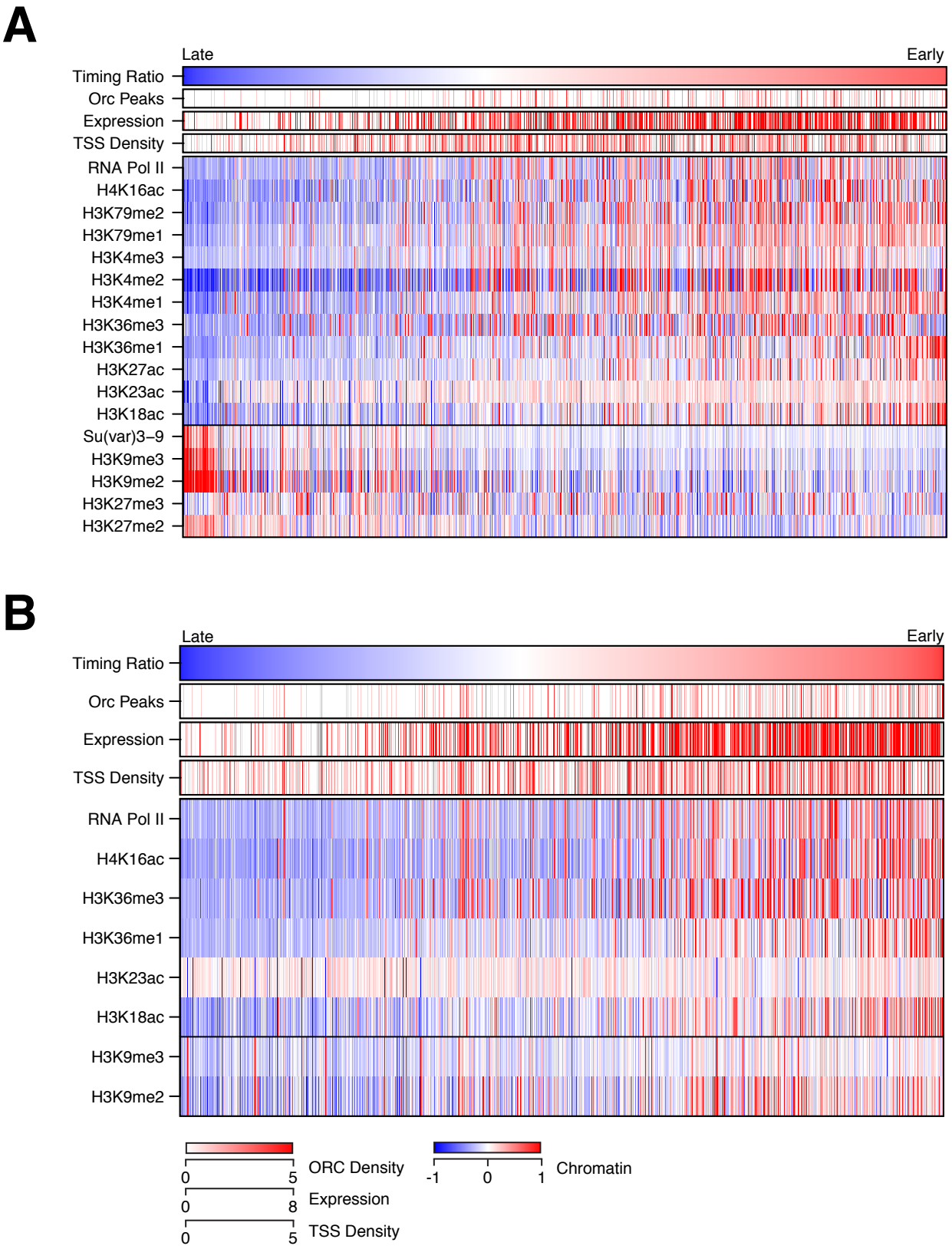
Lubelsky et al Supplemental Figure 1



Lubelsky et al Supplemental Figure 2

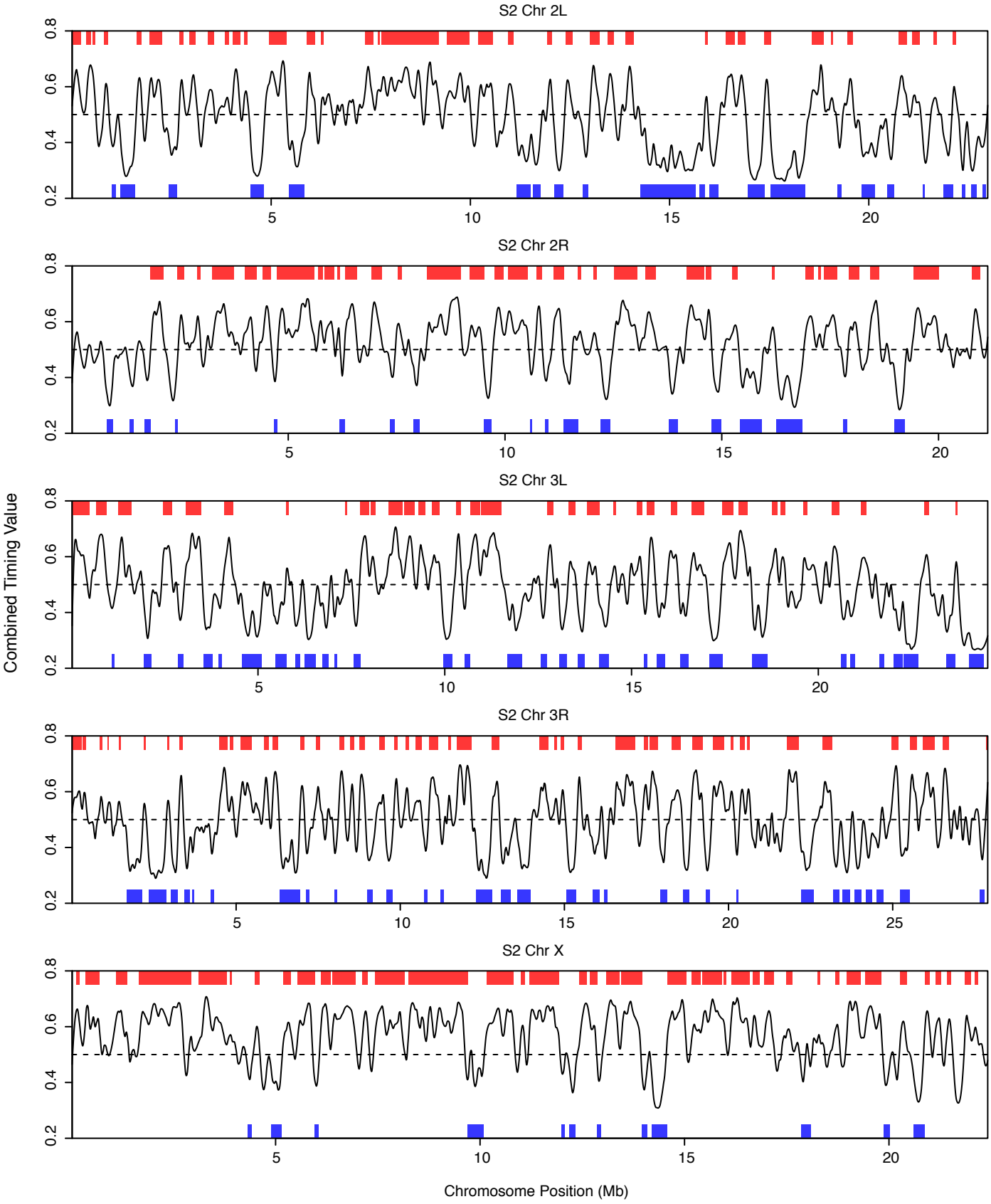


Lubelsky et al Supplemental Figure 3



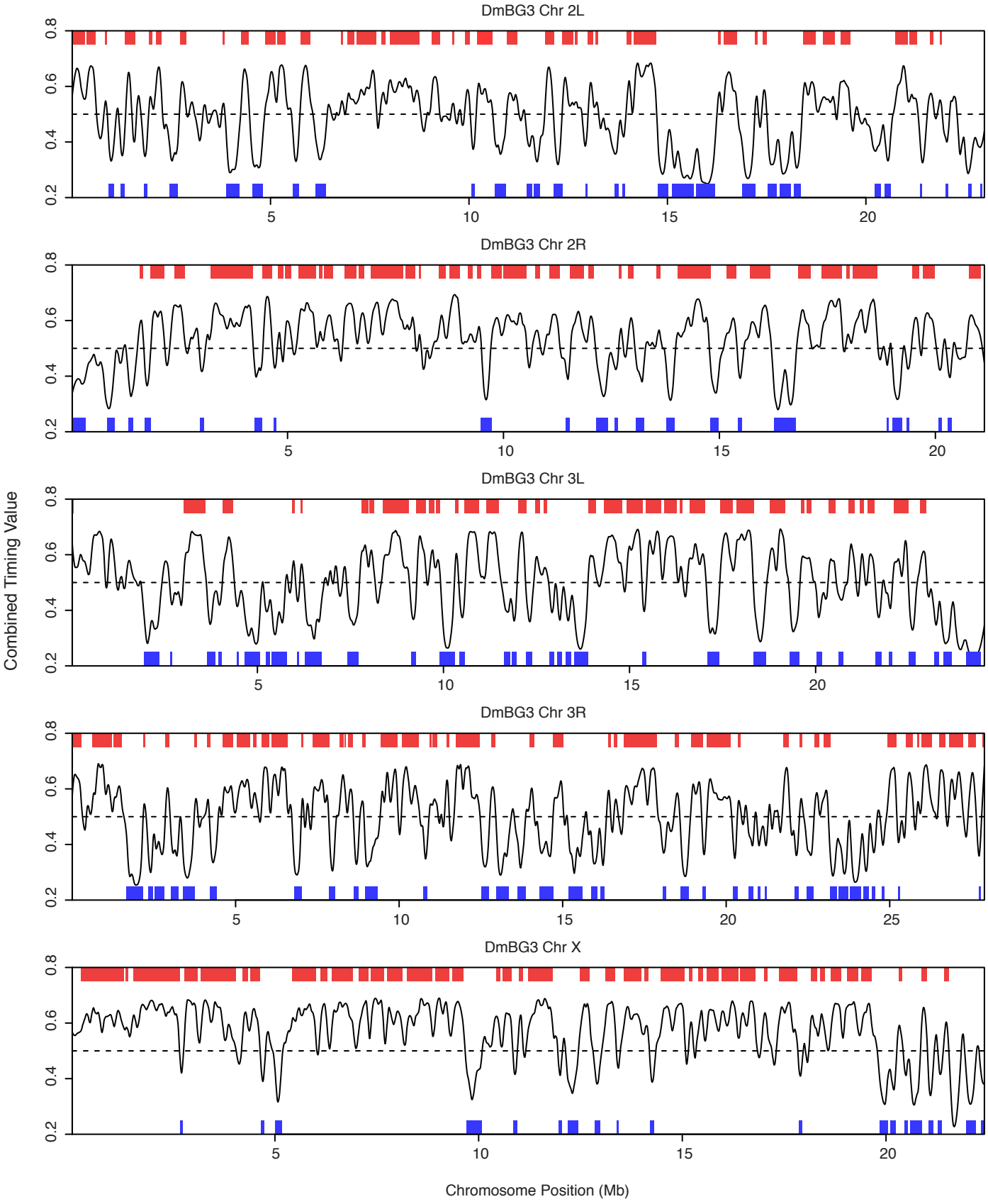
Lubelsky et al Supplemental Figure 4

A



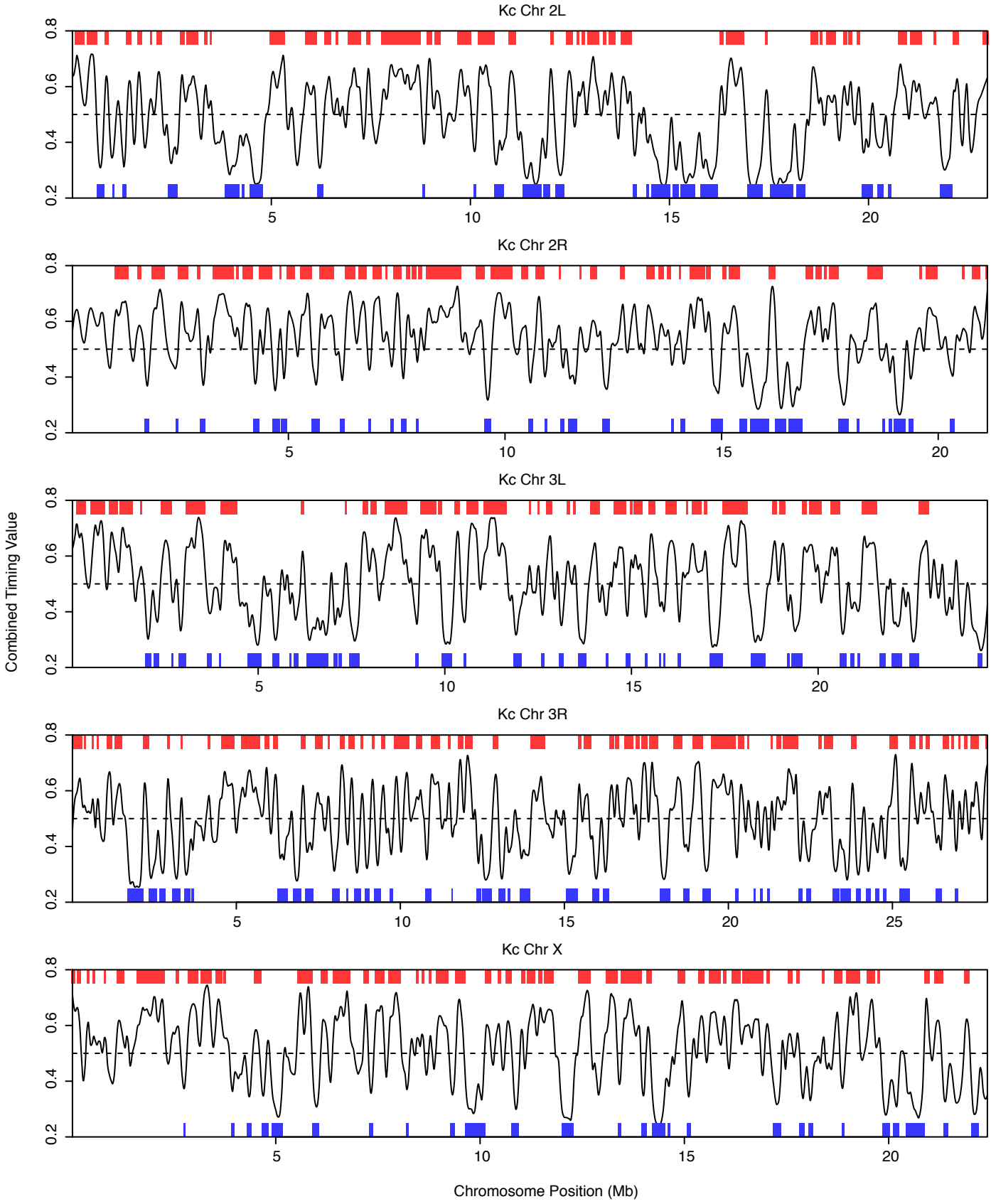
Lubelsky et al Supplemental Figure 5

B

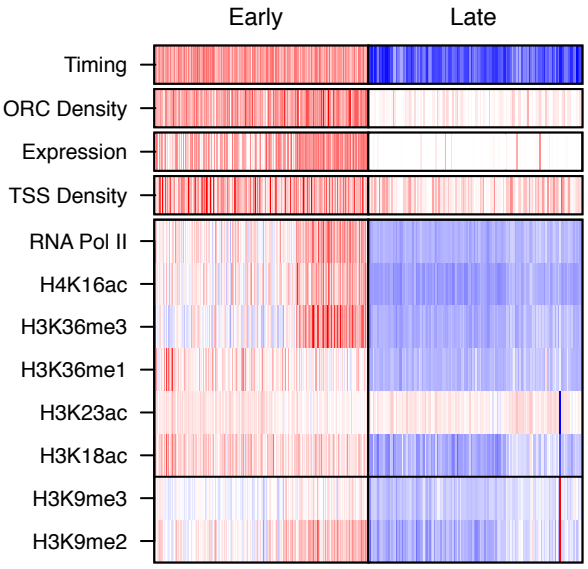


Lubelsky et al Supplemental Figure 5

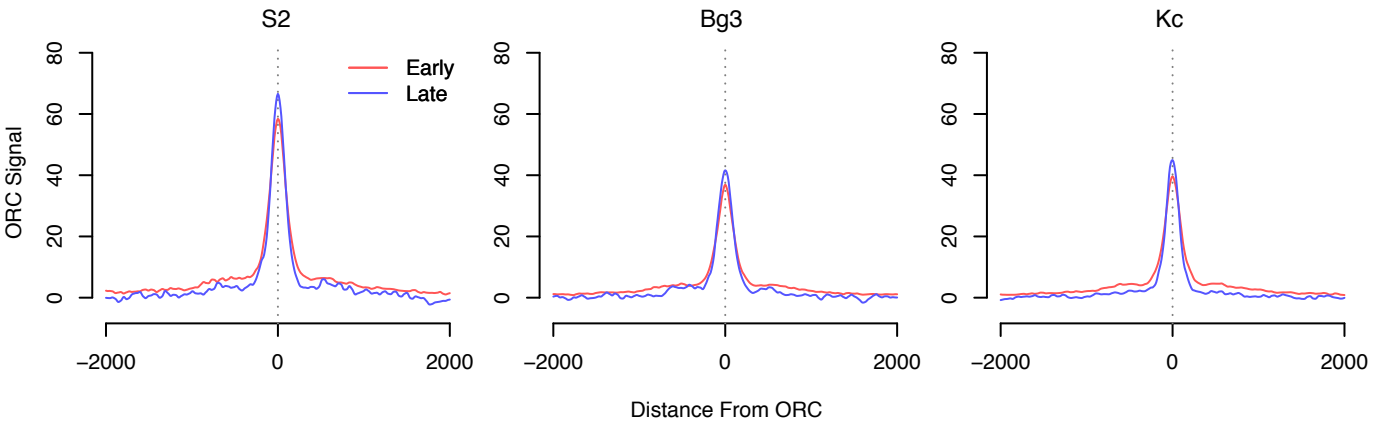
C



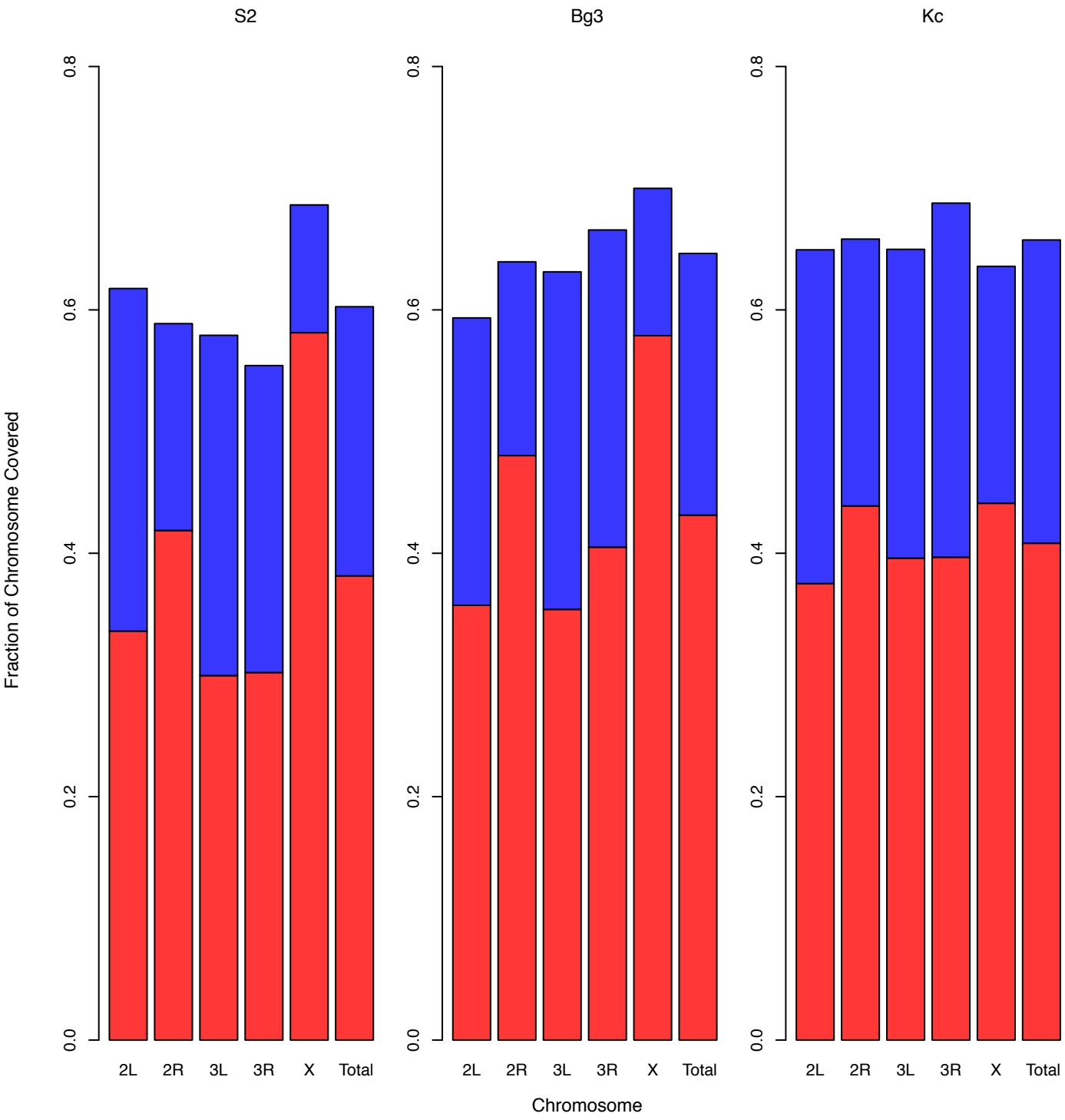
Lubelsky et al Supplemental Figure 5



Lubelsky et al Supplemental Figure 6



Lubelsky et al Supplemental Figure 7



Lubelsky et al Supplemental Figure 8