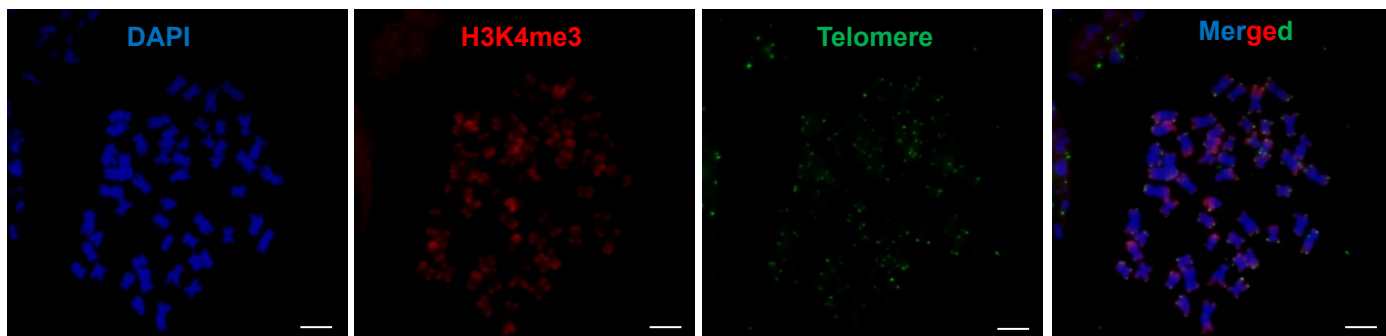
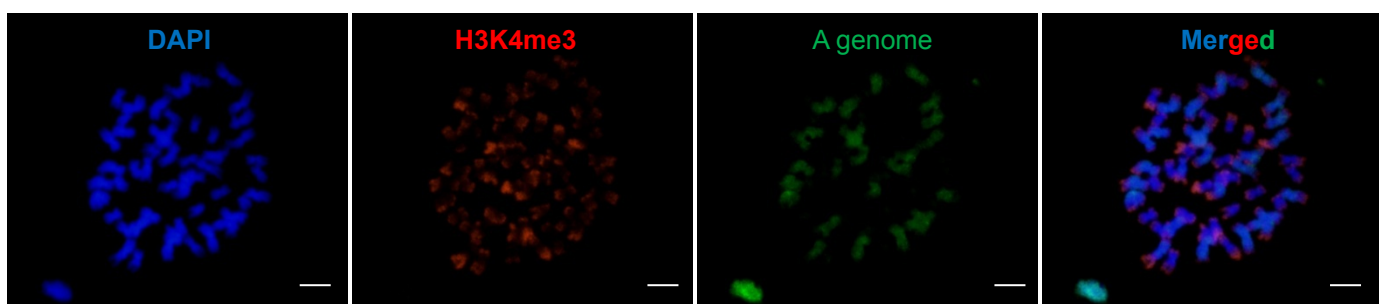


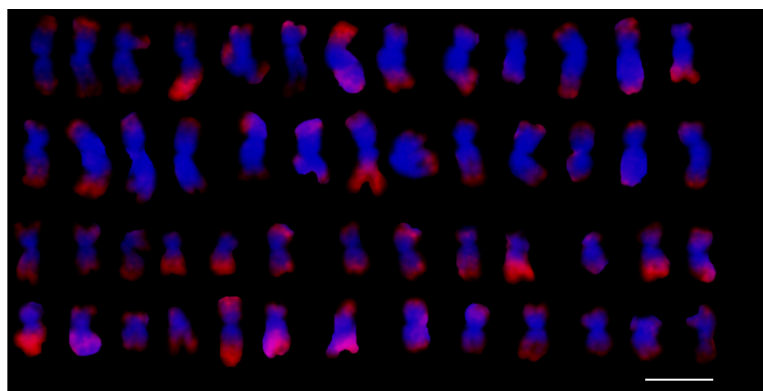
A



B

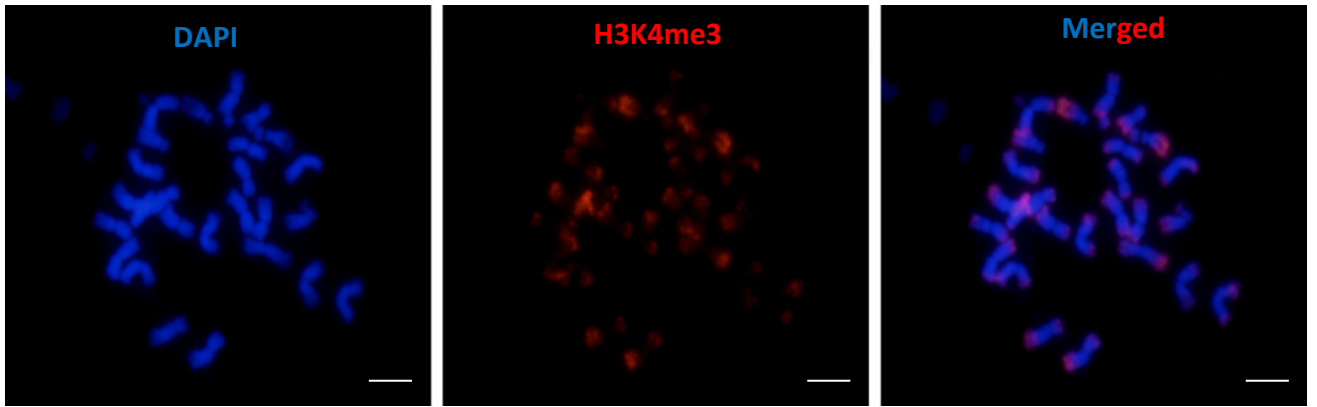


C

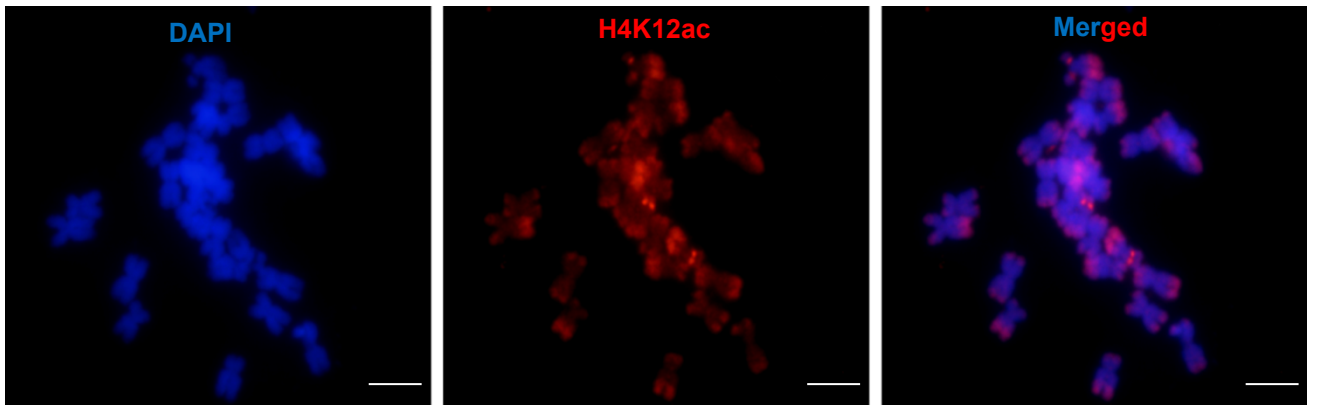
A homoeologous  
chromosomesD homoeologous  
chromosomes

**Supplemental Figure S1.** Antibodies against H3K4me3 Immunostaining and telomere locations of metaphase chromosomes in allotetraploid cotton. A and D homoeologous chromosomes were discriminated by genomic DNA *in situ* hybridization (GISH) using total genomic DNA of *G. arboreum* as a probe. A, Distribution of H3K4me3 and telomere across *G. hirsutum* metaphase chromosomes. Blue, red, and green indicate chromosomes (counterstained with DAPI), H3k4me3 (cy3), and telomeres (digoxigenin-labeled probes), respectively; bar=5 $\mu$ m. B, Discrimination between A and D homoeologous chromosomes by genomic *in situ* hybridization (GISH). Genomic DNA from *G. arboreum* was used as digoxigenin-labeled probe (fluorescein-conjugated anti-digoxigenin) in GISH analysis. Blue, red, and green signals indicate chromosomes, H3k4me3, A homoeologous chromosomes, respectively. C, A and D homoeologous chromosomes are arranged based on GISH, telomere, and immuno-staining patterns; bar=5 $\mu$ m.

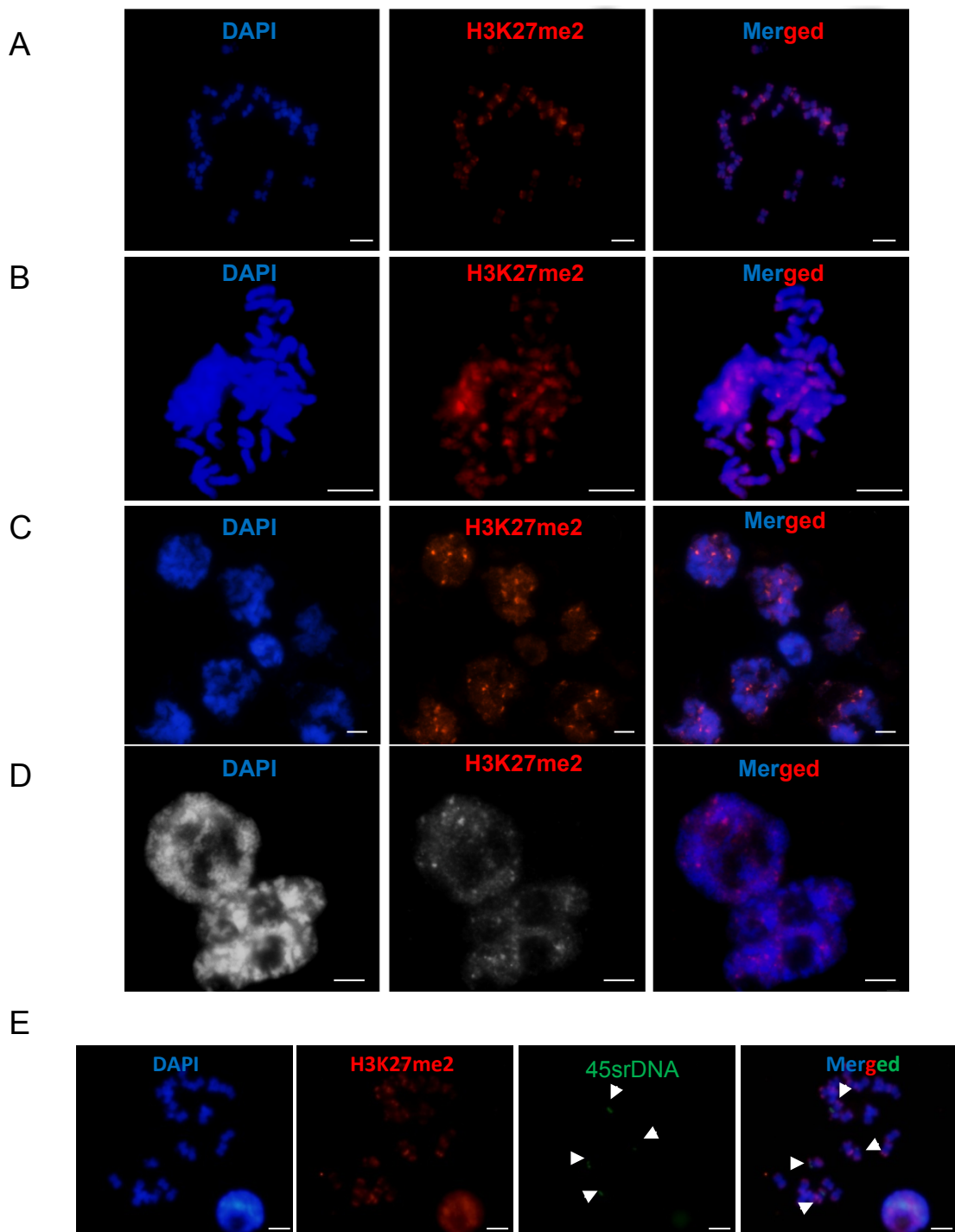
A



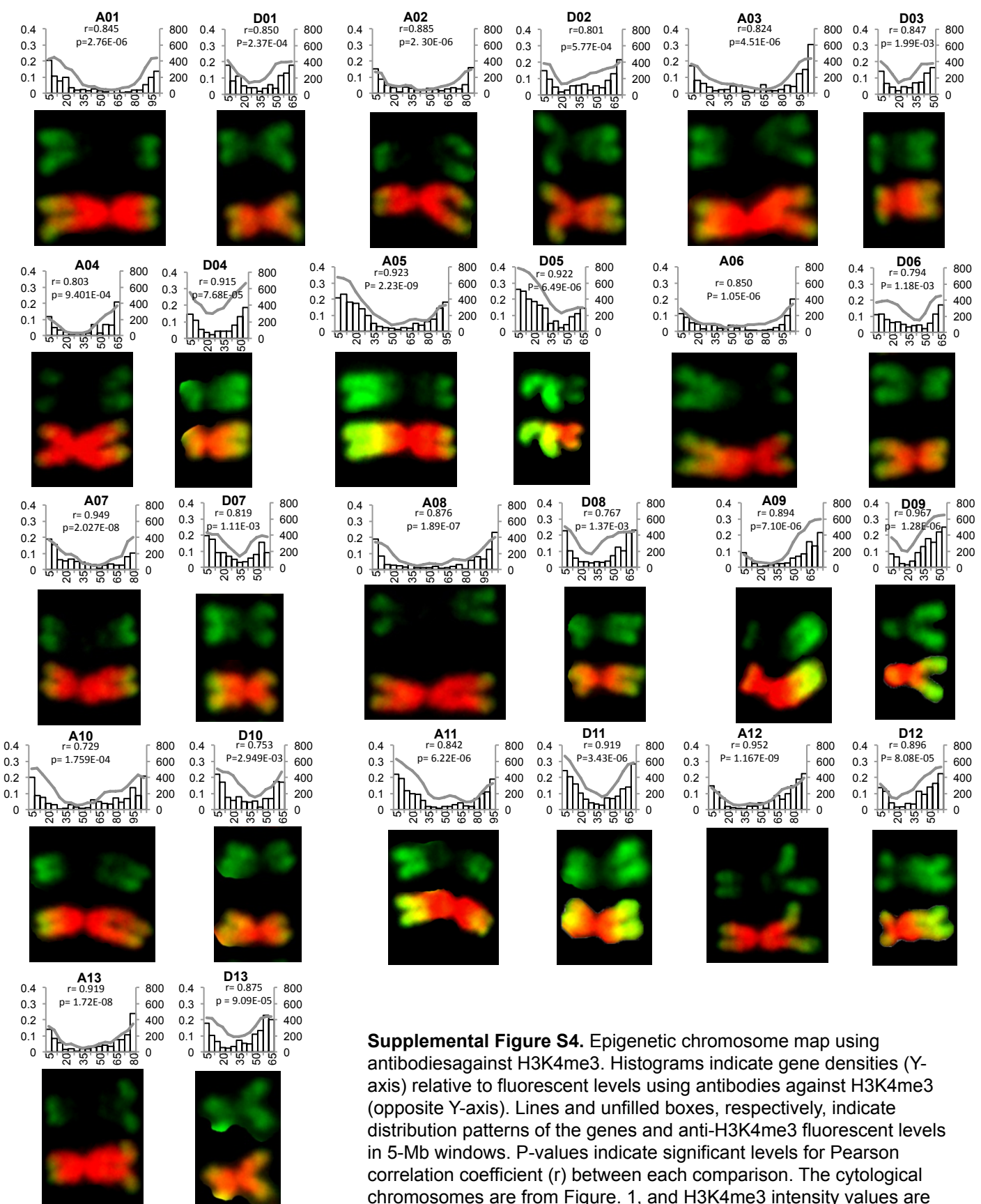
B





**Supplemental Figure S2.** Immunostaining images generated using antibodies against H3K4me3 and H4K12ac, respectively, in metaphase chromosomes of *G. arboreum*. A, Immunolabeling images using antibodies against H3K4me3 to metaphase of *G. arboreum*. Metaphase chromosomes counterstained with DAPI (blue) and antibodies against H3K4me3 (Cy3, red); bar=5 $\mu$ m. B, Immunofluorescence images using antibodies against H4K12ac to metaphase of *G. arboreum*. Metaphase chromosomes counterstained with DAPI (blue) and antibodies against H3K4ac12 (Cy3, red); bar=5 $\mu$ m.

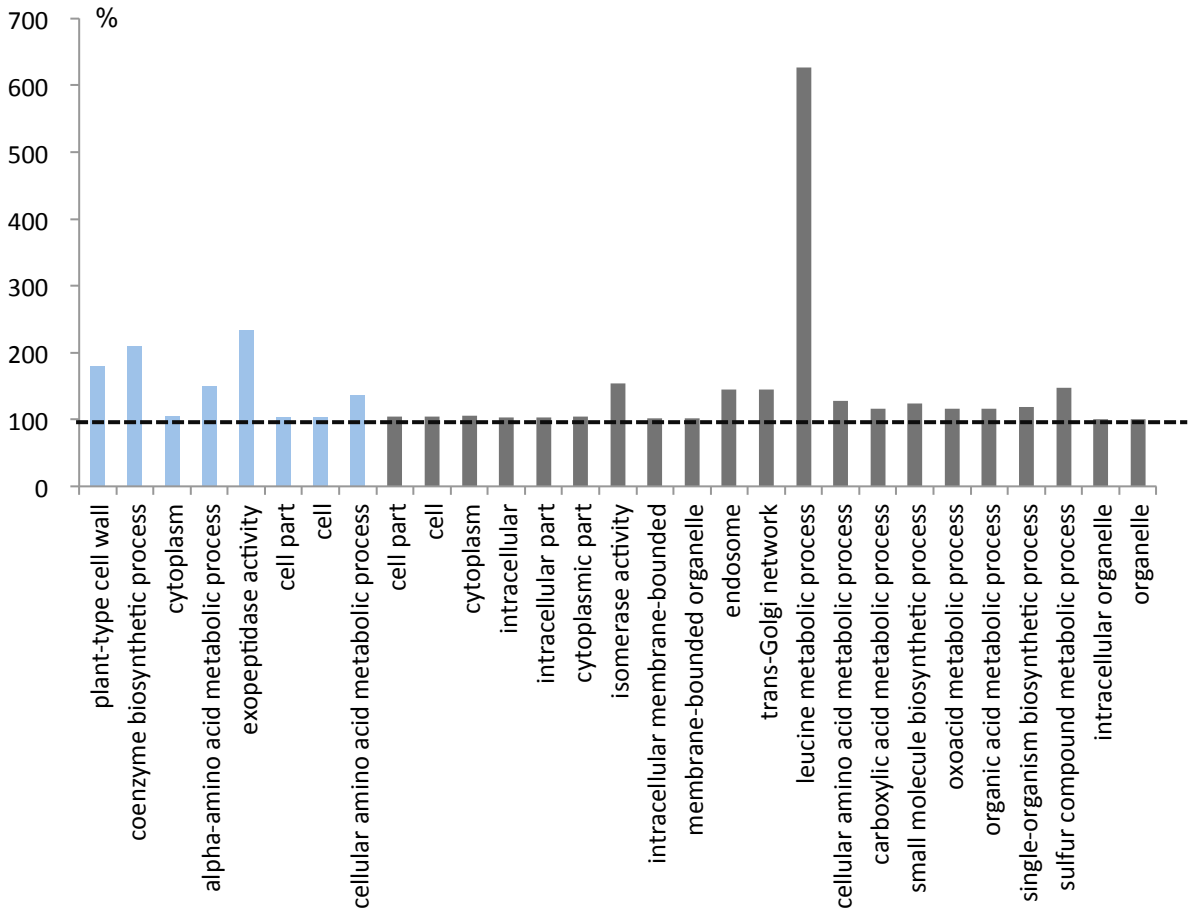


**Supplemental Figure S3.** Immunostaining images of interphase and metaphase chromosomes with antibodies against H3K27me2. A, Metaphase images of *G. arboreum*, showing chromosomes (blue, DAPI) and H3K27me2 (red, cy3); bar=5 $\mu$ m. B, Metaphase images of *G. hirsutum*, showing chromosomes (blue, DAPI) and H3K27me2 (red, cy3); bar=5 $\mu$ m. C, Interphase images of *G. arboreum*, showing chromosomes (blue, DAPI) and H3K27me2 (red, cy3); bar=5 $\mu$ m. D, Interphase images of *G. hirsutum*, showing interphase cell (left, DAPI) and H3K27me2 (middle, cy3); bar=5 $\mu$ m. E, The relationship between H3K27me2 and 45S rDNA in *G. arboreum*, showing chromosomes (blue, DAPI), H3k27me2 (red, Cy3), and 45S rDNA (green, digoxigenin-labeled probes were detected using fluorescein-conjugated anti-digoxigenin); bar=5 $\mu$ m.



**Supplemental Figure S4.** Epigenetic chromosome map using antibodies against H3K4me3. Histograms indicate gene densities (Y-axis) relative to fluorescent levels using antibodies against H3K4me3 (opposite Y-axis). Lines and unfilled boxes, respectively, indicate distribution patterns of the genes and anti-H3K4me3 fluorescent levels in 5-Mb windows. P-values indicate significant levels for Pearson correlation coefficient ( $r$ ) between each comparison. The cytological chromosomes are from Figure. 1, and H3K4me3 intensity values are shown in supplemental Data 2.

 Gene density  
 H3K4me3 level



**Supplemental Figure S5.** Gene Ontology (GO) analysis of the genes with homoeolog expression bias. Blue and grey histograms indicate enrichment of the genes with A-homoeolog expression bias ( $A > D$ ) and D-homoeolog expression bias ( $D > A$ ), respectively.