

Figure S1. Profiles and heatmaps showing read coverages for two epigenetic features across MorexV3 high-confidence genes, related to Figure 1B. Data from bisulfite sequencing show cytosine methylation in three sequence contexts in (A) 24DAP embryo and (B) leaf. (C) Profiles of ATAC-seq coverages around transcription start sites (TSSs) in the four developmental stages used in the study.

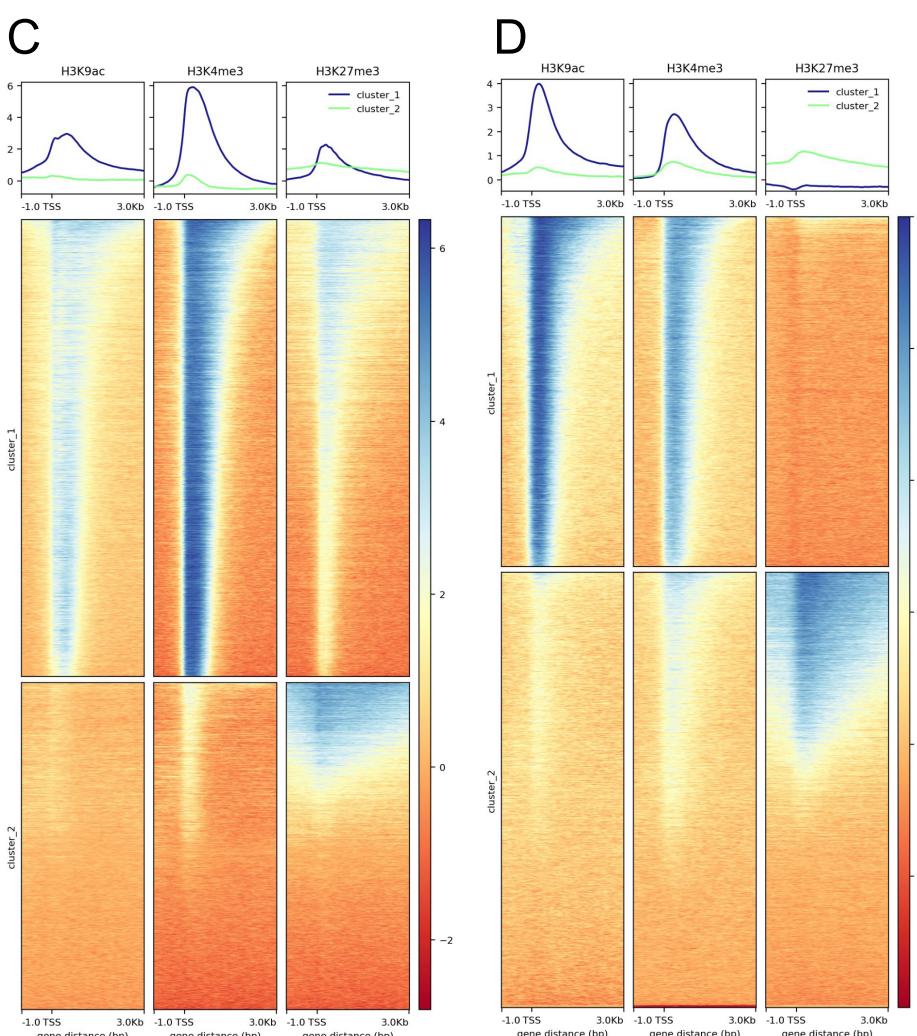
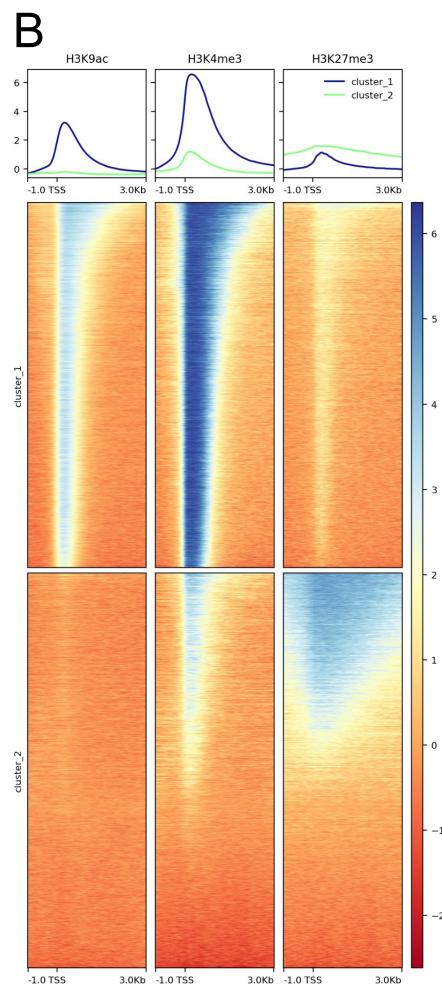
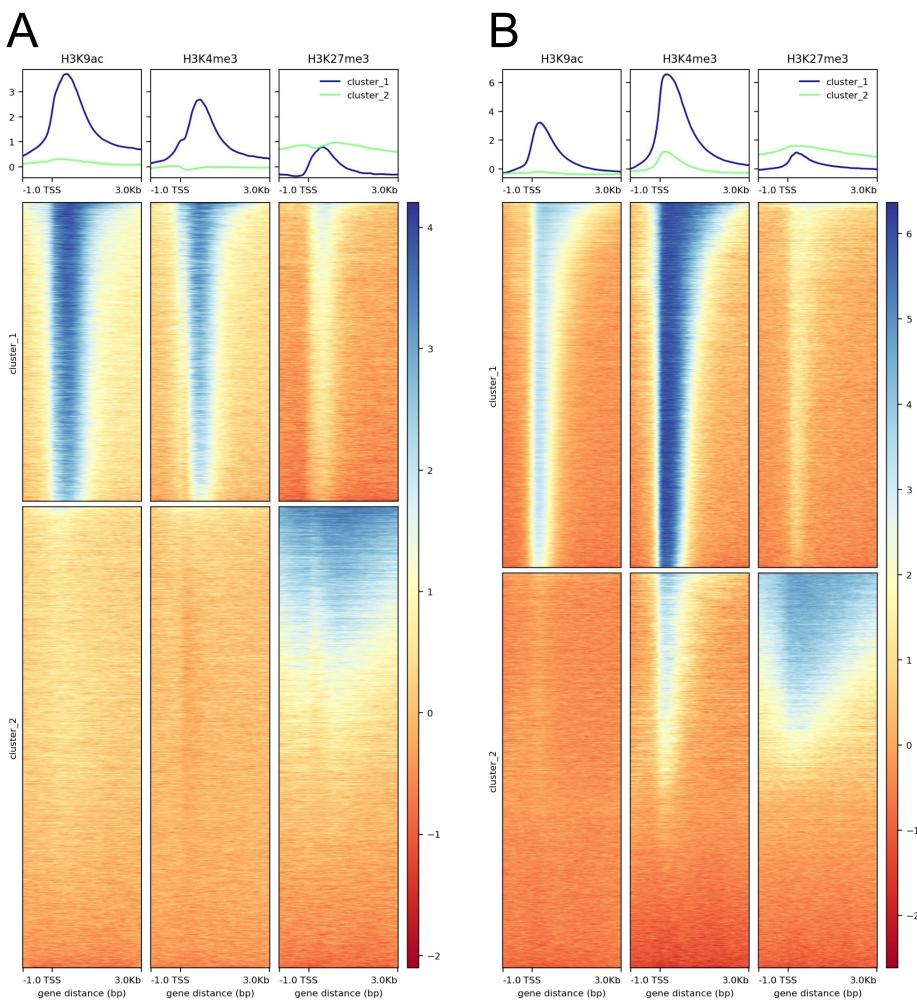


Figure S2. K-means clustered profiles of histone modifications around HC-gene TSSs from ChIP-seq, related to Figure 1B. (A) Profiles in 8DAP embryo. Cluster 1 (top) represents active genes while cluster 2 (bottom) shows profiles of silent genes. (B, C, D) Data from 24DAP (B), 4DAG (C) and leaf (D) samples. The k-means clustering separates two sub-profiles reflecting transcriptional activity.

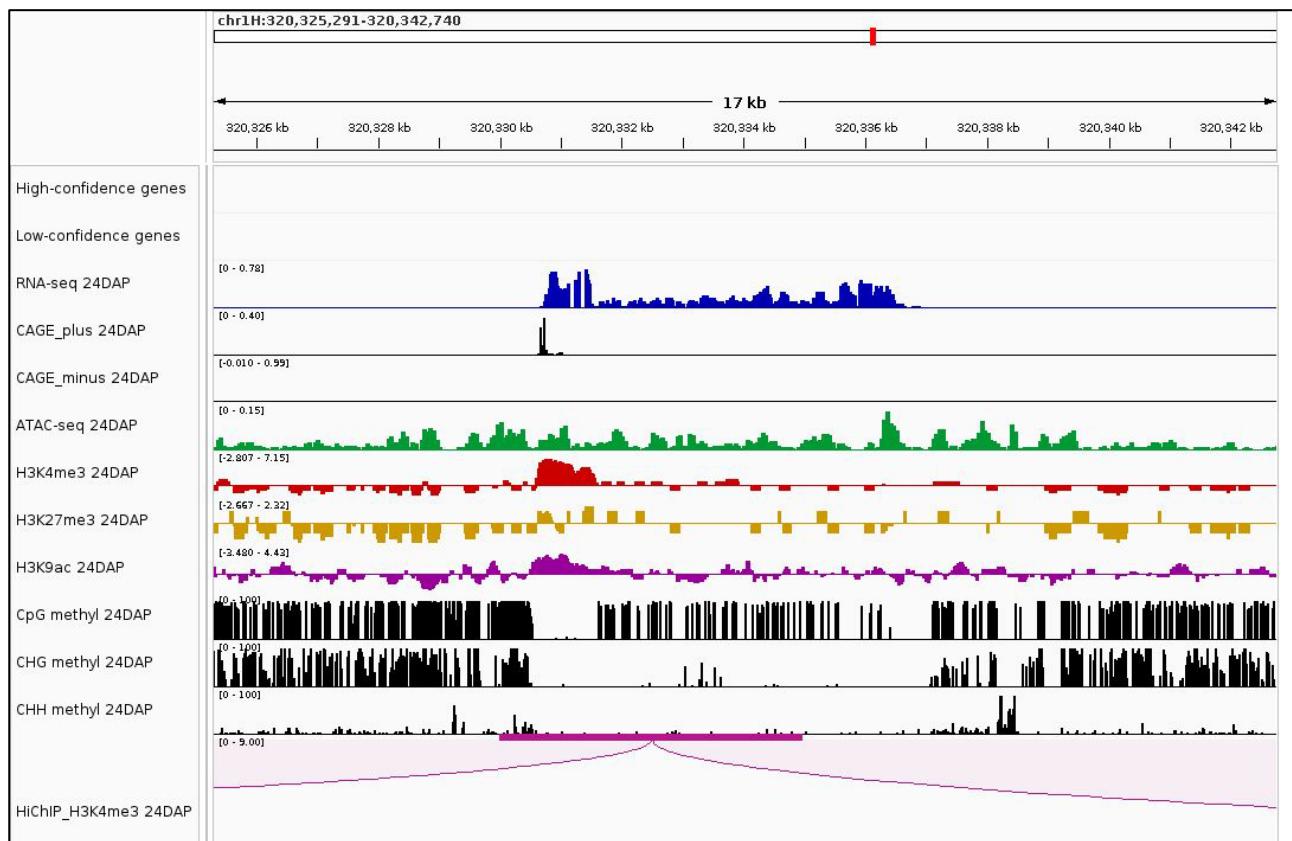
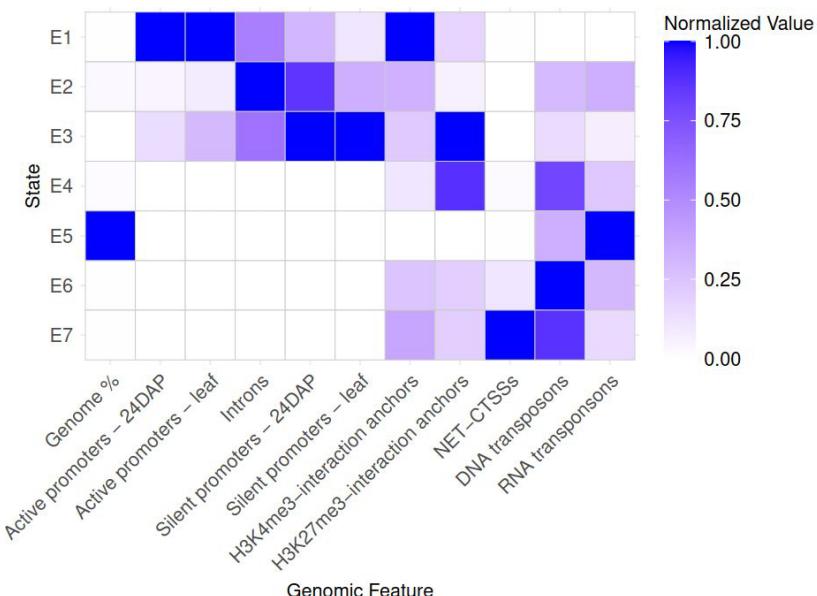
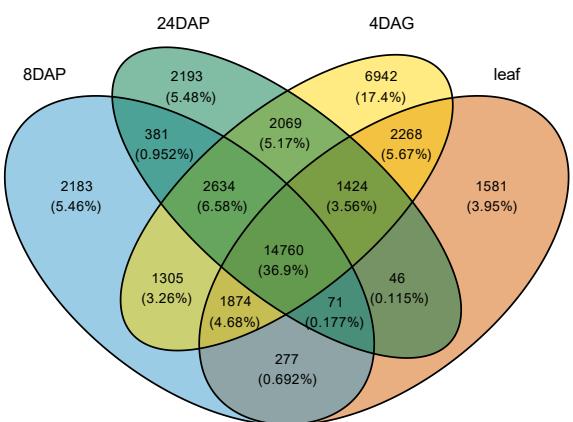


Figure S3. An example of a putative unannotated gene, related to Figure 1C. The occurrence of transcripts and features of active chromatin indicates the presence of an unannotated gene. To prevent misinterpretation of its promoter as an intergenic CRE, we excluded this and similar regions from the analysis of the non-coding genome.

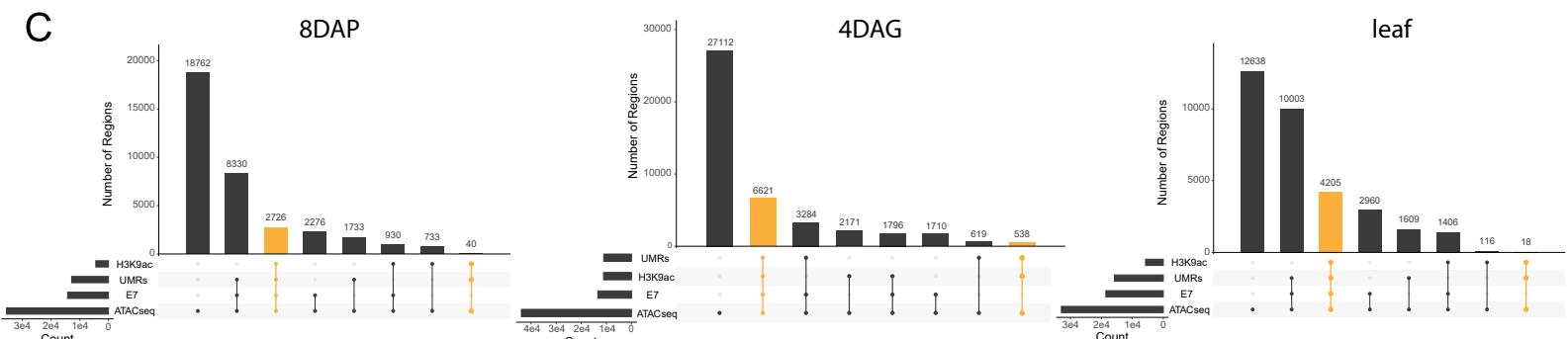
A



B



C



D

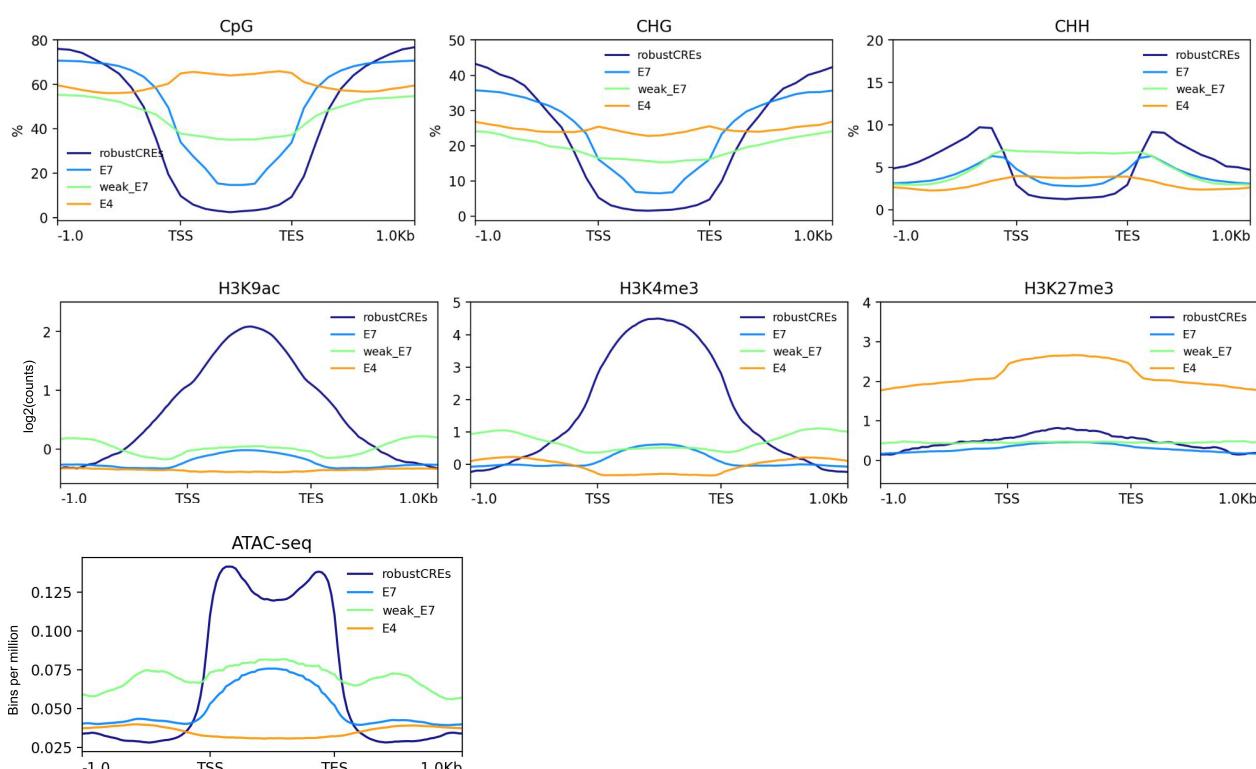
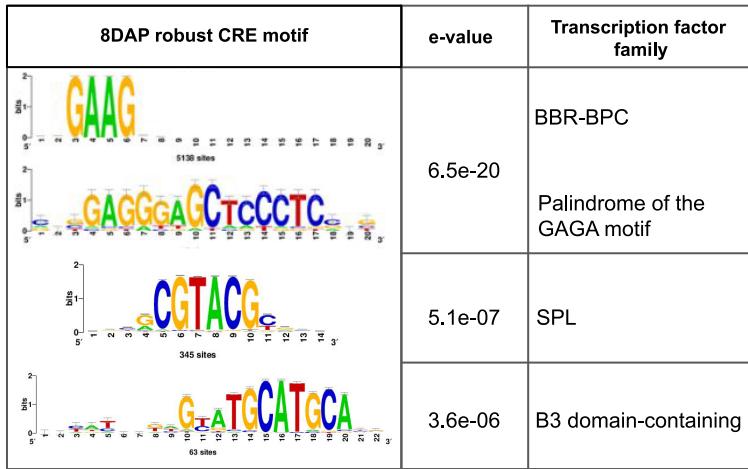
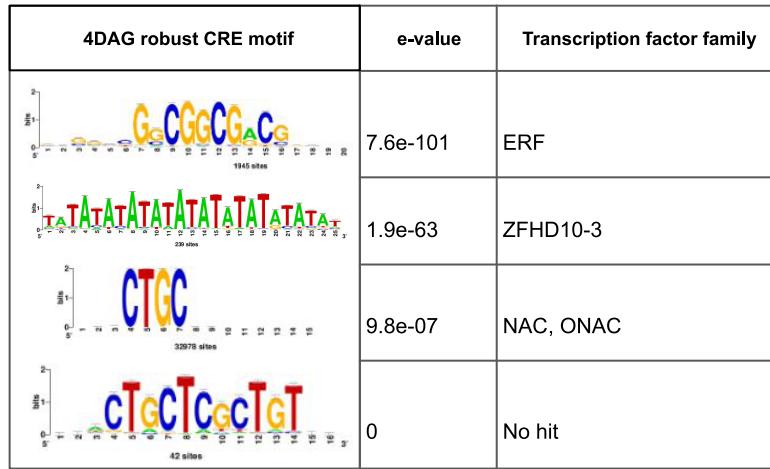


Figure S4. Chromatin state analysis is a sensitive method of cCREs detection, related to Figures 1C, D, and 2A, B. (A) An overlap enrichment of genomic features across 7 chromatin states in the leaf sample showing state dynamics following transcription dynamics between individual stages. (B) Dynamics of chromatin state-E segments, potentially comprising silenced cCREs. (C) The overlap of activating intergenic genomic features - peaks of ATAC-seq and H3K9ac and unmethylated regions (UMRs) - defines tissue-specific sets of robust cCREs (highlighted yellow), which shows a high overlap with E7 segments. ‘Count’ indicates the total number of peaks for ATAC-seq while for E7, H3K9ac and UMRs, overlaps of these datasets with ATAC-seq peaks are counted. (D) Comparison of main chromatin feature profiles across robust cCREs, states E4, E7 and the E7-state segments not overlapped with any of the epigenetic feature peaks (weak E7).

A



B



C

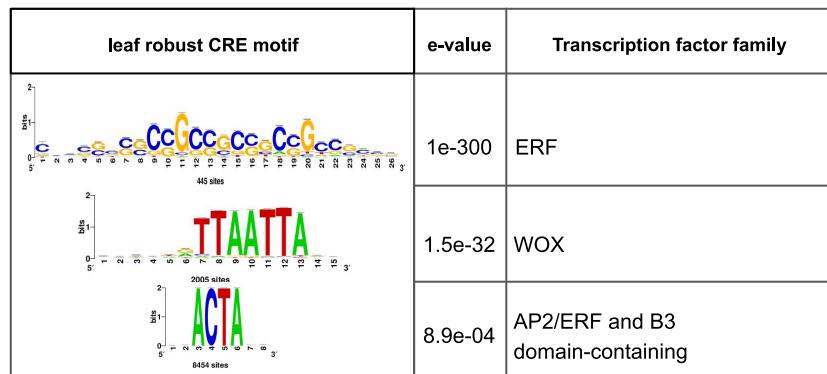
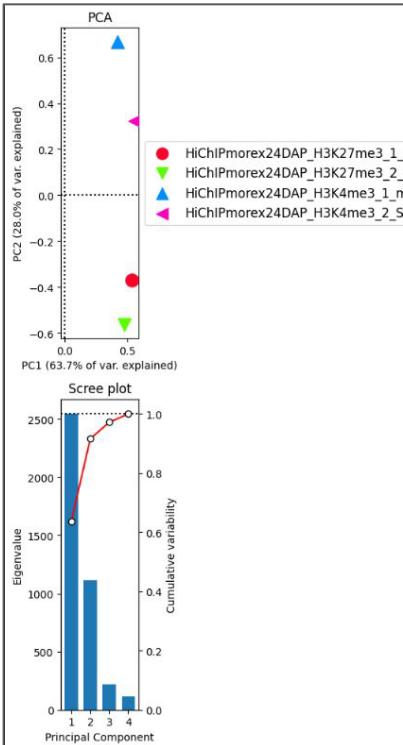
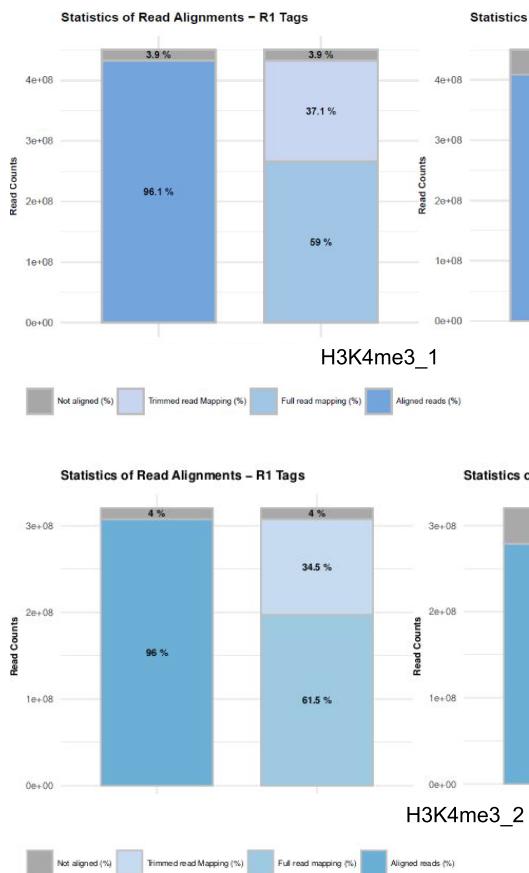


Figure S5. Transcription factor binding sites in robust cCREs, related to Figure 2D. Transcription factor binding sites enriched in (A) 8DAP, (B) 4DAG, and (C) leaf robust cCRE datasets.

A



B



C

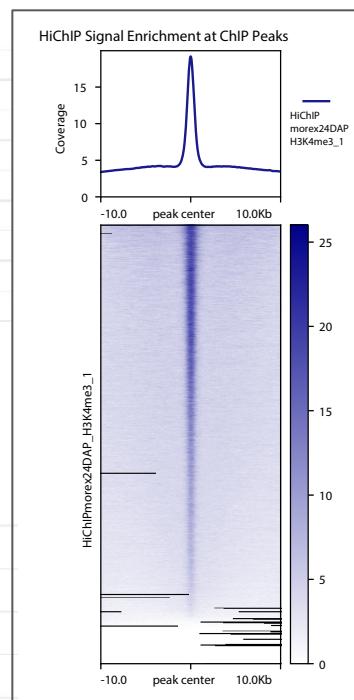
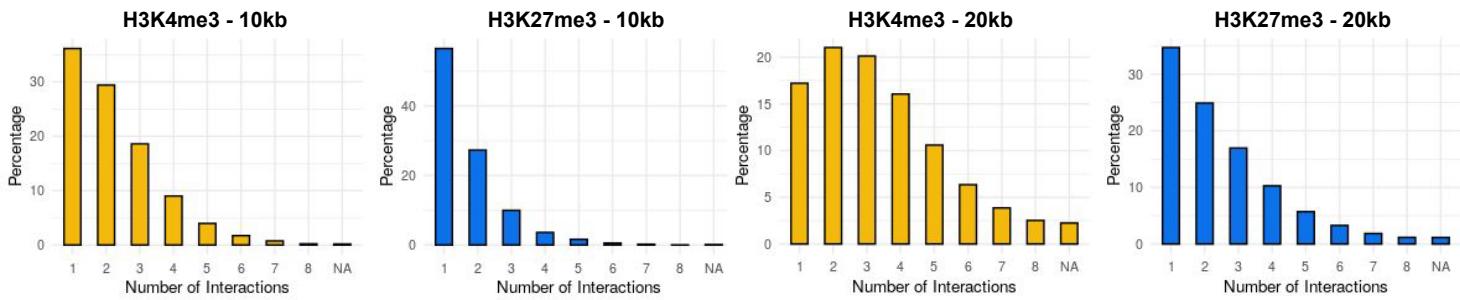
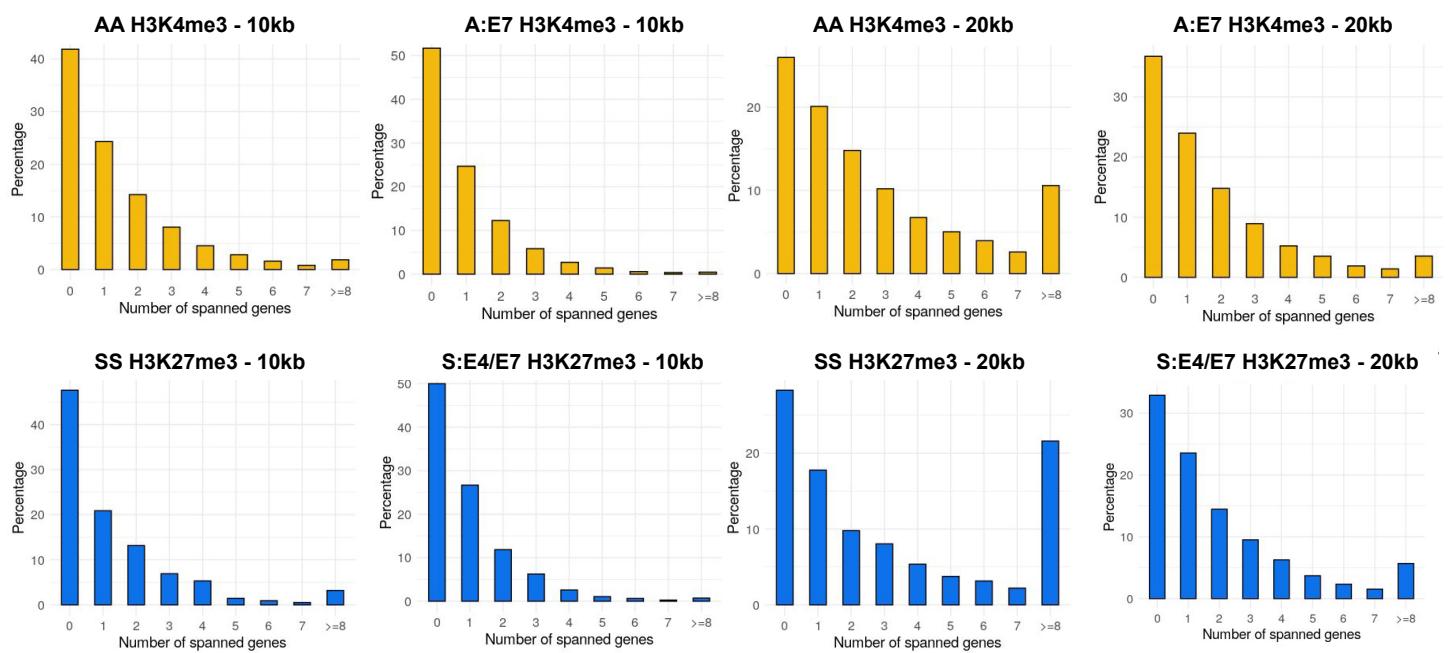


Figure S6. Quality Control of the 24DAP H3K4me3 HiChIP sequencing datasets, related to STAR Methods - HiChIP data analysis. (A) HiChIP replicate correlation of mapped data by Principal Component Analysis (PCA). (B) Mapping statistics of the two 24DAP H3K4me3 HiChIP replicates. (C) HiChIP signal enrichment at the H3K4me3 ChIP peaks as called from previously performed native ChIP-seq experiment.

A



B



C

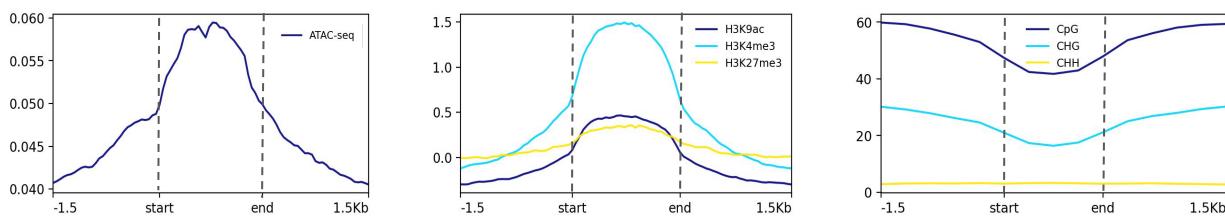
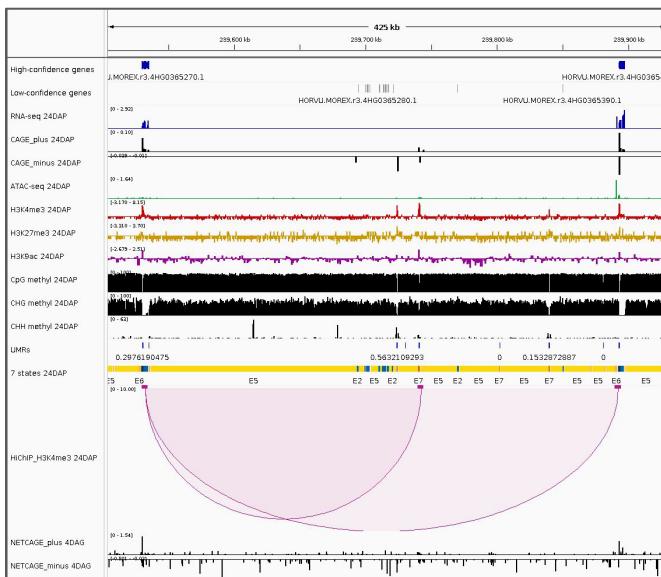
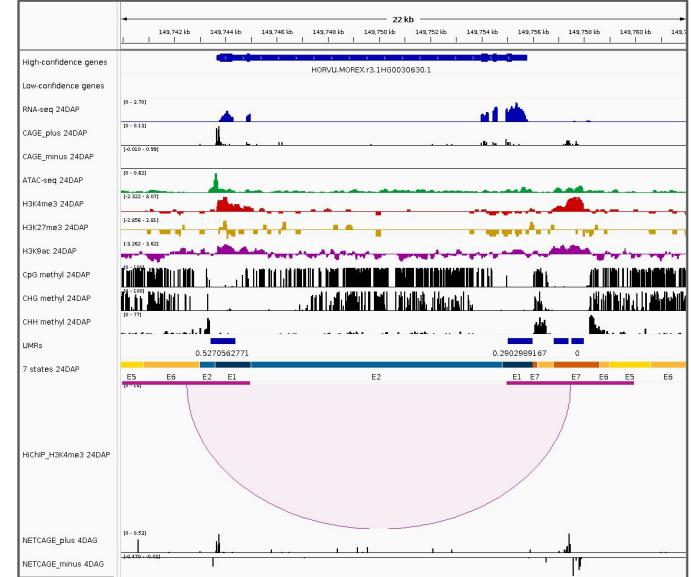


Figure S7. Analysis of the interactome in the 24DAP barley embryo, related to Figure 4. (A) Numbers of H3K4me3 and H3K27me3 HiChIP interactions per promoter at 10- and 20-kb resolution. (B) Numbers of genes spanned by H3K4me3 and H3K27me3 HiChIP interactions at 10- and 20-kb resolution. (C) Enrichments of selected epigenetic features across H3K4me3 HiChIP anchors at 5-kb resolution indicates that they are transcriptionally dense regions.

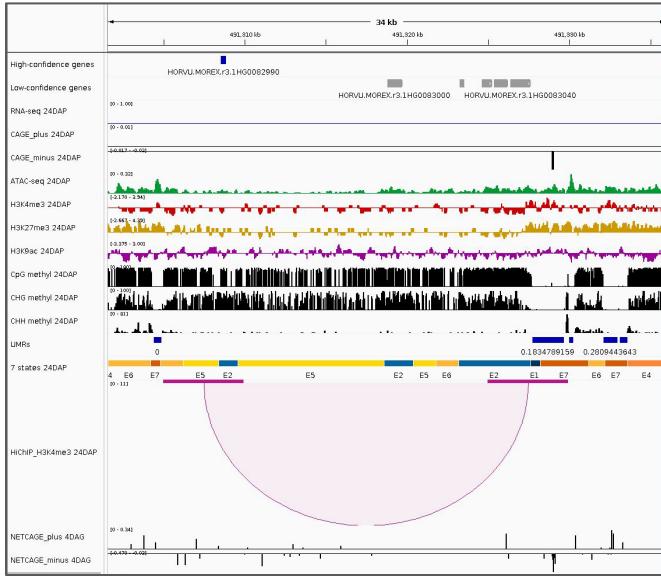
A



B



C



D

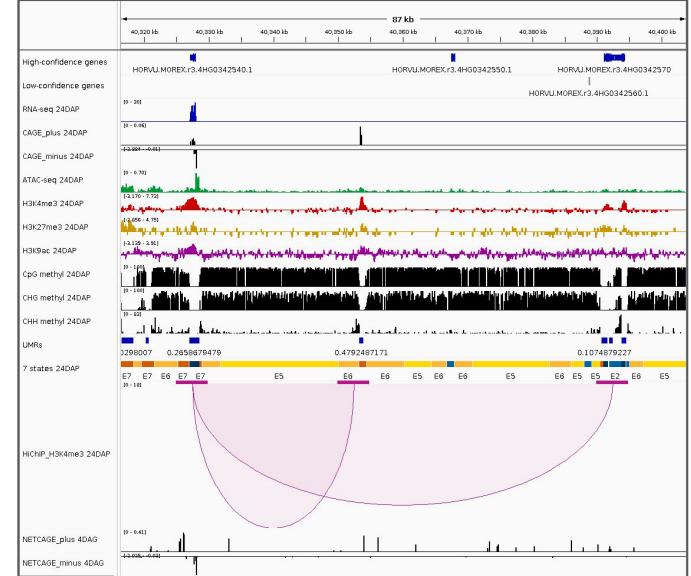
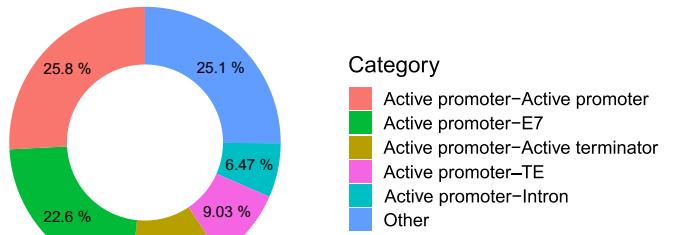


Figure S8. Examples of distinct interaction classes together with epigenomic features, related to Figure 4.
(A) Active promoter-active promoter, **(B)** active self-gene loop, **(C)** silent promoter-CREc, **(D)** active promoter-CREc and active promoter-silent gene.

A



B

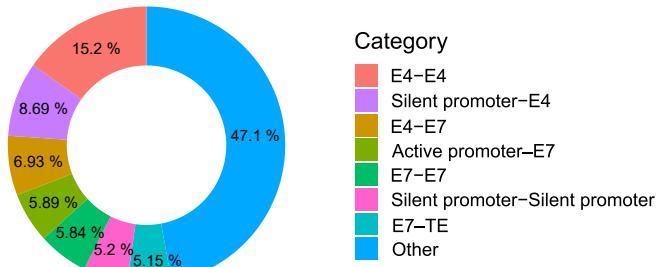


Figure S9. Proportions of interaction classes identified by HiChIP at 5-kb resolution in 24DAP embryo, focusing on high-confidence genes, related to Figure 5. Annotation of all significant interactions associated with (A) activating (H3K4me3) and (B) repressive (H3K27me3) histone mark. The category „Other“ includes all interaction classes with <5% frequency.

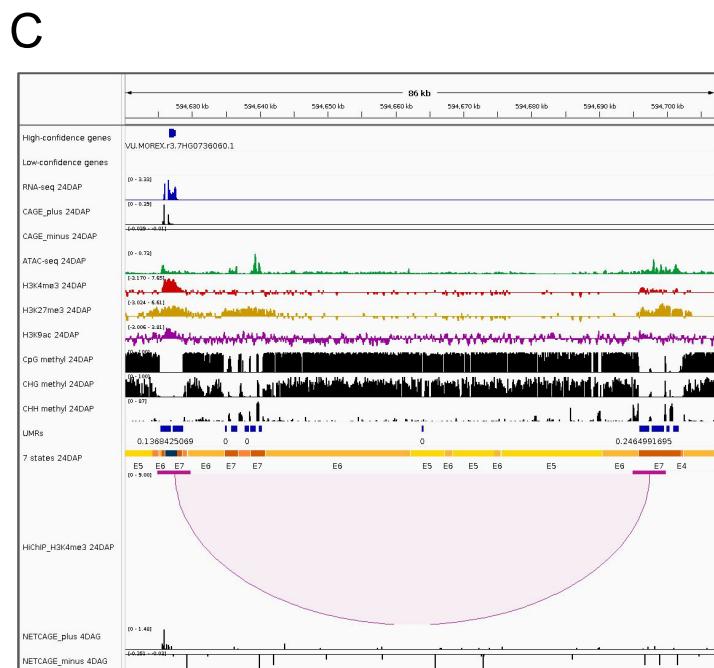
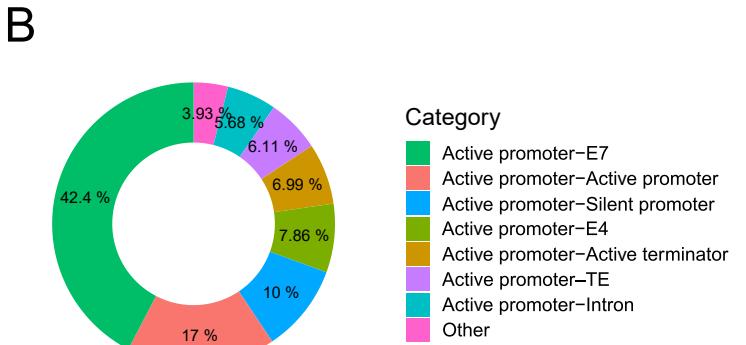
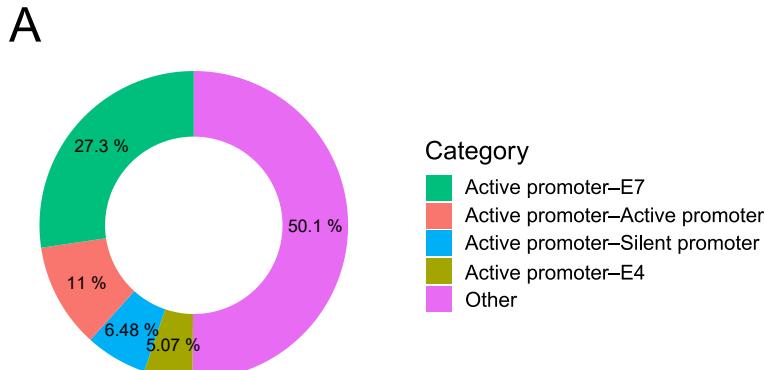


Figure S10. Features of bivalent interactions, related to Figure 5. Annotation of all bivalent interactions (A), and the same set from the active promoter-centric view (B). The category „Other“ includes all interaction classes with <5% frequency. (C) An example of a bivalent interaction.

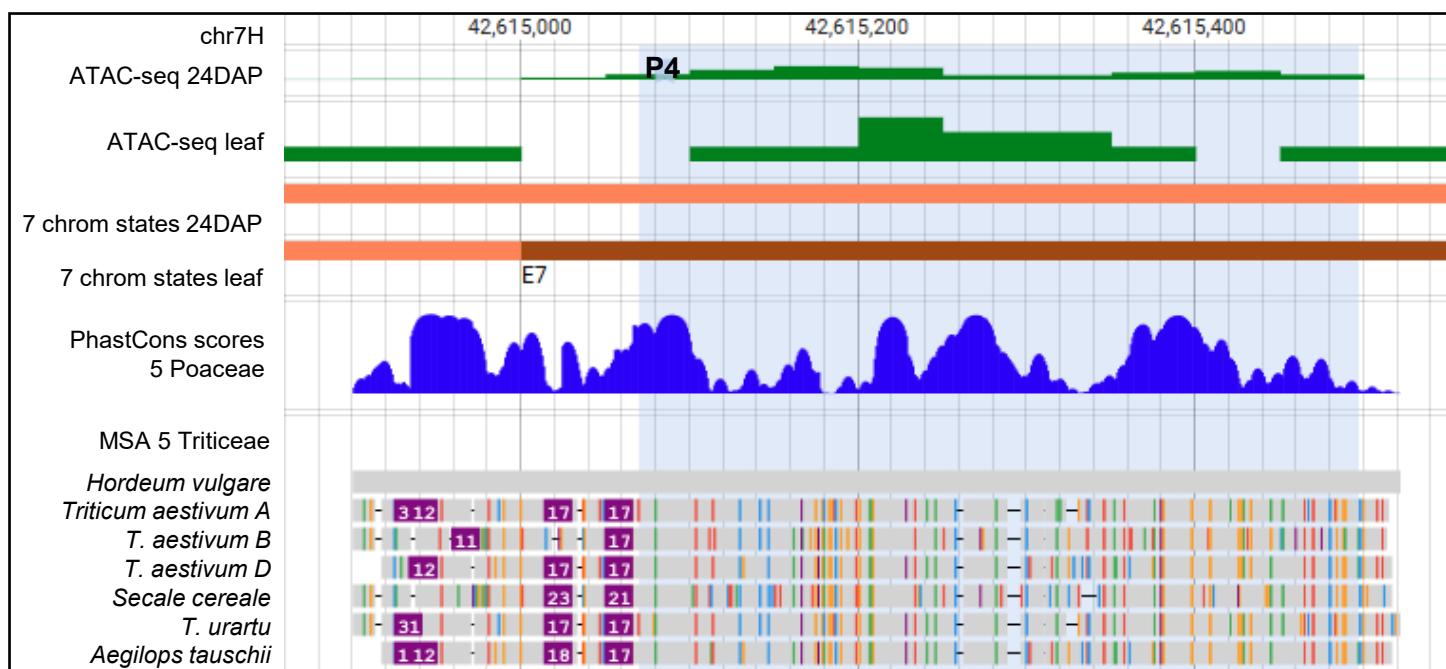
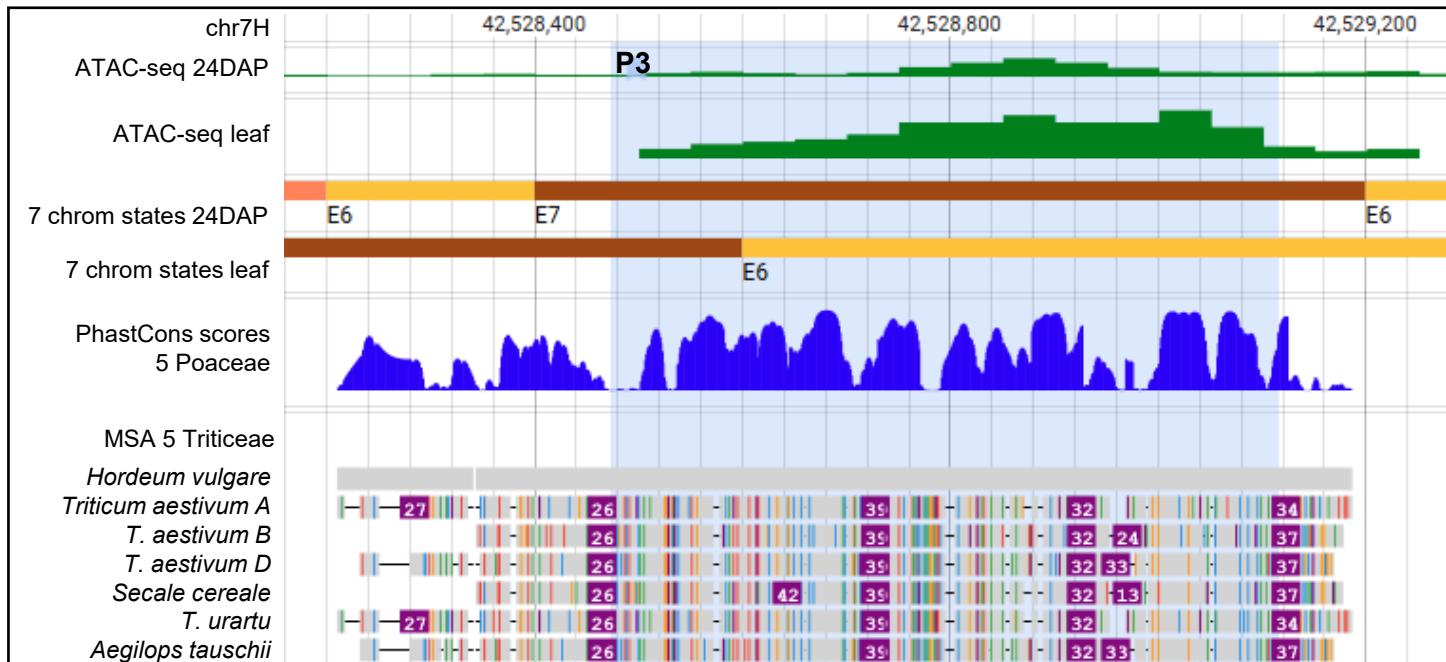


Figure S11. Regulome of the barley Vernalization 3 gene: evolutionary conservation, related to Figure 7.
 Regions of cCRE3 (top) and cCRE4 (bottom) with BLAST hits of bread wheat enhancers P3 and P4 (highlighted blue) show high sequence conservation (PhastCons scores, scale 0-1) among five *Poaceae* species (*H. vulgare*, *Triticum urartu*, *Aegilops tauschii*, *Secale cereale* and *Brachypodium distachyon*). Multiple sequence alignment (MSA) shows the conservation at single-base resolution.