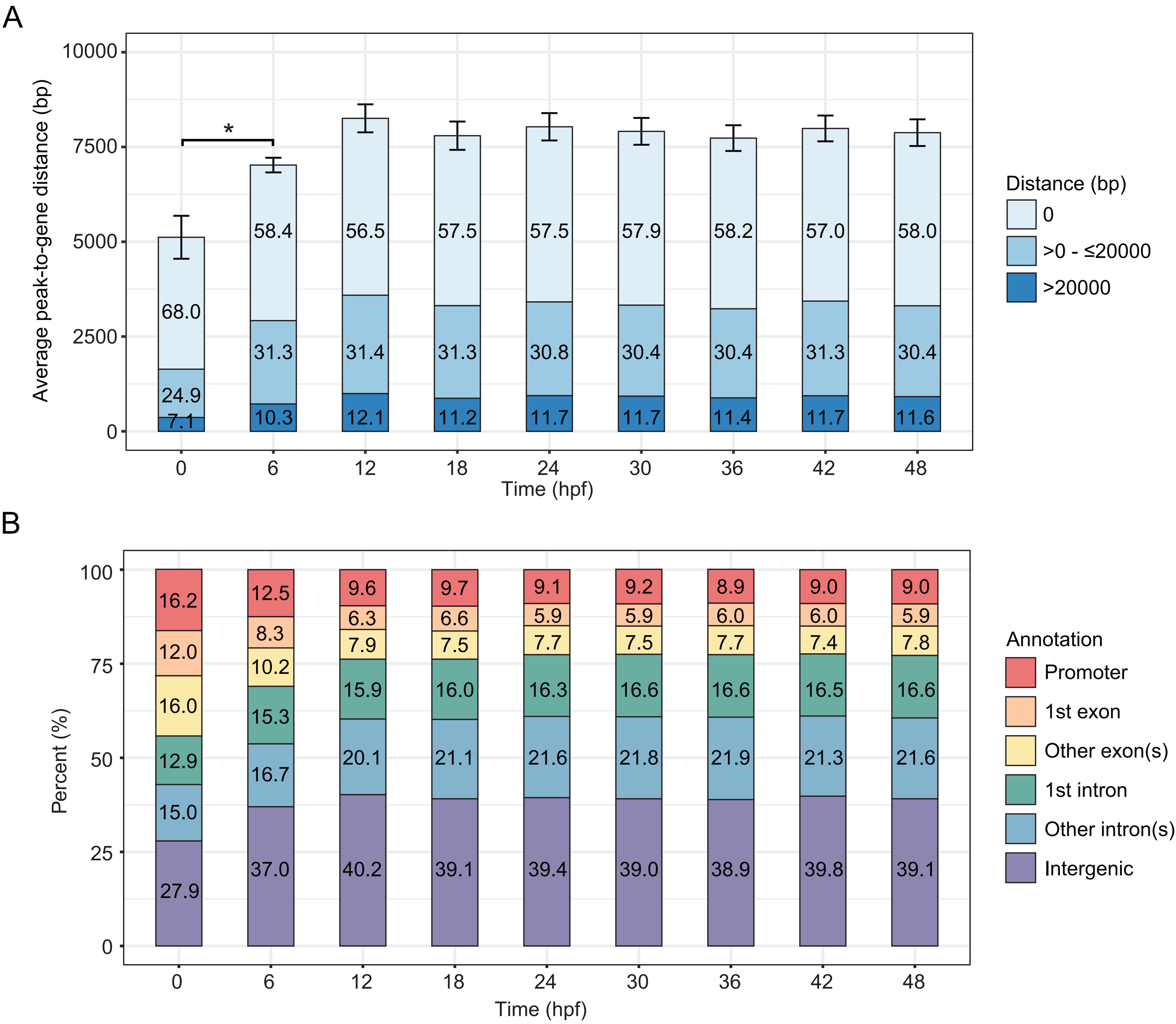
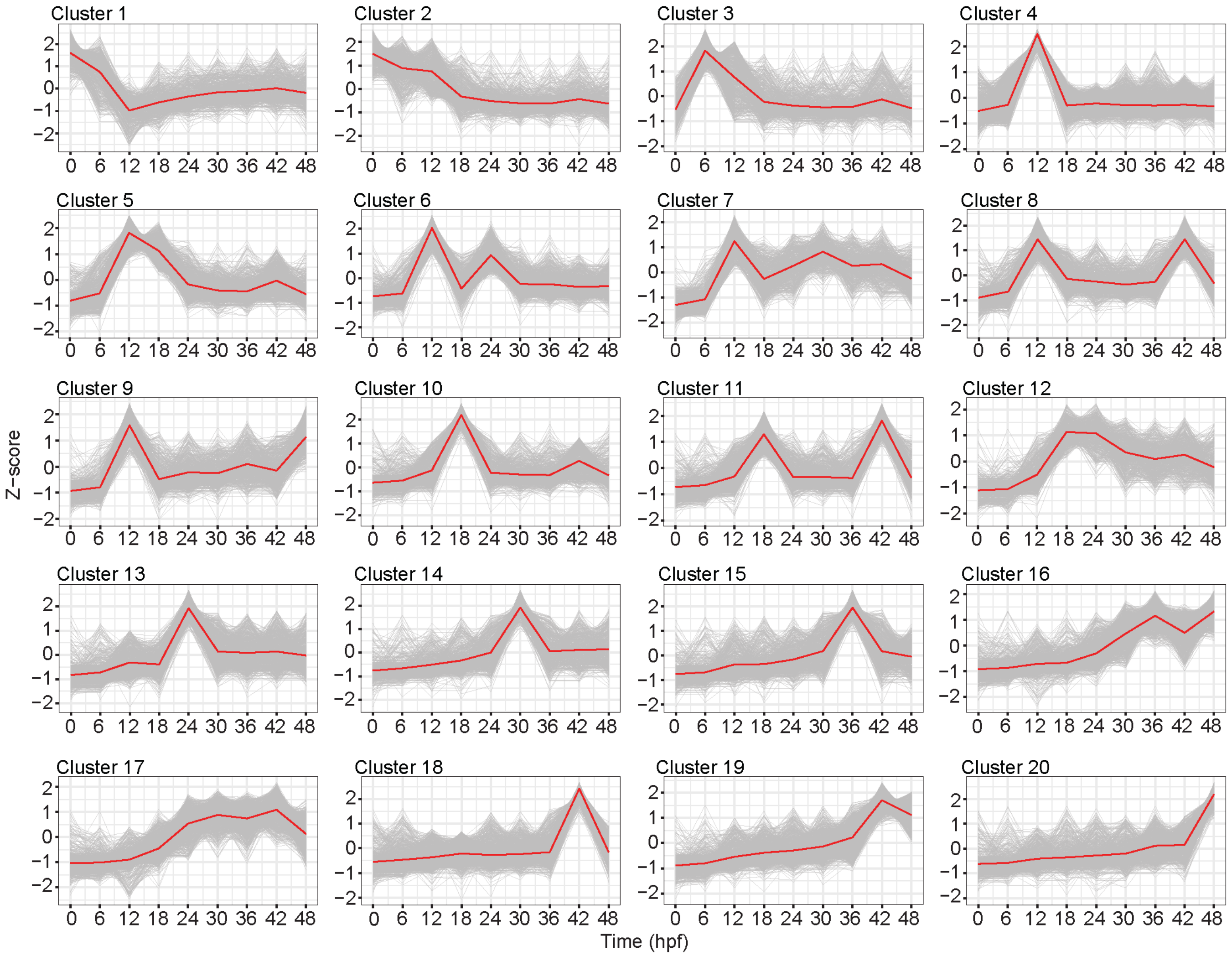
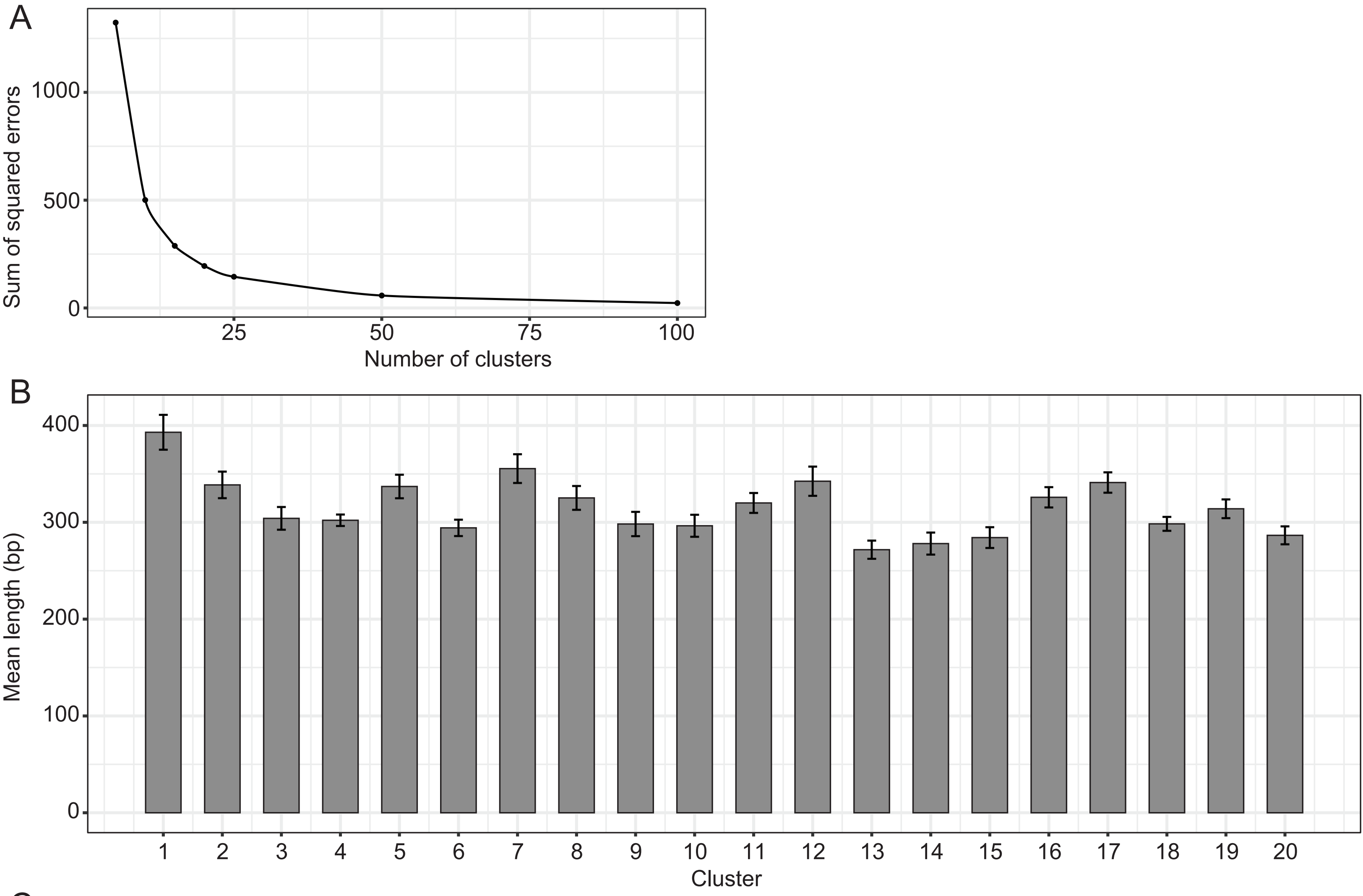
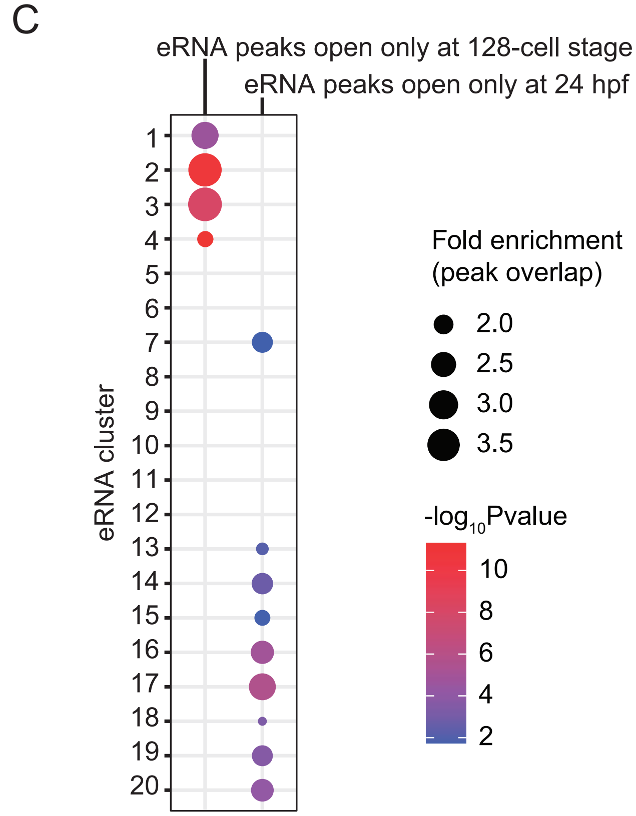


Supplemental Figure S1: Annotation and analysis of eRNAs across time points. (A) Average peak-to-gene distance (bp) of eRNAs at each time point (*P<0.001). Stacked bars represent the percentages of eRNAs in discrete distance bins. Error bars show 95% confidence intervals. (B) Stacked bar chart showing the location of eRNAs relative to the closest annotated gene.

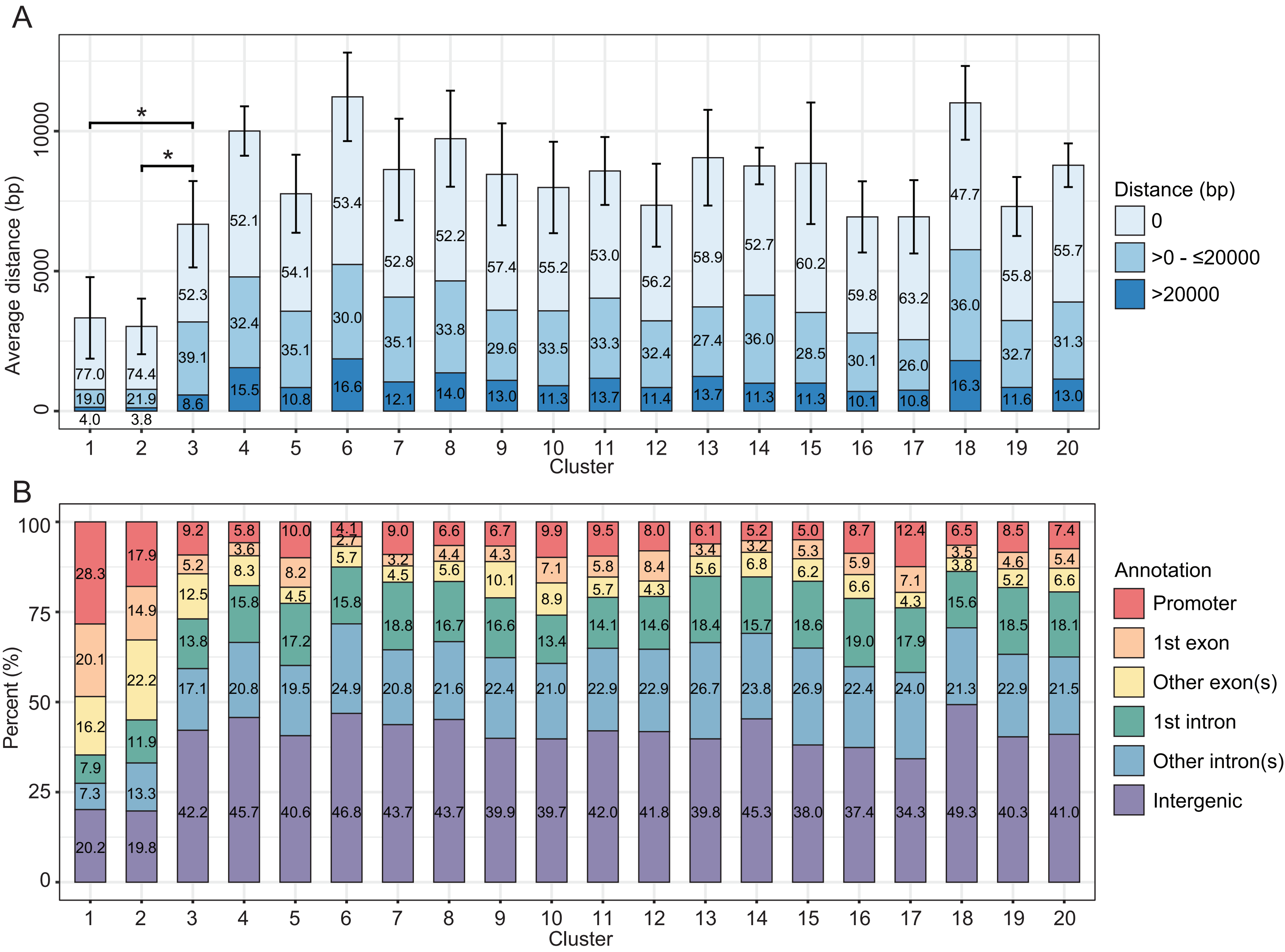


Supplemental Figure S2: Clustered eRNA expression profiles (same clusters as shown in Fig. 3). Gray lines show normalized expression profiles of individual eRNAs and red lines represent average expression patterns of eRNAs within clusters.

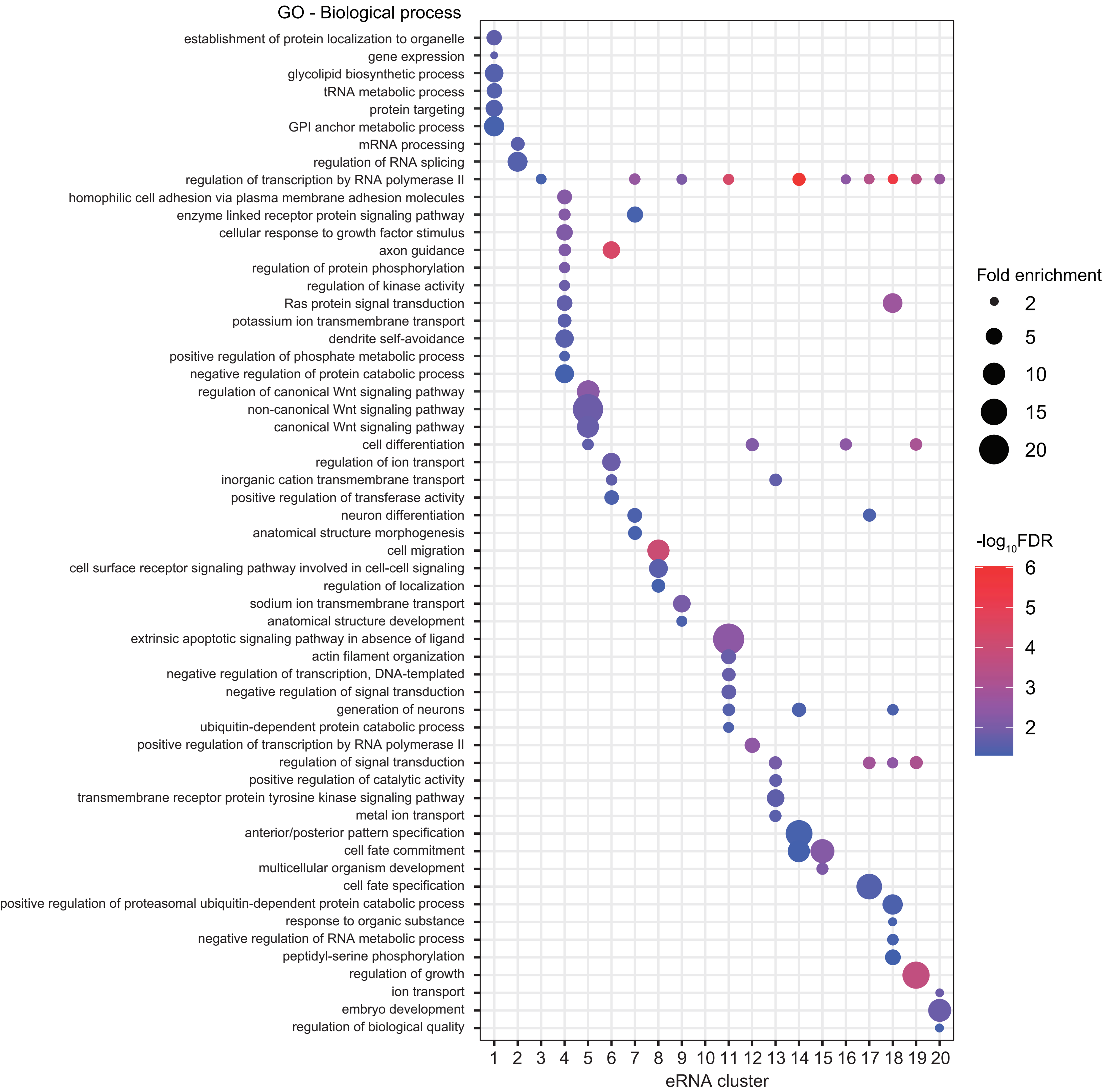




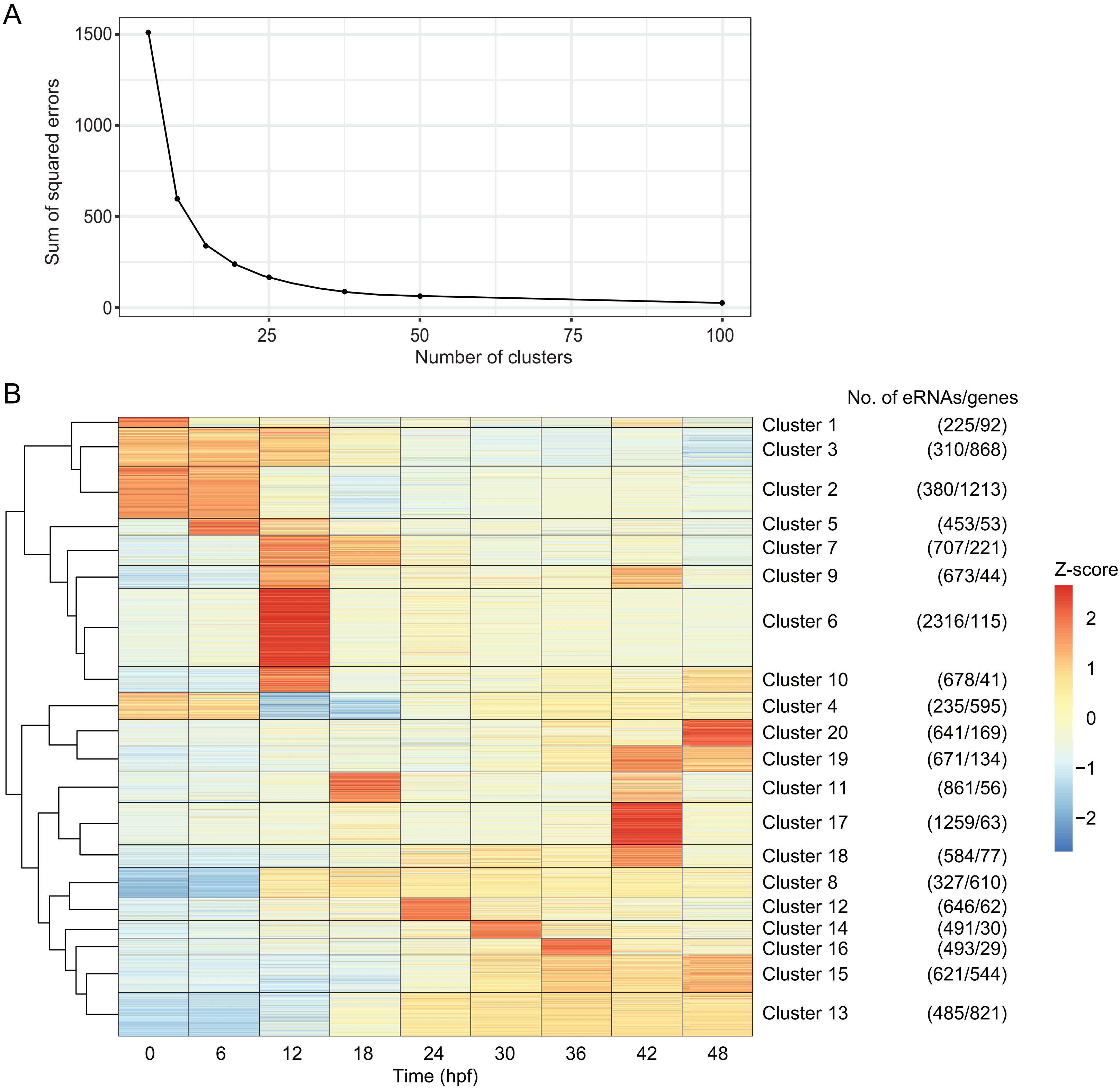
Supplemental Figure S3: (A) Plot of mean within-cluster sum-of-squared error (SSE) as a function of cluster number. (B) Mean length of eRNAs in each cluster. Error bars represent 95% confidence intervals. (C) Analysis of eRNA peaks that are exclusively accessible at 128-cell stage (128-cell eRNA peaks) or 24 hpf (24 hpf eRNA peaks) to overlap with eRNA peaks from different temporal expression clusters (Cluster analysis summarized in Fig. 3). Fold enrichment to overlap with clustered eRNA peaks is represented by the size of the circles, and the colors correspond to the significance of the enrichment, expressed as -log10(Pvalue). Only enrichments that show >1.5 fold enrichment and P<0.05 are shown.



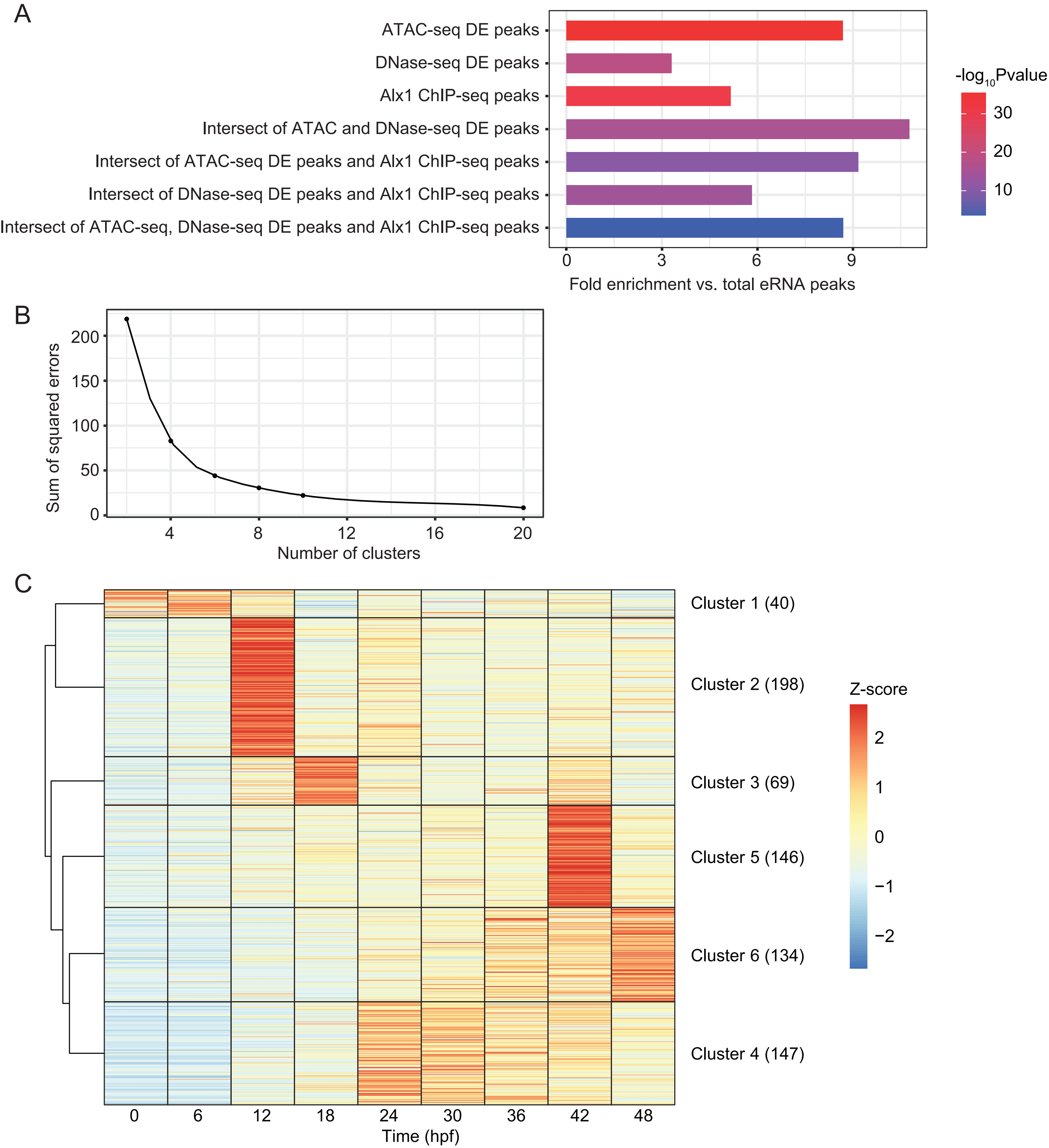
Supplemental Figure S4: Annotation and analysis of clustered eRNAs (Cluster analysis summarized in Fig. 3). (A) Average peak-to-gene distance (bp) of eRNAs in each cluster (*P<0.001). Stacked bars represent percentages of eRNAs in discrete distance bins. Error bars show 95% confidence intervals. (B) Stacked bar chart showing the location of eRNAs relative to the nearest annotated gene in each cluster.



Supplemental Figure S5: Gene ontology (GO) term enrichment analysis of genes within 20 kb of eRNA peaks in each expression cluster (Cluster analysis summarized in Fig. 3). Fold enrichment of each GO term is represented by the area of a circle, and the color of the circle corresponds to the significance of the enrichment, expressed as -log10(FDR).



Supplemental Figure S6: Cluster analysis of pooled eRNA/mRNA expression patterns. (A) Plot of mean within-cluster sum-of-squared error (SSE) as a function of cluster number. (B) Heatmap showing k-means clustering (K=20) of pooled eRNA/mRNA expression patterns. The total number of eRNAs and mRNAs in each cluster is indicated.


A
ATAC-seq DE peaks
DNase-seq DE peaks
Alx1 ChIP-seq peaks
Intersect of ATAC and DNase-seq DE peaks
Intersect of ATAC-seq DE peaks and Alx1 ChIP-seq peaks
Intersect of DNase-seq DE peaks and Alx1 ChIP-seq peaks
Intersect of ATAC-seq, DNase-seq DE peaks and Alx1 ChIP-seq peaks
0
3
6
9
Fold enrichment vs. total eRNA peaks
-log$_{10}$Pvalue
30
20
10
B
Sum of squared errors
200
150
100
50
0
4
8
12
16
20
Number of clusters
C
Cluster 1 (40)
Cluster 2 (198)
Cluster 3 (69)
Cluster 5 (146)
Cluster 6 (134)
Cluster 4 (147)
Z-score
2
1
0
−1
−2
0
6
12
18
24
30
36
42
48
Time (hpf)

Supplemental Figure S7: Analysis of eRNA peaks located within 20 kb of genes differentially expressed by PMCs. (A) eRNA peaks near PMC genes and their enrichment for overlapping with Alx1-bound regions and regions differentially accessible in PMCs compared to total eRNAs. The colors of the bars correspond to the significance of the enrichment, expressed as -log10(Pvalue). (B) Plot of mean within-cluster sum-of-squared error (SSE) as a function of cluster number. (C) Heatmap of k-means clustering of expression profiles of eRNAs near PMC genes (K=6). Clusters were used for HOMER de novo motif discovery (Supplemental Table S3)