Differences in firing efficiency, chromatin and transcription underlie the developmental plasticity of the *Arabidopsis* DNA replication origins

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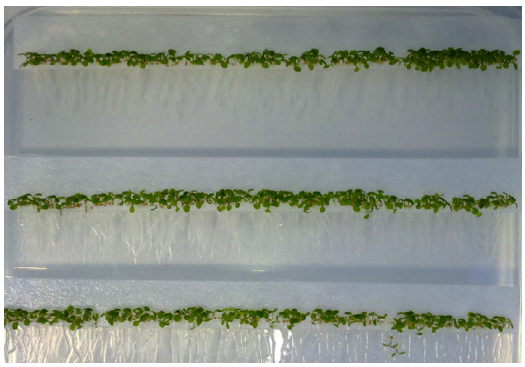
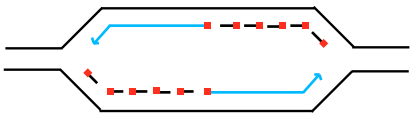


Arabidopsis thaliana seedlings (~12g)

Nuclear purification
Eliminate polyphenols
and polysaccharides

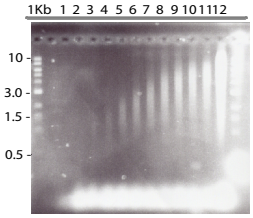
Extract genomic
DNA gently

ORI



Heat denaturation

Sucrose gradient



Process fractions
0.3 kb < NS < 2 kb

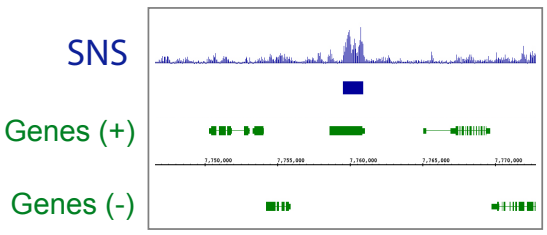
T4 PNK phosphorylation
λ-exonuclease (2 times)

Purify
nascent
strands

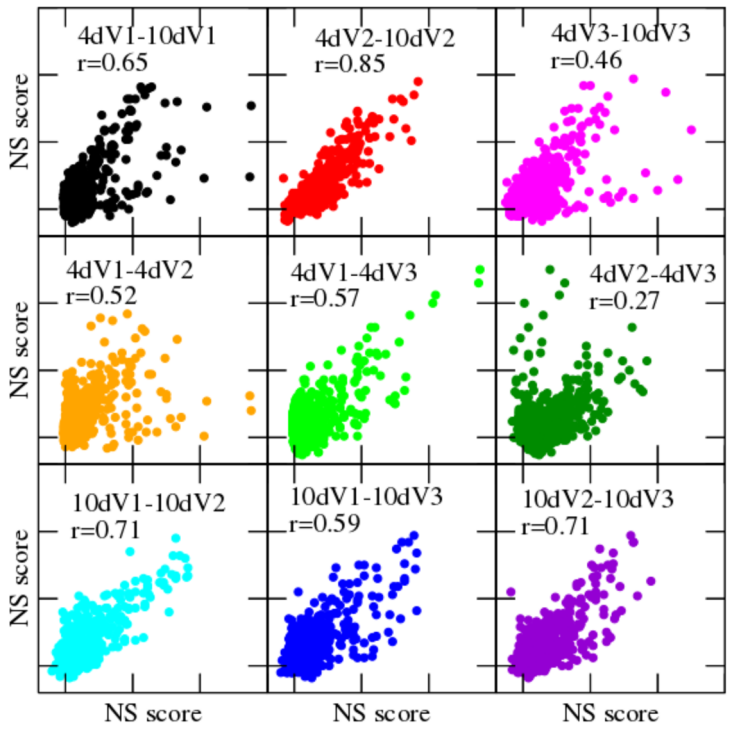
dsDNA
conversion
with slow-
annealing
of primers

Deep sequencing
Peak calling

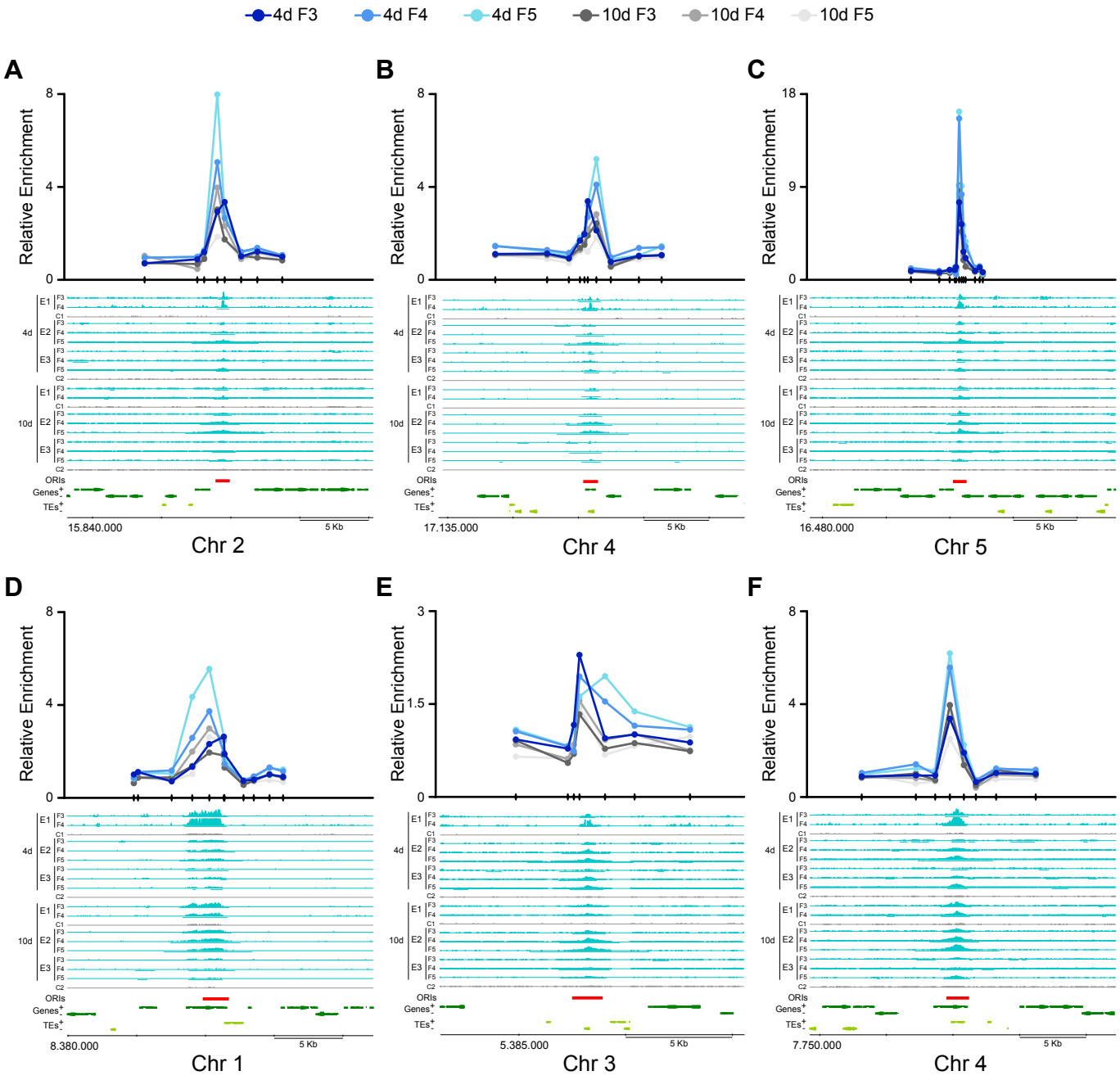
ORI



Supplemental Figure S1. Enhanced protocol for purification of nascent strands (NS) from whole developing seedlings

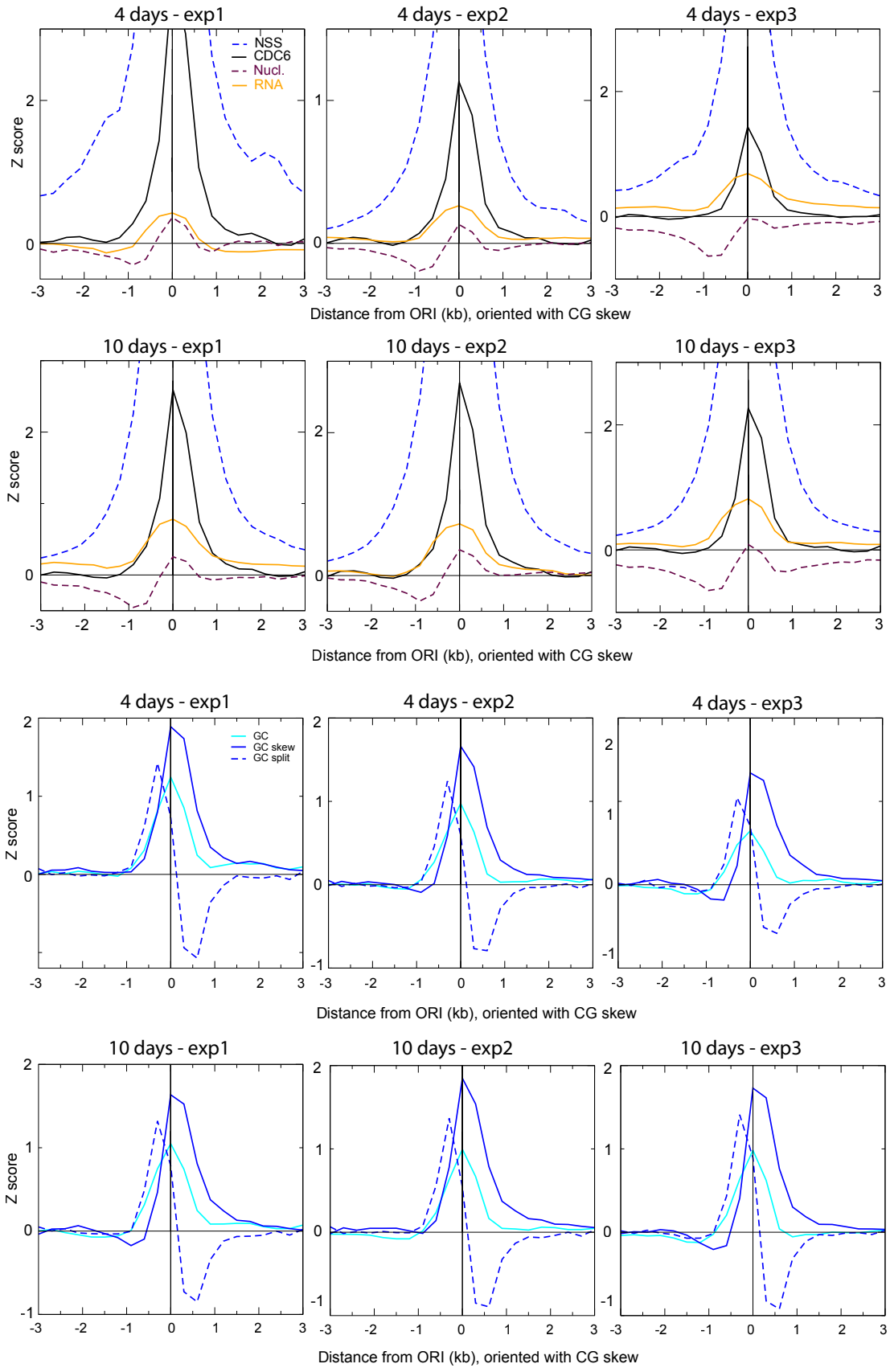


Supplemental Figure S2. Scatter plots of the nascent strand score (NSS) measured in different experiments. Each point represents one of the 2374 ORIs identified in this study. Only pairs corresponding to the same day or the same experiment are shown. V1, V2 and V3 refer to each of the three experiments.

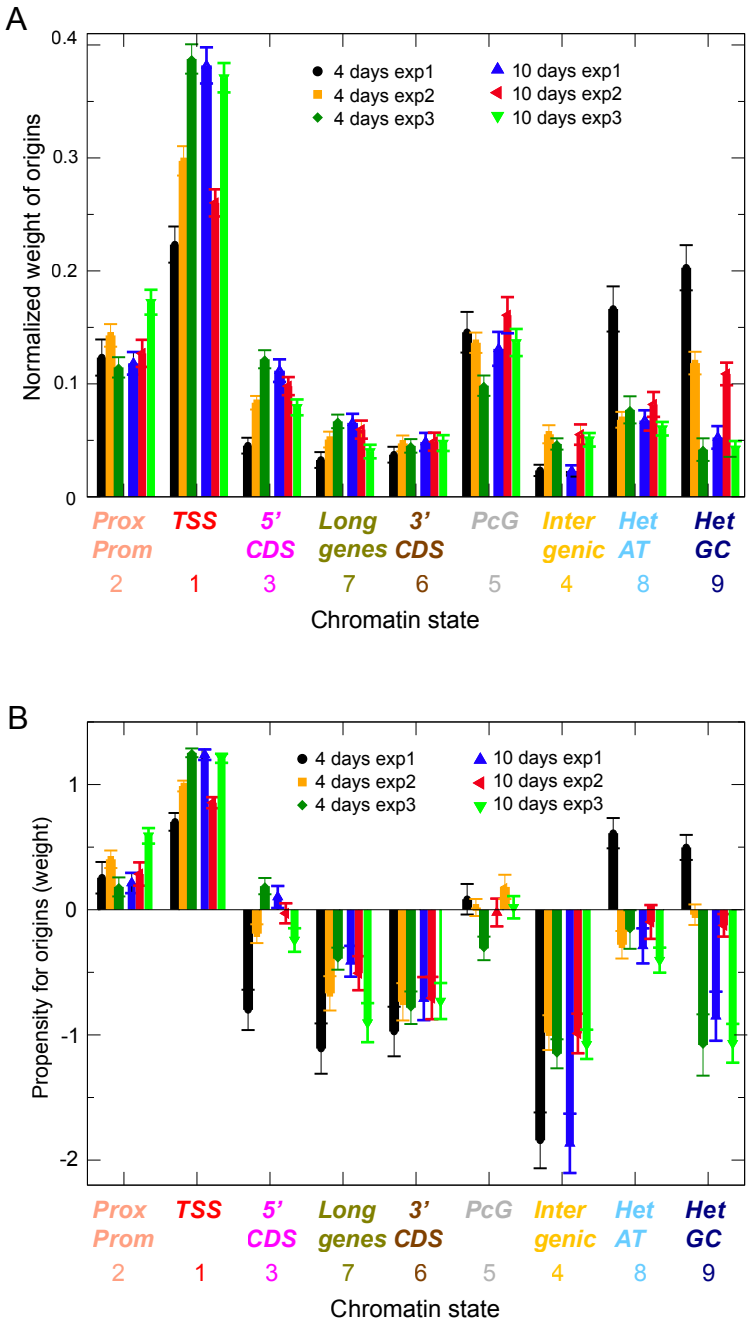


Supplemental Figure S3. Validation of DNA replication origin activity by measuring nascent strands abundance.

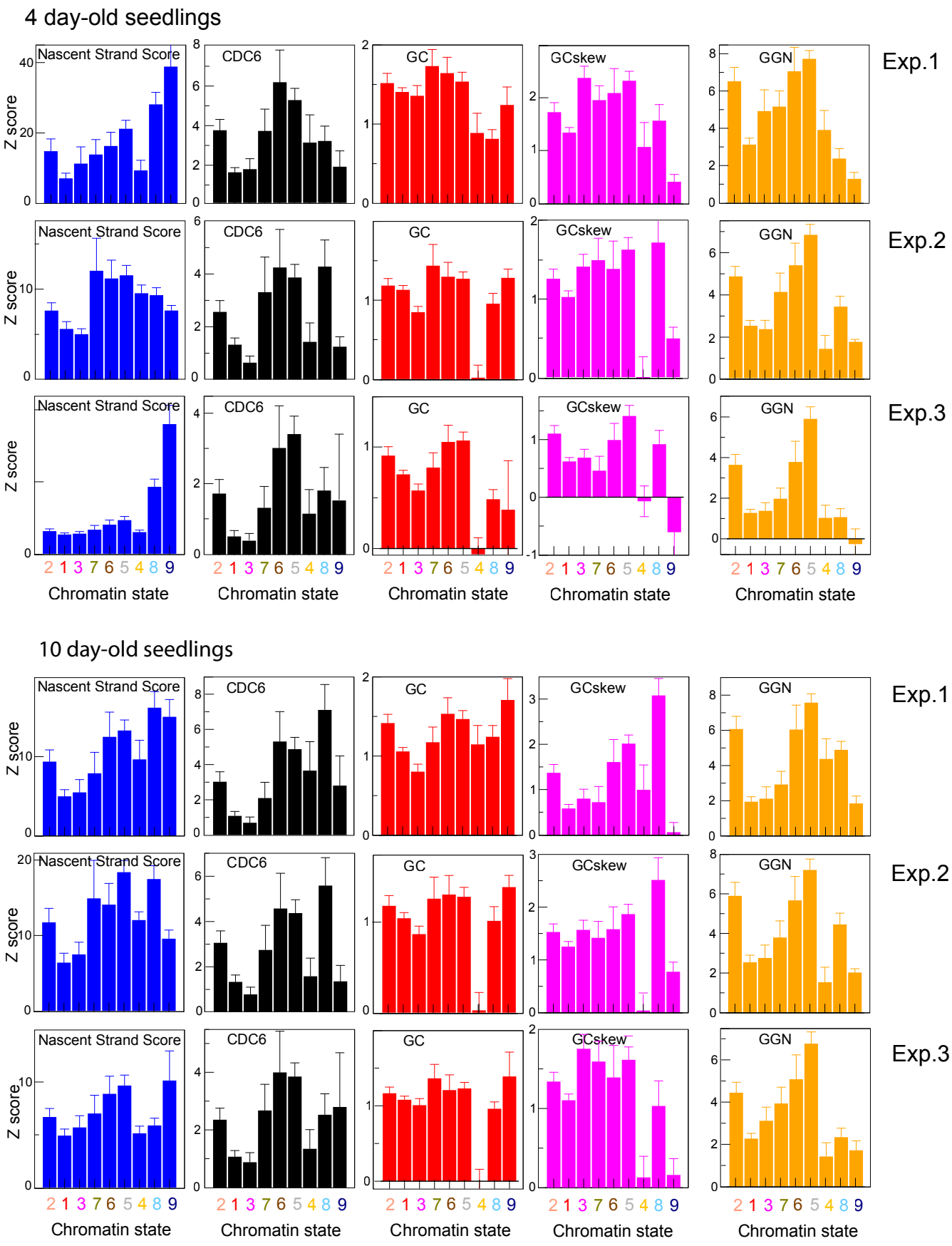
Several ORI-containing regions were chosen from the SNS-seq datasets belonging to ORIs (red bars at the bottom) that did not (A-C) coincide with biased regions (class 5 in Fig. 1C) and that colocalize with regions with some background in the controls (D-F; class 3 in Fig. 1C) to determine the abundance of nascent strands by qPCR. The sample was an independent preparation of nascent strands, purified by sucrose gradient centrifugation, treated with λ-exonuclease but not converted to dsDNA. The genomic regions under study are shown at the bottom of each panel and contain the read maps across them for the various sucrose gradient fractions used of the three independent experiments from 4 and 10 day-old seedlings, as indicated. Coordinates and location of primer pairs used to scan each region (small marks in the X-axis) are also indicated. A region lacking SNS-seq signal was used as a negative control and to normalize the qPCR values.



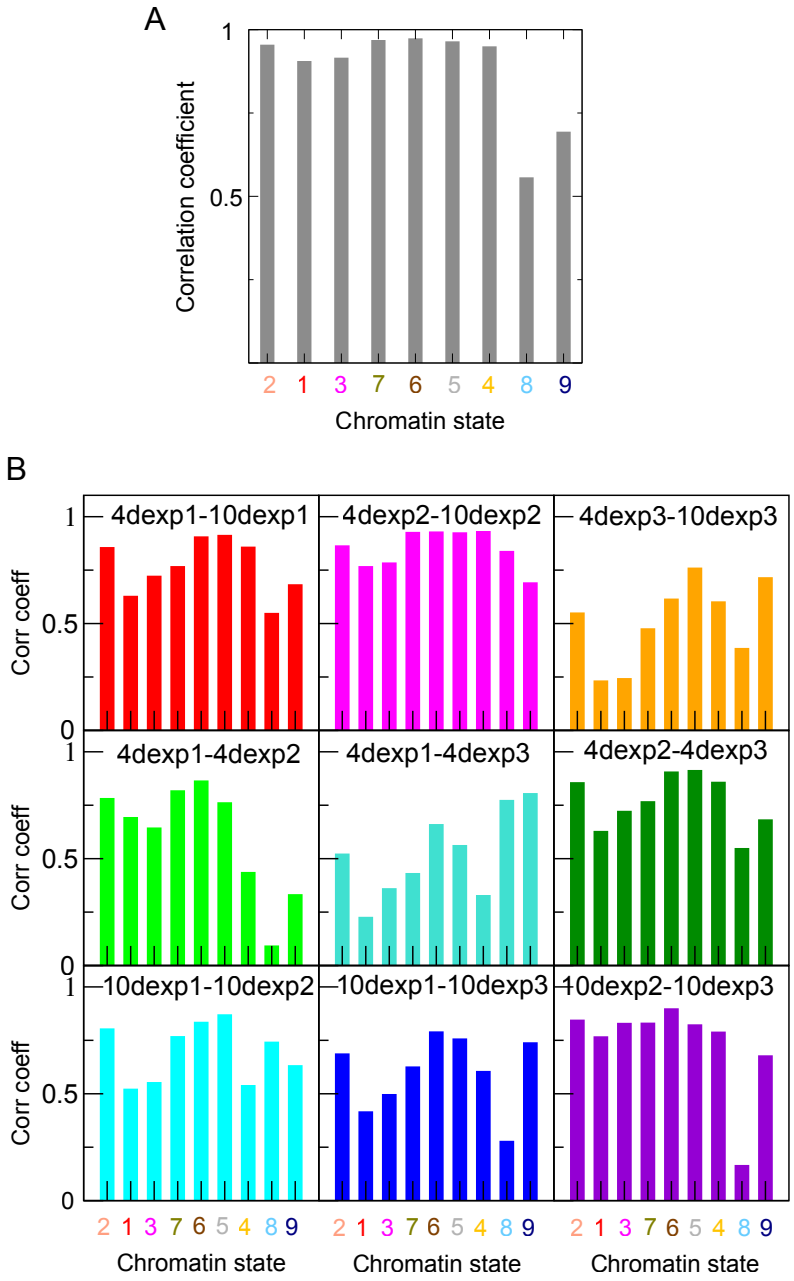
Supplemental Figure S4. Features of the local neighborhood of ORIs in whole Arabidopsis seedlings. Metaplots of NSS, CDC6, transcript content (RNA) and nucleosome (nucl.) content (upper two panels) and of GC, GC skew and GC split (lower two panels) of each of the three independent experiments in 4 and 10 day-old seedlings, as indicated.



Supplemental Figure S5. Association of ORIs with chromatin states.
A. Normalized weight of ORIs belonging to the 9 chromatin states in each of the three independent experiments of 4 day-old and 10 day-old seedlings, as indicated.
B. Same as in panel A, showing the propensity (instead of the cumulative weight) for ORIs in the 9 chromatin states is depicted for each of the three independent experiments of 4 day-old and 10 day-old seedlings, as indicated.



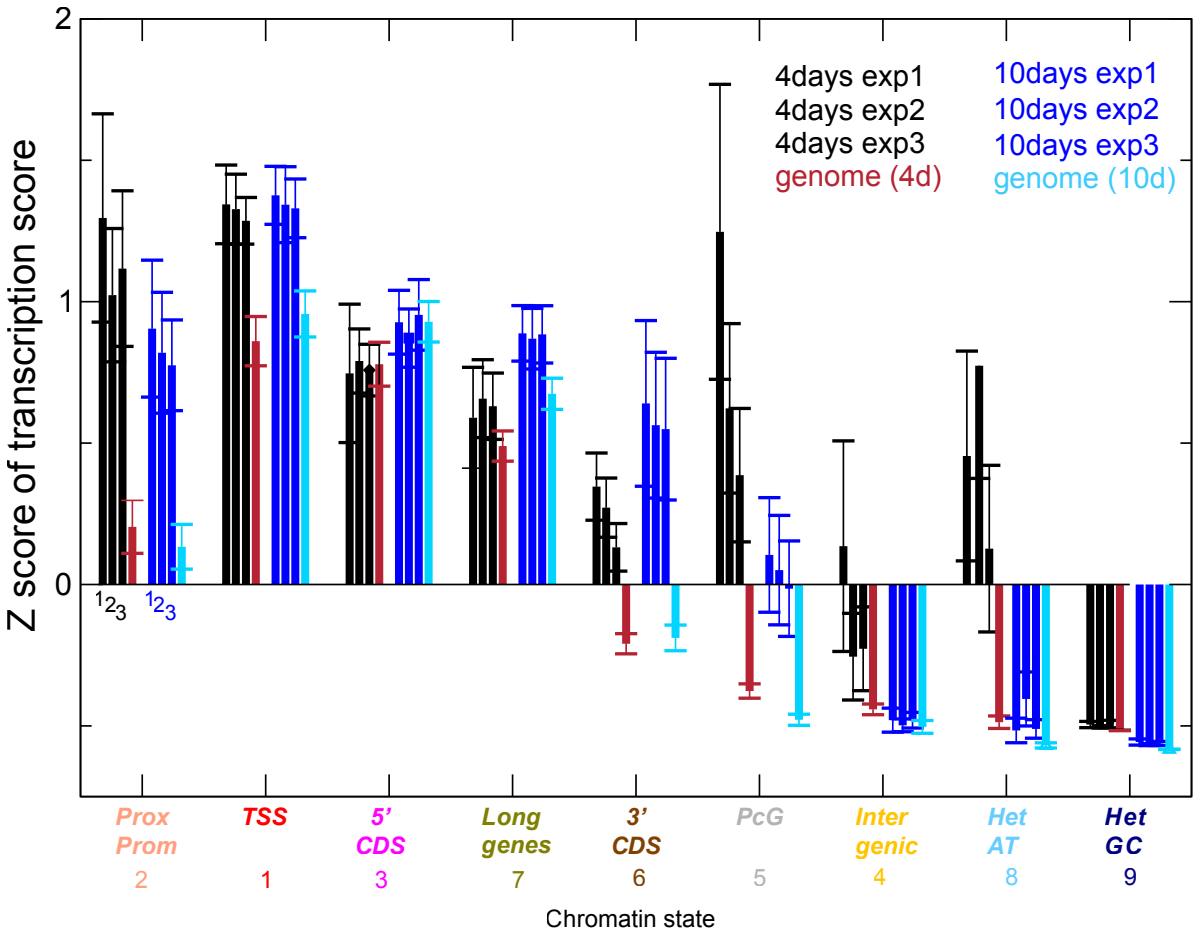
Supplemental Figure S6. Relevance of several genomic variables for ORI specification. The weighted averages of the Z scores for several variables (NSS, CDC6, GC content, GC skew and GGN trinucleotide) are shown for each of the three independent experiments in 4 day-old and 10 day-old seedlings, as indicated.



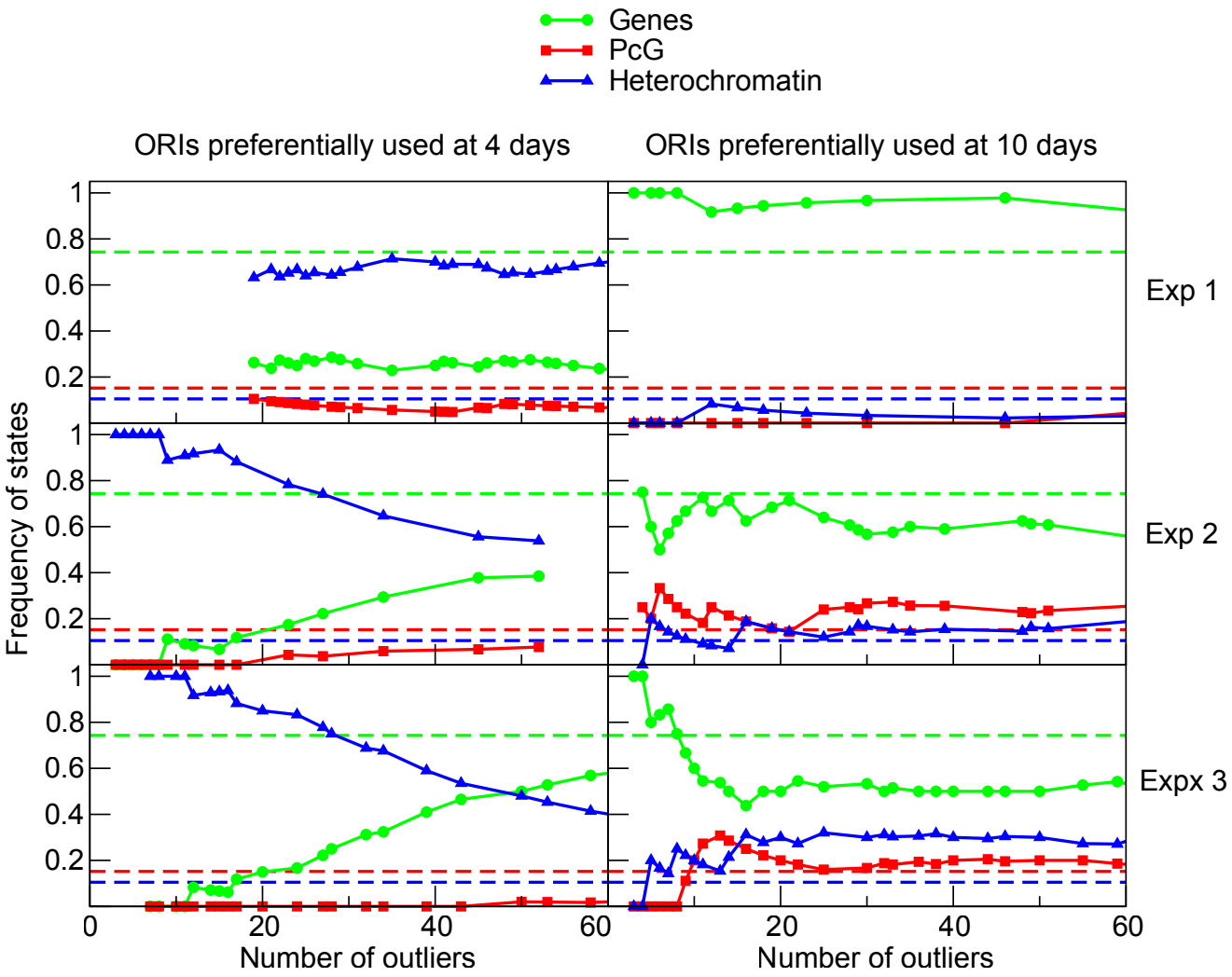
Supplemental Figure S7.

A. Correlation coefficients of the combined NSS of all ORIs identified in 4 day-old and 10 day-old seedlings, in each chromatin state.

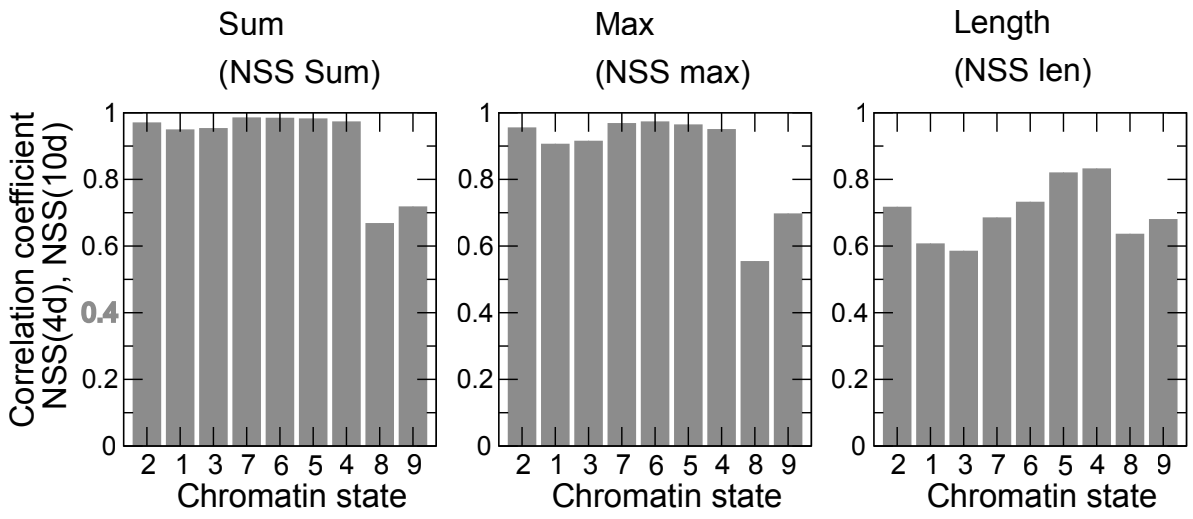
B. The correlations between the 6 individual NSS for the indicated experimental pairs.



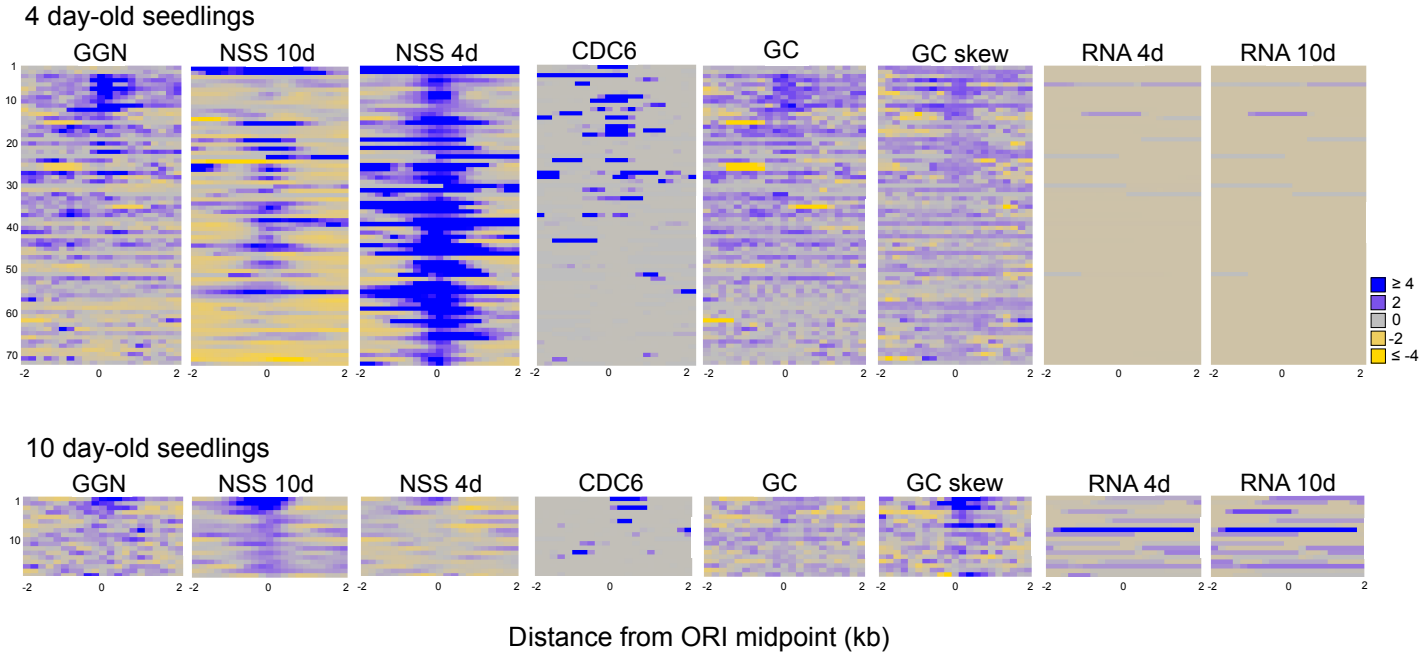
Supplemental Figure S8. Average Z score of the transcription score with respect to the average transcription scores of the entire genome at 4 days (brown bras) and 10 days (light blue bars) is shown for regions containing ORIs in each chromatin state for each of the three independent experiments of 4 day-old (black bars) and 10 day-old seedlings (dark blue bars). Note that the three replicas appear in order, as indicated below the bars corresponding to chromatin state 2 (Proximal promoters).



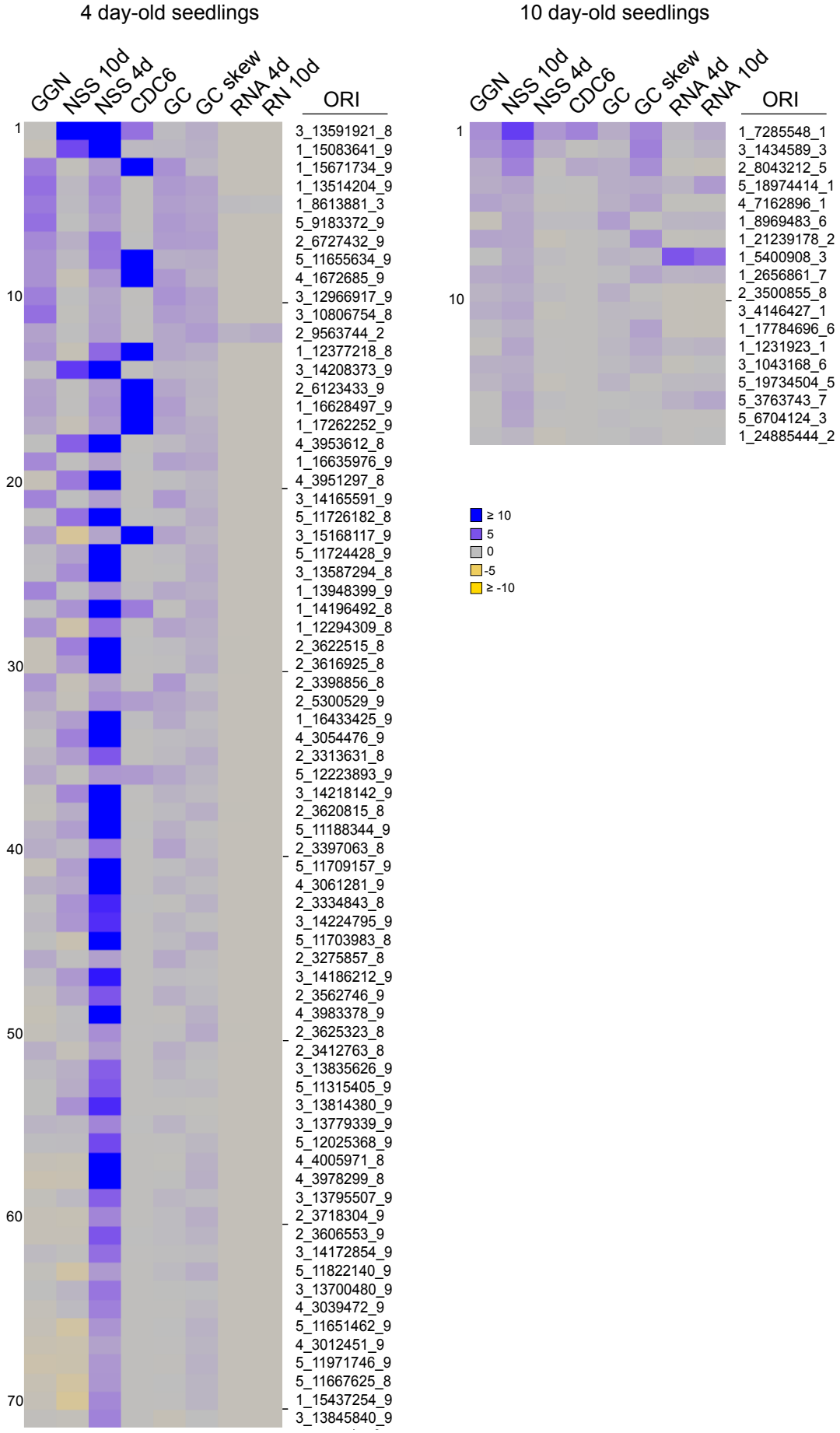
Supplemental Figure S9. Frequency of ORIs preferentially active in 4 day-old and 10 day-old seedlings, as indicated, in the three independent experiments, as a function of the number of ORIs obtained by varying the threshold. To simply the analysis, ORIs have been grouped as genes (chromatin states 2, 1, 3, 7 and 6), Polycomb (chromatin states 4 and 5) and heterochromatin (chromatin states 8 and 9). Broken lines represent the average values for the entire genome.



Supplemental Figure S10. The NSSmax score, defined as the maximum number of nascent DNA over all the 25bp windows that cover the ORI, and the NSSlen score, defined as the number of windows for which the Z score of the number of NS reads is larger than one. The NSSmax score assesses the frequency of origins activation, and the NSSlen score assesses the resistance of the ORI to failures.



Supplemental Figure S11. Heatmaps of the values of the indicated features in ±2 kb around the ORI midpoint (0) of the ORIs preferentially activated in 4 day-old (upper panels) and 10 day-old (lower panels) seedlings. In all cases ORIs are ranked according to values of the first principal component of the complete set of features, computed over the set of all 2374 ORIs. The color scale applies to both sets of panels.



Supplemental Figure S12. Heatmaps of the values of the indicated features at the ORI midpoint of those preferentially activated in 4 day-old (left panel) and 10 day-old (right panel) seedlings. In all cases ORIs are ranked according to values of the first principal component of the complete set of features, computed over the set of all 2374 ORIs. The color scale applies to both sets of panels. Each ORI is identified by the following code: chromosome_coordinate at ORI midpoint_chromatin state.