Nuclear purification
Eliminate polyphenols
and polysaccharides

Extract genomic
DNA gently

ORI
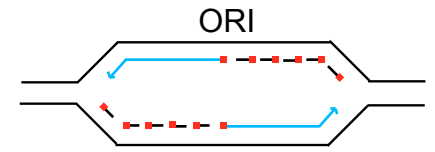
Heat denaturation

Sucrose gradient

10 day-old Arabidopsis thaliana
seedlings (~12g)

Deep sequencing
Peak calling

ORI

SNS

Genes (+)

Genes (-)

dsDNA
conversion
with slow-
annealing
of primers
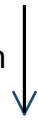
Purify
nascent
strands
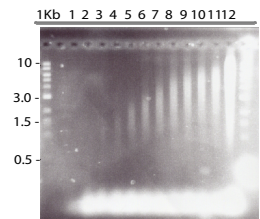
Process fractions
0.3 kb > NS > 2 kb
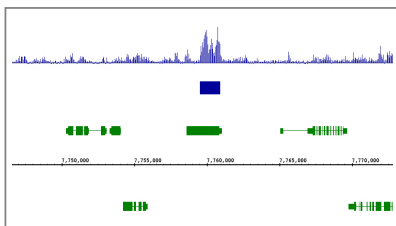
T4 PNK phosphorylation
λ-exonuclease (3 times)

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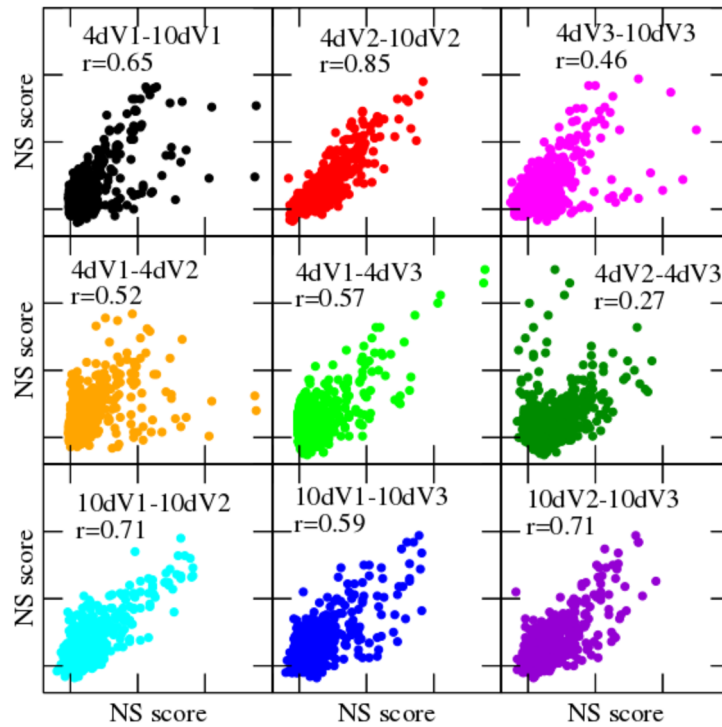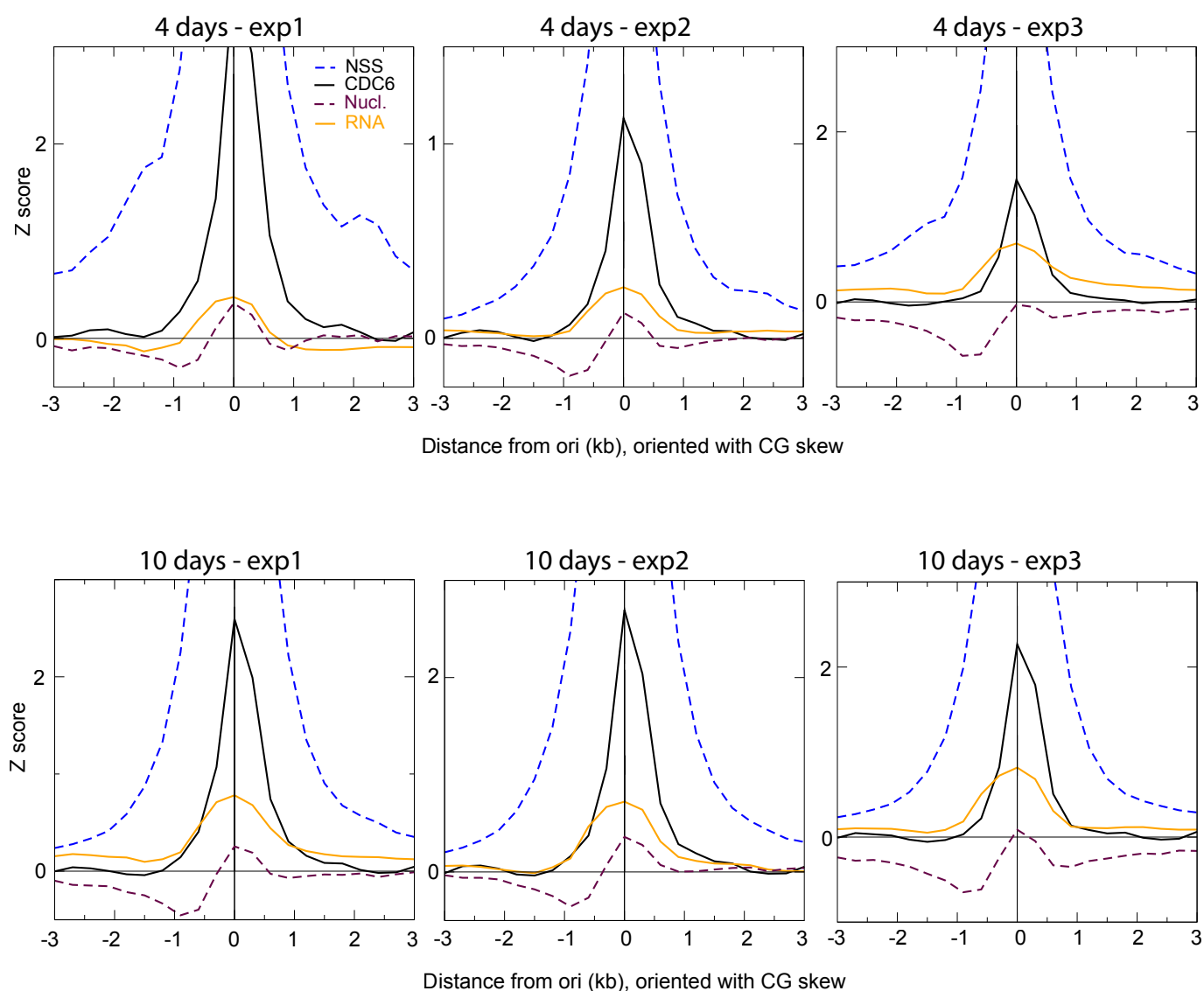whole developing seedlings

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. Features of the local neighborhood of ORIs in whole Arabidopsis seedlings. Metaplots of NSS, CDC6, transcript content (RNA) and nucleosome (nucl.) content of each of the three independent experiments in 4 day-old (top panels) and 10 day-old seedlings (bottom panels), as indicated.
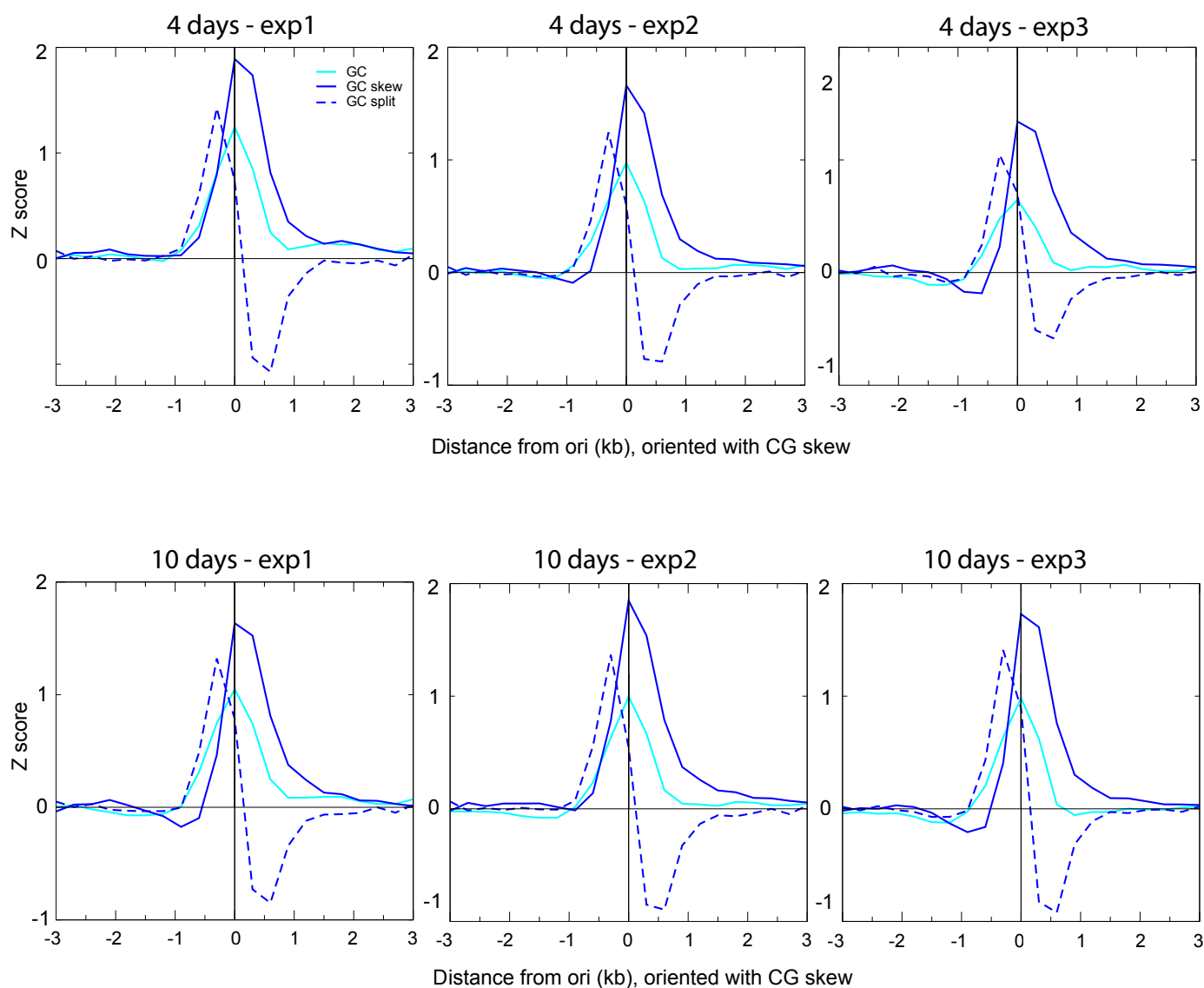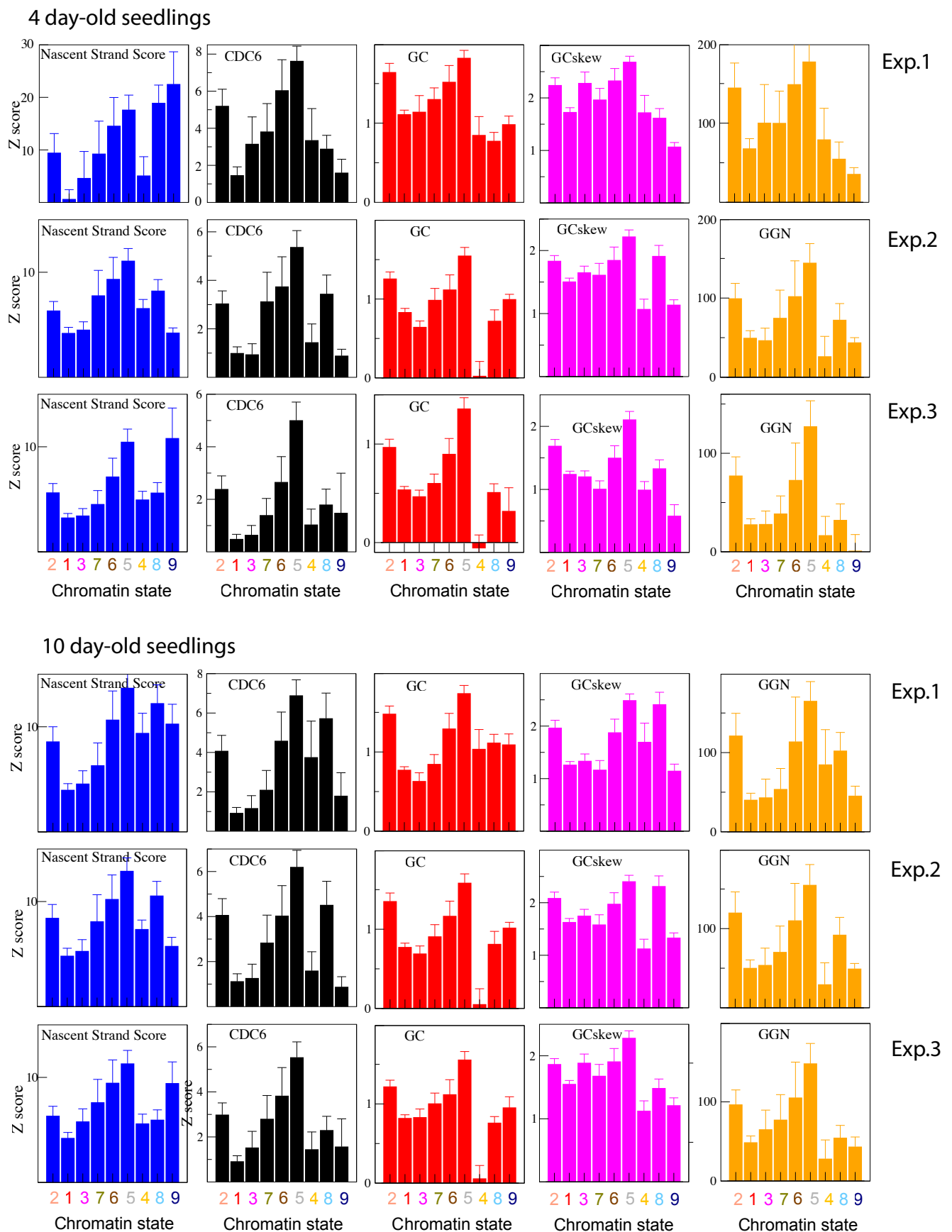
Supplemental Figure S4. Features of the local neighborhood of ORIs in whole Arabidopsis seedlings. Metaplots of GC, GC skew and GC split of each of the three independent experiments of 4 day-old (top panels) and 10 day-old (bottom panels) seedlings.
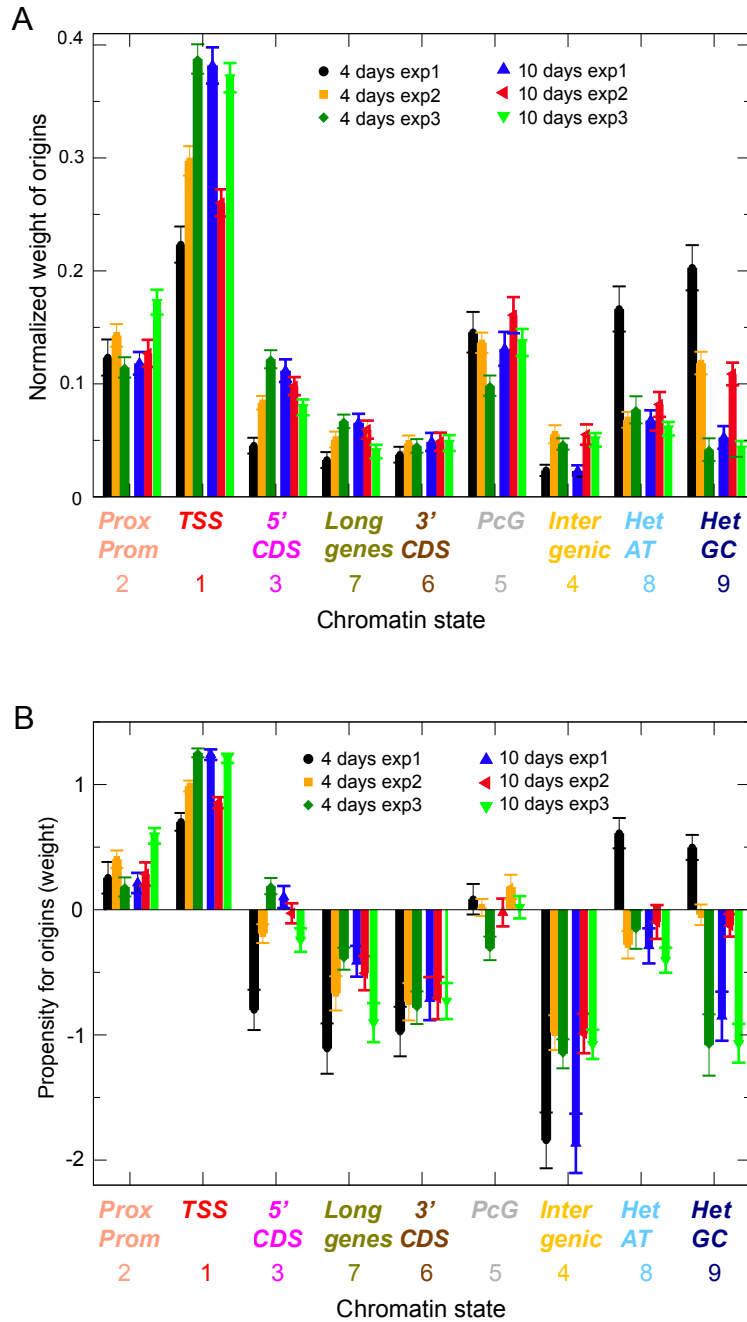
Supplemental Figure S5. Association of ORIs with chromatin states.
A. Normalized weight of ORIs belonging to the 9 chromatin states in each of the three independent experiments of 4 day-old and 10 day-old seedlings, as indicated.
B. Same as in panel A, showing the propensity (instead of the cumulative 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.
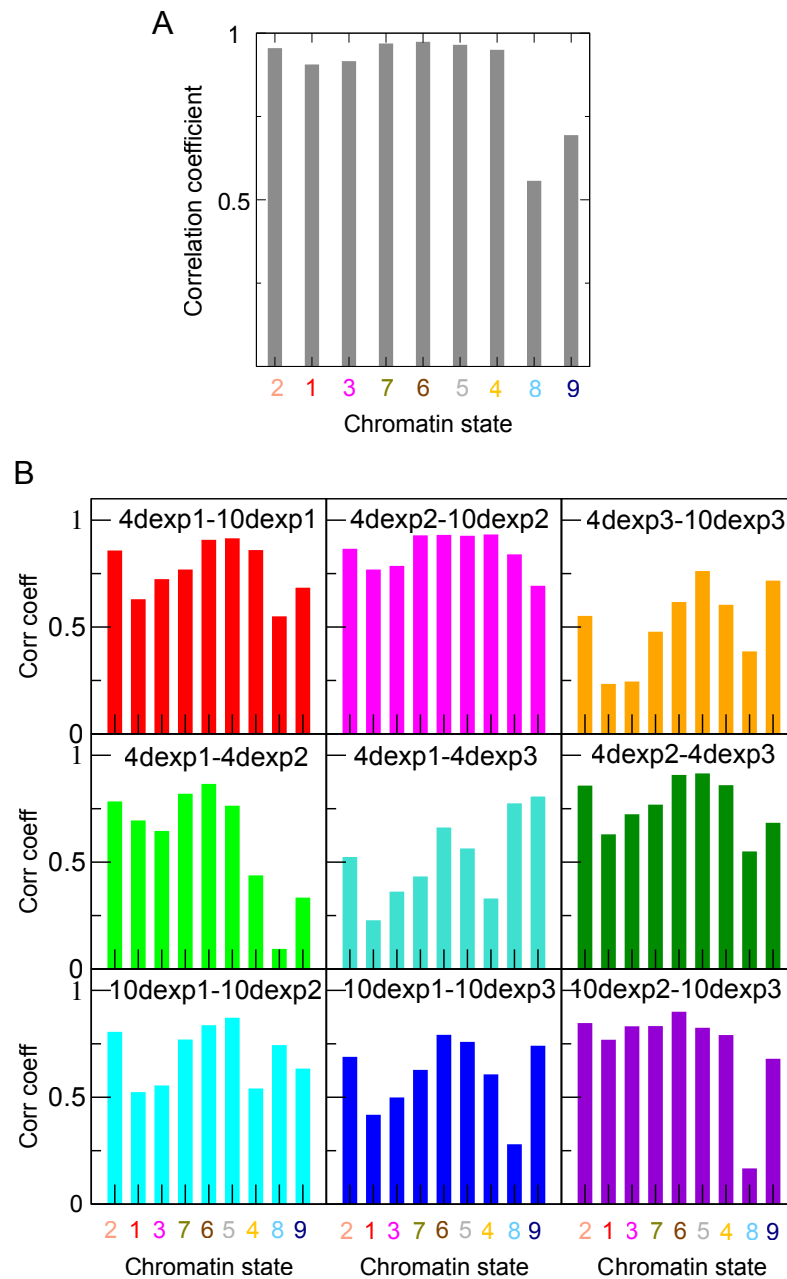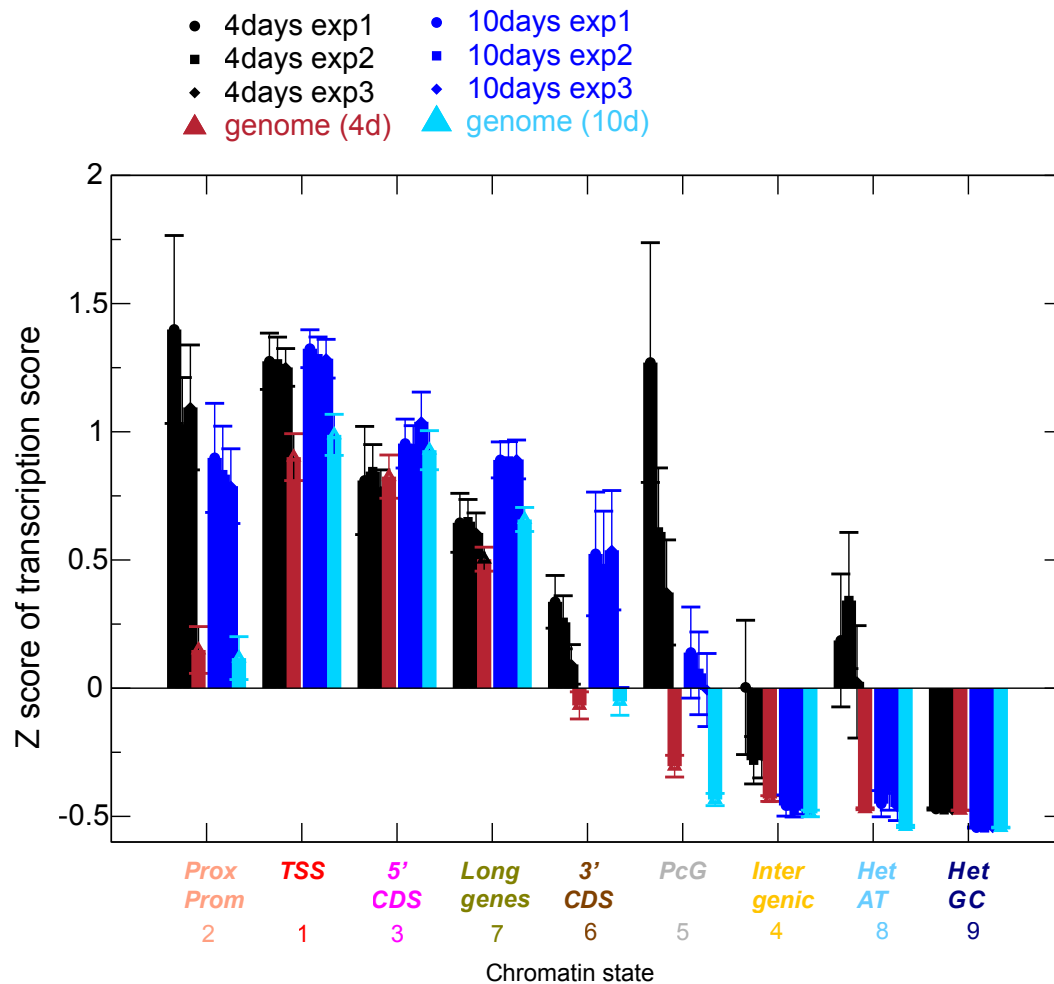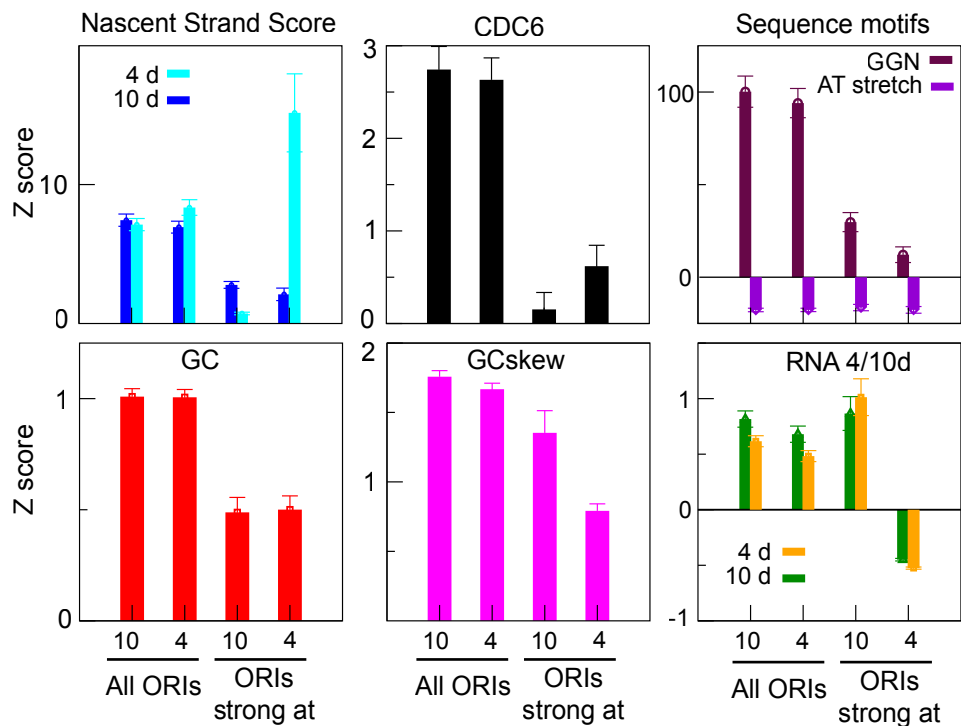
Supplemental Figure S7.
A. Correlation coefficients of the combined NSS of all ORIs identified in 4 day-old and 10 day-old seedlings, in each chromatin state.
B. The correlations between the 6 individual NSS for the indicated experimental pairs.

Supplemental Figure S8. Average Z score of the transcription score with respect to the average transcription score of the entire genome is shown for ORIs in each chroamtin state for each of the three independent experiments in 4 day-old (top panel) and 10 day-old seedlinsg (bottom panel), as indicated.

Supplemental Figure S9. Weighted average of several genomic and epigenomic properties, using as weights the combined NSS, for the set of all ORIs and the ORIs preferred at 4 or 10 day-old seedlings, as indicated. In all cases we have used the stronger weight. Measurements were transformed to Z-score with respect to the whole genome, which produces positive values when they are higher than a generic genomic region.