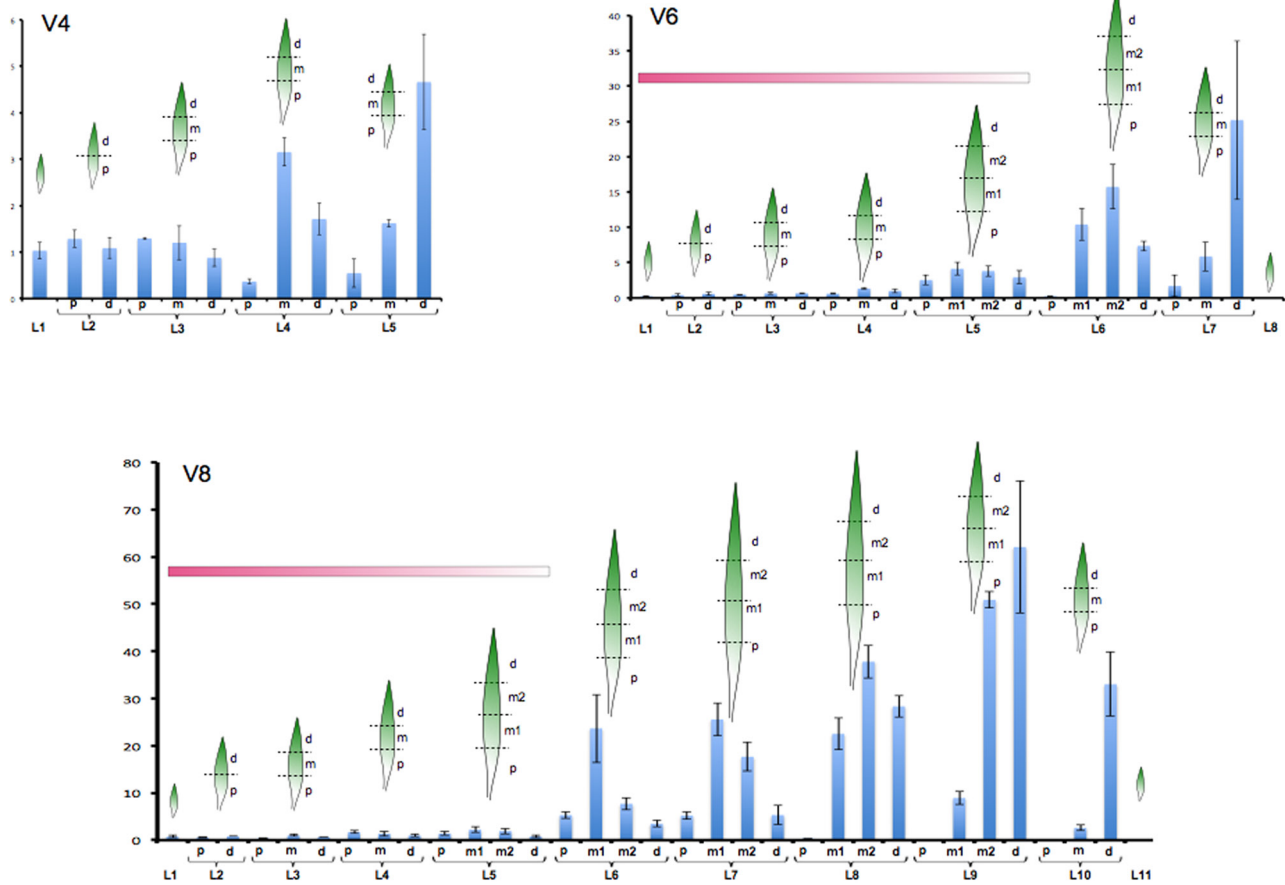
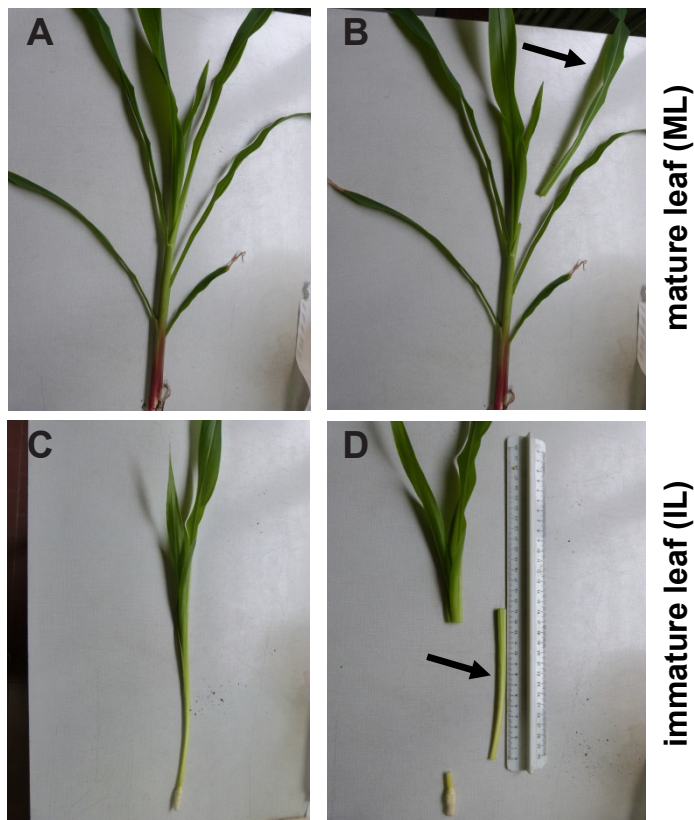


Supplemental Figure S1. Schematic depiction of *ZCN7* and *ZCN8* genes and transcripts of maize B73 inbred. Gene structure, genome location, and transcript isoforms of *ZCN7* (A) and *ZCN8* (B) in maize B73 inbred. The genome location of genes is based on the RefGen_v3 version of the B73 genome sequence (<http://www.maizegdb.org/>). Gene structure is schematized with black boxes and lines, representing exons and introns, respectively. The position of *ZCN7* and *ZCN8* start and stop codon, in accordance with experimental evidence arising from their cDNAs identification, is reported. Below the gene structure of *ZCN7* and *ZCN8* a schematic representation of the three transcript isoforms produced by these genes is reported.



Supplemental Figure S2. Examination of *ZCN7/ZCN8* mRNAs by qPCR in plants at pre-transition (V4), transition (V6), and post-transition reproductive stage (V8). Expression analysis is compared to lowest level (set to 1) and normalized with maize *gapc2* gene; relative expression is indicated on the y-axis. Error bars show standard error; $p < 0.01$. RNA from whole leaf blades for leaves under 10 cm was isolated and converted to cDNA. Primers used for the analysis are targeted to amplify sense transcript for *ZCN7* and *ZCN8*, without distinguishing the two paralogs. Larger leaves were divided into proximal (p) sections (nearest the shoot apex and younger) and distal (d) sections (near tip and oldest part of leaf). Leaves more than 30 cm long were further subdivided into middle sections (m, or m1 and m2 for largest leaves) as indicated. Shaded bar indicates leaves in juvenile stage (leaf 5 is a juvenile/adult transition leaf).



Supplemental Figure S3. Sampling strategy. The strategy employed to sample mature (ML) and immature (IL) leaf of maize B73 plants is illustrated. A similar strategy was employed also for teosinte plants (see Materials and Methods). The ML and IL samples were used for both expression and chromatin modification analysis. A, the B73 seedling at V6/V7 stage, corresponding to floral transition stage (see Fig. 1) was harvested. B, the entire leaf blades of the fully extended leaf (indicated with an arrow) was collected to represent the mature leaf. C, all the leaves of the same B73 seedling were eliminated to obtain the central cylinder of non-photosynthetic developing leaf. D, The central cylinder was cut from 2 cm to 12 cm above the shoot apex to obtain the immature leaf (indicated with an arrow).

>ZCN7_B73_AS

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>ZCN8_B73_AS

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>ZCN7_teosinte_AS

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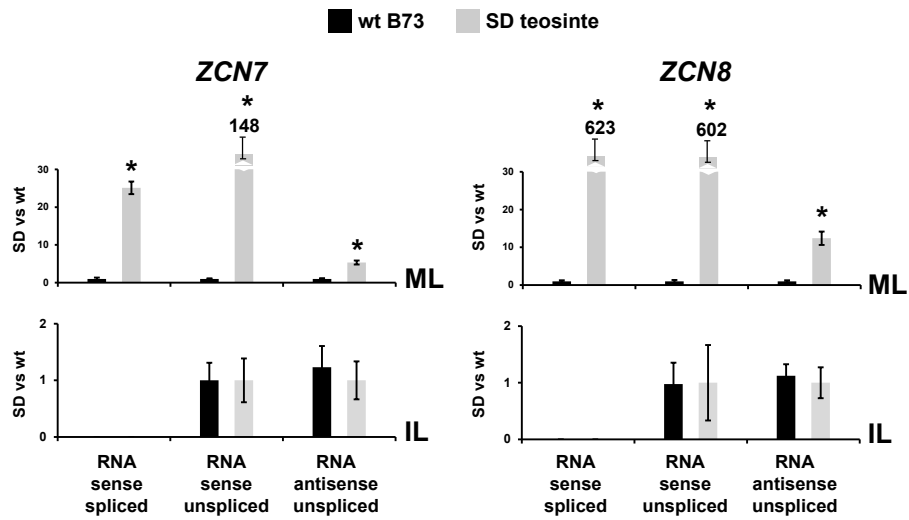
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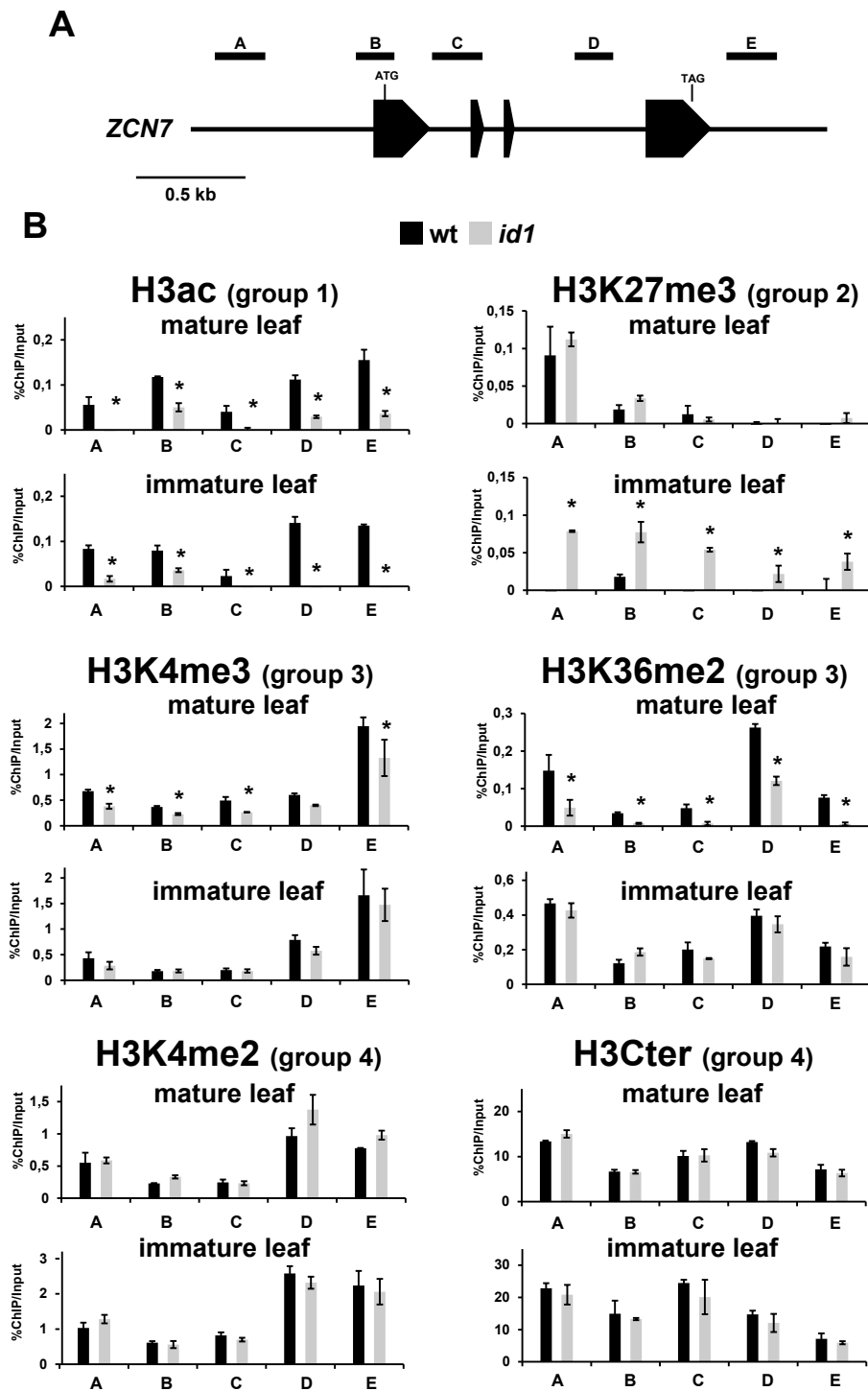
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Supplemental Figure S4. Nucleotide sequence of *ZCN7* and *ZCN8* antisense RNA strand. cDNAs amplified by means of strand specific RT-PCR with *ZCN7* and *ZCN8* specific primer combinations located in the 5'-end regions were cloned and sequenced (see Methods). ORFs that are at least 30 codons long were found using the ORF finder program (http://www.bioinformatics.org/sms2/orf_find.html), and they are indicated by underlining the first and last codon and highlighting them in light blue (ORF for reading frame 1), green (ORF for reading frame 2), or yellow (ORF for reading frame 3). The predicted amino acid sequence for each ORF was submitted as query in a BLASTp search using the “non-redundant protein sequence” database available at NCBI (<http://blast.ncbi.nlm.nih.gov/>) with an *e* value cutoff < 0.5.

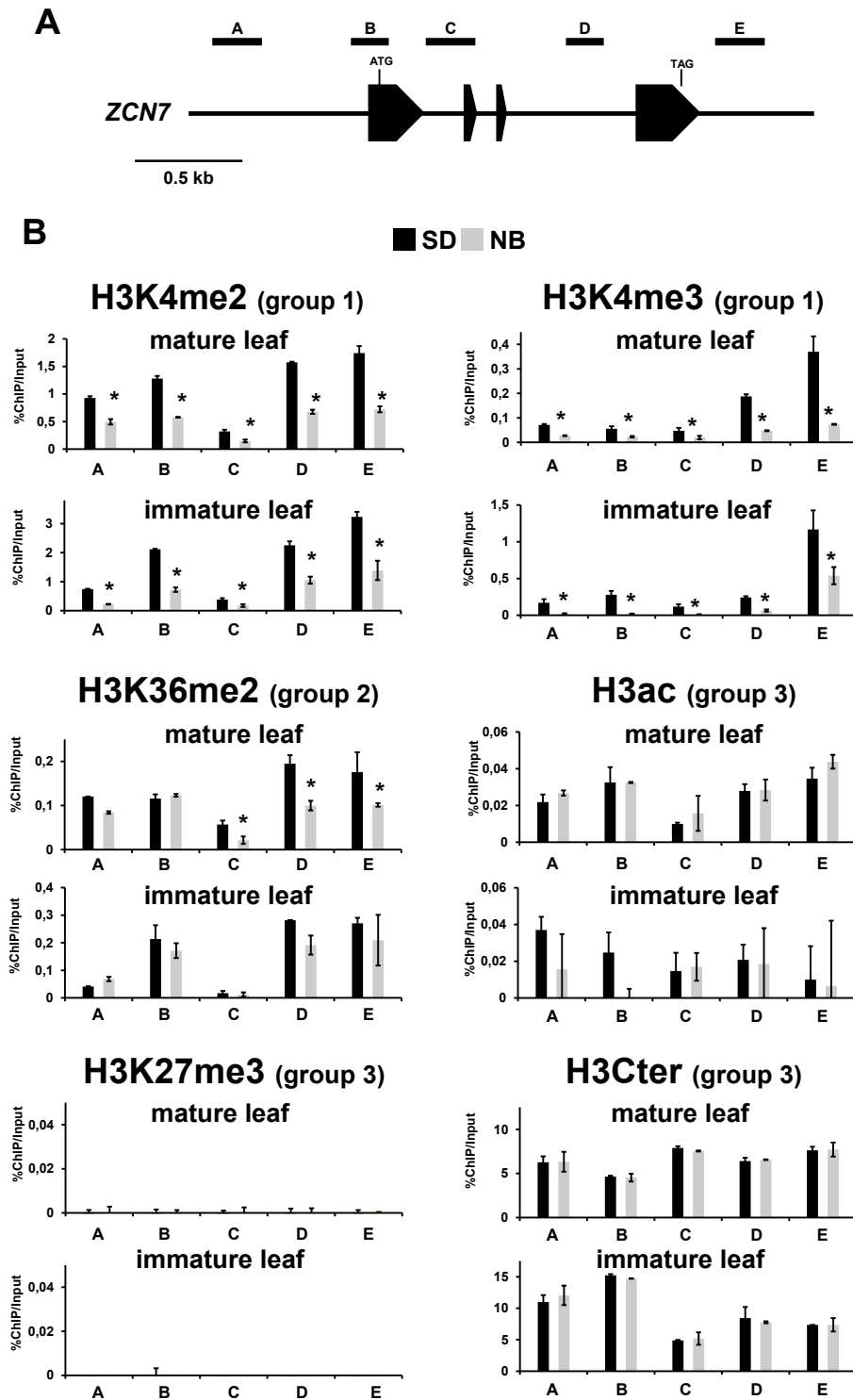
None of the ORFs identified had a hit, with exception of B73 *ZCN7* antisense ORF number 1 in reading frame 1, B73 *ZCN8* antisense ORF number 1 in reading frame 2, teosinte *ZCN7* antisense ORF number 1 in reading frame 1, and teosinte *ZCN8* antisense ORF number 1 in reading frame 1. All these ORFs encode for the same putative polypeptide of 80 amino acid in the B73 *ZCN7* and *ZCN8* antisense transcripts and of 83 amino acid in the teosinte *ZCN7* and *ZCN8* antisense transcripts. These polypeptides exhibit an identity with a maximum value of 35% (*e* value 0.001) with the amino acid sequence of the *Oryza sativa japonica* hypothetical protein NP_001066171.1 (185 amino acid length). However, no further homology with this protein was found in the *ZCN7* and *ZCN8* B73 genomic sequence, when 10,000 nucleotide located up-stream and down-stream of these ORFs were analyzed with BLASTx, suggesting that the ORFs very likely do not represent the maize homolog of the NP_001066171.1 rice protein.



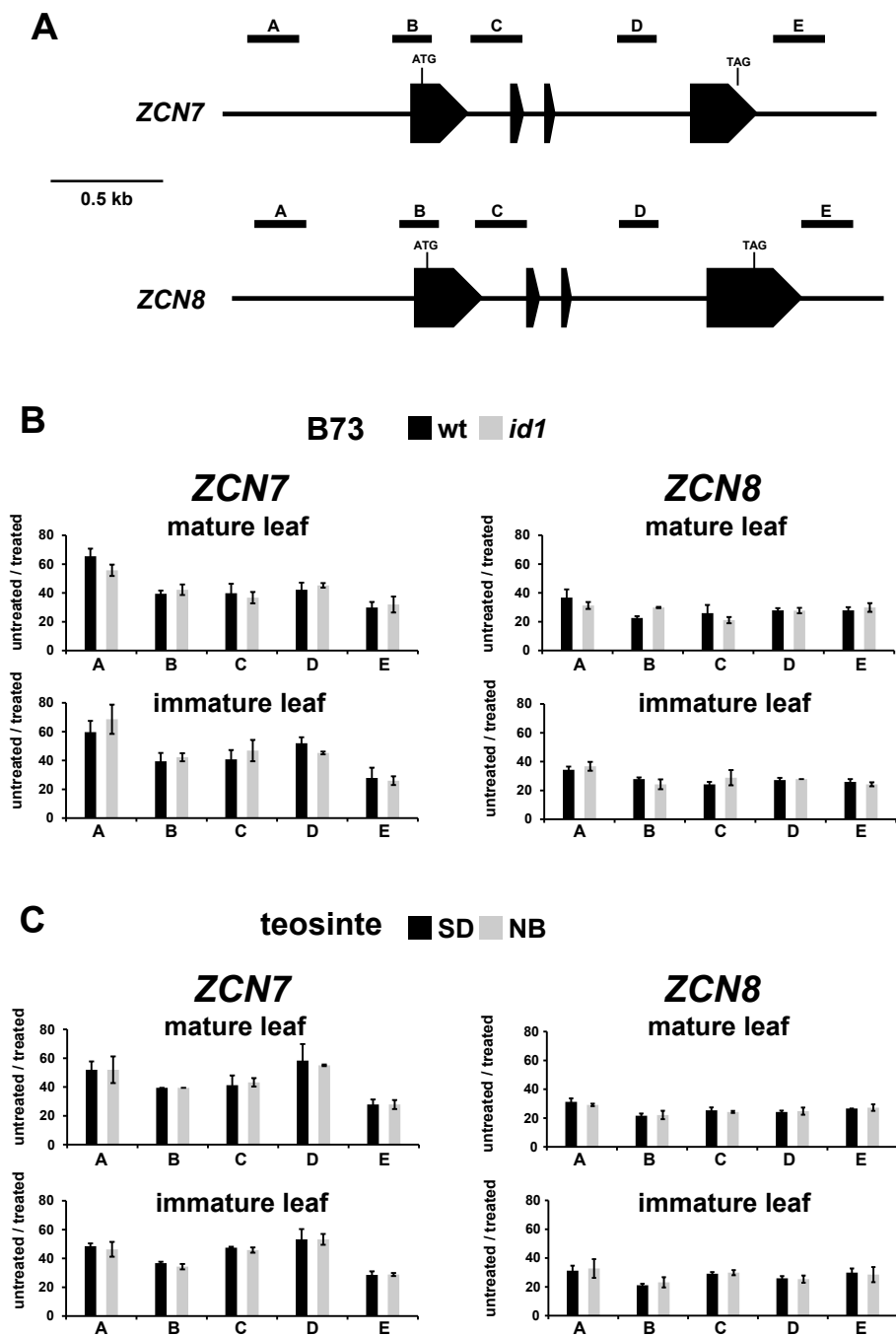
Supplemental Figure S5. Comparison of *ZCN7* and *ZCN8* transcript isoforms level in B73 and teosinte plants under inductive floral conditions. Comparison of real-time qRT-PCR data obtained using locus and strand-specific primers, with RNA extracted from mature (ML) and immature (IL) leaf tissues of wild-type (wt) and teosinte plants grown under short days (SD). The data are the same obtained in experiments illustrated in Figure 3, but these illustrate the comparison between wt B73 maize and SD teosinte. Bar diagrams are the mean value of transcript amount for one biological replicate, normalized to *gapc2* sense mRNA. For each of the comparison the sample with the lower amount of the RNA isoform has the value set to 1 and the amount of relative change for the other sample is calculated using the $2^{-\Delta\Delta C_t}$ method. Asterisk indicates statistically significant change ($p \leq 0.01$) when it was achieved in the separate analysis of the two biological replicates.



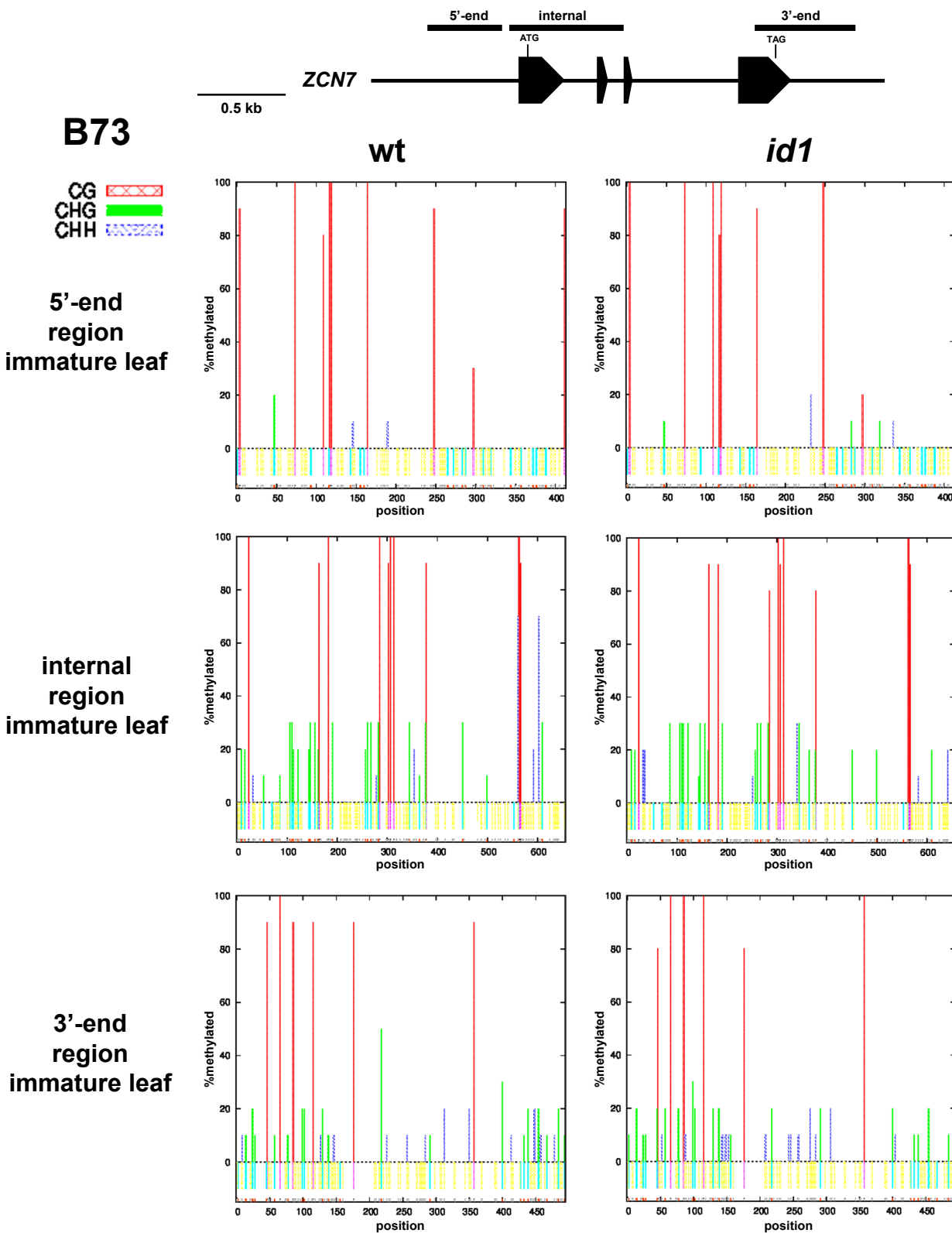
Supplemental Figure S6. Analysis of *ZCN7* histone modifications in B73 wild-type and *id1* mutant plants. A, Schematic depiction of *ZCN7* gene, with black boxes representing exons. Positions of the regions analyzed in ChIP assays are indicated. B, Bar diagrams represent real-time PCR quantification of ChIP DNA, reported as percentage of the chromatin input, from assays performed using the indicated antibodies. The data are average values from two independent ChIP assays and from three PCR repetitions for each ChIP assay and are reported by subtracting the background signal, measured by omitting antibody during the ChIP procedure. Asterisk indicates statistically significant change ($p \leq 0.01$) in *id1* mutant vs wild-type. The grouping of histone marks on the basis of how its variation associates with activity of *id1* gene is indicated by parentheses. Similar results were obtained after correction for nucleosome occupancy measured as reported by Rossi et al., (2007).



Supplemental Figure S7. Analysis of *ZCN7* histone modifications in teosinte SD and NB plants. A, Schematic depiction of *ZCN7* gene, with black boxes representing exons. Positions of the regions analyzed in ChIP assays are indicated. B, Bar diagrams represent real-time PCR quantification of ChIP DNA, reported as percentage of the chromatin input, from assays performed using the indicated antibodies. The data are average values from two independent ChIP assays and from three PCR repetitions for each ChIP assay and are reported by subtracting the background signal, measured by omitting antibody during the ChIP procedure. Asterisk indicates statistically significant change ($p \leq 0.01$) in SD vs NB plants. The grouping of histone marks on the basis of how its variation associates with SD floral induction is indicated by parentheses. Similar results were obtained after correction for nucleosome occupancy measured as reported by Rossi et al., (2007).



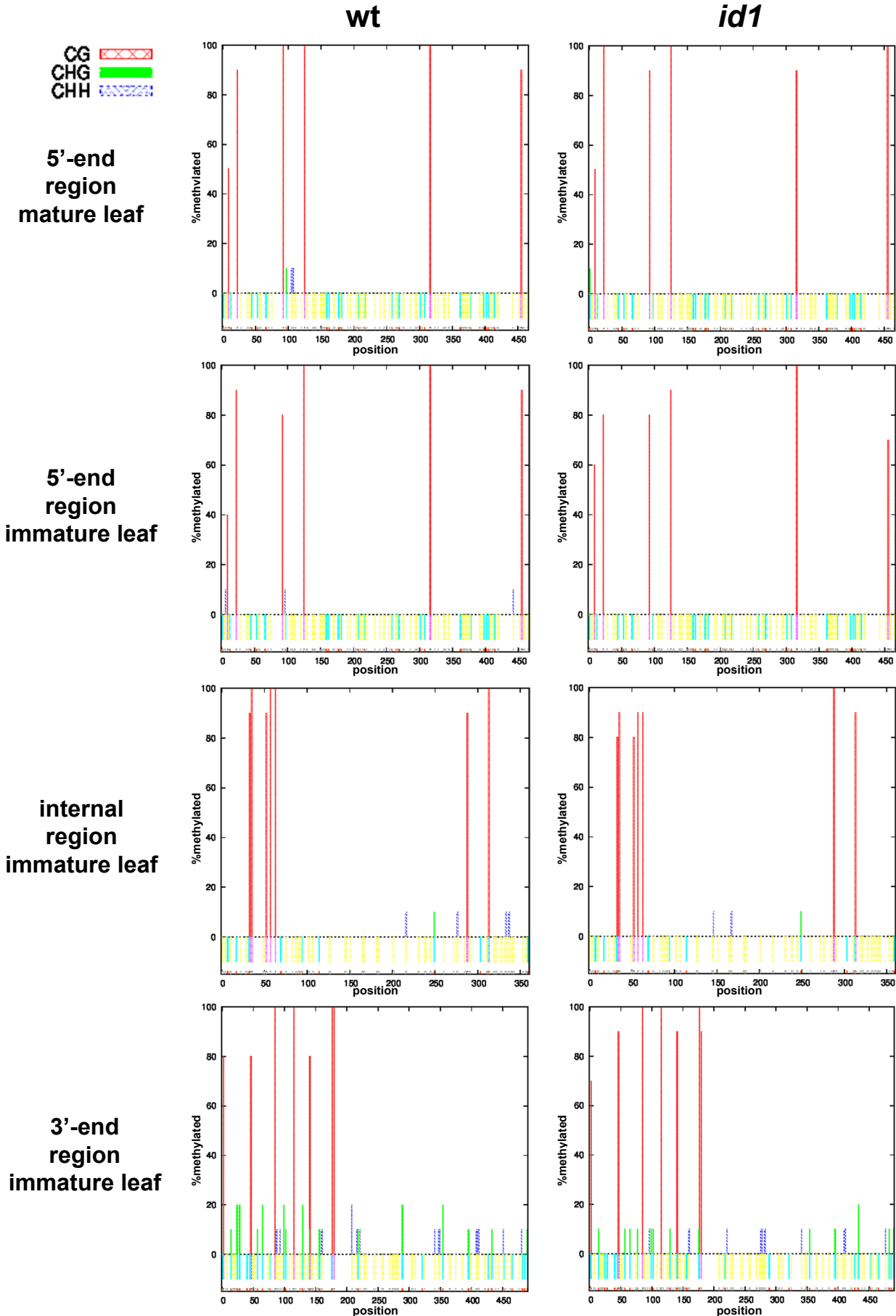
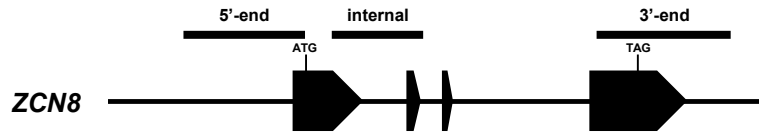
Supplemental Figure S8. Analysis of mC level at *ZCN7* and *ZCN8* by *Msp*JI restriction. A, Diagram of *ZCN7* and *ZCN8* genes, with black boxes representing exons. The structure of both genes is conserved between B73 and teosinte. Positions of the regions analyzed in *Msp*JI assays are indicated and are the same analyzed in ChIP assays. B, Bar diagrams represent PCR quantification of B73 wild-type (wt) and *id1* mutant genomic DNA extracted from mature and immature leaf and untreated and treated with the *Msp*JI restriction enzyme. Ordinates are the untreated/treated ratio calculated as the main values from two independent *Msp*JI treatments with three PCR repetitions for each treatment. This ratio represents an estimation of the mC level, with higher values that indicate increased methylation within the sequence. Standard errors are reported. In all regions analyzed, no statistically significant changes ($p \leq 0.01$) between wt vs *id1* were observed. C, Like in B, but the genomic DNA was extracted from mature and immature leaf of teosinte plants grown under SD and NB condition. In all regions analyzed, no statistically significant changes ($p \leq 0.01$) between SD vs NB were observed.



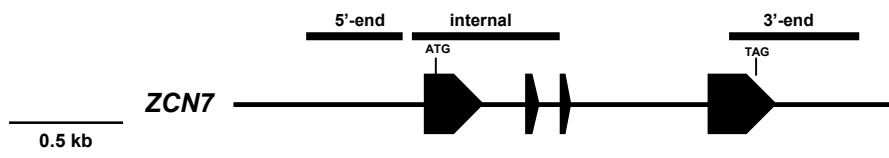
Supplemental Figure S9. Bisulfite sequencing analysis of mC at B73 *ZCN7*. The *ZCN7* gene structure and the positions of the regions are indicated. The data are illustrated using the Kismeth web-based tool (<http://katahdin.mssm.edu/kismeth/revpage.pl>; Gruntman et al., 2008). The fraction of times the cytosine at each location is methylated is reported as colored bars above the x-axis and the three colors represent the three sequence context (CG, CHG, and CHH). The number of times each cytosine is sampled in the sequenced reads is reported as colored bars below the x-axis. For all regions analyzed we used ten independent bisulfite treated and sequenced clones.

B73

0.5 kb



Supplemental Figure S10. Bisulfite sequencing analysis of mC at B73 *ZCN8*. The *ZCN8* gene structure and the positions of the regions are indicated. The data are illustrated using the Kismeth web-based tool (<http://katahdin.mssm.edu/kismeth/revpage.pl>; Gruntman et al., 2008). The fraction of times the cytosine at each location is methylated is reported as colored bars above the x-axis and the three colors represent the three sequence context (CG, CHG, and CHH). The number of times each cytosine is sampled in the sequenced reads is reported as colored bars below the x-axis. For all regions analyzed we used ten independent bisulfite treated and sequenced clones.



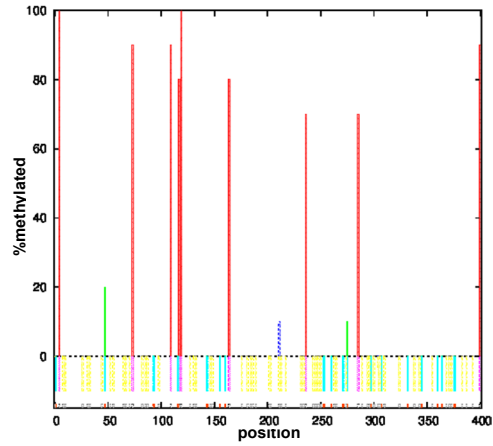
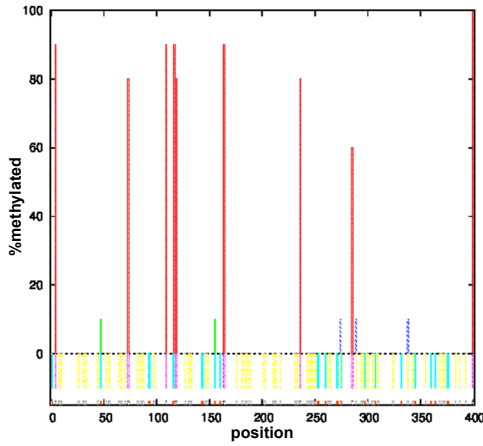
teosinte

wt

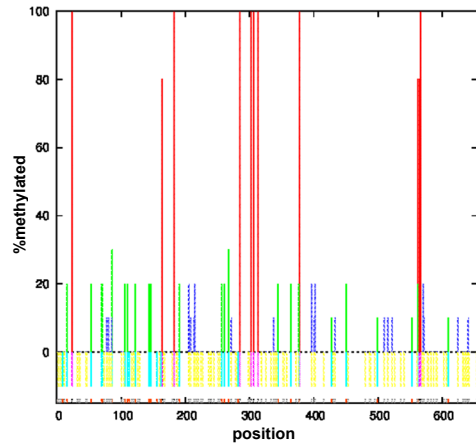
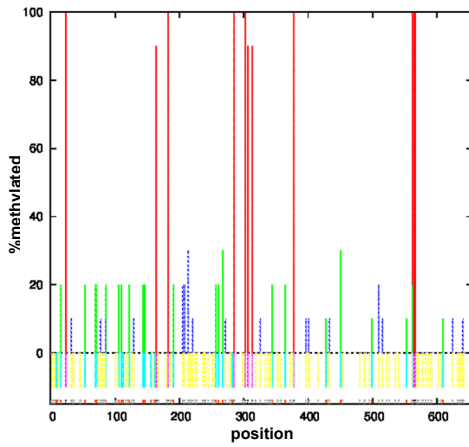
id1

CG
 CHG
 CHH

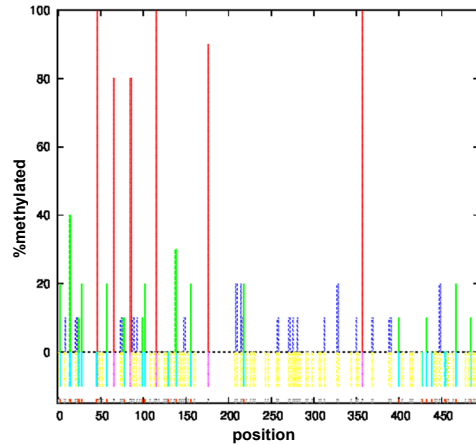
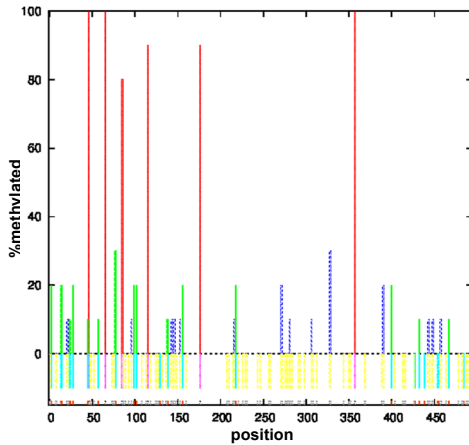
5'-end region
immature leaf



internal region
immature leaf



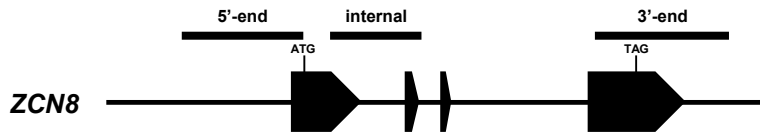
3'-end region
immature leaf



Supplemental Figure S11. Bisulfite sequencing analysis of mC at teosinte *ZCN7*. The *ZCN7* gene structure and the positions of the regions are indicated. The data are illustrated using the Kismeth web-based tool (<http://katahdin.mssm.edu/kismeth/revpage.pl>; Gruntman et al., 2008). The fraction of times the cytosine at each location is methylated is reported as colored bars above the x-axis and the three colors represent the three sequence context (CG, CHG, and CHH). The number of times each cytosine is sampled in the sequenced reads is reported as colored bars below the x-axis. For all regions analyzed we used ten independent bisulfite treated and sequenced clones.

teosinte

0.5 kb



wt

id1

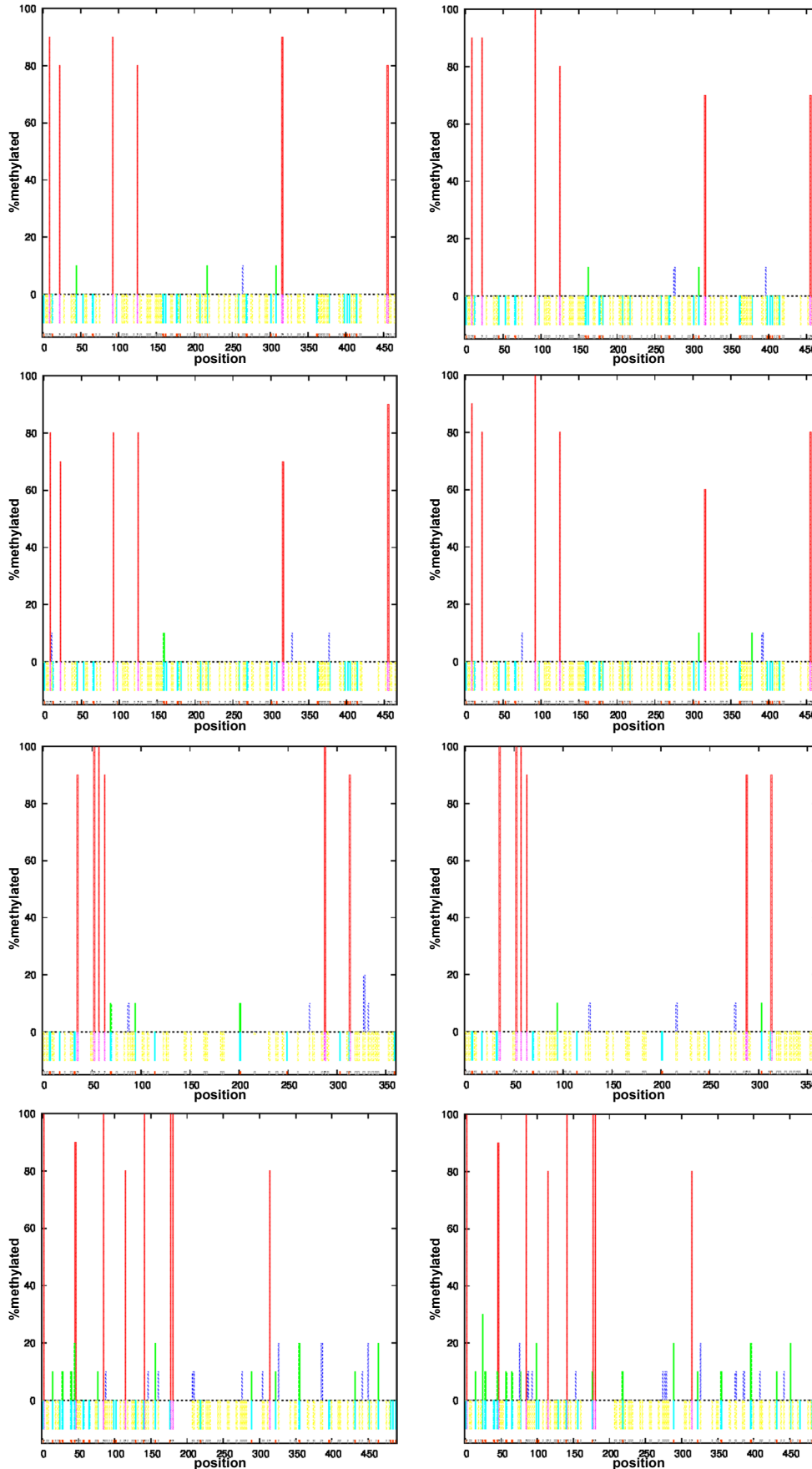
CG 
CHG 
CHH 

5'-end region
mature leaf

5'-end region
immature leaf

internal region
immature leaf

3'-end region
immature leaf



Supplemental Figure S12. Bisulfite sequencing analysis of mC at teosinte *ZCN8*. The *ZCN8* gene structure and the positions of the regions are indicated. The data are illustrated using the Kismeth web-based tool (<http://katahdin.mssm.edu/kismeth/revpage.pl>; Gruntman et al., 2008). The fraction of times the cytosine at each location is methylated is reported as colored bars above the x-axis and the three colors represent the three sequence context (CG, CHG, and CHH). The number of times each cytosine is sampled in the sequenced reads is reported as colored bars below the x-axis. For all regions analyzed we used ten independent bisulfite treated and sequenced clones.