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Supplemental Material to:

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Epigenetic switches of tobacco transgenes associate with transient redistribution of histone marks in callus culture

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Figure S1

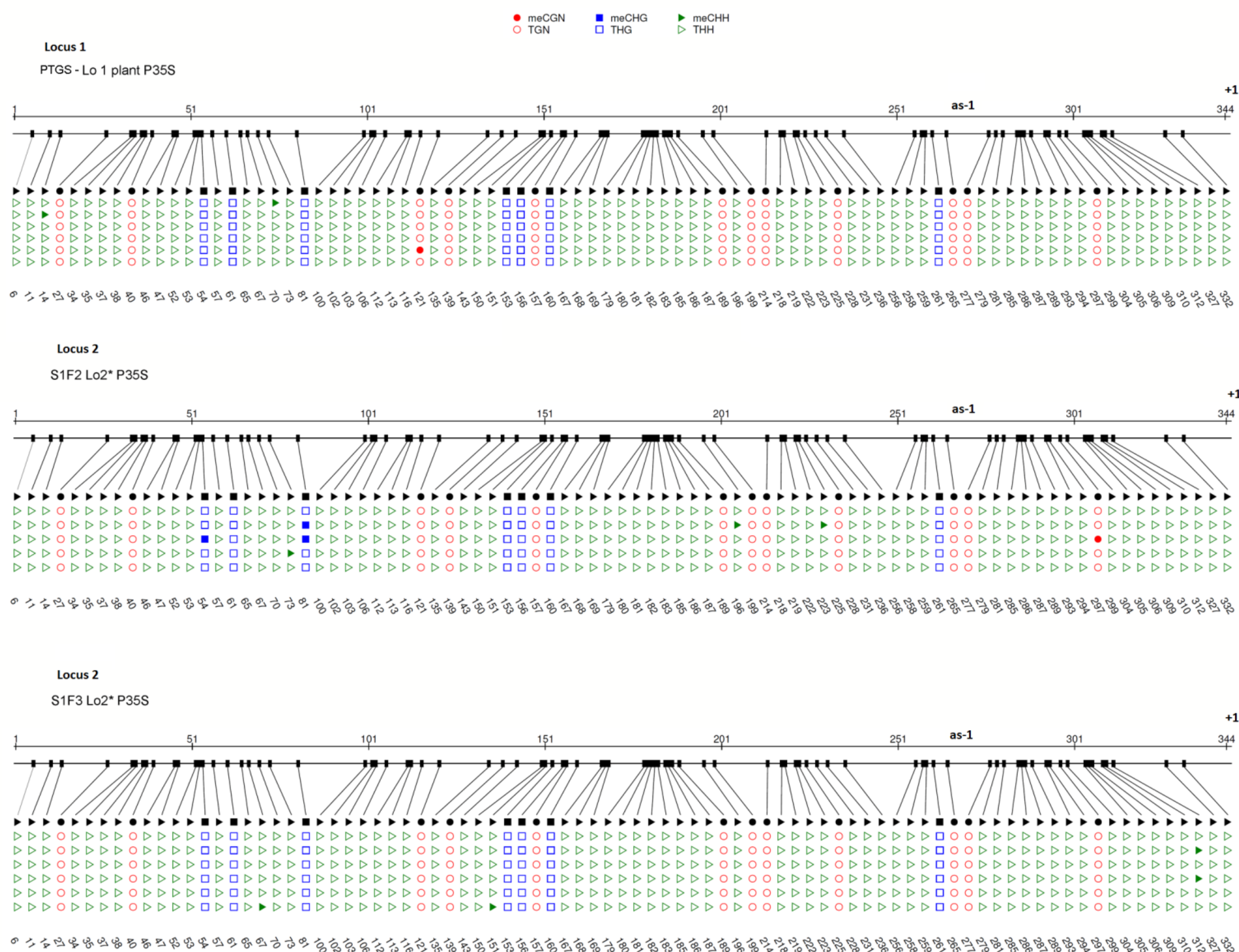

Figure S1. The CyMATE graphical outputs from bisulfite genomic sequencing of active P35S promoters in PTGS locus 1 and a non silenced locus 2. The locus 2 analysis shows the situation in two lines (S1F1 Lo2* and S1F2Lo2*) obtained after the segregation of the silencer locus 271. Both silencing[46] and methylation were not meiotically inherited. The sequenced region includes the P35S and a part of the upstream sequence. Empty symbols – non methylated Cs, Filled symbols – methylated Cs. The position of *as-1* element is indicated.

Figure S2

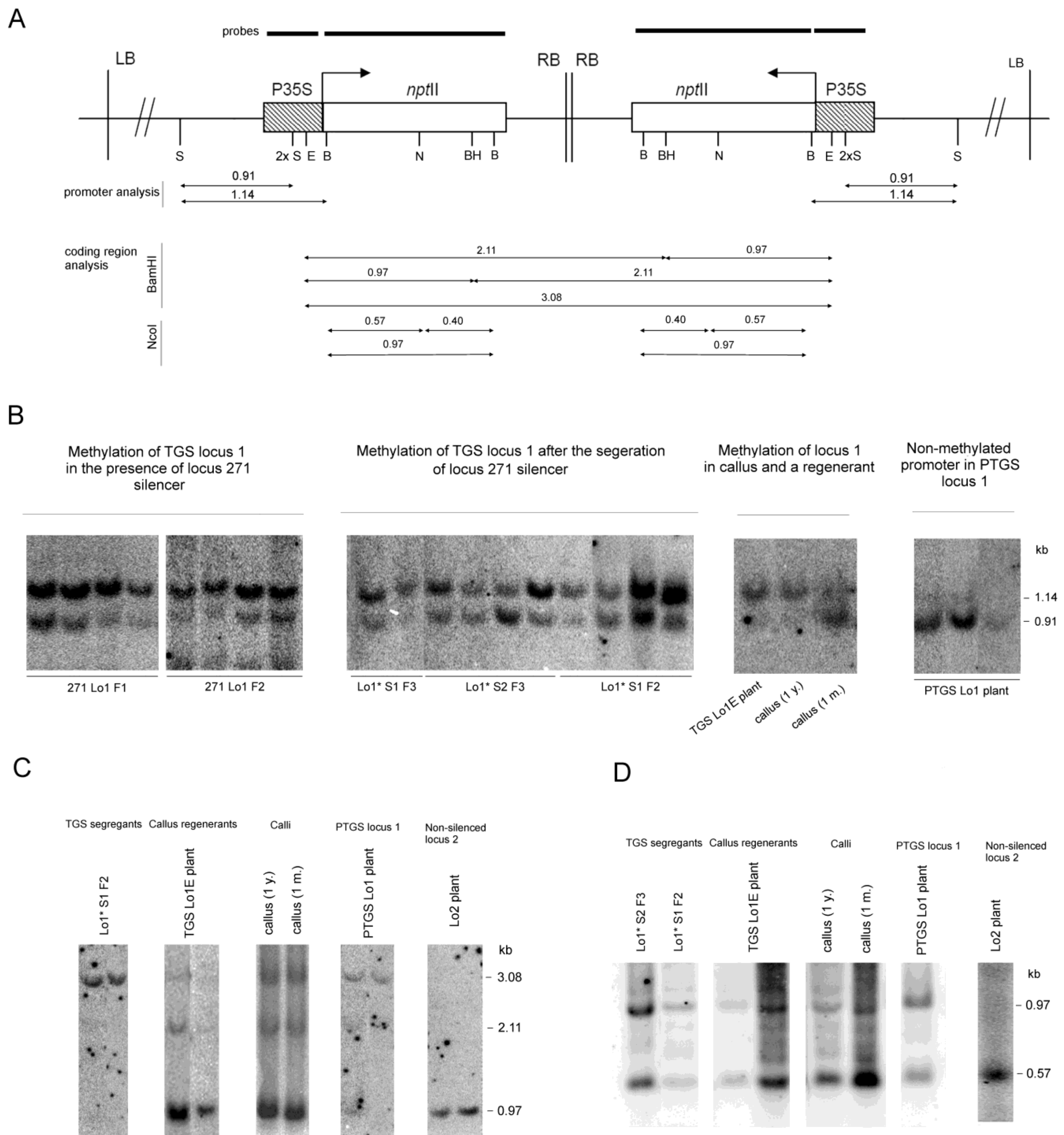

Figure S2. Southern blot hybridization analysis of cytosine methylation in the promoter (B) and 3'coding region (C,D). The genomic DNAs were digested with enzymes recognizing nonsymmetrical sites in the P35S (*Sau*96I) and *npt*II coding region (*Bam*HI, *Nco*I) and hybridised on blots with relevant probes. Positions of restriction sites and regions of probe hybridisation are diagrammatically depicted in (A): B, *Bcl*I site; S, *Sau*96I site; E, *Eco*RV site; BH, *Bam*HI site; N, *Nco*I site. Black lines above the schematic outline the locus 1 highlight the region of probe hybridisation. Black arrows below the scheme indicate length and position of restriction fragments from individual experiments. (B) Analysis of the 35S promoter methylation in the Lo1 plant, derived callus, Lo1E regenerant, 271 Lo1 hybrid plants and segregating progenies. *Bcl*I enzyme was used for the dissection of the particular regions of T-DNA. Methylation analysis of the central (D) and 3' (C) regions of the *npt*II coding region. *Bam*HI (C) and *Nco*I (D) were used as methylation-sensitive enzymes.

Figure S3

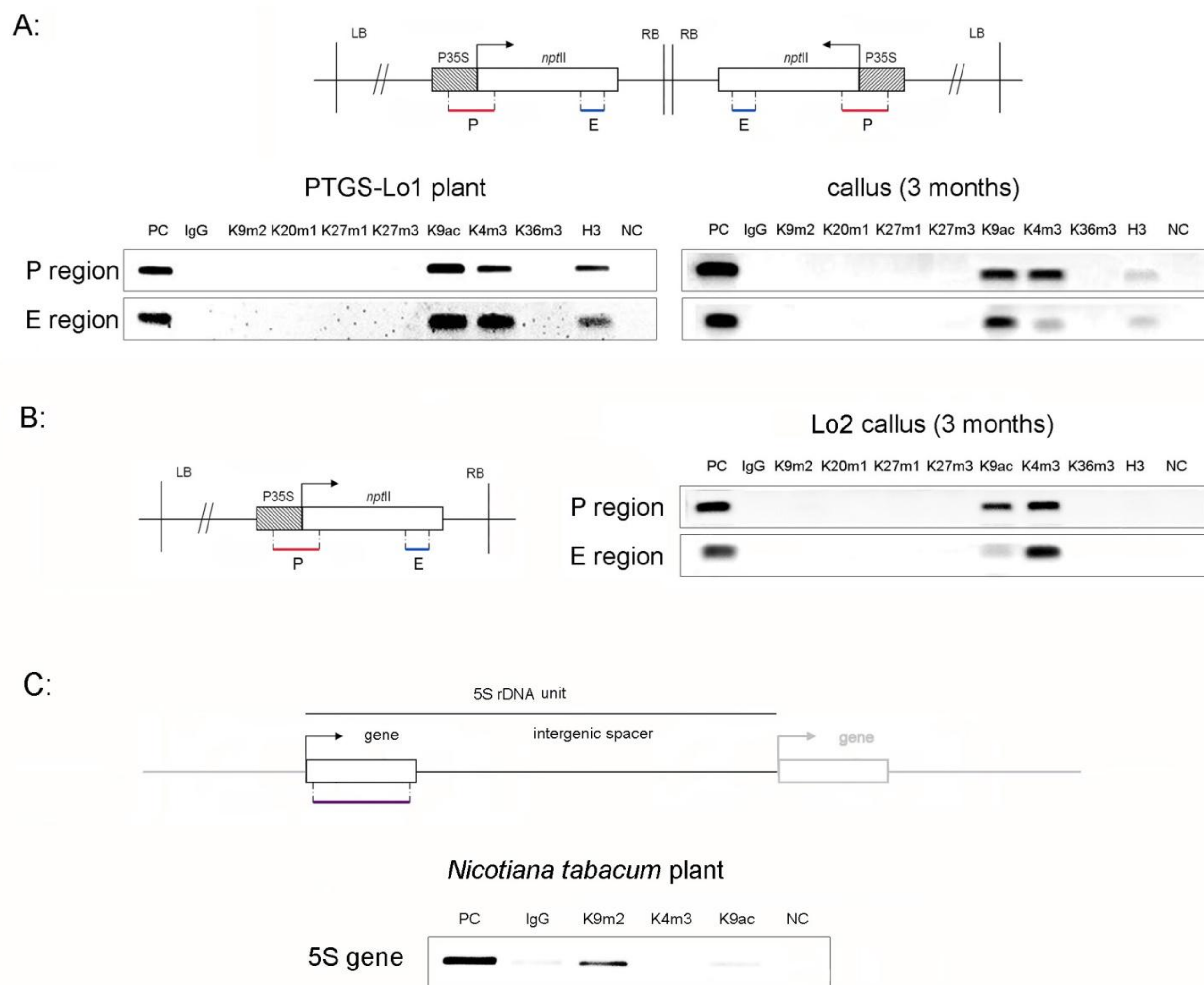

Figure S3. Chromatin immunoprecipitation profiles obtained by a classical PCR on DNAs extracted after ChIP on leaf and callus tissues. (A) Schematic outline of transgenic locus 1; subregions P (promoter) and E (3‘ end of *npt*II) analysed by ChIP. In addition to previously used set of antibodies, samples were immunoprecipitated with antibodies against positive-acting H3K36m3 and mostly repressive H3K27m1, H3K27m3 and H4K20m1 marks. Anti-H3 was used as a control. (B) Schematic outline of transgenic locus 2 together with ChIP results of three months old callus. (C) Schematic outline of 5S rDNA unit and electrophoretic profiles of immunoprecipitated 5S chromatin.