

# Supplementary Material

## Role of CG and Non-CG Methylation in Immobilization of Transposons in *Arabidopsis*

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### Supplementary Results

#### Flanking Sequences of the Transposed *CACTA1* Element

Transpositions were confirmed by directly sequencing flanking regions of *CACTA1* for *cmt3* single and *met1-cmt3* double mutants. The experimental conditions were as described in Miura et al. [S1].

#### *cmt3-met1* Double Mutant in F<sub>2</sub> Families in Figure 2C

##### Plant 1 in Figure 2C

ATTTCTAATCGAAGTGTCTAAGATTTTCAGCGTCTTTTCTTTGT  
TTTCTCTTTGAATAAATTTATCCTTGCTTCTGCTTTGTATGTTCCG  
ATAGTCGAACCTCTTATTTTATTCGTACGTATAATCGAACTATTCT  
TCAACATGAATAAAACAATTTTATTAATAGCTGAACTCGTTTCCG  
AGATGCCTACATACCCATTTTCGAGATCAAGCCACACCGTAGTCA  
TGACAAAGAATGC (Complete match to AB010068, 17517–17272,  
on chromosome 5.)

##### Plant 4 in Figure 2C

AAGGATAAGTGGAAAAGACTATTCAGGAAGGAAAGTTTGCACAAA  
AGAGATCACACACTGTTGGGGTGAAGGGCTTTGCCATTTTAGAG  
ATAAAAGAGTGTAAATACACTTTTTCTGTGATTTCGAACTTTCTTA  
GTCTTGTTCACGAGCTTGATTTCTACGTATATTGTAACGTTCTGTA  
ACGGATTGCTGGAAAGATTTTGACCAAGACGTAGATTGGTGCAC  
ATTTATTGCTATGAACTCGTAGATTTTGTATAATTTTCTGTTCTAT  
TTCTTTTGTGTCGGTGTGTTTCTCAACAATTCAGACGGTCCG  
AGATACAGTCTAGAG (Complete match to AL163832.1, 24913–  
25246, on chromosome 3, except 1 base of INDEL.)

##### Plant 4 in Figure 2C

TGACTCTTCTTTACCTGTTATCCAATTCGTATTCAATAAAGTGA  
TTTTTTTTTGTGGTGAAGAACATTCATAAATGTCTGAAGTTATT  
TATGGATTTTGTATCTTTTGTAGTCCGATAACGAAT (Complete  
match to AB026658, 22974–23102, on chromosome 3.)

##### Plant 18 in Figure 2C

TGTCATAACCCACATTAGATTTTTCGCCGAGTGAATTAAGTTGTG  
CTTGTGTTGACGATTCAACGACTCCGACATTAGTCTGTTAGAAAG  
GTTAAGTCACTCATCGTTTCAGCCAAACGACAAAAGTAGACTAA  
GCCACCCCAAGACTTGAATAAATAAAGTCTTCTATTTCAGAGACAA  
AATAATGGAATTGTATGTAAGCGATGTGGTTGATCAC (Complete  
match to AB005232, 11543–11760, on chromosome 5.)

##### Plant 21 in Figure 2C

TCATTAGGAAAAAATAAGCAAGGACACAAGCAAATCATCCCCGG  
AGAATAGTCACTCTTGCAGGATTAGTTACTGCAACAACATAGAA  
AATTAGTCAAAATATCGCAGTACCCATTCATATCGTAATCGTT  
AATTAGTCTGAATCCTCCAAGAAATAATGTAATTTTTGTTGG  
TGGGAAACTGATTG (Complete match to AC011809, 58380–58189,  
on chromosome 1.)

#### *cmt3* Mutant in F<sub>3</sub> Families in Figures 3C and S2

##### Progeny of Plant 9 (Sixth Lane from Right)

AAAGGATTTGAACTTACTTGCGAATGAATTGACCTTTGACGTGAG  
GCCGTTGCTCAGCTAGTTTCTCCTGCTATGGTACCTAACCTAAA  
GGATACCAAAAAAAGCTTGTAGTTTCTGCTCAGGAAACAAGAGCTT  
AAGCTTGTGTTAGAGCTTACCTTTTTCTCAAACATCGCTTTTAC  
GTTAAGGCGAACTTCATCAAAGCAGCTTCTTTGTGCCATC  
GGTCACTTCCACTAGACTGCTGCTTTAAAGGATTGCTACTGCTTG  
AACTCGAACCCAAACAGTTTTGCAATAGGACTGTGATGAGGAGAA  
CAACTTTCCACATTTTCTTCTGAGCCCTAACTCAGCAAAAGTTCA  
AAGTTTTGATGAAACCGGCATAAATTTCAAAGACAGCGCCAAG  
CATCATGAAAAGTTACCTTCTTAGCAGAAGTAGCACCAATTATTGT  
ATCTGCATATCACAAGAGAACCAATAAATCCTCAAATCAGTCACAGG  
TTCGACATTTTCAATTTGAGTTAATGTCAAGAACTTACTTTGAGAAT  
GCAGAGAGATTTGAATGCCTCAACACACTTCGATCACCGACTTTG  
TAATCTCTATCCTCTCTTGTGTTTT (Complete match to AB019231,  
27471–28078, on chromosome 5.)

##### Progeny of Plant 9 (Fourth Lane from Right)

ACTGTTCTCAGCAGTAAGATCTTTGGAGGGTGAAGCCTTAAAGCT  
TCTTTTGCAGTGCAGAAACAGAAAATCCATCTCAGACAAAACATCG  
TAGTCGATCTTGTCCCATGTTTCCAAATCAGTTTCTTCTGCTCG  
TCCAAAGCCGCGGACCACAGTGTGTTTGAATAGATAAGCA  
CCGTCATGTGGCAGTGATAGAGCTTGATGTTGTCTGCAAAAC  
AAACCAGCAATGAGCAAACAGTTACTTCAGATTGATGTTCTCT  
CTACCGTCTTTGTACTTTGAGTCGATGAAACATTGTAGCATGTCG  
TTCTCTGATTTGTCACCAGAGCGTTTTCTTGTGCTATGATGTTTG  
AAAAGATTTTTGCAATCTTTCCGCGAGCAGGTCACGACAATTGT  
GAGCTGGAATGGGGAGCTTTGGGAAGATGACAACTTATGGGTTGC  
ATGCTTTATCAAGGTCATGGAACAATGGAGCAACATCATCAAAA  
AGTTGGTACGACTTCTCGACCCAATAGACATCTACTAGCAGTC  
AAGGTGATTAGACGCTCTAACTCGGCCTTAGATCCACTTCCCCA  
CTTTCTCCCCATTTTGAGAAGTAATCCTGCAAAACATTACA (Com-  
plete match to AC002329, 37846–38470, on chromosome 2.)

##### Progeny of Plant 33 (Fourth Lane from Left)

AATCCTTAAGAAAAAGATGAATTTCCGAACATAACCGAAAGTAT  
GATAAAAAAAGATACTTGAACAGTTAAACACAATATAATAGTA  
GATCCTCGTTCAGTACACGAAACATAAAATAAGCCAAAACCTT  
TGTTTCGTTTTATTGAAGTAATCTCATAAATGACAATGACTTTGTG  
AACGTGAGCCAACACAACAAAAGAAAATAGAAAGCTTATAAAGAA  
ACTTCGGTCCACCGAATACATATTTTTAAAAAATAAATAAAGAAAG  
CAAACCATGTTTCATAAAAAGAACTTATTCGAAACGCACTCACTTA  
TTGAAAAAGAACTTATTGGATGGAGACTCTATTTTAGAGGTGTAG  
TATGAAAAAATAAAGTTTA (Complete match to AC007293, 18904–  
18524, on chromosome 2.)

##### Progeny of Plant 33 (14<sup>th</sup> Lane from Left)

CATATAATATTAATGCGAATTAAGTAGTAGAGAGTATAATAATAGTA  
ATAACAAAATGCTTGGTTACTGTATGTAATCCCTAGACAAAAGAA  
ATAAGAAATAAAAAGAGCAAAATTTTCATCCCAAGATCCATCAGG  
AGAGTCAGAGTCAGACCCCATCTCTCATTTCCCATCTCTCTTTTT  
CACATTTATTTCTTTTTTTTTCTTTCTAGA (Complete match to  
AC001229, 77642–77853, on chromosome 1.)

### Supplementary References

1. Miura, A., Yonebayashi, S., Watanabe, K., Toyama, T., Shimada, H., and Kakutani, T. (2001). Mobilization of transposons by a mutation abolishing full DNA methylation in *Arabidopsis*. *Nature* 411, 212–214.
2. Paulin, R., Grigg, G.W., Davey, M.W., and Piper, A.A. (1998). Urea improves efficiency of bisulphate-mediated sequencing of 5'-methylcytosine in genomic DNA. *Nucleic Acids Res.* 26, 5009–5010.
3. Jeddeloh, J.A., Bender, J., and Richards, E.J. (1998). The DNA methylation locus DDM1 is required for maintenance of gene silencing in *Arabidopsis*. *Genes Dev.* 12, 1714–1725.

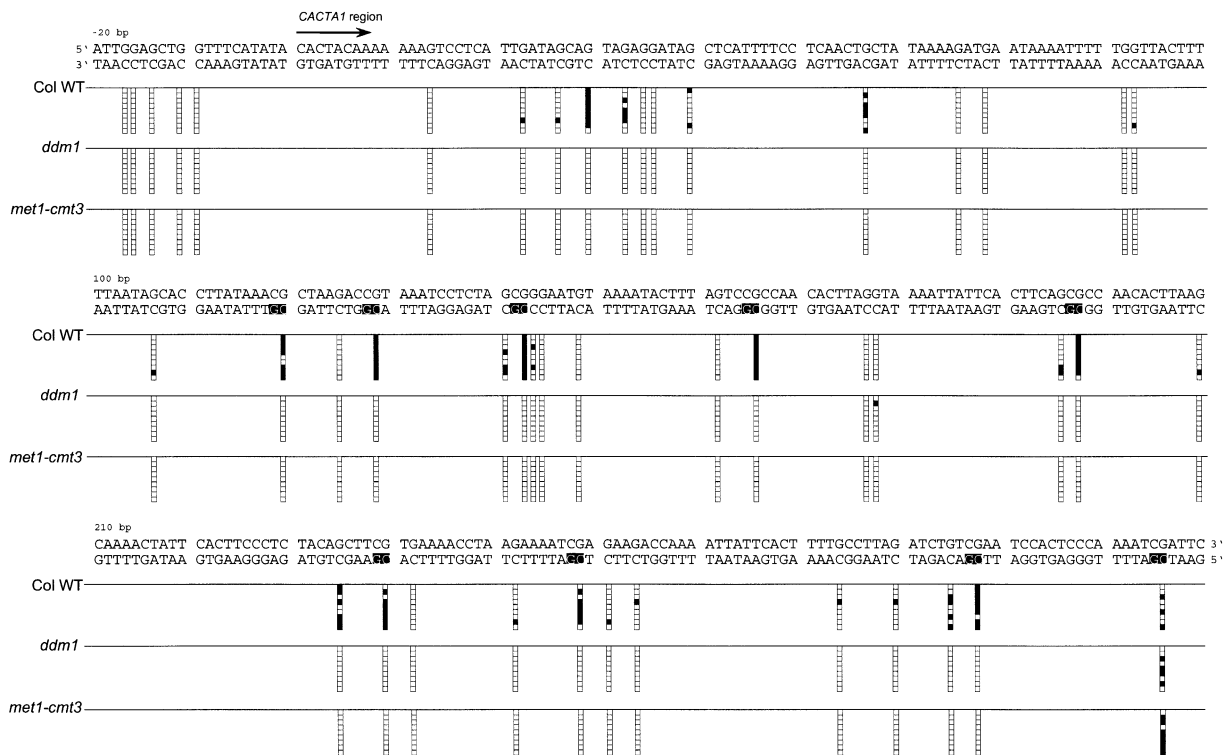


Figure S1. Methylation Patterns at the 5'-Terminal Region of *CACTA1*

Genomic DNA was isolated from leaf tissue of the indicated strains and digested with *Dra* I and *Hind* III. Conditions for bisulfite treatment are as described by Paulin et al. [S2]. The primer pairs used for the first and the second PCR are BS-F1 (5'-TTCCCTTAAATATCCACATAATAAT TACT-3') + BS-R12 (5'-TGAATGGATTAATAAGGAATAAGGAAGAAAGA-3') and BS-F2 (5'-TTTTCTTTTAATTCAAACATACACCATTCCAAA-3') + BS-R13 (5'-TTTTAGGTTGAATCGATTTTGGGAGTGGA-3'), respectively.

The obtained PCR products were cloned into pT7 Blue T-Vector (Novagen) and sequenced with the primer BS-F4 (5'-TACTCATTCTCATGA TACATCAAA-3').

In each plant, the completeness of bisulfite treatment was confirmed by directly sequencing the PCR product from the control *ASA1* sequence [S3].

Data are shown for nine independent clones of each sample, with the monitored cytosines indicated by squares. Filled squares indicate methylation and empty squares indicate no methylation. CG cytosines are highlighted in black. In wild-type Col, both CG and non-CG (especially CNG) sites are methylated.

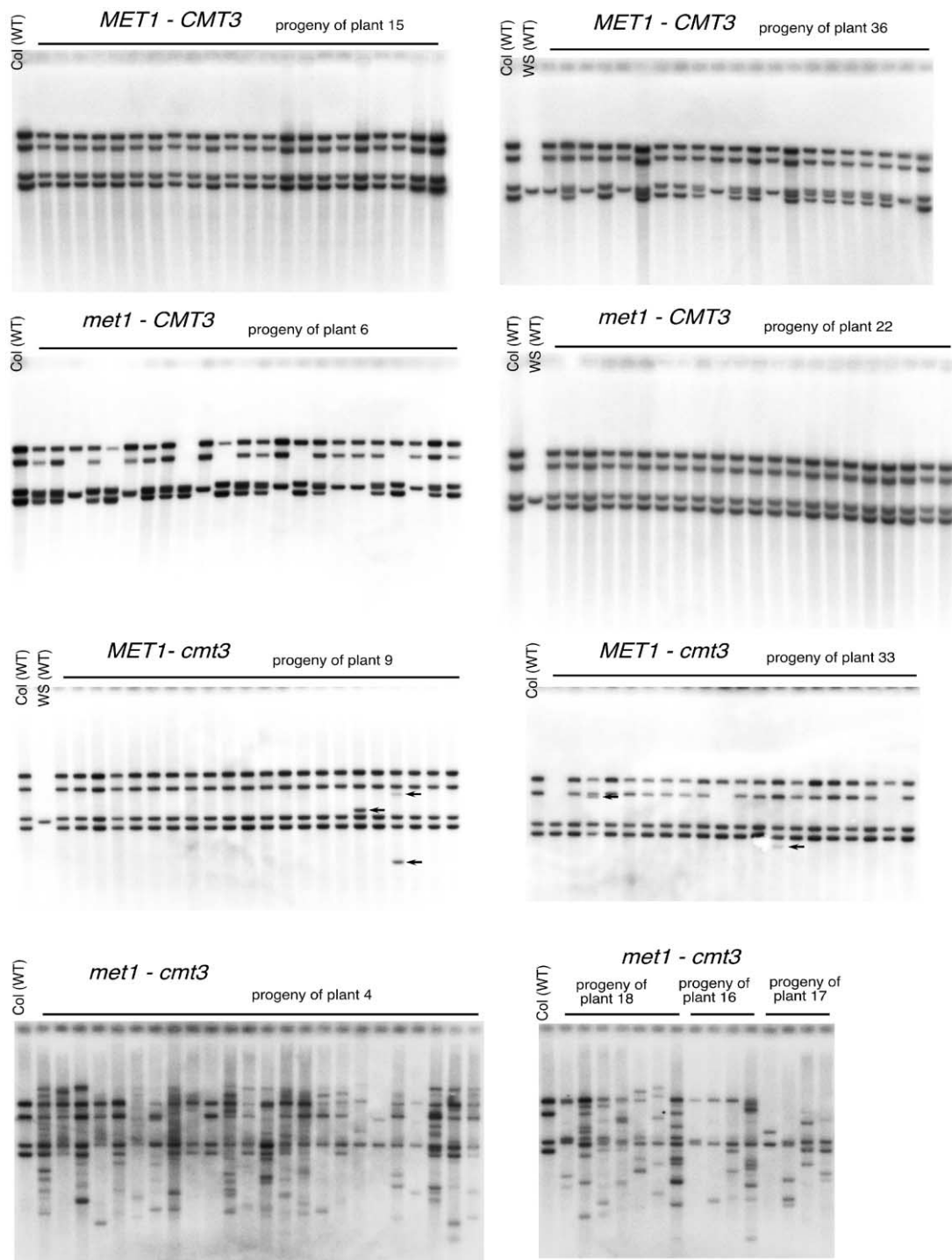


Figure S2. Transposition of CACTA Elements in the  $F_3$  Generation of Families 3 and 4

Self-pollinated progeny from  $F_2$  plants of each genotype class in Figure 2C are shown. As the parent plants 6, 4, 33, and 36 were heterozygous for some of the CACTA copies, segregation of bands was observed in their progenies.

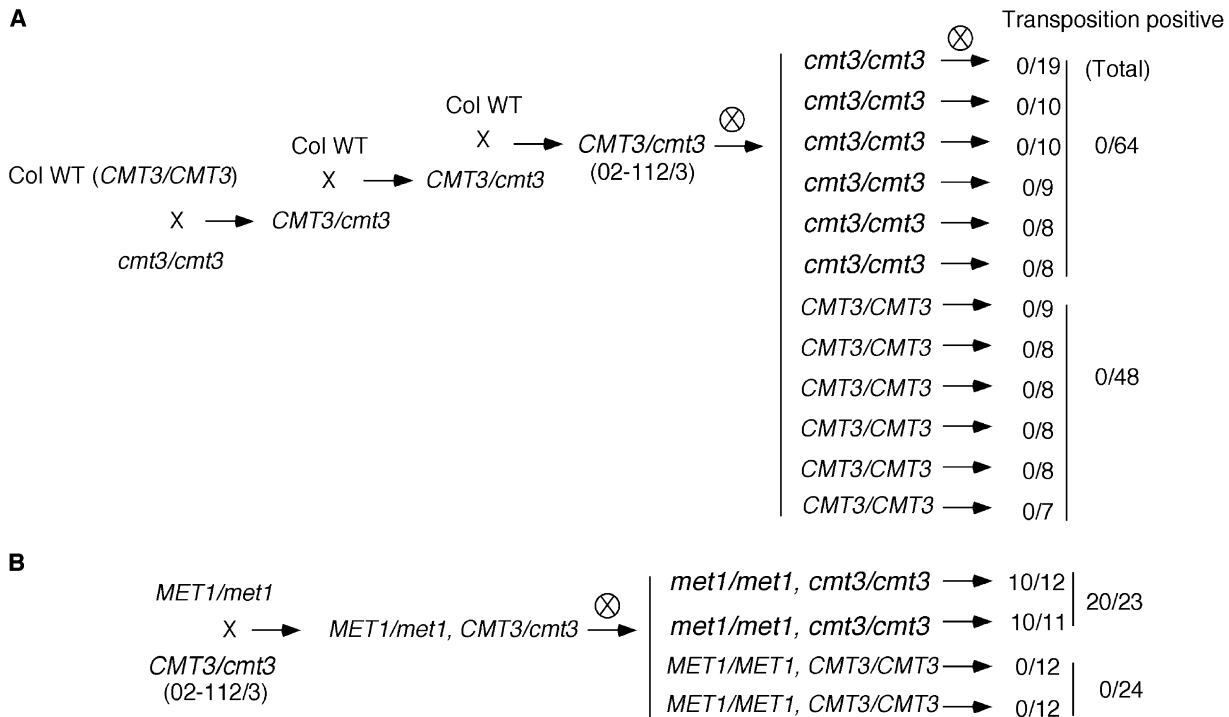


Figure S3. Effect of the *cmt3-i11* Mutation on Transposition of CAC Elements Introduced from Wild-Type Col Plants

The transposition was monitored as described in Figure 1.

(A) A *cmt3-i11* homozygote in WS background was backcrossed three times to wild-type Col, resulting in a *CMT3/cmt3* plant homozygous for all the CAC copies present in Col background (plant #02-112/3). From self-pollinated progeny of this plant, *cmt3/cmt3* and *CMT3/CMT3* homozygotes were selected and transposition was scored after one more self-pollination. No transposition was observed in 64 self-pollinated progenies from the *cmt3* mutants. This contrasts with the low-frequency CAC transpositions in *cmt3* mutants in families 3 and 4 (Table 1, Figure 3C), where the transposons were introduced from *MET1/met1* heterozygote. The results suggest that when CAC elements are introduced from wild-type instead of *MET1/met1* heterozygote, the *cmt3* single mutation may not be sufficient for the mobilization. This difference may reflect heritable partial demethylation of CAC elements that segregated from the *MET1/met1* background.

(B) In order to confirm effect of *cmt3* mutation in this family in combination with *met1* mutation, the same parental *CMT3/cmt3* plant (02-112/3) was also crossed to a *MET1/met1* plant to generate a double heterozygote *MET1/met1, CMT3/cmt3*. From self-pollinated progeny of the double heterozygote, the *met1/met1, cmt3/cmt3* double mutants were selected and transposition was scored after one more self-pollination. High-frequency transposition was observed, confirming the results for families 3 and 4. Taken together, the results further support our proposal that *CMT3* and *MET1* function redundantly in the immobilization of the CAC elements.