

Figure S1

**A**

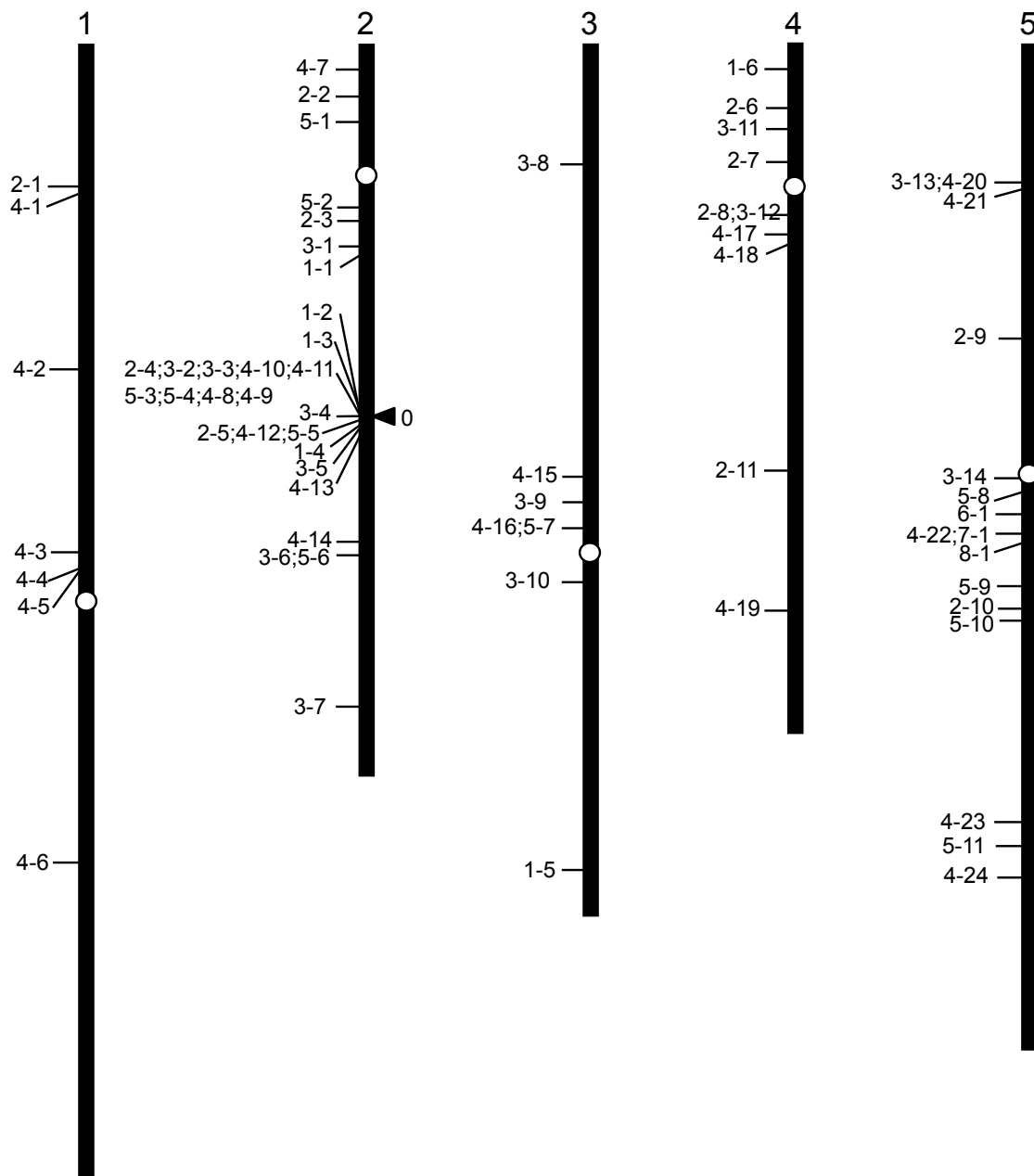
5' - GAGGGATCATCTCTTGTGCCCTTTGTGGCACAAATAATCTGCAAAACCACTT - TAGAGTCTTTATATCCCTTTTCACTTAATGA  
 3' - GAATAATCGTCTGGCCAGTCCCTTTTGT - AAAGAGAAAGCCAAAAATCCTTGTAAAAACTGAAATAATTTTGTTTTTTTT

**B**

Original locus	ctcc <u>TCACAGAAC</u>	<u>GAGGGATCAT..(Hi)..ACGATTATTC</u>	<u>TCACAGAAC</u> cgag
De novo insertions in <i>ddm1</i>	taga <u>CTTTTGAG</u>	<u>GAGGGATCAT..(Hi)..ACGATTATTC</u>	<u>CTTTTGAG</u> gttt
	tttt <u>GTTTCTTT</u>	<u>GAGGGATCAT..(Hi)..ACGATTATTC</u>	<u>GTTTCTTT</u> cgat
	tgga <u>CTCGGAAGG</u>	<u>GAGGGATCAT..(Hi)..ACGATTATTC</u>	<u>CTCGGAAGG</u> gaaa
	atgc <u>AATCGATCT</u>	<u>GAGGGATCAT..(Hi)..ACGATTATTC</u>	<u>AATCGATCT</u> ttcc
	gata <u>ATTCCAATT</u>	<u>GAGGGATCAT..(Hi)..ACGATTATTC</u>	<u>ATTCCAATT</u> ccag
	gtca <u>CCTTTAGTC</u>	<u>GAGGGATCAT..(Hi)..ACGATTATTC</u>	<u>CCTTTAGTC</u> ggac
	acgc <u>TTTCCTCA</u>	<u>GAGGGATCAT..(Hi)..ACGATTATTC</u>	<u>TTTCCTCA</u> aggg
	cagc <u>AAGTCAGTG</u>	<u>GAGGGATCAT..(Hi)..ACGATTATTC</u>	<u>AAGTCAGTG</u> taag
	ggcg <u>GTTGAAGG</u>	<u>GAGGGATCAT..(Hi)..ACGATTATTC</u>	<u>GTTGAAGG</u> ccta
	tata <u>CATACGATT</u>	<u>GAGGGATCAT..(Hi)..ACGATTATTC</u>	<u>CATACGATT</u> aaag
	aagc <u>ACTTCAGTC</u>	<u>GAGGGATCAT..(Hi)..ACGATTATTC</u>	<u>ACTTCAGTC</u> acac
	agag <u>AGTGGAAAC</u>	<u>GAGGGATCAT..(Hi)..ACGATTATTC</u>	<u>AGTGGAAAC</u> gcta
	ctga <u>AATAAAAAAG</u>	<u>GAGGGATCAT..(Hi)..ACGATTATTC</u>	<u>AATAAAAAAG</u> agta
	cata <u>GTTTACAACC</u>	<u>GAGGGATCAT..(Hi)..ACGATTATTC</u>	<u>GTTTACAACC</u> atta (10 bp TSD)
	ttggttttttag <u>G</u>	<u>GAGGGATCAT..(Hi)..ACG-----</u>	<u>G</u> tcactgctccga (1 bp TDS)

**Figure S1. Terminal sequences and integration products of *Hi*.** (A) Terminal inverted repeats of *Hi* are much degenerated. Remaining complementary regions are shaded blue after alignment. (B) Integration of *Hi* occurs precisely at the terminal regions shown in (A). In both original locus and *de novo* insertions, terminal regions of *Hi* are flanked by target site duplications (TSDs; underlined), most of which are 9-bp. Two exceptional insertions, with TSDs of 10-bp and 1-bp (or 2-bp) are shown in the bottom. The latter may reflect deletion of the 3' terminal region of integrated *Hi*.

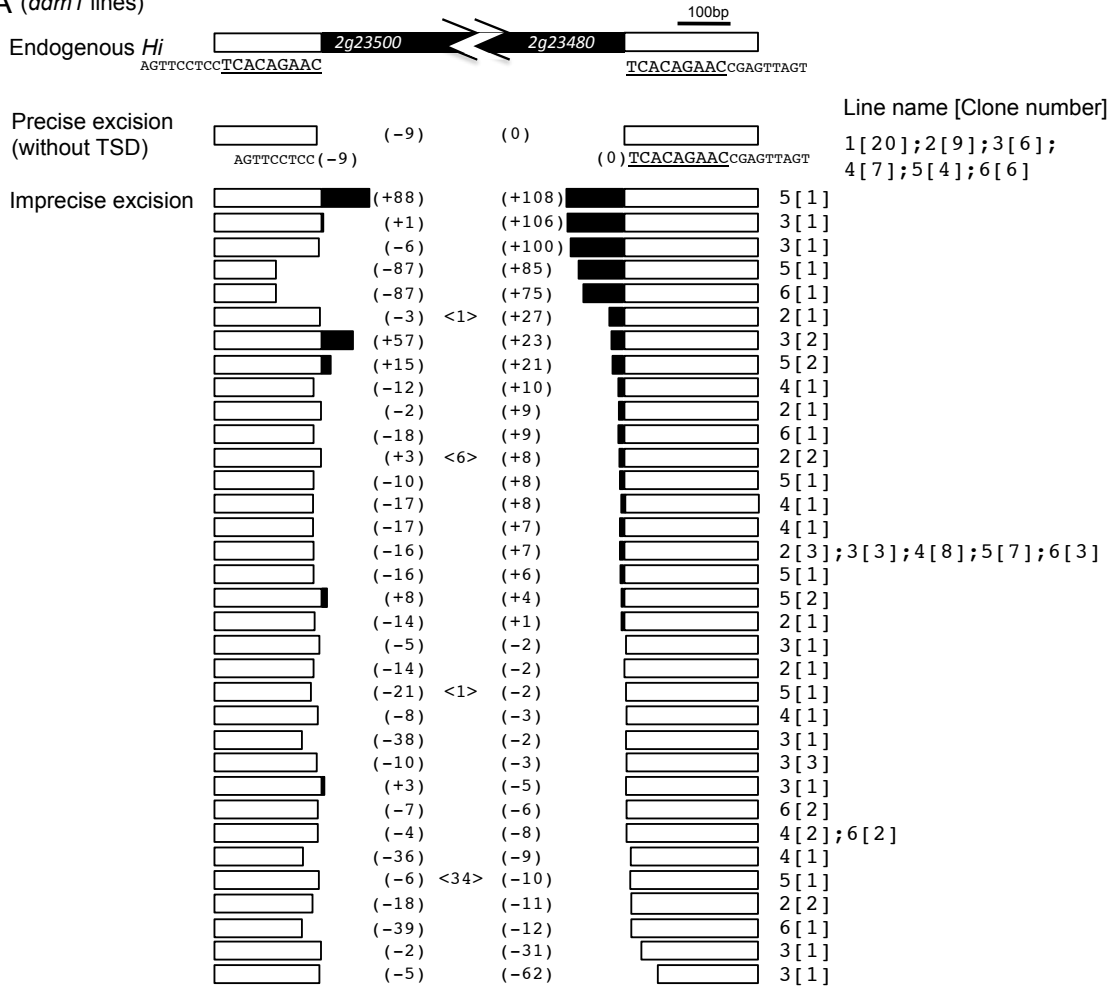
Figure S2



**Figure S2. *De novo* integration sites of *Hi* within the *Arabidopsis* genome.** Centromere is shown by circle for each of the five chromosomes. Arrowhead written at right side of chromosome 2 represents the original *Hi* locus. Loci for *de novo* integrations are shown in the left side of chromosomes. The first number (from 1 to 8) reflects different *d<sub>dm1</sub>* lines examined.

Figure S3

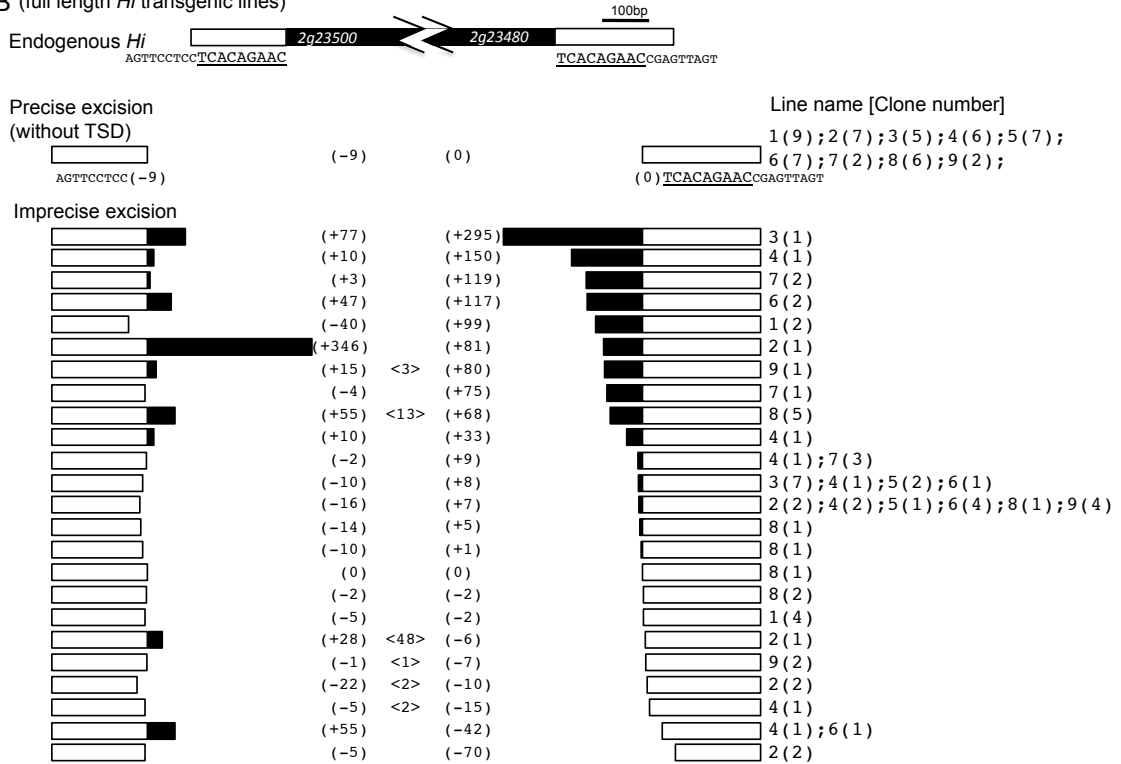
A (*ddm1* lines)



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Figure S3 (continued)

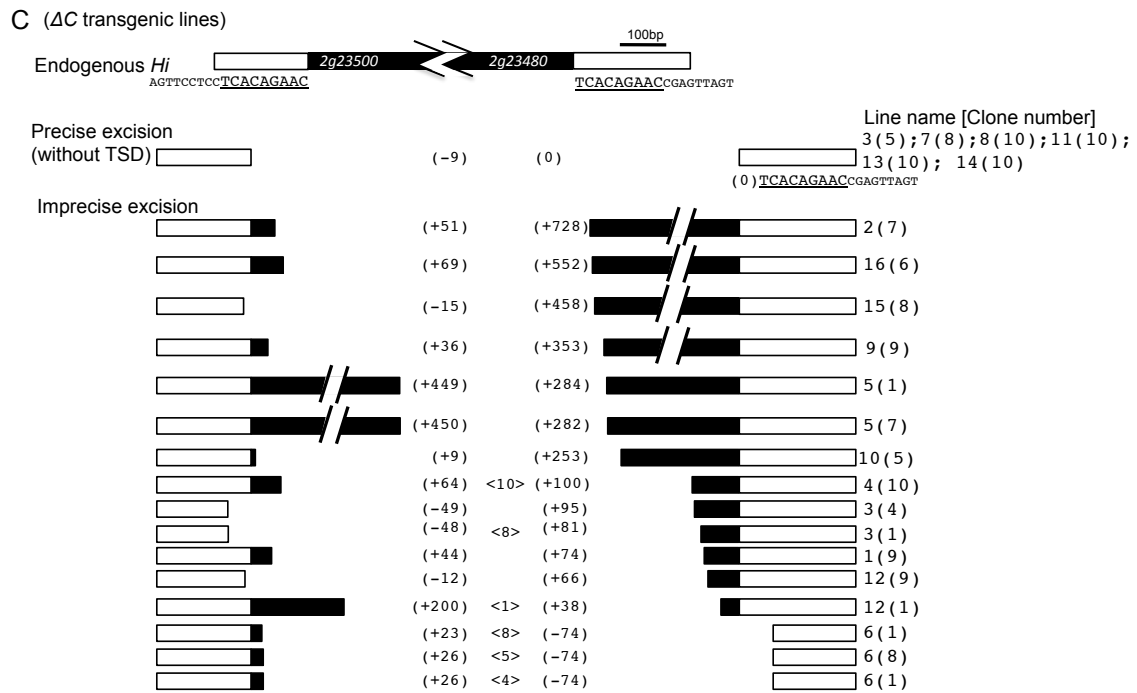
B (full length *Hi* transgenic lines)



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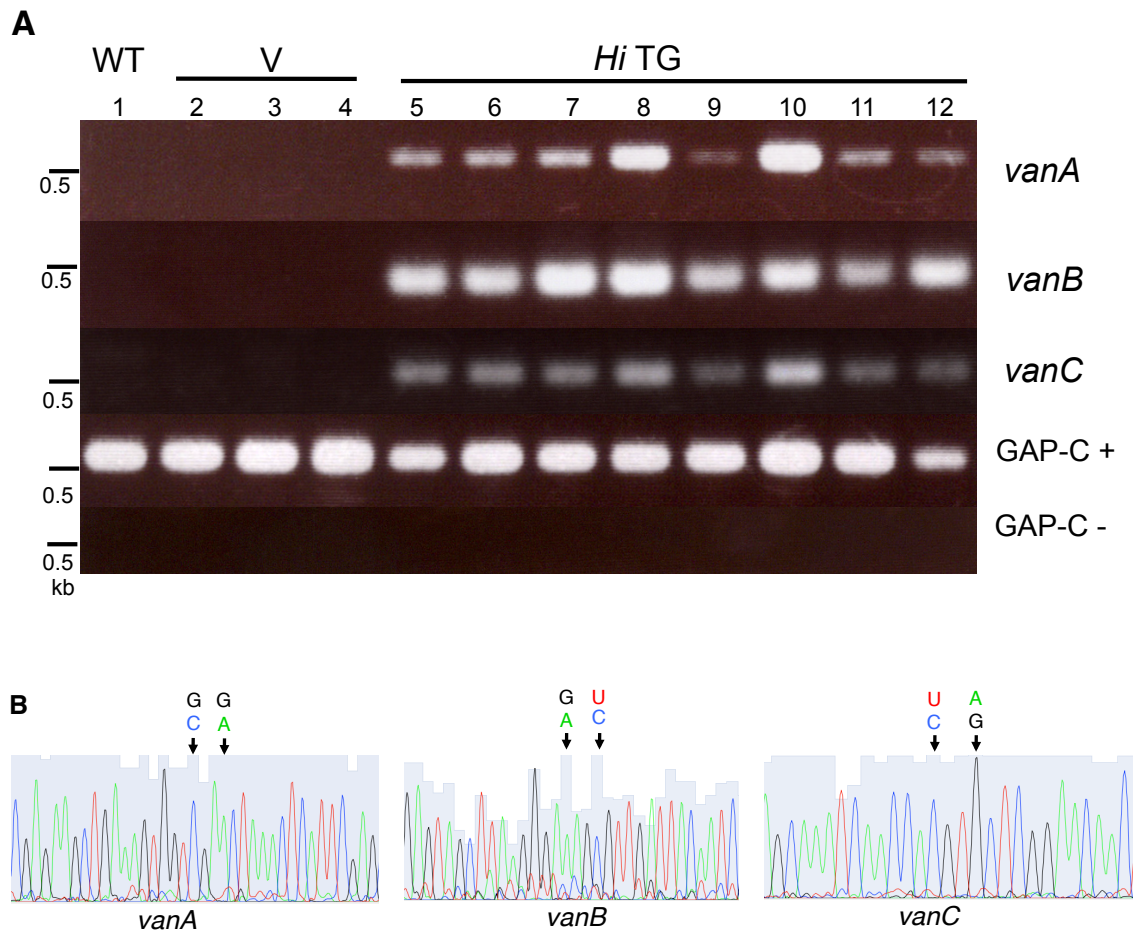


Figure S3 (continued)



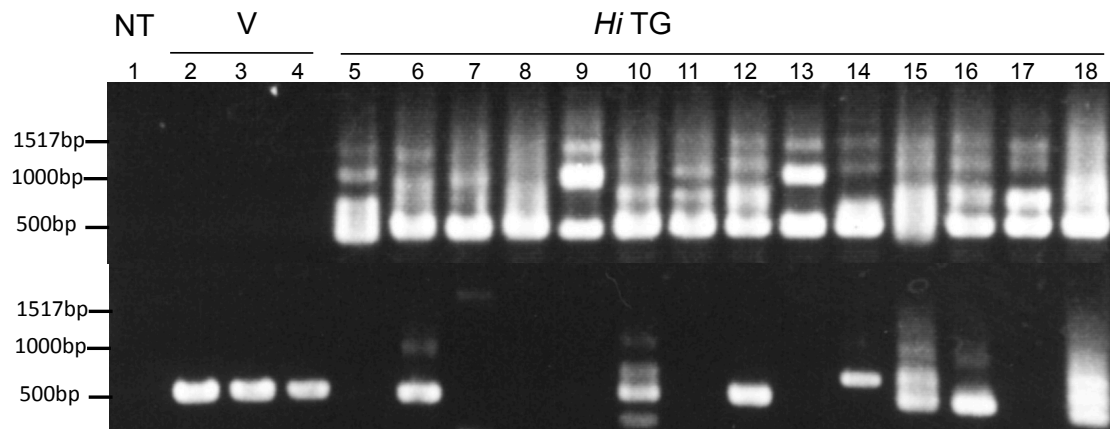
**Figure S3. Excision products of endogenous *Hi* at *ddm1* lines (A), full length *Hi* transgenic lines (B), and  $\Delta C$  transgenic lines (C).** Excision patterns were categorized into two groups, “Precise excision” and “Imprecise excision”. The former represents excision to recover sequence before integration, which does not have the target site duplication (TSD). Flanking sequences of *Hi* are shown below the diagrams for the original *Hi* locus and “Precise excision”, with the TSDs emphasized by underlines. For the “Imprecise excisions”, length of remaining *Hi* sequence is shown by black bar, and deletion of flanking sequence is shown by shorter white bar. Number with plus sign within ( ) indicates length of remaining terminal sequences of endogenous *Hi*. Number with minus sign in ( ) indicates length of deletion in the flanking sequence. Number within < > indicates length of sequence of unknown origin found in the excision product. We sequenced 120 clones of excision products from six independently self-pollinated *ddm1* plants (A), 116 clones from 9 independent full length transgenic lines (B), and 140 clones from 16 independent  $\Delta C$  transgenic lines. The line name and number of clones read are shown in right side of each excision product.

Figure S4



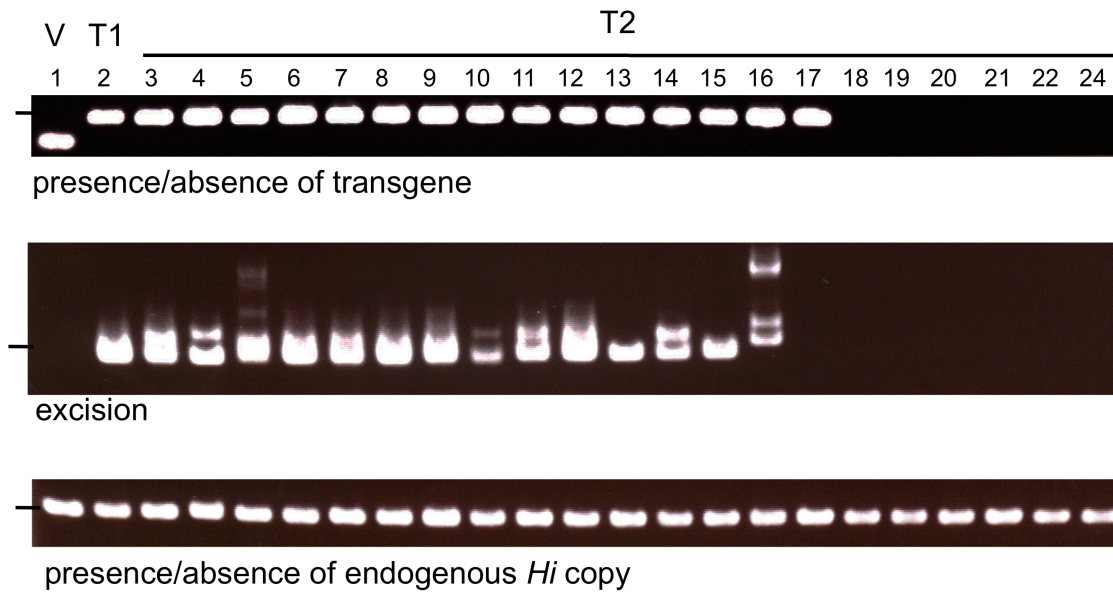
**Figure S4. Expression of ORFs in *Hi*.** (A) In endogenous *Hi*, three ORFs (*vanA*, *vanB* and *vanC*) are silent in wild type (lanes 1-4), but they are transcribed in *Hi* transgenic lines (lanes 5-12). WT: wild type; V: transformant lines with empty vector. For the transformants, each lane represents independent transgenic line. (B) Origins of the transcripts from the transgene and from original endogenous locus were distinguished between by direct sequencing. Sites of synonymous mutations (two for each ORF) are indicated by arrows, with the nucleotides for the original locus (top) and transgene (bottom). For all of the three ORFs, the signals indicate that most of the transcripts are from the transgene. The result for the transgenic line in lane 5 of panel A is shown. Transcripts from seven other lines showed essentially the same results.

Figure S5



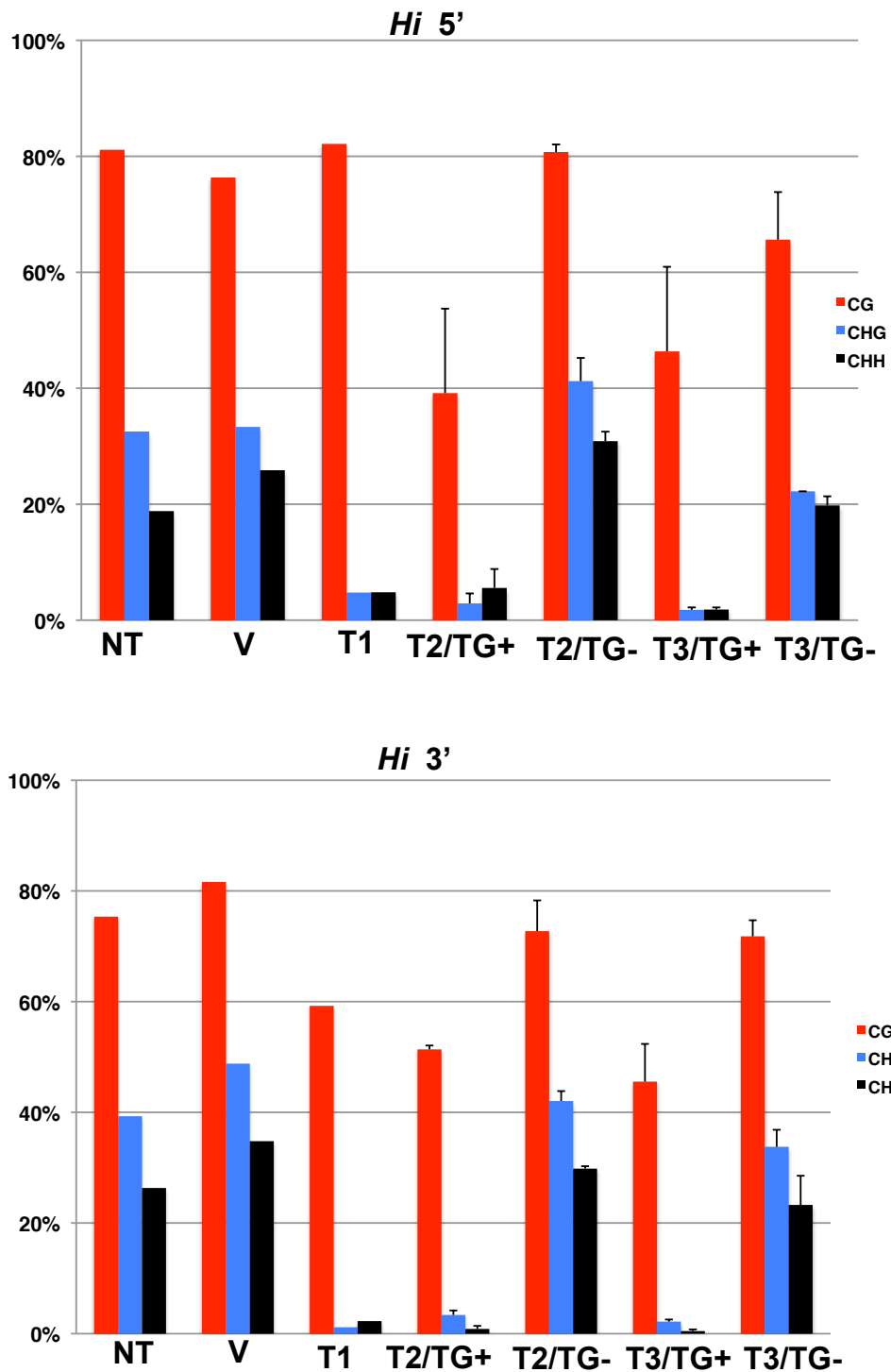
**Figure S5. Excision of *Hi* in the transgene.** Top panel is reproduction of Figure 2A. For each of the transgenic lines, we also examined excision of *Hi* in the transgene (bottom panel). Excision was detected in some, although not all, of the transgenic lines. Transgenic lines for the empty vector also showed the band, because primers for the vector sequence were used to detect excision of *Hi* in the transgene.

Figure S6



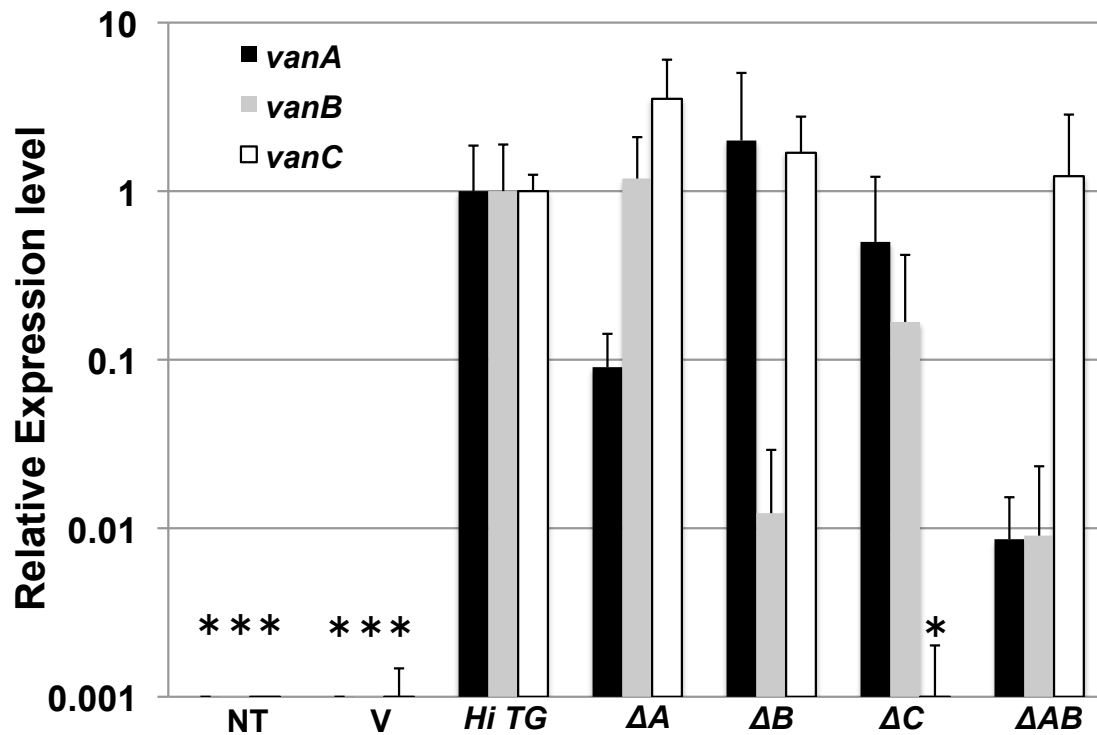
**Figure S6. Excision of endogenous *Hi* in T2 plants.** In a T2 family, presence/absence of the *Hi* transgene segregated (top panel). For the T2 plants with the transgene, 14 out of 15 plants showed excision, while none of the T2 plants without the transgene showed the signal (middle panel). Bottom panel shows presence of the remaining endogenous *Hi* copy. In each panel, length of molecular weight marker is 0.5kb.

Figure S7



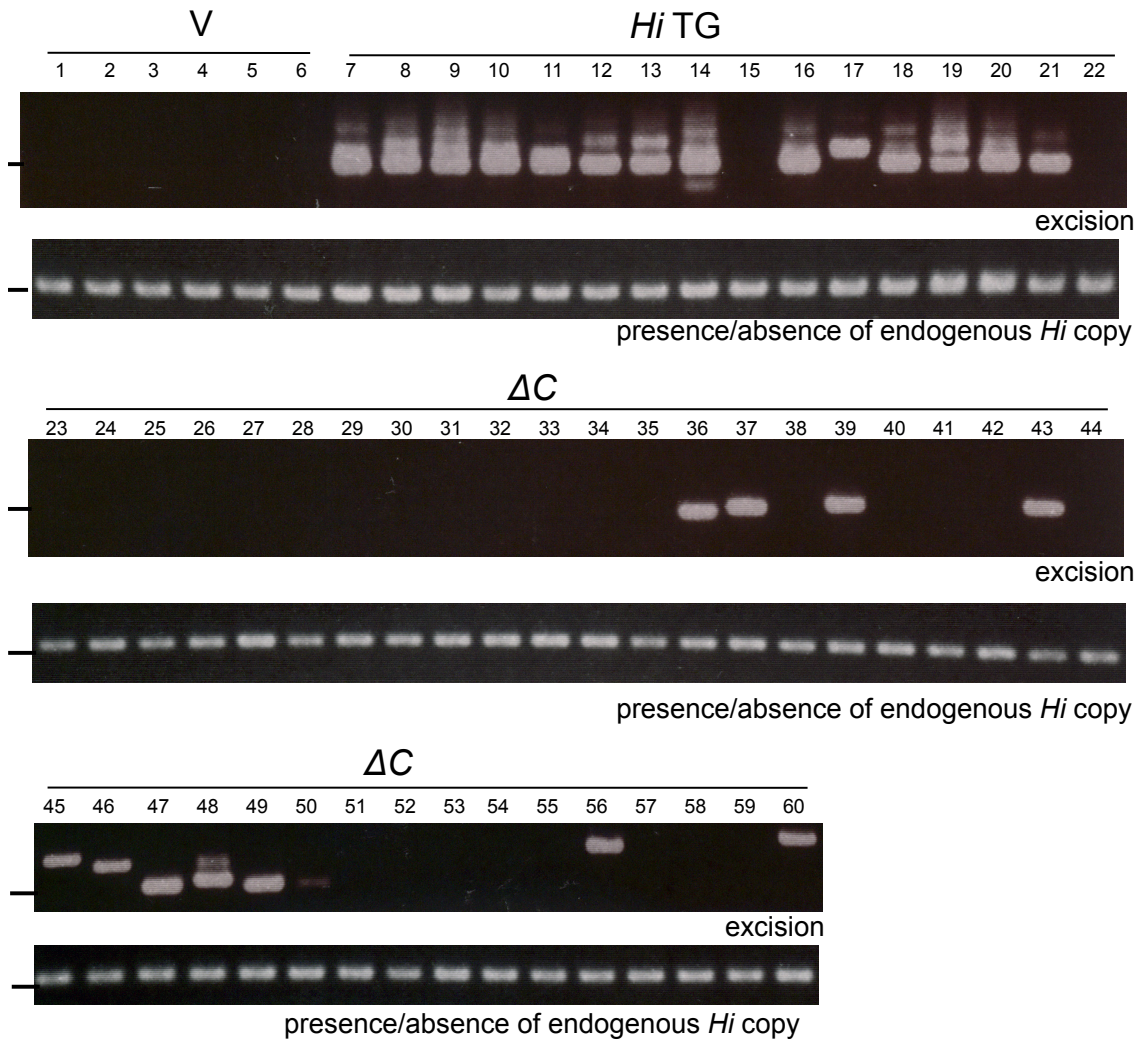
**Figure S7. Remethylation of endogenous *Hi* in the T3 generation.** DNA methylation in terminal regions of *Hi* was examined in a T3 progeny segregating the transgene. Two plants were examined for both TG+ and TG- plants in T3 generation. Others are reproduction of results shown in Figure 2B.

Figure S8



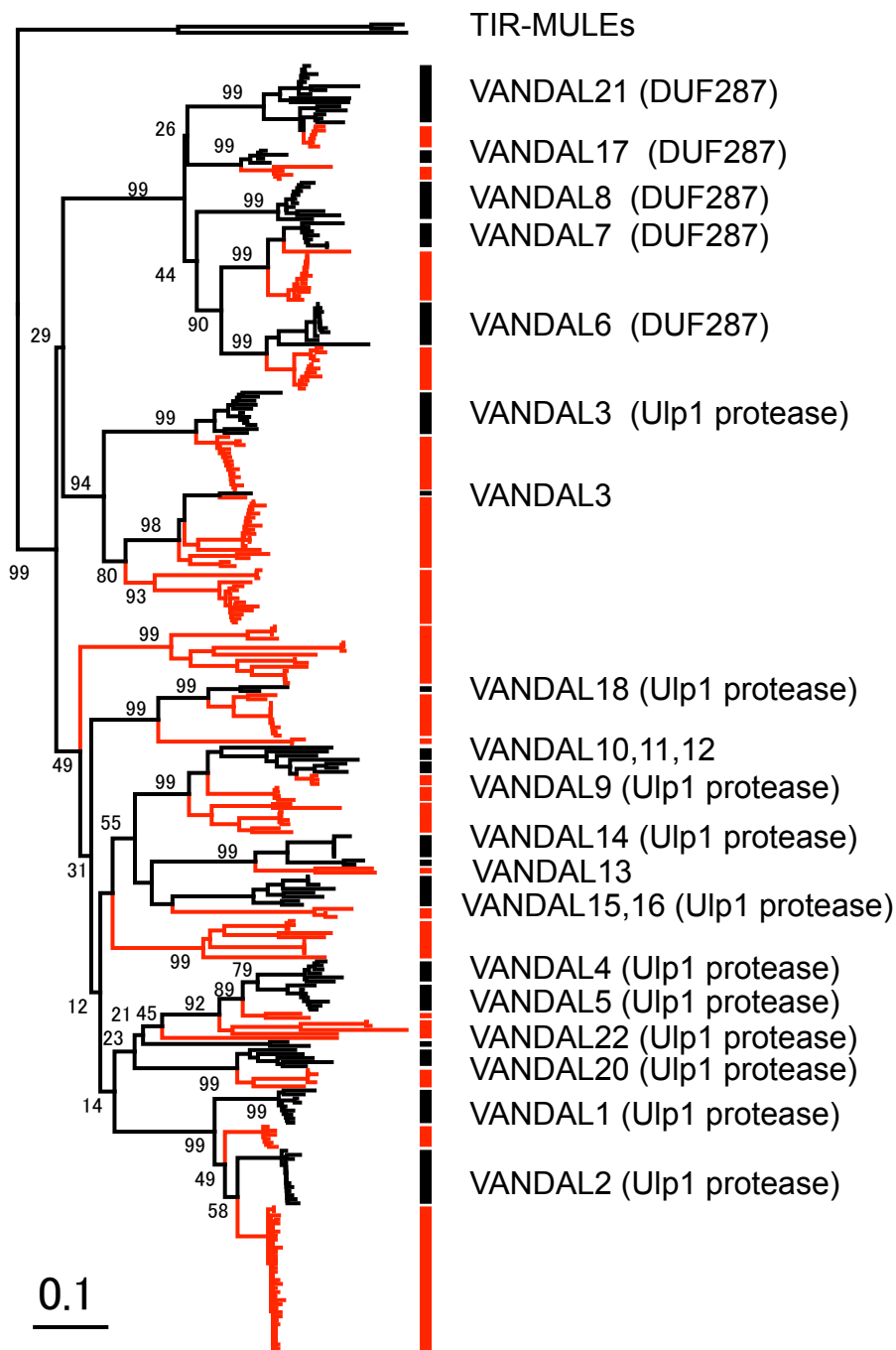
**Figure S8. Transcripts of three ORFs of *Hi* in transgenic plants for full length *Hi* and its deletion derivatives.** The transcript levels were measured by quantitative RT-PCR. For each ORF, the value was normalized by the transcript level in the transgenic line with full length *Hi*. We examined three transgenic lines for empty vector, four lines for full length *Hi*, 12 lines for  $\Delta A$ , six lines for  $\Delta B$ , 12 lines for  $\Delta C$ , and 18 lines for  $\Delta AB$ . Each bar indicates standard deviation among the values for different transgenic lines. Asterisks indicate averages less than 0.001.

Figure S9



**Figure S9. Excision of endogenous *Hi* in full length and  $\Delta C$ -TG lines.** Experiments in Figure 5A are repeated with additional  $\Delta C$  transgenic lines and full length *Hi* transgenic lines. Excision was detected in 14 out of 16 independently-transformed full length transgenic lines. On the other hand, only 11 out of 38  $\Delta C$  transgenic lines showed the excision. Together with the results in Figure 5A, the results suggest that the excision efficiency is less in  $\Delta C$  transgenic lines. Structures of excision products of these  $\Delta C$  transgenic lines are shown in Supplementary Figure S3C. In each panel, length of molecular weight marker is 0.5kb.

Figure S10

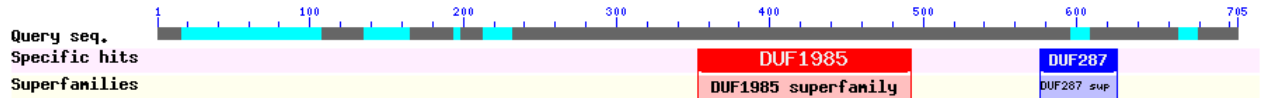


**Figure S10. Evolution and proliferation of VANDAL families.** Phylogenetic relationship among VANDAL families in genomes of *A. thaliana* and *A. lyrata*. *A. lyrata*-specific lineages are shown by red lines. A NJ tree made by JC distance is shown. Scale bar is shown below the tree. Bootstrap probabilities (%) with 1000 replications for major clusters are indicated beside the branches.

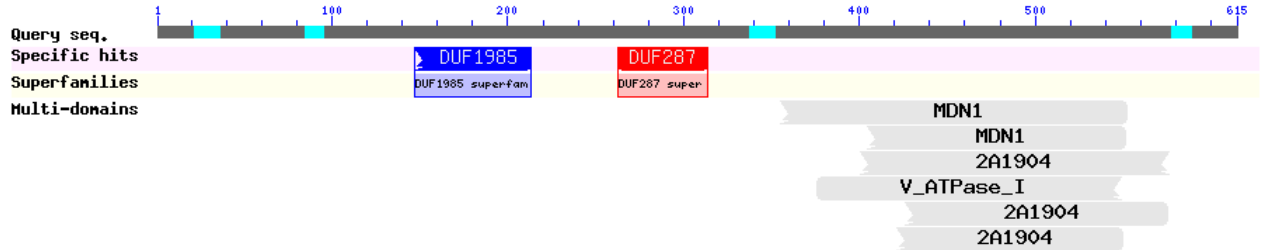


# Figure S11

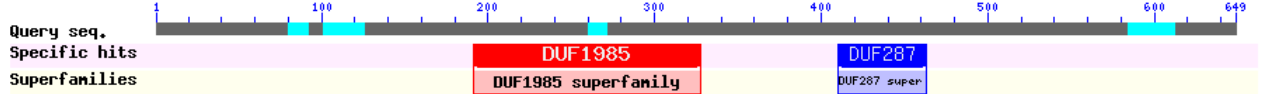
VANDAL21 (At2g23480: hiC)



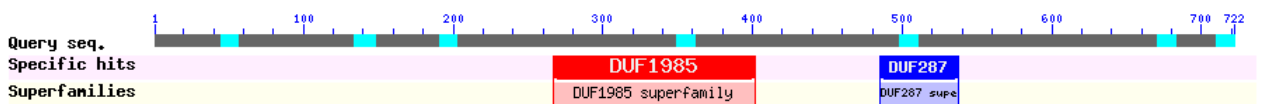
VANDAL17 (At3g43530)



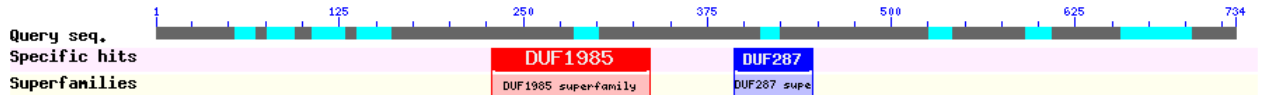
VANDAL6 (At1g23930)



VANDAL7 (At3g45380)



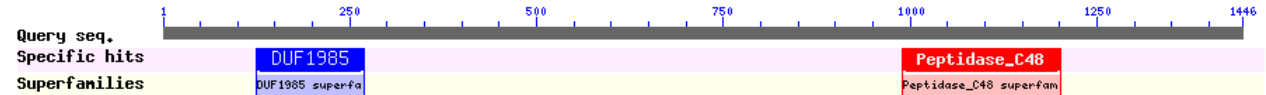
VANDAL8 (At4g07520)



VANDAL1 (At1g45090)



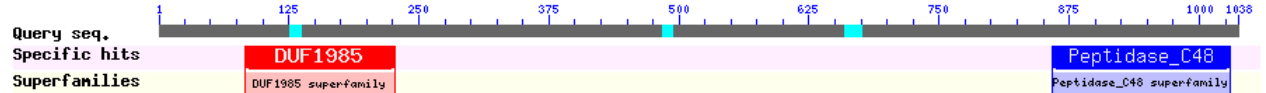
VANDAL2 (At1g27780)



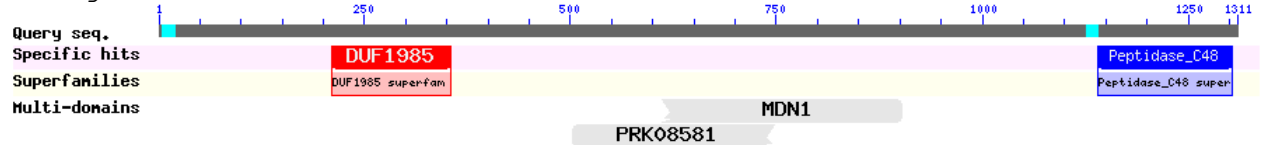
VANDAL3 (At2g24930)



VANDAL4 (At1g44880)

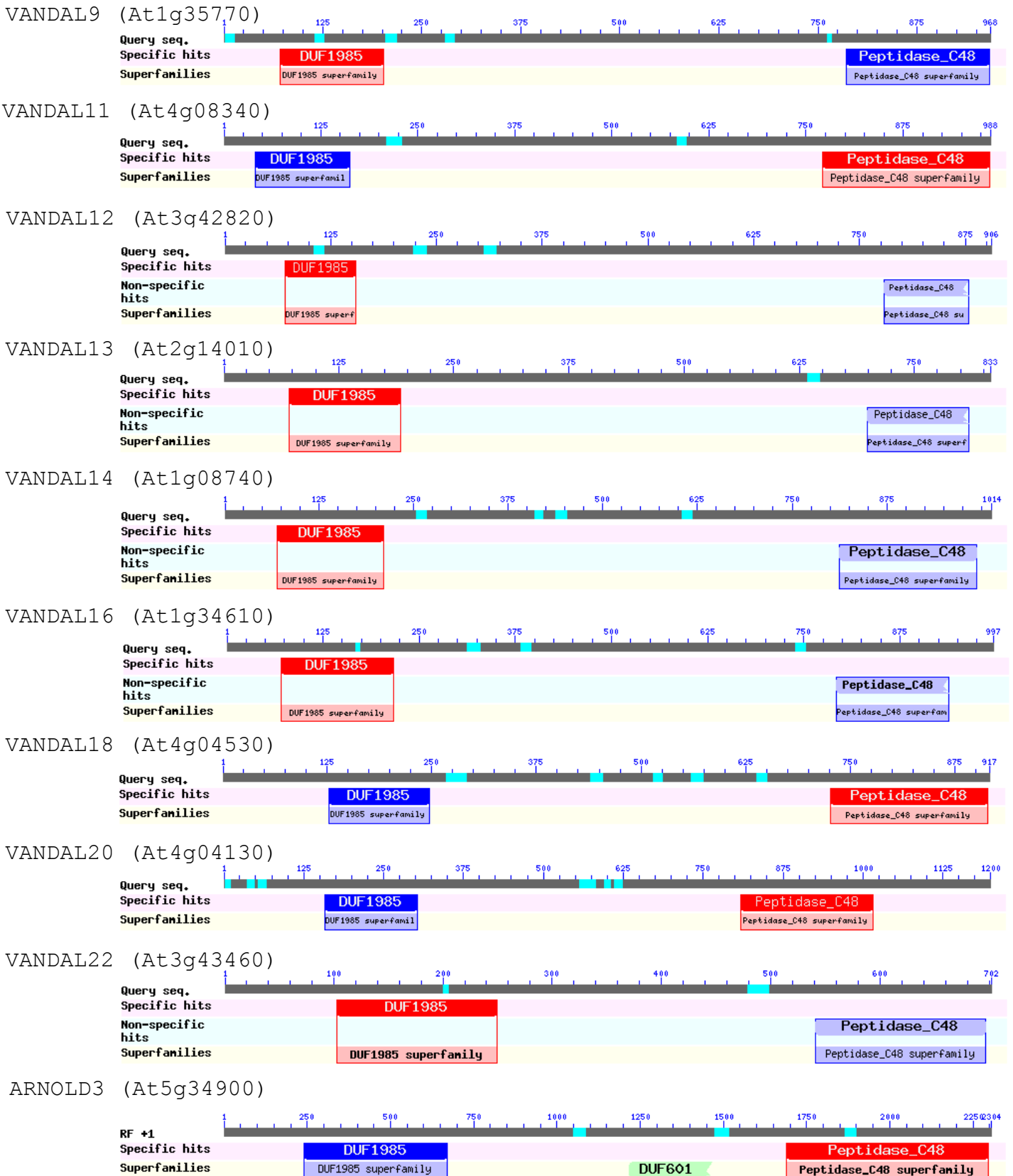


VANDAL5 (At1g35110)



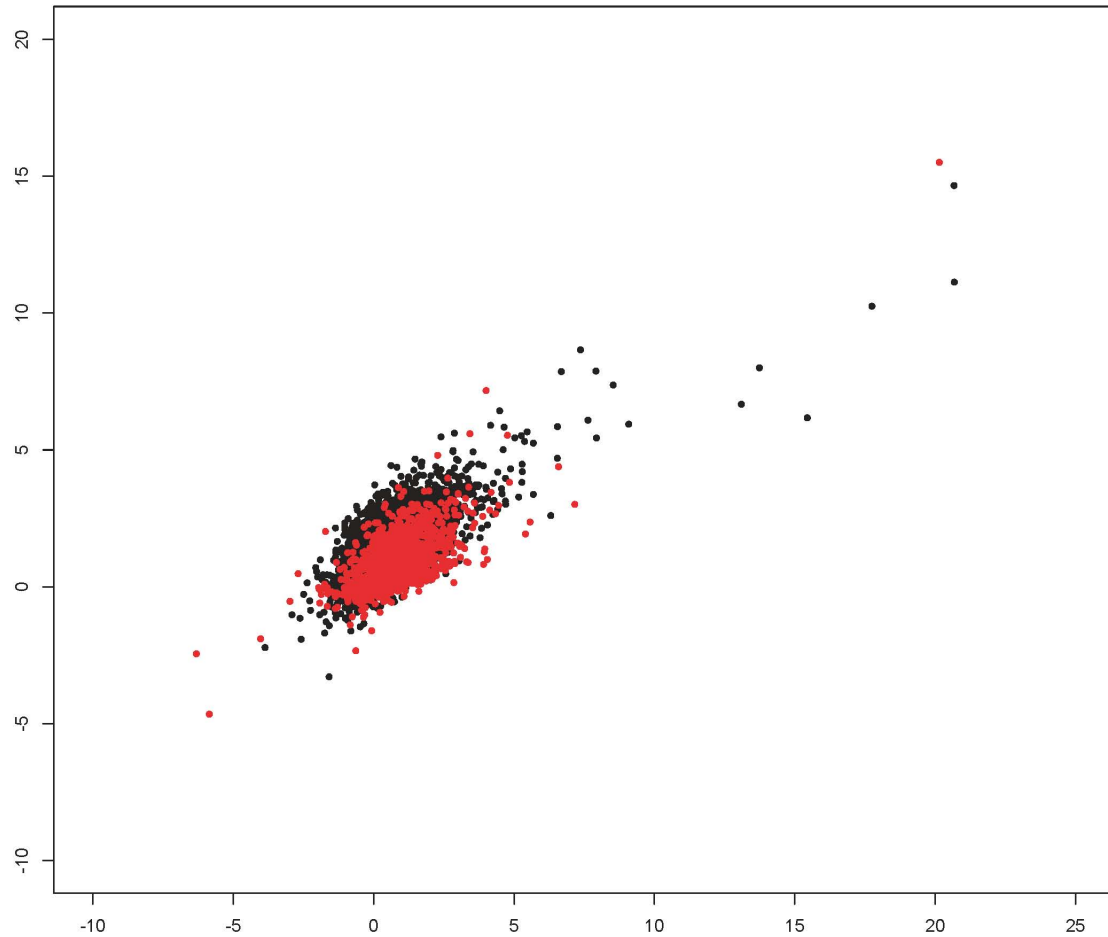
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## Figure S11 (continued)



**Figure S11. Conserved domain structure of products of *vanC* and related genes.** Conserved domain was searched by NCBI conserved domain search site (Marchler-Bauer et al. 2011; <http://ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi>). One gene was examined for each cluster. CDS sequences were obtained from TAIR. *VANDAL10* and *VANDAL15* families were not analyzed, because no gene with complete structure was found.

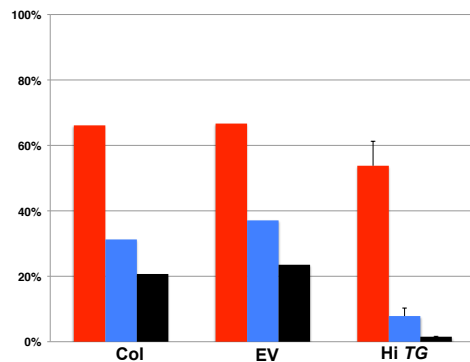
Figure S12



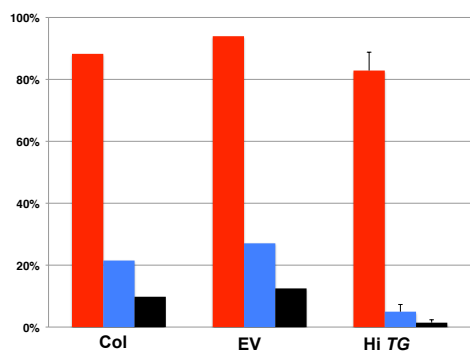
**Figure S12. *Hi* transgene affects DNA methylation in transposons rather than genes.** Changes of methylation at CpHpG sites and CpHpH sites are plotted for genes (red dot) and transposon genes (black dot). The conditions are as described in Figure 9. A red dot in top right is AT3G14670. This does not seem to be a real normal gene, because it is included in a *VANDAL21* transposon, AT3E20780 (shown in Figure 9B).

Figure S13

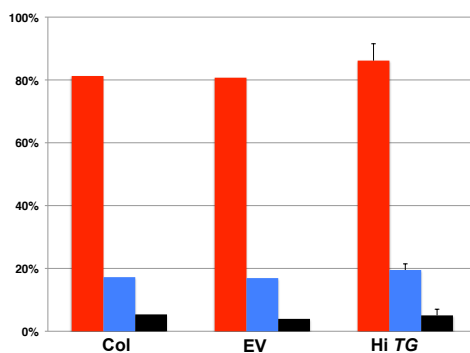
AT2TE06955 (*VANDAL21*)



AT3TE48070 (*VANDAL21*)



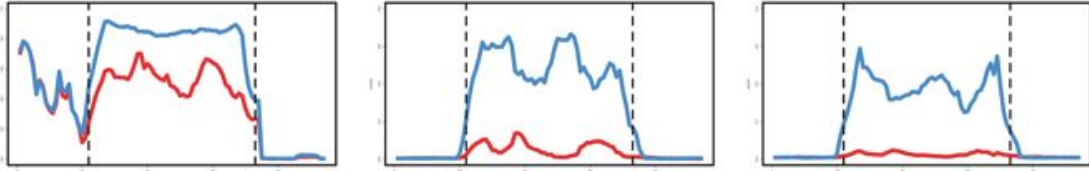
AT1ETE42210 (*CACTA2*)



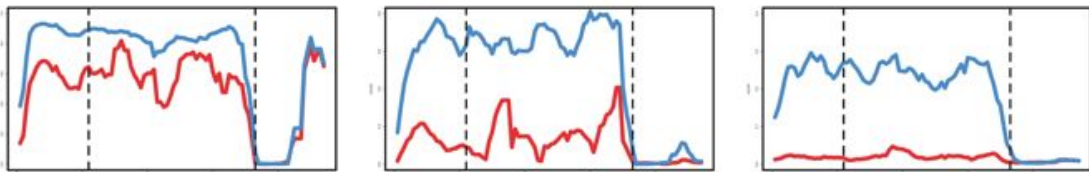
**Figure S13. Reduced DNA methylation in terminal regions of *VANDAL21* members.** The methylation status was examined for two *VANDAL21* members by conventional bisulfite sequencing using primers internal and flanking the TE. Results for three independent *Hi* transgenic lines are shown with control non-transgenic plant and transgenic plant with empty vector. *CACTA2* was also examined as a negative control.

Figure S14

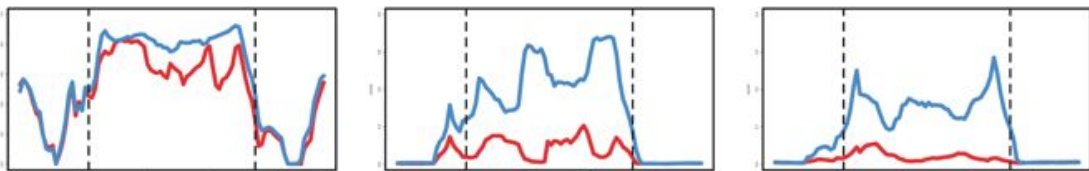
AT2TE42810 (*Hi*)



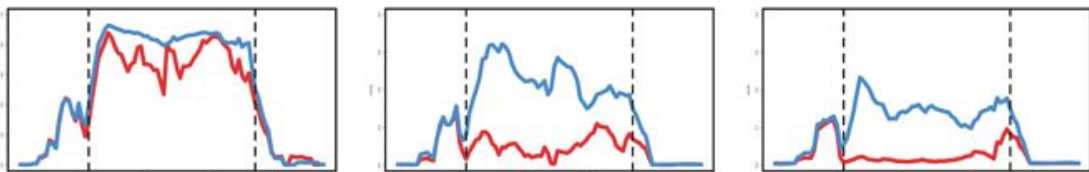
AT3TE20780 (*VANDAL21*)



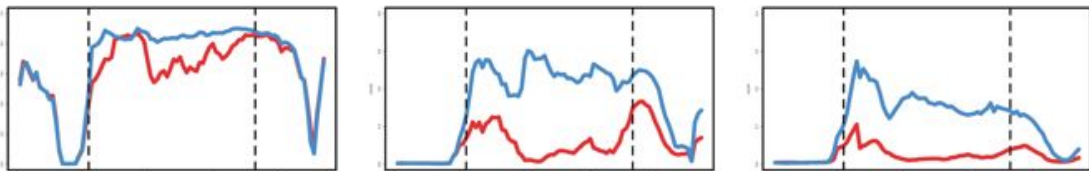
AT2TE06955 (*VANDAL21*)



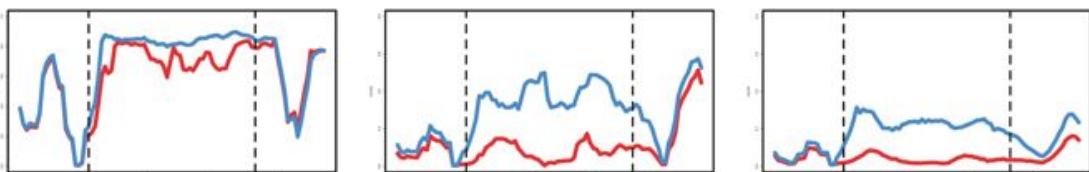
AT2TE05755 (*VANDAL21*)



AT5TE49755 (*VANDAL21*)



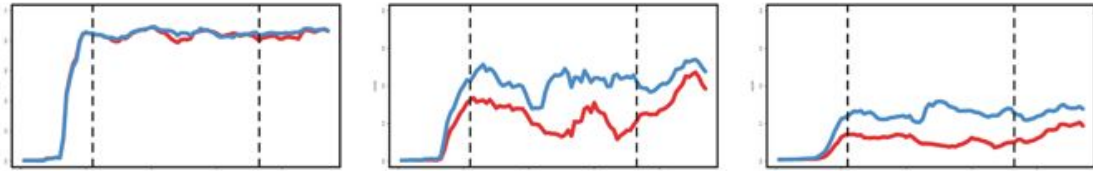
AT3TE48070 (*VANDAL21*)



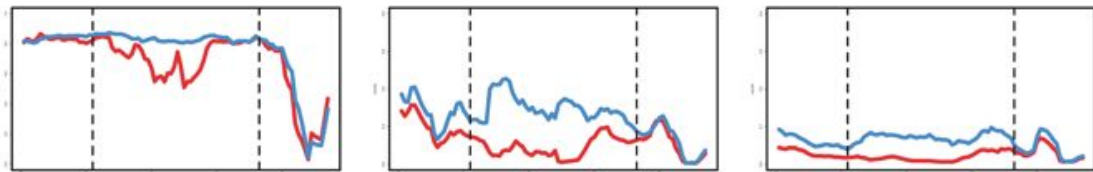
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Figure S14 (continued)

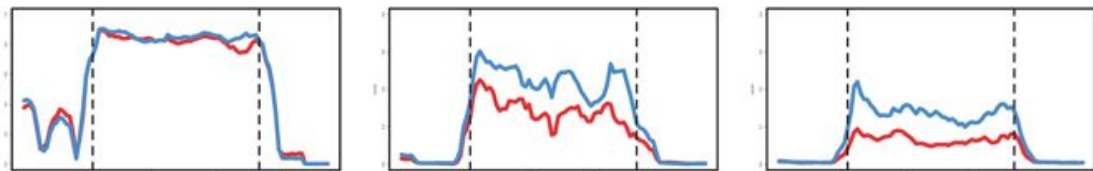
AT2TE47555 (*VANDAL21*)



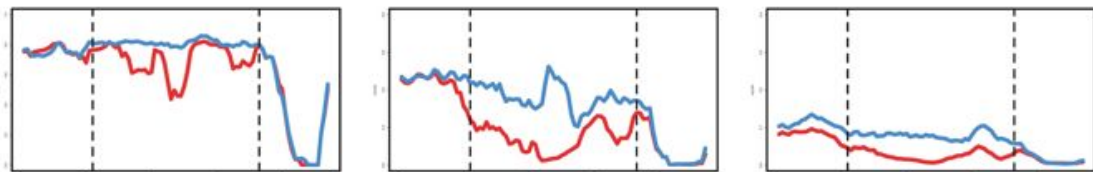
AT2TE20140 (*VANDAL21*)



AT4TE23345 (*VANDAL21*)



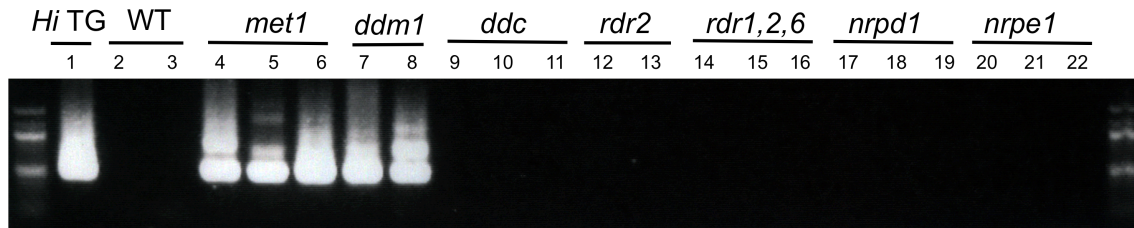
AT4TE16990 (*VANDAL21*)



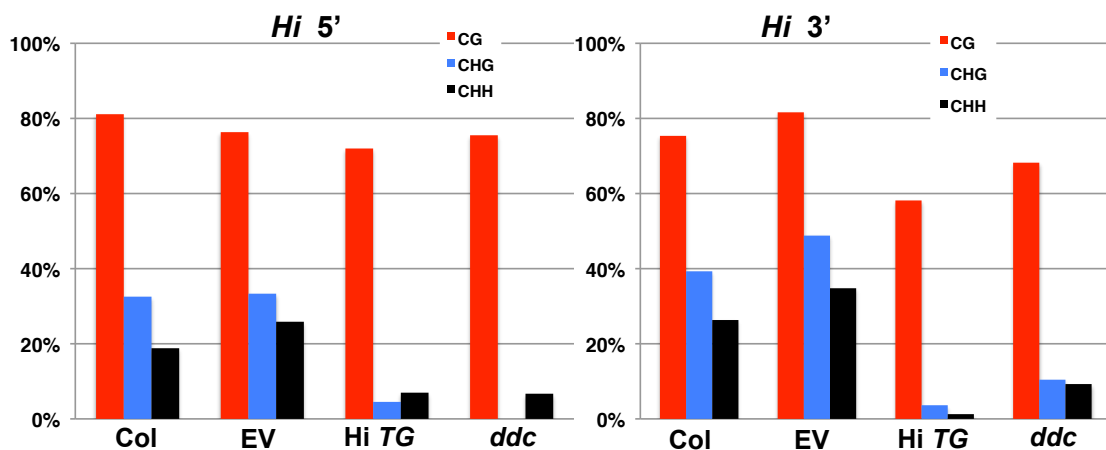
**Figure S14. Demethylation of *VANDAL21* members induced by *Hi* transgene.** *Hi* transgene affects not only terminal regions but also internal regions of *VANDAL21* members. *VANDAL21* members shown in Figure 9 are shown with additional six members. For each TE, three panels are CpG, CpHpG, and CpHpH contexts of methylation, from left to right. Conditions are the same as those of Figure 9B.

Figure S15

A



B



**Figure S15. Effects of mutations in DNA methylation machinery on *Hi* mobility.** (A) Mutation in the maintenance DNA methyltransferase gene *MET1* induced mobilization of *Hi*, indicating that DNA methylation at CpG sites is necessary for the silencing. On the other hand, triple mutation in non-CpG methyltransferases *drm1-drm2-cmt3* (*ddc*) did not induce the mobilization. Mutations in components of RdDM (RNA-directed DNA methylation) machinery did not induce the mobilization either. Therefore, loss of non-CpG methylation in terminal regions does not seem to be sufficient for mobilizing *Hi*. Still, the possibility remains that non-CpG methylation in internal region plays a role, because, according to a recent report (Zemack et al Cell 153, 193-), another DNA methyltransferase CMT2 plays the major role in methylation at CpHpH sites of internal regions of TEs. This could be important, because *Hi* affects not only terminal regions but also internal regions of related TEs (Figure 9; Supplementary Figure S14). (B) The *ddc* mutation induced reduction of DNA methylation at non-CpG sites in terminal regions of *Hi*.

Supplementary Table S1

Flanking sequences for *de novo* insertions of *VANDAL21* copies

ID <sup>a</sup>	Flanking sequence	Read <sup>b</sup>	Side <sup>c</sup>	TSD <sup>d</sup>
1-1	TTTGGACAGAGCCAAGGTCA	2	5'	
1-2	CACTGACTTGCTGATCGTAT	24	5'	AAGTCAGTG
	AAGTCAGTGTAAAGATTGATT	38	3'	
1-3	TGAGGAAAAGCGTGGGAAGATTAATAATGACGGATATGTCCTTATCATGGT	-	5'	TTTTCTCA
	TTTTCTCAAGGGCTTATTC	7	3'	
1-4	CTCCTTTCACCACCAATCTCTCGTTTCATTTACTACAACACTACAACACTCT	-	5'	
1-5	AGTTTCAGATGAGAGAATAT	1	5'	
1-6	TACTTAAAGTGCTACGAAGA	1	5'	
2-1	TTTGCAAGATCTACTAAATT	2	3'	
2-2	ATTTAGATGCTAGGTGATTT	2	3'	
2-3	TATTTAAAGCTTGTGAATTC	1	3'	
2-4	TGTGAGAATAATCGTCTGGC	2	5'	
2-5	TGTGAGGAGGAACTAAGAGT	1	5'	
2-6	CAAAATATTAGGGTTTAATT	2	5'	
2-7	GTCAAGTACGCGGTCGAGTGAAACGGTCGAGTATA	7	5'	
2-8	CCTTCAAACCGCCTAGGATCAGAGATCCGGGACG	7	5'	GTTTGAAGG
	GTTTGAAGGCCTAGGTCCT	13	3'	
2-9	TAATATAGAACGCTTAATAT	1	5'	
2-10	CTACGTCGACCCGTGAATCTGTTGGCCGAAC	12	5'	
2-11	CTCAAATACTCTTCTGCAGTGACAAATCCATAAACTCTACCAATAAGAT	-	5'	
3-1	AAAGAAAACAAAAGTTAAAA	12	5'	GTTTTCTTT
	AAAGAAAACAAAAGTTAAAACGGAATATAAATATACTGTATCGAACTAA	-	5'	
	GTTTTCTTTGATAAAGATC	10	3'	
3-2	TTCGGATGGTTTGGTTCCGGA	2	3'	
3-3	TGTGAGAATAATCGTCTGGC	5	5'	
3-4	GATGAGGGAGAAGAGAGATG	1	3'	
3-5	CCTCCGAGTCCACTAAGTA	44	5'	CTCGGAAGG
	CTCGGAAGGAGAAGGGAAAT	24	3'	
	CCTCCGAGTCCACTAAGTAGCTTCTCG	-	5'	
3-6	ACTTCAGTCACACGTTTTTC	2	3'	



3-7	CATACGATTAAGAAACCCA	15	3'	CATACGATT
	AATCGTATGTATACTCACCC	12	5'	
3-8	AATTAAGAATCCCTGGAC	4	3'	
3-9	AGATCGATTGCATAAGAAATGGAAGAAGAAGACTGCGAAGGTTTCGAAGA	-	5'	AATCGATCT
3-10	CGAATCATTCTTGAGGAGAT	3	5'	
3-11	AATTGGAATTATCTATCAGTCGCGTCAATTTCTGAGTGTAATAGATAGAA	-	5'	ATTCCAATT
3-12	ACCGCCTAGGATCAGAGATCCGGGACGAGCGCTGATGTAGTACGTCCCCT	-	5'	
3-13	GTGTGCGACTGTTACAGTAACACTGAAG	-	5'	
3-14	GTTTTGAGTCGAATATGACTTGTTGTCA	1	5'	
4-1	AAACATAACATGAGTGTACG	2	3'	
4-2	GAGATTGGAAGAACTTGACA	1	5'	
4-3	CTCAAATACTCATCTGTAAG	1	5'	
4-4	CGGGATCCACTTTGTTATAAACCTAAGTATCTGCAATTAGGGTTGTTGCTACTT CAC	1	3'	
4-5	TTCATTCCGTTAGAAAAGTG	2	3'	
4-6	ATAATCAAGTGTGTTGTTG	1	5'	
4-7	CCTAAAAACCAAAAAGTGGTTACAGTGAGAAATTACACTCACCGTTTGT	-	5'	G
4-8	GGCCAGACGATTATTCTCAC	1	5'	
4-9	TCTGGCCAGTCCCTTTTTTGTA	1	5'	
4-10	TGTGAGAATAATCGTCTGGC	4	5'	
4-11	GAACCGAGTTAGTAGTTACA	1	3'	
4-12	TGTGAGGAGGAACTAAGAGT	2	5'	
4-13	TAAATAACATCAACATGCAT	3	3'	
4-14	TTCAAAATCAGATGTTCTTG	1	5'	
4-15	ATTTCTCAATCGAAACAGAATGTACA	2	5'	
4-16	GACTAAAGGTGACTATGAAAAGTTGACGAATGGGAAACAGATACGGGTTG	-	5'	CCTTTAGTC
4-17	CAAATACTCTCTTTTCTA	1	5'	
4-18	GAGACACACGCTCTGCCATT	1	5'	
4-19	GTTCTTTCCCTCTCTGAAA	1	3'	
4-20	GTGTGCGACTGTTACAGTAA	2	5'	
4-21	GTGAAGGAGGGGAAGGCAGG	2	5'	
4-22	CTCCAAAAGTCTATTCAAAT	21	5'	CTTTTGGAG
	CTTTTGGAGGTTTGTGGAGA	5	3'	

4-23	TTCAAAAAACCCACACACAC	1	3'	
4-24	CTTTCTCAGTCTCTCTGT	1	5'	
5-1	GTTTTCGTAGCCATGGCTTC	1	3'	
5-2	GCCTTAGCAAGTGCATCGGC	2	3'	
5-3	GTTCTGTGAGAATAATCGTC	1	5'	
5-4	TGTGAGAATAATCGTCTGGC	2	5'	
5-5	TGTGAGGAGGAACTAAGAGT	1	5'	
5-6	GACTGAAGTGCTTTTTGAT	33	5'	ACTTCAGTC
	ACTTCAGTCACACGTTTTTC	22	3'	
5-7	GACTAAAGGTGACTATGAAA	58	5'	CCTTTAGTC
	CCTTTAGTCGGACCGAACAC	35	3'	
5-8	GTCGGTCGACACTGTCGCGTTCTGTAGTGTCTACCGTGGCTGG	2	3'	
5-9	GTTTCCACTCTCTCAAGGTG	1	5'	AGTGGAAC
	AGTGGAACGCTACAGAGTT	1	3'	
5-10	CTTATGCGACCTGTTGTTAC	2	5'	
5-11	CTTTTTATTTTCAGAGCAAAA	16	5'	AATAAAAAG
	AATAAAAAGAGTAAGAGAAG	10	3'	
6-1	GGTTGTAAACTATGGTTAGCTTGGATTGGTTAGGTTGGGTTAGGTTGGGT	-	5'	GTTTACAACC
7-1	CTCCAAAAGTCTATTCAAAT	-	5'	CTTTTGGAG
8-1	CATTCATCTCCCTAACTTTGTTAACTTTGC	-	5'	
1-B1 <sup>e</sup>	TATAGCTCAAATCGCCTTAA	1	5'	
2-B1 <sup>e</sup>	TGATCTTTCCCCTTCTCTGACAAGGATCA	1	5'	
4-B1 <sup>e</sup>	GATATGAAGACTACAAAAGGATTTTACA	1	5'	

<sup>a</sup> Names of insertions shown in Supplementary Figure S2. Number before “-” indicates specific *ddm1* line self-pollinated independently. <sup>b</sup> Number of reads found in the genome re-sequencing. Number is not shown for insertions identified by suppression PCR. <sup>c</sup> This indicates which side of the TE the flanking sequence was found in. <sup>d</sup> TSD (target site duplication) could be defined for insertions in which flanking sequences of both sides were identified (Supplementary Figure S1B). For some of the insertions identified by suppression PCR, flanking sequences of the other side was identified by direct PCR amplification and sequencing. <sup>e</sup> Insertions for AT4TE15615 (a *VANDAL21* copy different form *Hi*). All other insertions are those of *Hi*. Read numbers for the original locus in the five self-pollinated *ddm1* (plant #1-5) were 50, 50, 41, 28, and 41 for the 5' regions and 0, 41, 30, 33, and 27 for the 3' regions, suggesting that the empty locus was not fixed in most of these lines, even after repeated self-pollinations. The plant#1 seems to have fixed rearrangement around 3' region of the original *Hi* locus

## Supplementary Table S2

### List of primers used in this study

Purpose of the PCR	Target sequence	Primer pairs
Cloning <i>Hi</i> for transformation	Endogenous <i>Hi</i>	TACGGGCCCCGAATAATCGTCTGGCCAGTCCCTT + ATCGTCGACGAGGGATCATCTCTTGTGTCCCT
Introducing a silent mutation into <i>Hi</i> transgene	<i>At2g23500(vanA)</i>	AGTGGTCGAACTAAACTCATTTCGAGCGTGA + TTTAGTTCGACCACTTTCAGCTTCTCGGCA
	<i>At2g23490(vanB)</i>	AAGGGAAAGCGTTGATTACAATCGGAAGA + TCAACGCTTCCCTTAAGCTACTCACCTCT
	<i>At2g23480(vanC)</i>	CAACCACGTGCTCGGAACAACTGAGGTTAG + TCCGAGCACGTGGTTGATTGCTCAAGGGT
Constructing $\Delta A$ transgene	Cloned <i>Hi</i>	GCAGATTACAGTTTTTAACCTTGTCTGC + AGTTAAAACTGTAATCTGCCAAAACAATA
Constructing $\Delta B$ transgene	Cloned <i>Hi</i>	TCTCTCACATTGTGTTATCCTATTGTTCT + GGATAACACAATGTGAGAGAATTCGAGTCG
Constructing $\Delta C$ transgene	Cloned <i>Hi</i>	ATATTACCAAGACTGATTCGAATCGGAAA + GAAATCAGTCTTGGTAATATCGCGTAATAC
Constructing $\Delta AB$ transgene	Cloned <i>Hi</i>	TCTCTCACATTGTGTTATCCTATTGTTCT + GGATAACACAATGTGAGAGAATTCGAGTCG
Suppression PCR (first PCR)	Flanking sequence of <i>Hi</i>	GGATCCTAATACGACTCACTATAGGGC + CAAAGCTTTTGAAGCTCTCTCCATACC
Suppression PCR (second PCR)	Flanking sequence of <i>Hi</i>	AATAGGGCTCGAGCGGC + GCTTGCAGGAGGAGAAAAACGACAATG
Sequencing the other side of flanking sequences for identifying target site duplication	<i>At2g13290</i>	GATTAAGAAATGAGAACACACG + CTGGAAAACATCATGACCTTA
	<i>At2g23450</i>	TGCGAAATAACAATCAGAGTA + GATATCCCAATTGCTCGTTGA
	<i>At2g23830</i>	GTTCCATGTTGAATAATCAGC + GACGCTTATCCGCATAGTTCT
	<i>At3g30851</i>	GTTTTGAAATCGAAGAGAGC + GGACATTTTAGCGACTAACT
	<i>At3g32111</i>	AGAAAGCTGGAGAGGCTAATG + TCCATCAACCACCGTTCTGGT
	<i>At5g33405</i>	TCAATTAGGCAATTGAGCACT + AAGTGAAGAGATAGATCGATT
	<i>At4g04720</i>	GTAAAGGAGGAGACTTTCGTT +

		TCAGCAAATTGACAAAGACA
PCR to detect excision of <i>Hi</i> (original locus, first PCR)	Flanking sequences of <i>Hi</i>	ACGAGCAGAAAACATGCCACCA + TGCTCTAAACATTGCCTGAAGC
PCR to detect excision of <i>Hi</i> (original locus, second PCR)	Flanking sequences of <i>Hi</i>	CGACGAGCTACGTTACTGGG + AGTCTATTCACCATCGCCTAGTT
PCR to detect excision of <i>Hi</i> (transgene, first PCR)	Vector sequence	TCCCACTATCCTTCGCAAGA + GACCGAGCGCAGCGAGTCAG
PCR to detect excision of <i>Hi</i> (transgen, second PCR)	Vector sequence	TCGCCATTCAGGCTGCGCAA + AGGCACCCCAGGCTTTACTACT
RT-PCR	At2g23500( <i>vanA</i> )	CAGGAGTTAAGTCGGGTCTAC + TGCACCTATCCGGAACAAGA
RT-PCR	At2g23490( <i>vanB</i> )	GACCCCTACTACGATGATATG + CCATAGGATTACGGAATACCA
RT-PCR	At2g23480( <i>vanC</i> )	ACAGCTGTGGGAACTTCCTCT + AACACTCAGTCACCATGGCCT
RT-PCR	At3G04120(GAPC)	CACTTGAAGGGTGGTGCCAAG + CCTGTTGTGCGCAACGAAGTC
qRT-PCR	At2g23500( <i>vanA</i> )	GATGGTGCCTTTGGTCGAGA + TTTCAAAAGCAAGCTCACCGT
qRT-PCR	At2g23490( <i>vanB</i> )	TAGCATTGTCGAGACGCGAA + ATCCCAAAGTTTACGGATGTGC
qRT-PCR	At2g23480( <i>vanC</i> )	AGGATGTGCAAGGTGAGTTTCA + ACTCCCGTGATTTACGCCAA
qRT-PCR	At5g25760(UBC)	CTGCGACTCAGGGAATCTTCTAA + TTGTGCCATTGAATTGAACCC
Bisulfite-sequencing	3' terminal sequence of <i>Hi</i> (At2g23480 side)	CTTTCTTCRCCRRCACCTTCTCCTTCACTTTCTCA + ATGGGTATTGAAAAAGTYGAGAGYTTTGATTYGTG
Bisulfite-sequencing	5' terminal sequence of <i>Hi</i> (At2g23500 side)	AATCTCAACATCCTCAAAATATRTAATTCAAARCT + GTTAGAAGAAAAAAAAYTAAATGGGYAAGTGTGT
Bisulfite-sequencing	3' terminal sequence of AT2TE06955 ( <i>VANDAL21</i> )	GAGGGGAYTGAGTAGAYAAAAGAGAYTGTTTTGAT+ CTTCTATCTCCTTTCTTTTCCCTTRACCTCCTCCAC
Bisulfite-sequencing	3' terminal sequence of AT3TE48070 ( <i>VANDAL21</i> )	GAGTTGAGTGAAGYGAAYATYAAGAAAYTTAAAGAA+ CTTCTATCTCATTTCTTTTCCCTTRACCTCCTCCAC
Bisulfite-sequencing	AT1TE42210 ( <i>CACTA2</i> )	CATATAAACCCCAAAATCAAATC + ATGGAAAAGGAGAAGGAGGTAT
qPCR after McrBC digestion	5' of <i>Hi</i>	TTTTTGGTTTCAAAATGTTTTCTACA + TCTTAGTTCCTCCTCACAGAACG

Quantification of copy number of <i>Hi</i>	Internal region of <i>Hi</i>	CGAGTGACCCGTTCAACC + TCCCTATGCTTTGTAAGACTTCTC
	AT5G13440 (internal control)	ACAAGCCAATTTTTGCTGAGC + ACAACAGTCCGAGTGTGTCATGGT
	AT5G36220 (internal control)	CCGAACACTTCACCAGATCA + CAGACCCGGGTAACCTTTGA
Presence of <i>Hi</i> in the original locus	5' of <i>Hi</i>	CTTGCAGGAGGAGAAAAACG + TCTTAGTTCCTCCTCACAGAACG