SUPPLEMENTARY INFORMATION

Methods

Plant Material and Growth Methods. Plants were grown in soil in long-day conditions (growth chamber, 16 h light, 8 h dark, 22°C). The epiRIL F_2 population was derived from one self-fertilized F_1 plant resulting from the cross of a wild-type (WT) Col-0 accession (Col) and a *met1-3* mutant (Col-0 background) as the maternal and paternal parents, respectively [1]. Based on previous results showing *EVD* activity in epi12 ([2] and Fig. 1), F_3 generation epi12 siblings were screened for *EVD* retrotransposon activity (see below) and selected plants were used first for inter-accession reciprocal crosses to the WT Landsberg *erecta* (L*er*) accession. A second screen for further *EVD*-active individuals led to the identification of one donor (epi12.38) that was used for intra-accession reciprocal crosses to WT Col-0, as well as selected mutants (see Table S1).

Genomic DNA Analysis. The Southern blot experiments were done as previously described [2] using 5 µg of genomic DNA. To assess the penetrance of *EVD* replication within epi12 F_4 generation siblings, $n = 6$ individuals were analyzed. For the reciprocally crossed F_1 entries and the L*er* parental control, bulk DNA samples from 8 individuals were analyzed.

DNA Quantification. DNA quantification was performed for each sample using SYBR Gold staining (Molecular Probes) with a Synergy 96-well fluorescent plate reader (Biotek). Each DNA sample (5 µl) was diluted in 25 µl TE buffer, mixed with 20 µl of the SYBR Gold solution (1:1000 dilution in "TE" buffer: 10 mM Tris pH 7.5, 1 mM EDTA, stored in darkness until used), and incubated in the dark (5 min). DNA fluorescence was measured at excitation levels of 485/20 nm and emission levels at 528/20 nm; DNA quantities were estimated relative to a dilution series of calf thymus DNA. Each sample was normalized to 10 ng/µl in TE buffer.

***EVD* Circular LTR-PCR Assay**. PCR reactions consisted of 4 µl of 5X Green GoTaq Flexi Buffer, 0.4 µl PCR Nucleotide Mix (10 mM each), 0.4 µl each primer (10 µM each), 0.1 µl of GoTaq DNA Polymerase (5 u/µl) mixed with 0.8 µl of DNA (10 ng/µl) and sterile, deionized water to a final volume of 20 µl. The assay mixtures were heated to 95°C for 3 min, followed by 35 cycles of amplification at 95°C (30 s), 55°C (30 s) and 72°C (2 min). The amplification product was visualized on an ethidium bromide-stained gel illuminated with UV light.

***EVD* DNA Content Analyses.** From each experimental crossing, 12 seeds per entry were planted in soil, stratified in the dark for 2 days at 4°C, and 8 seedlings per entry were selected after germination. Assays to amplify *EVD* and *ACTIN 2* (*ACT2*) loci were designed using Primer Express (see Table S2). Copy analyses were performed using Taqman assays on an Applied Biosystems 7900HT Fast Real-Time PCR System (qPCR). Copy levels were calculated using the relative quantification method [3] for each duplex

reaction. Duplex qPCR reactions were performed in MicroAmp Optical 96- or 384-well reaction plates with 10 µl final volumes comprising 5 µlQuantiFast Multiplex PCR Master Mix (Qiagen), 0.5 µl of combined q*EVD* assay reagents (8 µM forward primer, 8 µM reverse primer, 4 µM probe), 0.5 µl of combined qACT2 assay reagents (8 µM forward primer, 8 µM reverse primer, 4 µM probe), 2 µl sterile water, and 2 µl of DNA (diluted to 1 ng/µl in sterile water). The qPCR reactions were run for 95°C for 10 min followed by 40 cycles at 95°C for 15 s, 55°C for 30 s, and 72°C for 30 s. Two technical replicates were amplified for each DNA sample to calculate the midpoint comprising each biological replicate's datapoint. Comparisons of *EVD* levels were then normalized to *ACT2* for each replicate using the delta-delta Ct. *EVD* levels per entry (e.g. per hybrid cross, per self-fertilized parent, *etc*) were calculated as the mean with standard error (+/- SEM) of all DNA samples (n = 8, unless otherwise noted) measured within the entry. The relative *EVD* copy number variation is the fold-change in each entry relative to the *EVD*-silent controls (mid-parent Col and L*er* values for inter-accession crosses; Col value for intra-accession crosses), which are represented with an *EVD* copy number value of 1. F_1 and reciprocal F_1 generation entries within each population were compared using a Student's *t*-test.

***EVD* Transposon Display.** Genomic DNA (300 ng) was digested with 25 units of *Dra*I restriction enzyme (New England Biolabs) for two hours at 37°C and purified using a QIAquick purification kit (Qiagen). The eluted DNA was ethanol precipitated and resuspended in 10 µl of sterile water and ligated to the GenWalk adapter (Clonetech), following the manufacturers protocol. The ligation reaction product was diluted 20-fold

and 1 µl aliquots were PCR reactions comprised of 12.5 µl of GoTaq PCR Mastermix (Promega), 9.5 µl sterile water, 1 ul of AP1 primer (GTAATACGACTCACTATAGGGC ; 10 mM initial concentration) and 1 ul of an *EVD*-specific primer (CACAAAGGTTACTATCTAATTCTATCAAT ; 10 mM initial concentration) were amplified with 33 cycles of denaturing at 94°C for 25 seconds, annealing at 58°C for 30 seconds, and elongation for 1 minute at 72°C. PCR reaction products were electrophoresed in an agarose gel stained with ethidium bromide and visualized under UV light.

Figure Legends

Fig S1. *EVD* ecDNA quantification. *EVD* ecDNA signal intensity was measured on Southern blots hybridized with a specific *EVD* probe [2] . Quantification was performed relative to the total signal of integrated *EVD* copies. Bars represent standard errors for *n* independent biological samples as indicated.

Fig S2. Molecular diagnostic for *EVD* activity. (**A**) PCR assay design to detect circular forms of activated *EVD* ecDNA. Design of a reverse primer (*green arrow*, position 529) upstream of the forward primer (*red arrow*, position 3931) can amplify circular forms of extrachromosomal *EVD* DNA with either 1 or 2 LTR (2.0 and 1.6 kb amplicons, respectively). Minimal PCR amplification was observed in the *met1-3* and *nrpd2a-2* mutants, whereas increased amplification was detected for the *EVD*-active F_8 generation of epi12 and the *met1*/*nrpd2a* double mutant. (**B**) Diagnostic detection of *EVD* activity

using the circular PCR assay in 24 epi12 F_3 generation individuals (*Y* axis) along with *ACT2* control amplification. (**C**) Positions for the *EVD* real time quantitative PCR (qPCR) assay. To confer sequence specificity, the qPCR assay was designed to amplify *COPIA93* LTRs.

Fig S3. Screening and development of intra-accession *EVD* populations. (**A**) Initial F_3 epi12 levels of *EVD* activity. The relative *EVD* DNA levels are expressed as described in Fig 2, but relative to Col (set to 1 on the *Y* axis). (**B**) Molecular diagnostic for *EVD* activity as described in Fig S2A. To confirm the specificity in selecting for *EVD* activity, two additional F_3 generation epiRILs, epi07.2 (*epi07*), and epi17.4 (*epi17*) previously shown to be *EVD*-silent [2] were also crossed as negative controls to the respective mutants, as described in Fig 3. (**C**) Initial F_1 hybrid levels of *EVD* activity in each respective cross. All mutants are in the wild-type Col accession. The relative *EVD* DNA levels are expressed as described in (*A*). The black and gray arrows identify the F_1 individuals selected as the *EVD*-active and *EVD*-silent sources, respectively, used for intra-accession population development.

Fig S4. *EVD* transmission rates in *EVD*-silent first-generation reciprocal backcross (BC_1) populations. (**A**) Crossing scheme between one epi12 F_3 generation individual (epi12.38, paternal parent) to each *ago5*, *kyp*, and *nrpd2a* mutants (maternal parent) to create *EVD*-silent backcross populations. (**B**) Relative *EVD* copy number levels (*Y* axis) are shown for each entry (*X* axis) for different mutant populations: *ago5* (*upper panel*), *kyp* (*middle panel*), and *nrpd2a* (*lower panel*), as described in Fig 3.

Fig S5. Transposon display showing *EVD* neo-insertions in a subset of lines analyzed in Fig 3. White stars indicate lines in which *EVD* relative DNA levels are higher than the control, as measured by qPCR, suggesting *EVD* activity (see Fig 3B).

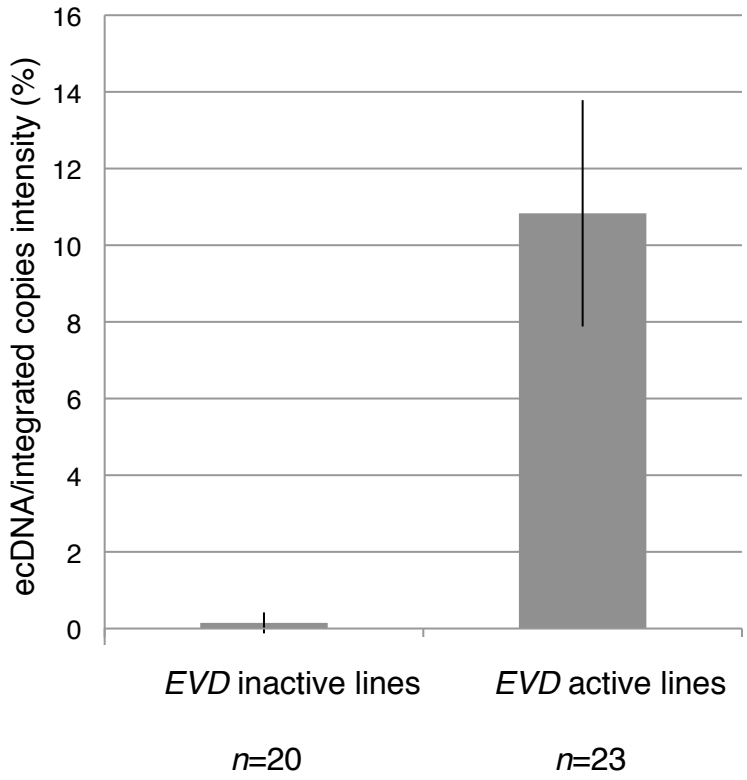
Table S1. Genetic material evaluated for *EVD* parent-of-origin effects. The Arabidopsis Genome Initiative (AGI) locus identifier, gene symbol, and mutant alleles evaluated in this study are listed with respect to the genomic background and the source of the mutant allele.

Table S2. Assays used in this study. The genotyping assays used to detect wild-type and mutant alleles for each locus are provided, with the mutant assay utilizing the T-DNA specific primer LBb1.3 (http://signal.salk.edu/tdnaprimers.2.html). The circular *EVD* assay and qPCR are also provided. See Methods for further details.

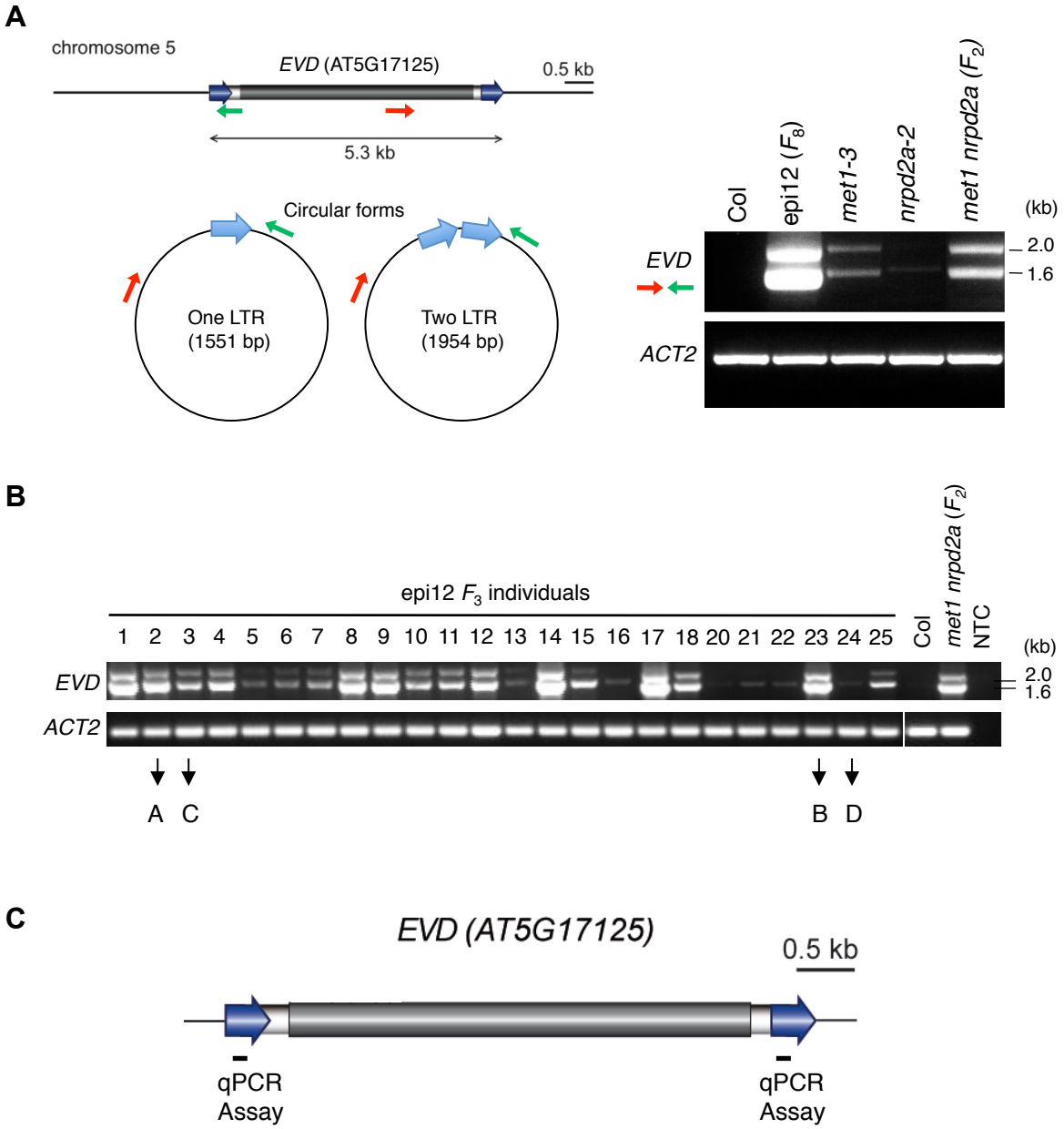
REFERENCES

1. Reinders J, Wulff BB, Mirouze M, Mari-Ordonez A, Dapp M, Rozhon W, Bucher E, Theiler G, Paszkowski J (2009) Compromised stability of DNA methylation and transposon immobilization in mosaic Arabidopsis epigenomes. *Genes Dev* **23:** 939-950
2. Mirouze M *et al* (2009) Selective epigenetic control of retrotransposition in Arabidopsis. *Nature* **461:** 427-430
3. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* **25:** 402-408

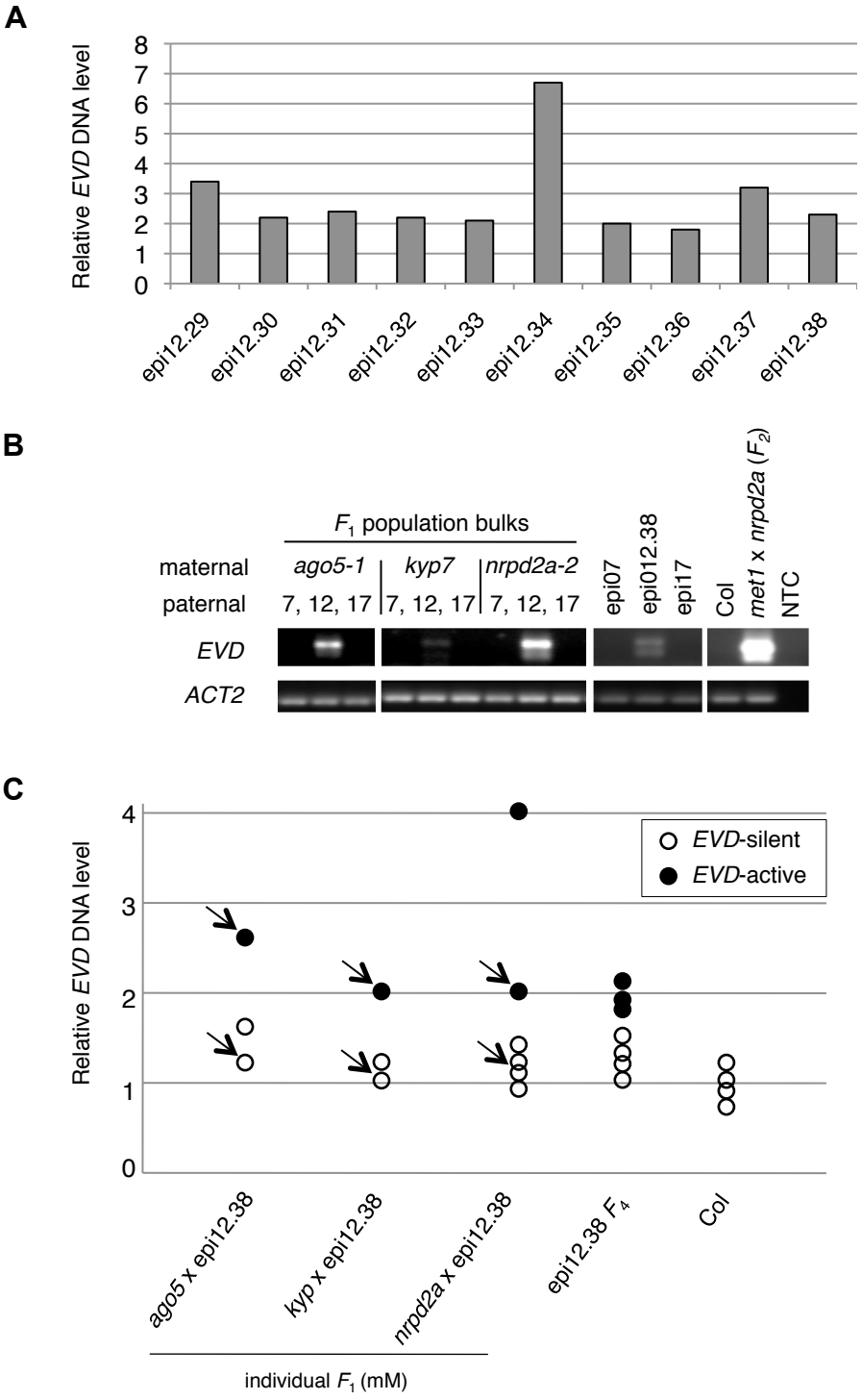
Supplementary Figure 1



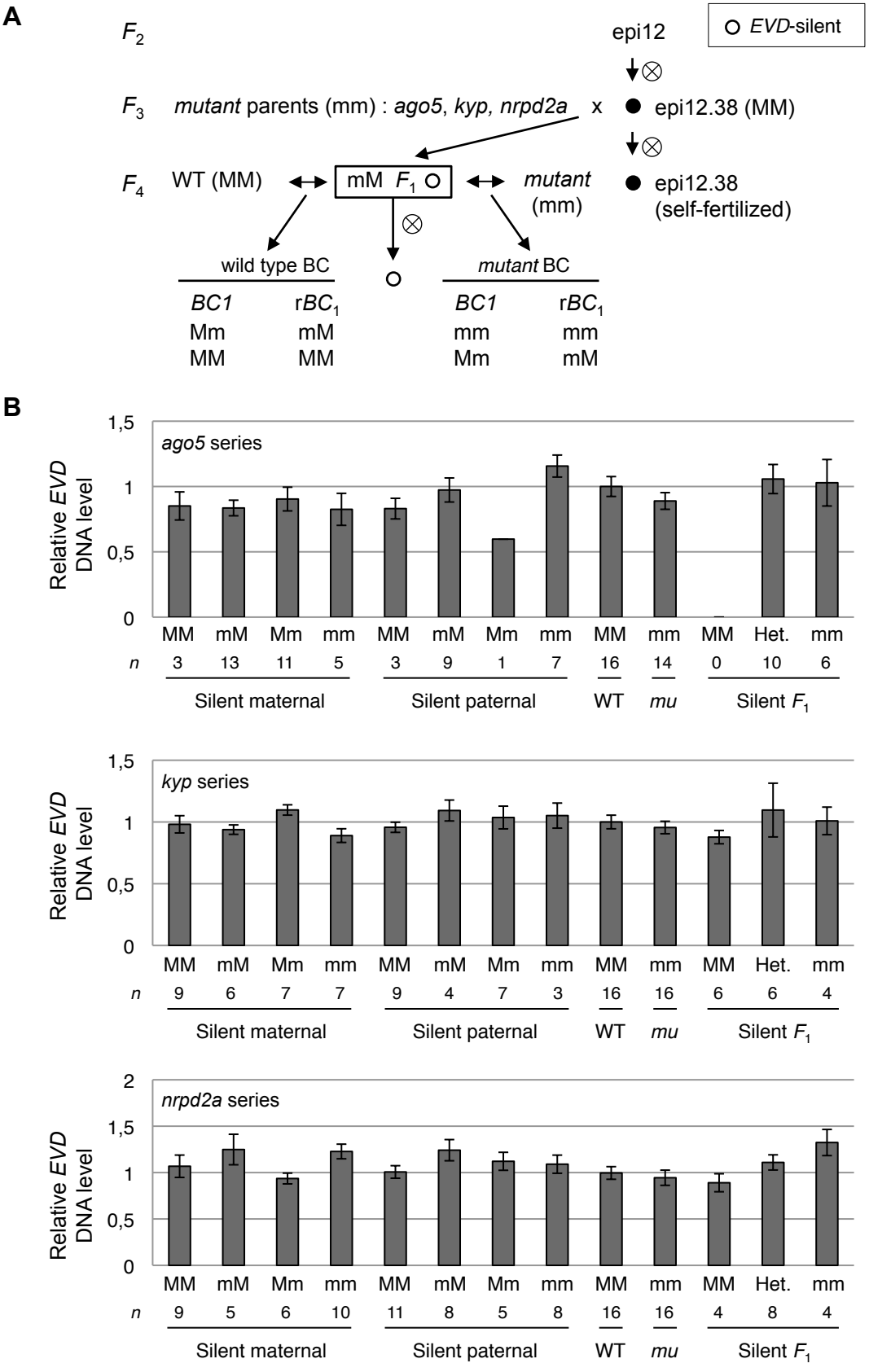
Supplementary Figure 2



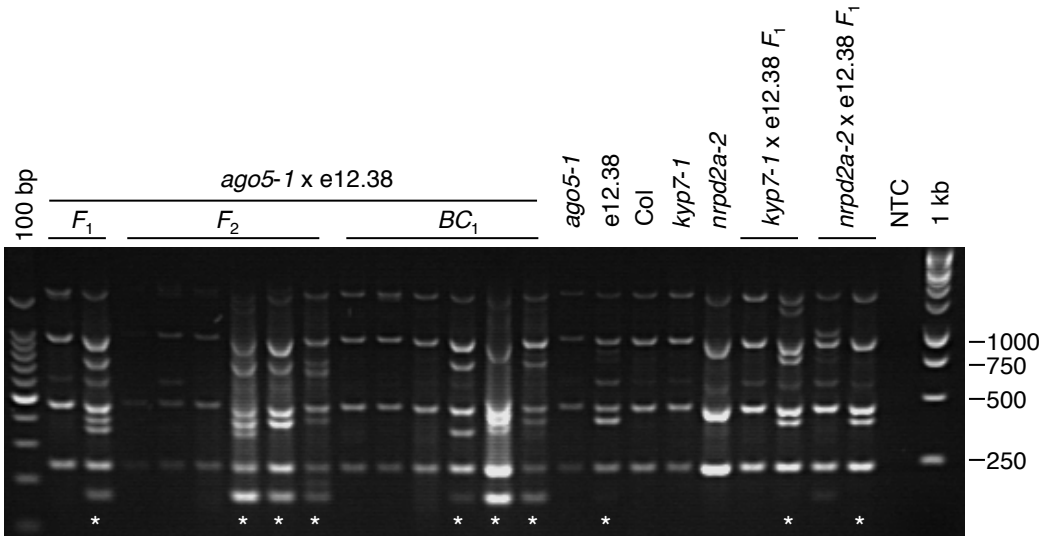
Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5



Supplementary Table 1

Locus	Gene symbol	*Mutant* allele	Accession	Mutagen, Stock reference
AT2G27880	AGO5	*ago5-1*	Col	T-DNA, SALK_063806
AT5G13960	SUVH4	*kyp-7*	Col	T-DNA, SALK_069326
AT3G23780	NRPD2	*nrpd2a-2*	Col	T-DNA, SALK_046208

Supplementary Table 2

Genotyping Assays

	Wild Type Assay	
Assay Name	Forward Primer	Reverse Primer
AGO5	GGATTGTCTCTCAGTGTTGCC	TGAGCATTTGCAACTGATCAG
KYP	CGGGAAAGAAAGAGGACAAAC	ACTACCAACCAACCTGGAAGG
NRPD2A	GCTTGATTTCCATCTGCAGAG	AGAAGAAAGCTCGATTCCTGG

	mutant Assay	
Assay Name	LBb1.3 (T-DNA)	RP (reverse)
ago5-1	ATTTTGCCGATTTCGGAAC	TGAGCATTTGCAACTGATCAG
kyp-7	ATTTTGCCGATTTCGGAAC	ACTACCAACCAACCTGGAAGG
nrpd2a-2	ATTTTGCCGATTTCGGAAC	AGAAGAAAGCTCGATTCCTGG

Circular *EVD* Assay

Assay Name	Forward Primer	Reverse Primer
c*EVD*	TTGAAGTGTGTCGCTCTAATGCTGG	GCACAAACGGACTGATGAATAAAGC

Quantitative PCR Assays

Assay Name	Forward Primer	Reverse Primer
qEVD29	CCGTTGAAGAGGACTCACGAG	TTCCTTGGTCTTGGTGATGAGA
qACT2	GCCAATCTACGAGGGTTTCT	TTACAATTTCCCGCTCTGCT

	Probe Sequence	Modifications
qEVD29_probe	TCTTTAGCAACAAGGTCAACCAATGACCAGTC	5'Fam - 3'BHQ-1
qACT2_probe	TCCGTCTTGACCTTGCTGGACG	5'Joe - 3'BHQ-1

Methylation Assay

Assay Name	Forward Primer	Reverse Primer
m*EVD*	AGGGGGAAACTACCTAACTTGGTC	CACCCGAGTCTATCACAATTGAAC