Supplementary Information for

Mutations in *EDM2* selectively affect silencing states of transposons and induce plant developmental plasticity

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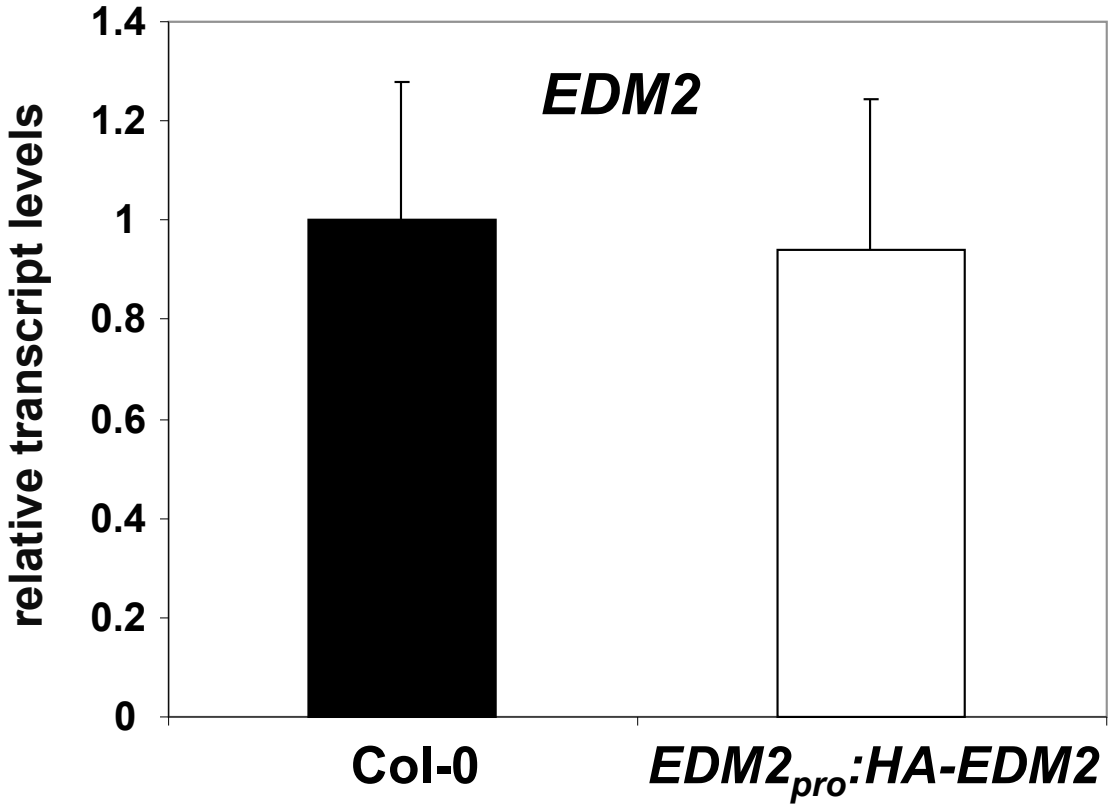
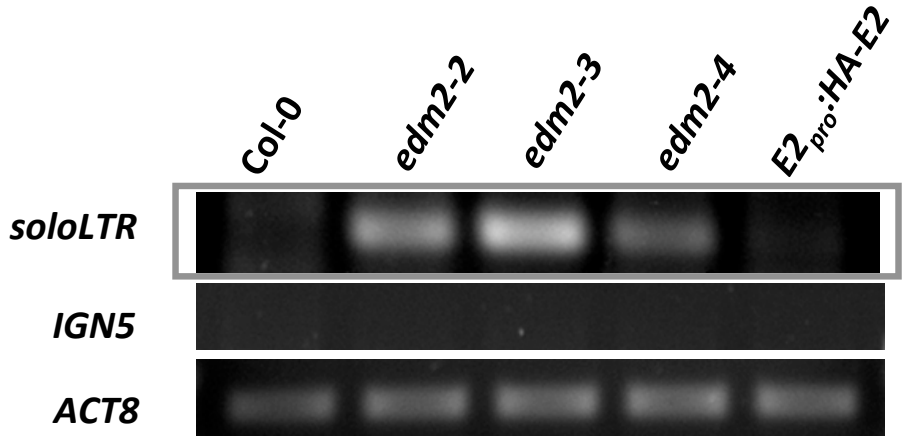
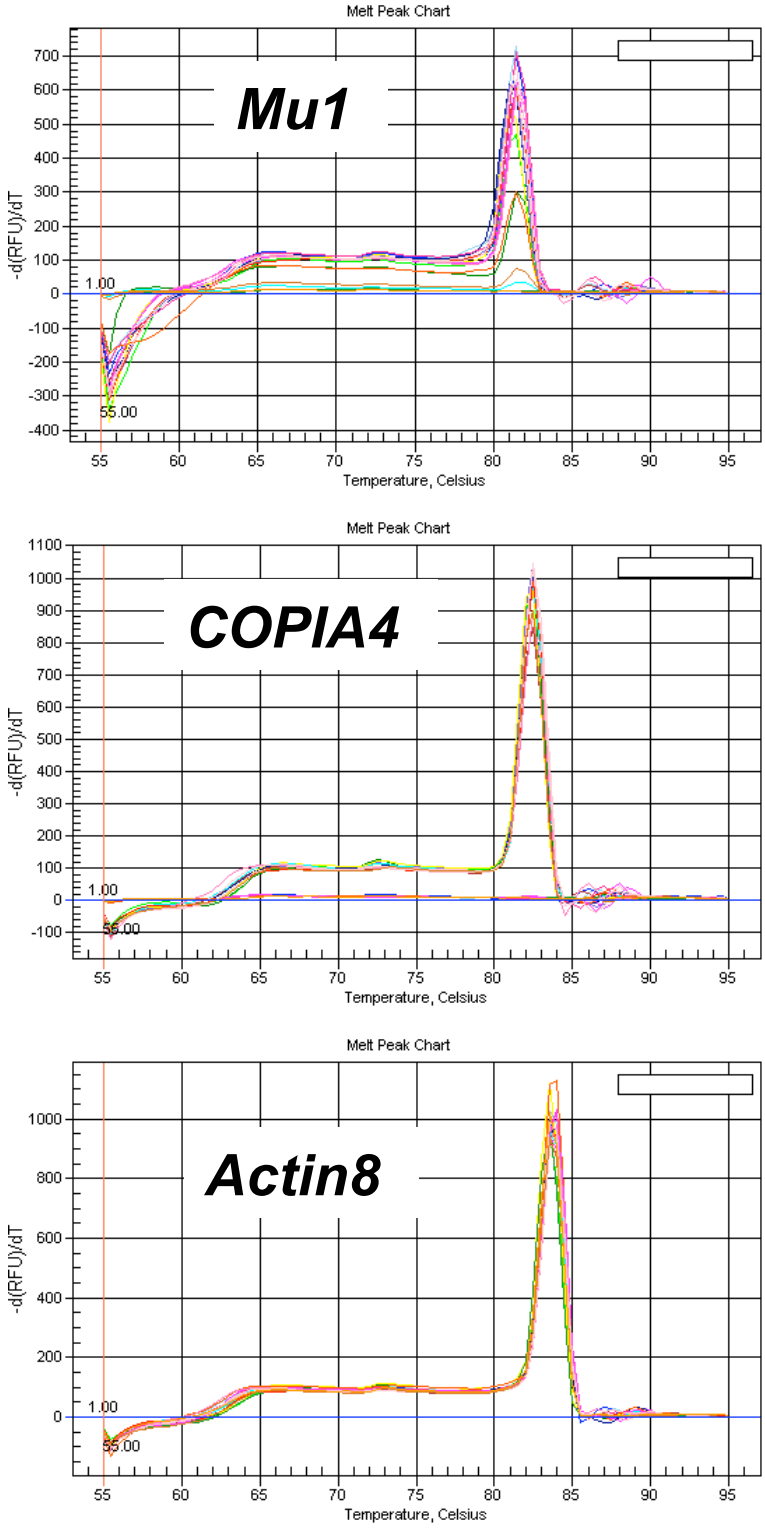


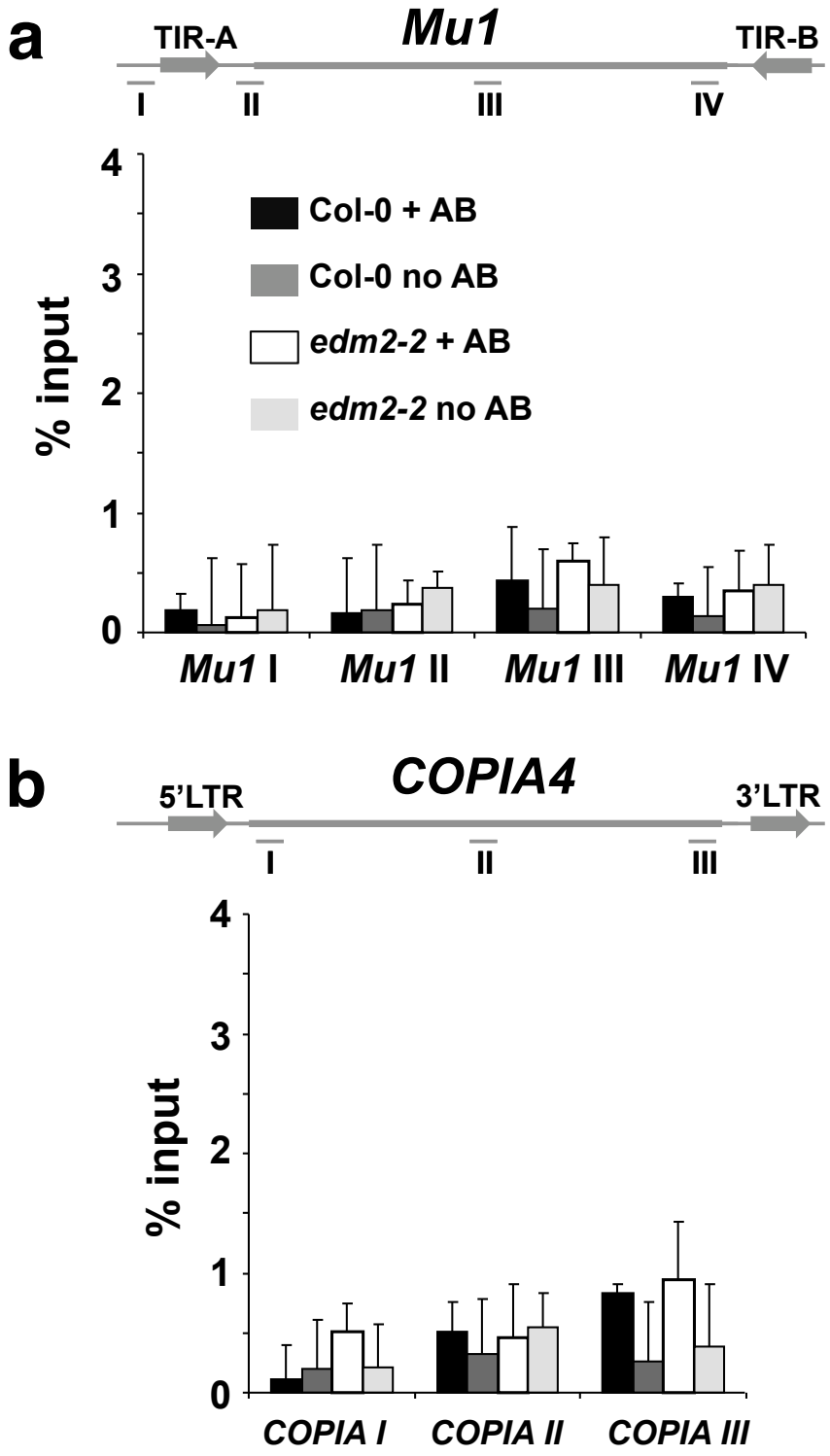
Supplementary Figure S1. Transcript levels of *EDM2* in *EDM2$_{pro}$:HA-EDM2*. Transcript levels of *EDM2* were determined by qRT-PCR.



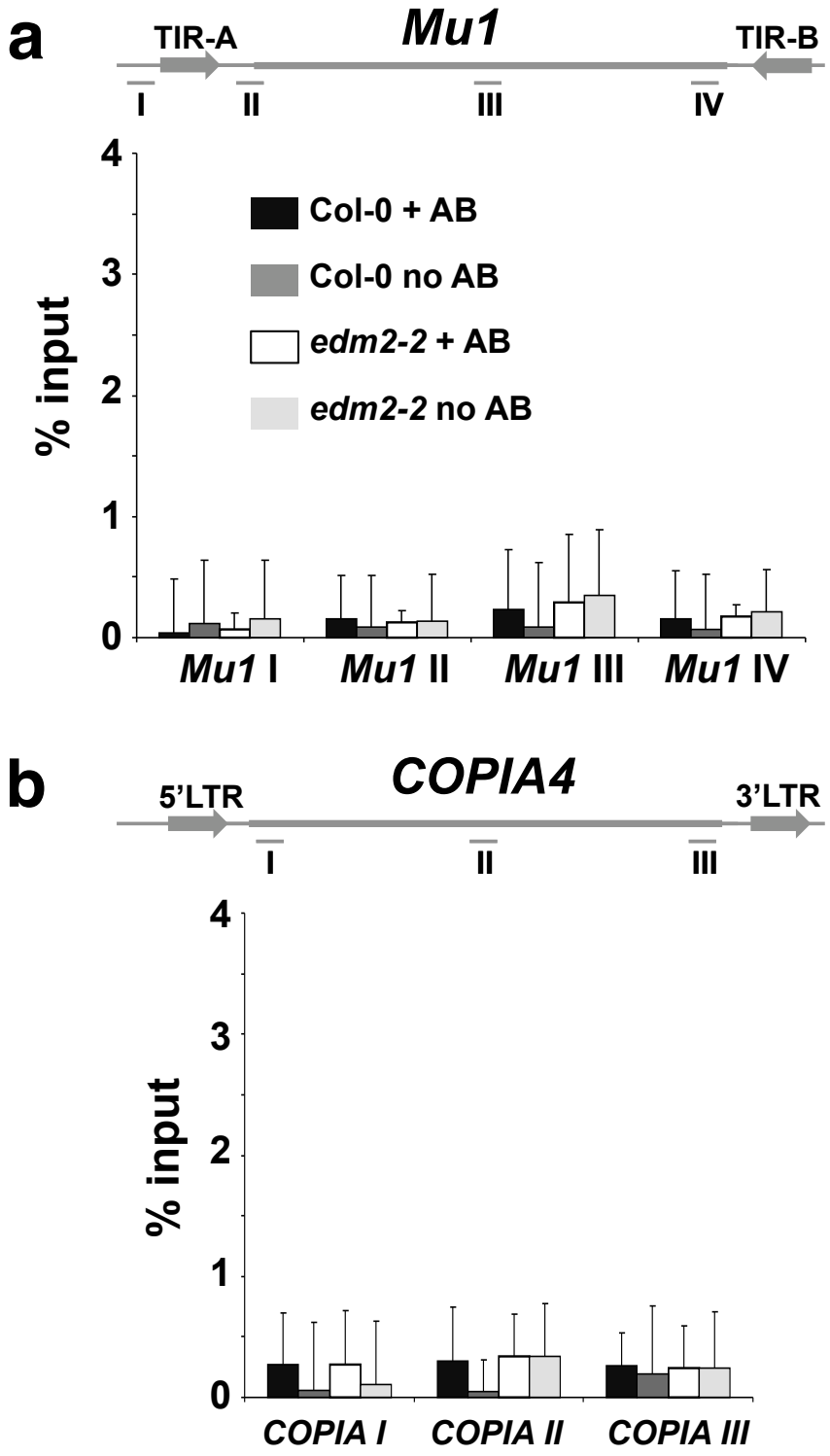
Supplementary Figure S2. Effects of *EDM2* on transcriptional silencing in *soloLTR* and *ING5* loci. Transcript levels of the respective loci were determined by RT-PCR. *ACTIN 8* (*ACT8*) served as an internal control. Transcriptional repression of *soloLTR_A* (framed by grey line) are clearly affected by EDM2. *E2pro:HA-E2*: *EDM2* complementation lines expressing in the *edm2-2* background HA-tagged EDM2 driven by the native *EDM2* promoter (*E2pro*). *soloLTR*, intergenic transcript initiated from a flanking solo retroposon long terminal repeat[1]. *IGN5*, INTERGENIC REGION 5[1].



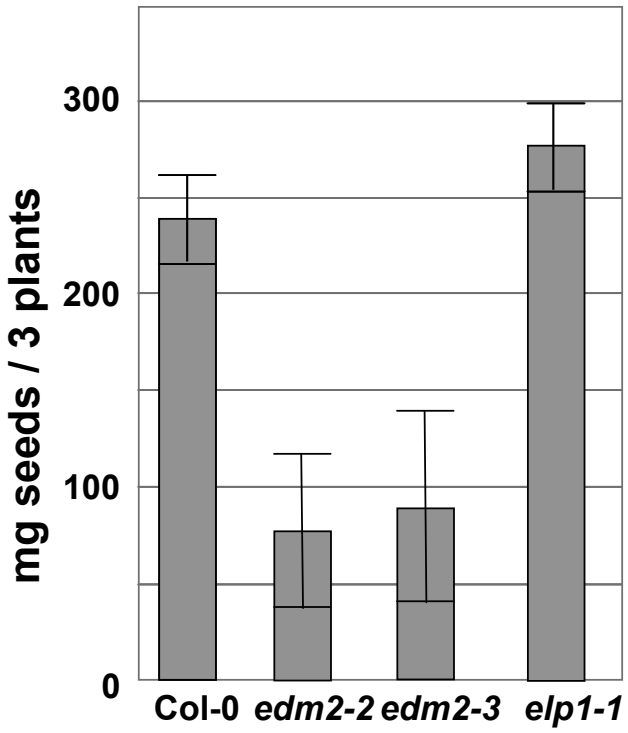
Supplementary Figure S3. Melting curves for *Mu1*, *COPIA4* and *Actin8* in qRT-PCRs. Melting curves were generated with PCR amplicons incorporating SYBR green dye at the end of the real-time PCR measurements shown in Figure 2a and d.



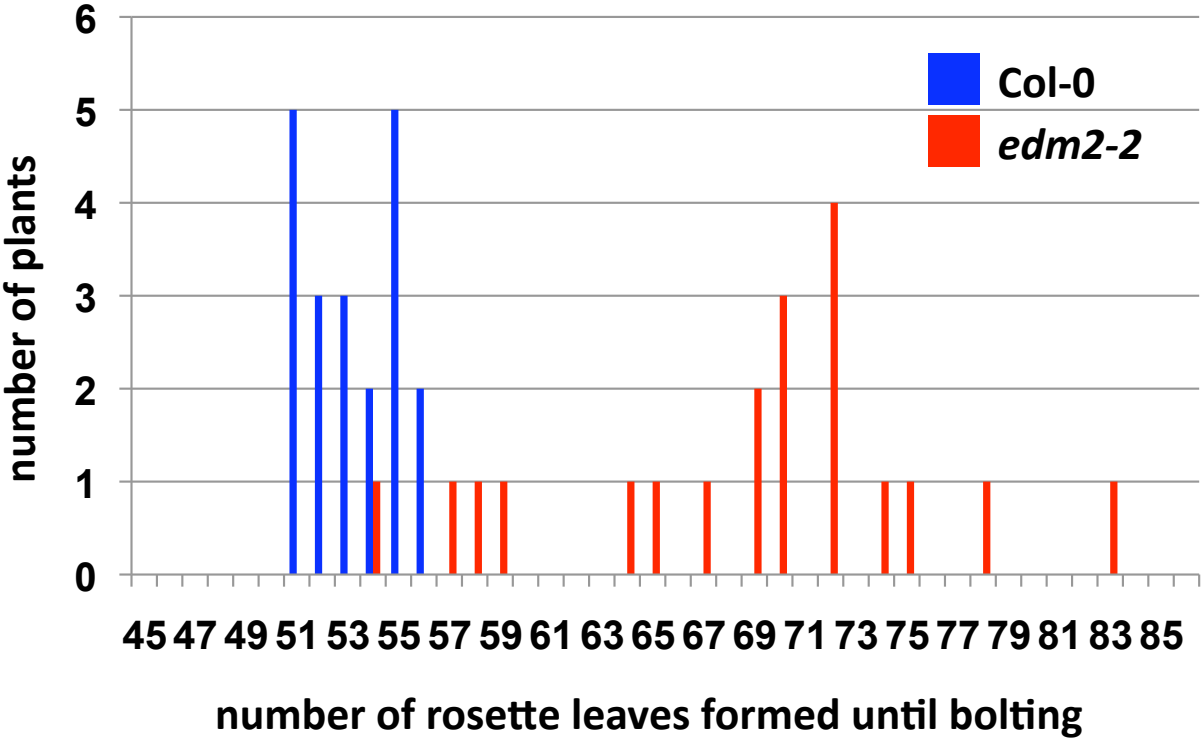
Supplementary Figure S4. H3K4me3 levels at *Mu1* and *COPIA4* determined by ChIP. ChIP combined with qPCR to measure H3K4me3 levels at *Mu1* (a) and *COPIA4* (b). In *Mu1* four and in *COPIA4* three different regions marked by roman numbers were tested. TIR: terminal inverted repeat, LTR: Long Terminal Repeat. Y axes in (a) and (b) represent "% input". Two biological replicates gave similar results.



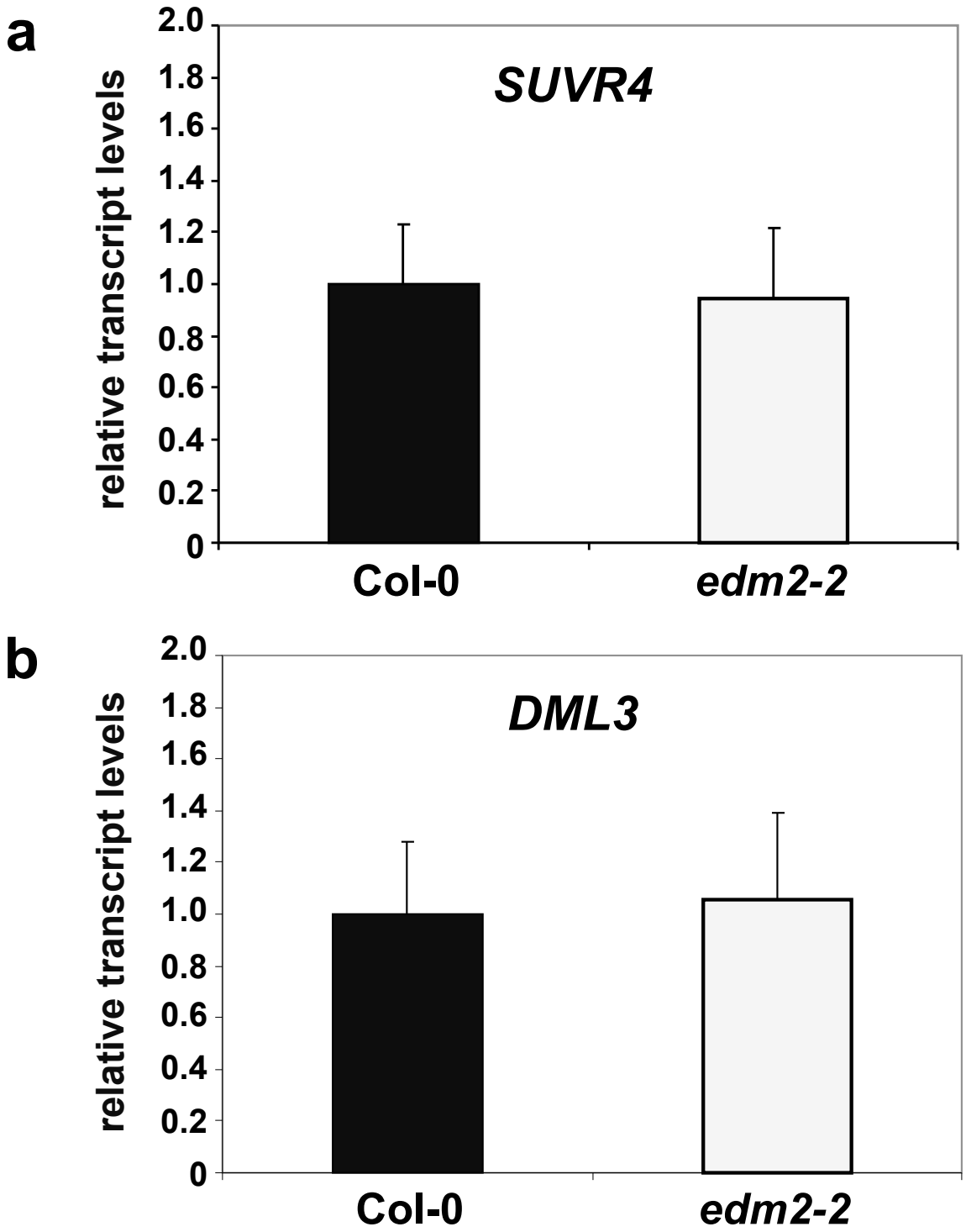
Supplementary Figure S5. H3K27me3 levels at *Mu1* and *COPIA4* determined by ChIP. ChIP combined with qPCR to measure H3K27me3 levels at *Mu1* (a) and *COPIA4* (b). In *Mu1* four and in *COPIA4* three different regions marked by roman numbers were tested. TIR: terminal inverted repeat, LTR: Long Terminal Repeat. Y axes in (a) and (b) represent "% input". Two biological replicates gave similar results.



Supplementary Figure S6. Reduced fertility of *edm2* mutant plants documented by reduced quantity of seeds. Seeds were harvested from a bulk of 3 plants per replicate. Total of 6 replicates was taken and average weight is shown. Error bars represent standard deviations.



Supplementary Figure S7. Variation in the timing of floral transition of *edm2* mutant plants. The number of rosette leaves formed until bolting is used as a proxy for the transition from vegetative to reproductive growth. The shown data are from experiments described previously[2], where they were, however, displayed as averages with Standard errors of the mean.



Supplementary Figure S8. Transcript levels of *SUVR4* and *DML3* in *edm2-2*. Transcript levels of *SUVR4* (a) and *DML3* (b) were determined by qRT-PCR.

References for supplementary figures

1. Wierzbicki, A.T., Haag, J.R. & Pikaard, C.S. Noncoding transcription by RNA polymerase Pol IVb/Pol V mediates transcriptional silencing of overlapping and adjacent genes. *Cell* ***135, 635-48 (2008).***
2. Tsuchiya, T. & Eulgem, T. The Arabidopsis defense component EDM2 affects the floral transition in an FLC-dependent manner. *Plant J* ***62, 518-28 (2010).***

Supplementary Table S1

Gene	AGI number	ATH1 probe	Fold change	P-value (FDR)
H3K9 methyltransferases and demethylase				
KYP/*SUVH4*	AT5G13960	250212_at	1.1278	0.5288
SUVH5	AT2G35160	266539_at	0.9238	0.8010
SUVH6	AT2G22740	265347_at	1.0910	0.8801
SUVR4	AT3G04380	no probes on ATH1		
SUVR5	AT2G23740	267290_at	1.1398	0.7869
		267291_at	1.0573	0.9144
		267292_at	0.9851	0.9709
SUVH2	AT2G33290	255796_at	1.0787	0.8270
IBM1	AT3G07610	259252_at	1.0261	0.9487
H3K27me1 methyltransferases				
ATXR5	AT5G09790	250492_at	1.0654	0.7945
ATXR6	AT5G24330	249788_at	1.1307	0.4572
DNA methyltransferases, demethylases and related				
MET1	AT5G49160	248597_at	0.9717	0.9471
CMT3	AT1G69770	260417_at	1.2581	0.2309
DRM2	AT5G14620	250139_at	0.9797	0.9694
		250140_at	0.8686	0.6982
DRM3	AT3G17310	258416_at	1.2473	0.5781
DME	AT5G04560	250834_at	1.0289	0.9130
		250835_at	1.1950	0.5535
		250836_at	1.0771	0.9418
ROS1	AT2G36490	263909_at	1.1341	0.7536
DML2	AT3G10010	258931_at	0.9673	0.9189
DML3	AT4G34060	no probes on ATH1		
ROS3	AT5G58130	247849_at	1.1278	0.6582

P-value (FDR): False Discovery Rate adjasted P-value.
No significant changes of transcript levels in *edm2-2* are represented by P-values > 0.05).

Supplementary Table S2. Oligonucleotides used in this study.

Primer name	Sequences (5'->3')	Comments
	Primers for genotyping of T-DNA mutants	
SALK_014520 LP	TAGCCGTCTTGTGACACAGTG	*edm2-2* genotyping
SALK_014520 RP	CCGATGAAGATGAATTGGATG	*edm2-2* genotyping
SALK_145992 LP	ATCGCCATTGTAGGTGAAGTG	*elp1-1* genotyping
SALK_145992 RP	TCTTCTTTACCTTGATCCATCG	*elp1-1* genotyping
SALK_041474 LP	ACTGGTGAACCAGCTGGTATG	*kyp-6* genotyping
SALK_041474 RP	TGAGGGGTACCTGTTCAATTG	*kyp-6* genotyping
LB salk	ACCACCATCAAACAGGATTTTC	T-DNA left border primer for SALK lines
	Primers for *EDM2$_{pro}$:HA-EDM2* construct	
HA:EDM2GW-F1	ATGTACCCATACGATGTTCCAGATTACGCTATGACGTTCGTTGACGATGA	Amplify full-length *EDM2* cDNA containing the HA-tag sequence. Used with EDM2GW-R in primary PCR
HA:EDM2GW-F2	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCACAATGTACCCATACGATGTTCCAGA	Amplify full-length *EDM2* cDNA containing the HA-tag sequence. Used with EDM2GW-R in secondary PCR
EDM2GW-R	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTAGTCATTAATCCAACCGCC	Amplify full-length *EDM2* cDNA containing the HA-tag sequence.
NOS$_{term}$-F	AAAACTAGTGAATTTCCCCGATCGTTCAAACAT	Amplify NOS terminator sequence. Contains *Spe*I site.
NOS$_{term}$-R	AAGAGCTCAGTAACATAGATGACACCGCGC	Amplify NOS terminator sequence Contains *Sac*I site.
EDM2$_{pro}$-F	AAAAAAGCTTGAAGCAGCTAACGAATAGAGCA	Amplify native EDM2 promoter sequence. Contains *Hind*III site.
EDM2$_{pro}$-R	TTGGCGCGCCTCAATCCCAAAATTCCCTTAACC	Amplify native EDM2 promoter sequence. Contains *Asc*I site.
	Primers for RT-PCR	
Mu1-F	TTGAATGAGGAACCACATACTTG	RT-PCR for *Mu1* Real-time PCR
Mu1-R	GAATTAATGACTTCGCTCTTGCT	RT-PCR for *Mu1* Real-time PCR
COPIA4-F	GCAAACGCTGCATCTGAAGTT	RT-PCR for *COPIA4* Real-time PCR
COPIA4-R	TGTTGCGAACGAAATGGTAGTC	RT-PCR for *COPIA4* Real-time PCR

JP1565	GATTCTTACTGTAAAGAACATGGCATTGAGAGA	RT-PCR for *Ta3* [1] Real-time PCR
JP1566	TCCAAATTTCCTGAGGTGCTTGTAACC	RT-PCR for *Ta3* Real-time PCR
SP15	AACAAAAGCATCATTCTACTTAAC	RT-PCR for *CACTA1* [2]
SP40	AGGCCTACAATGGAAATGACG	RT-PCR for *CACTA1*
ATS15	ACCAACGTGCTGTTGGCCCAGTGGTAAATC	RT-PCR for *SN1* [3]
AtSN1-F4	AAAATAAGTGGTGGTTGTACAAGC	RT-PCR for *SN1*
LINE1-4-F	ATATTGCAGGGGAAGAAAAACCA	RT-PCR for *LINE1-4*
LINE1-4-R	GACTGTGCCGTGTTGTGTGAGA	RT-PCR for *LINE1-4*
TSR1	CGTGAATCAAACAATGCATC	Reverse-transcription for *TSI* [4]
TSP1F	GAACTCATGGATACCCTAAAATAC	RT-PCR for *TSI*
TSP1R	CTCTACCCTTTGCATTCATGAATC	RT-PCR for *TSI*
IGN5_B-F	CGCAGCGGAATTGACATCCTATC	RT-PCR for *IGN5* region B [5]. Used for reverse-transcription
IGN5_B-R	TCGGAAAGAGACTCTCCGCTAGAAA	RT-PCR for *IGN5* region B
soloLTR_AA221	ATCAATTATTATGTCATGTTAAAACCGATTG	RT-PCR for *soloLTR* region A [5]. Used for reverse-transcription
soloLTR_AA222	TGTTTCGAGTTTTATTCTCTCTAGTCTTCATT	RT-PCR for *soloLTR* region A
FWA-F	CGACGCTGCAGAGACACTGC	RT-PCR for *FLOWERING WAGENINGEN*
FWA-R	AGTTGAGAGTCATTCGCTGTGCT	RT-PCR for *FLOWERING WAGENINGEN*
BNS-F	TACGGCTGCATTGTTTCTACCAGTA	RT-PCR for *BONSAI*
BNS-R	AGGCAAAGTGTCCCTCATCCAC	RT-PCR for *BONSAI*
RTPCR5S1	GGATGCGATCATACCAG	RT-PCR for *5S rDNA* [6]
5SUNIV2	CGAAAAGGTATCACATGCC	RT-PCR for *5S rDNA*. Used for reverse-transcription
106B-F	TTGATTGATAGATCCCTTCTGGA	RT-PCR for *106B* repeats [6]
106B-R	CGAGGATGGGGTAATTGAGT	RT-PCR for *106B* repeats. Used for reverse-transcription
180(all)-F	ACCATCAAAGCCTTGAGAAGCA	RT-PCR for 180bp centromer repeat [6]. Used for reverse-transcription
180(all)-R	CCGTATGAGTCTTTGTCTTTGTATCTTCT	RT-PCR for 180bp centromer repeat
SUVR4-F	TATTGACCAACACGGAGTTATACGAT	RT-PCR for *SUVR4* Real-time PCR

SUVR4-R	TGTGATTGATAAACCTTGCGACA	RT-PCR for *SUVR4* Real-time PCR
DML3-F	TTTAAACTTTTAGACACAAGACGGAT	RT-PCR for *DML3* Real-time PCR
DML3-R	TTTGGCCCTCTCTCATCAGG	RT-PCR for *DML3* Real-time PCR
EDM2-F	CTTGGACCCCATGTTAGCTC	RT-PCR for *EDM2* Real-time PCR
EDM2-R	TTAAGAGATTATGTCCGCTAGGTT	RT-PCR for *EDM2* Real-time PCR
ACT8-F1	ATGAAGATTAAGGTCGTGGCAC	RT-PCR for *ACTIN8*
ACT8-R1	GTTTTTATCCGAGTTTGAAGAGGC	RT-PCR for *ACTIN8*
ACT8-F2	CAGTGTCTGGATTGGTGGTTCTATC	RT-PCR for *ACTIN8* Real-time PCR
ACT8-R2	ATCCCGTCATGGAAACGATGT	RT-PCR for *ACTIN8* Real-time PCR
	Primers for chop-PCR	
Mu1 meth-F1	TAGGCAACTGTTTGTGGTTAAGACATC	Chop-PCR for *Hpy*CH4IV and *Bgl*II sites in *Mu1*
Mu1 meth-R1	GACACCATTTTCCAACGCTCT	Chop-PCR for *Hpy*CH4IV and *Bgl*II sites in *Mu1*
Mu1 meth-R2	TGGGTTTTGAGAATGAATTTCTTCAACAT	Chop-PCR for *Afl*II site in *Mu1*. Used with Mu1 meth F1
COPIA4 meth-F1	ACCACTTTCGTCTATGTACTTGTCT	Chop-PCR for *Hpy*CH4IV and *Bgl*II sites in *COPIA4*
COPIA4 meth-R1	TTTGTTCGGGTTGCTTCTATACCAA	Chop-PCR for *Hpy*CH4IV and *Bgl*II sites in *COPIA4*
COPIA4 meth-F2	CTCACTCAAGCTTCGGTTCC	Chop-PCR for *Hae*III site in *COPIA4*
COPIA4 meth-R2	TACTCGTAGACGCTCATAATTCGG	Chop-PCR for *Hae*III site in *COPIA4*
	Primers for Bisulfite sequencing	
Mu1 ConF1	TTTAGTTATGGGTTGGTAATTGTA	Bisulfite sequencing for *Mu1* [7]
Mu1 ConR	CTTCTTAACCTTCTTTTCAATCTAAA	Bisulfite sequencing for *Mu1*
COPIA4-BS-F	TTTGAAYAGAGTTTTTCGYCGTTAGTTACG	Bisulfite sequencing for *COPIA4*
COPIA4-BS-R	ATAACTRAACCACARATTCARACCCATTTTCATTT	Bisulfite sequencing for *COPIA4*
	Primers for ChIP	
Mu1 ChIP I_F	CACAGGTCAAGCCCTCGATT	ChIP for *Mu1* region I
Mu1 ChIP I_R	CAGCCCTTTTAAGATATCACTCGTTG	ChIP for *Mu1* region I
Mu1 ChIP II_F	GATTTTGTATTAATTTTATCTTGTTCGGA	ChIP for *Mu1* region II
Mu1 ChIP II_R	CGCTCTTCACTCTCTGATTCGTT	ChIP for *Mu1* region II

Mu1 ChIP III_F	ACATTAGTCTGGAAATTGGCTT	ChIP for *Mu1* region III. Also used for Chop-PCR internal control
Mu1 ChIP III_R	CAACTACCGAGCTTATAGAAACACC	ChIP for *Mu1* region III. Also used for Chop-PCR internal control
Mu1 ChIP IV_F	AATTCCCTAAAGAGAAGGTACCGAG	ChIP for *Mu1* region IV
Mu1 ChIP IV_R	AGCCTTCTTTTCAATCTGAGTAAGCAA	ChIP for *Mu1* region IV
COPIA4 ChIP I_F	GCAAACAATCTTCAACATTAACACGTCT	ChIP for *COPIA4* region I
COPIA4 ChIP I_R	AGCAGGAGTCTCGATAGAACCATC	ChIP for *COPIA4* region I
COPIA4 ChIP II_F	ATGGCTCAGACCTTACACTCACA	ChIP for *COPIA4* region II. Also used for Chop-PCR internal control
COPIA4 ChIP II_R	AAAGGAGGCTTCATCAAACACGAC	ChIP for *COPIA4* region II. Also used for Chop-PCR internal control
COPIA4 ChIP III_F	GCAAACGCTGCATCTGAAGTT	ChIP for *COPIA4* region III
COPIA4 ChIP III_R	TGTTGCGAACGAAATGGTAGTC	ChIP for *COPIA4* region III
ACT8 ChIP _F	CTAAAGAGACATCGTTTCCATGACGG	ChIP for *ACT8*
ACT8 ChIP _R	TCCTTAGACATCTCTCCAAACGC	ChIP for *ACT8*

References for supplementary tables

1. Johnson, L., Cao, X. & Jacobsen, S. Interplay between two epigenetic marks. DNA methylation and histone H3 lysine 9 methylation. *Current biology : CB* **12**, 1360-7 (2002).
2. Kato, M., Miura, A., Bender, J., Jacobsen, S.E. & Kakutani, T. Role of CG and non-CG methylation in immobilization of transposons in Arabidopsis. *Current biology : CB* **13**, 421-6 (2003).
3. Herr, A.J., Jensen, M.B., Dalmay, T. & Baulcombe, D.C. RNA polymerase IV directs silencing of endogenous DNA. *Science (New York, NY)* **308**, 118-20 (2005).
4. Numa, H. *et al.* Transduction of RNA-directed DNA methylation signals to repressive histone marks in Arabidopsis thaliana. *EMBO J* **29**, 352-62 (2010).
5. Wierzbicki, A.T., Haag, J.R. & Pikaard, C.S. Noncoding transcription by RNA polymerase Pol IVb/Pol V mediates transcriptional silencing of overlapping and adjacent genes. *Cell* **135**, 635-48 (2008).

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7. Zhu, J., Kapoor, A., Sridhar, V.V., Agius, F. & Zhu, J.K. The DNA glycosylase/lyase ROS1 functions in pruning DNA methylation patterns in Arabidopsis. *Curr Biol* **17**, 54-9 (2007).