Supplementary Material

Supplementary Table I List of primers.

Gene	Name (abbreviation)	Sequence
SUVH1	Su1start	ATAGTCGACCGCATGGAAAGAGGTGGTCAC
	Su1-stop	ATAGTCGACTCCAAATGAGCCACGGCAATACGC
	Su1-Start/NheI	ATAGCTAGCATGGAAAGAAATGGTGGTACA
	Su1-Stop	ATACTCGAGAAATGAGCCACGGCAATACGC
	Su1r908 (O)	TAACGCATCAAACCGCATAA
	Su1f592 (P)	ACCAAGCTCAGAACCCACCG
SUVH2	Su2Start (F)	ATAGTCGACATGAGTACATTGTTACCA
	Su2Stop	ATAGTCGACCTAGTTGCAGATGGCGAG
	Su2StartNheI	ATAGCTAGCATGAGTACATTGTTACCA
	Su2Xho	ATACTCGAGGTTGCAGATGGCGAGCTTG
	Su2-1631B (H)	ATAACATCCCCATTGATTGACAG
	mycSal1	ATAGTCGACATGGGCGGACGCGAACAAAAGTTG
	2RNAi3X/Bstop	ATAGGATCCCTCGAGGCTCGTTCACTTCAT
	2RNAi5-BamH1F	ATAGGATCCCTCAATCCTCCACCG
	2RNAi5XB	ATACTCGAGGGATCCCCCAAAACCAGACTTCCCC
	2RNAi3BamH1	ATAGGATCCTTTGCTGTGGGGAAGTCT
	Su2-1290F (K)	GATCAGCAGGACTGGCTGCGAG
	Su2r545 (G)	TCTCGCTAGGTCTCTCGGTC
	Su2f527	GACCGACGAGAGACCTAGCGAGA
SUVH3	Su3r1447 (R)	AGCAGGCCCTTTATCCTCAT
	Su3f1150 (S)	GAGGATGCAGCTTCGAAAAC
SUVH4	Su4r1227 (M)	CTCGGCAGTTACACCCAGTT
	Su4f1010 (N)	TGGACGCATACCAACGAGTA
	A	AGAAATTGAGTGTTTCTCTGAATGG
	B	GCATTGTCTAGATCATCCTCGTACT
	C	TGGTTCACGTAGTGGGCCATCG
	D	CAATTGATTTCCATGTGGTATTGTA
	E	AGATTGTGATTAAGCTCAGATTTGG

Gene	Name (abbreviation)	Sequence
T-DNA	35S-1HindIII	GAAAGAATGCTAACCCACAGATG
	35sd2fwd	AGGGTAATAT(CT)GGGAAA(CT)
MET1	Met1-6f	GTCAGGTATATATTTCAGATTGTGG
	Met1-6r	TGTATAAGACTGACCTTTCTCTTTG
CMT3	CMT3-eF	TGGTCTGTTTTAGTGTCTAGGAATA
	CMT3-eR	GGTCTAAAGATAAATCCTAGGAAAA
Actin	Actinf250	ATGGAAAAGATATGGCATCACAC
	Actinr520	GAAGAGCATACCCCTCGTAGATT
GFP	GFP-NheIStop	GCTAGCTTATTGTATAGTTCATCC
	GFP-Start	GGATCCATGAGTAAAGGAGAAG
DDM1	DDM1f	GAGATCTCTACCCTCCTGT
	ddm1-2dRSa	TGAGCTACGAGCCATGGGTTTGTGAAACGTA
Athila	Athila 817	TGAGGATGGGATAGAATAG
	Athila back	ATTAATCCCTAAAACACTATCTT
Luciferase	Lu1626B	GGCCTTTATGAGGATCTCTCTG
	LU8F	ACGCCAAAAACATAAAGAAAGG
	lucd2back	AACT(AG)CAACTCC(AG)ATAAT
GUS	Gus-start	ATAGGATCCATGTTACGTCCTGTAGAAACCC
	Gus-stop	ATAGGATCCTTGTTTGCCTCCCTGCTG
	GUS1212	GTGGTGATGTGGAGTATTG
	GUS 1576 rev	ATACTCTTCACTCCACAT
18SrDNA	18SrDNA R478	TTAGCGACAAAGGGCTGAAT
	18SrDNA F214	CGCCTCTAAGTCAGAATCCG

Underlined letters indicate restriction sites used for subcloning.

Supplementary Figure 1. Molecular characterisation of *SUVH1 and SUVH2* mutant lines. (**A**) Loss-of-function and antisense lines of *SUVH1* and *SUVH2* do not affect transcription of other *SUVH* genes. Results of RT-PCR analysis with the primers indicated or listed in Supplementary Table I. (**B**) PCR analysis of the *suvh2* T-DNA insertional mutation SALK 079574. Specific PCR fragment amplification with primer pair CG in the SALK 079574 homozygous mutant line. The *SUVH2* specific primers F and G only amplify a *SUVH2* specific fragment in wild-type plants but not in the homozygous SALK 079574 line.

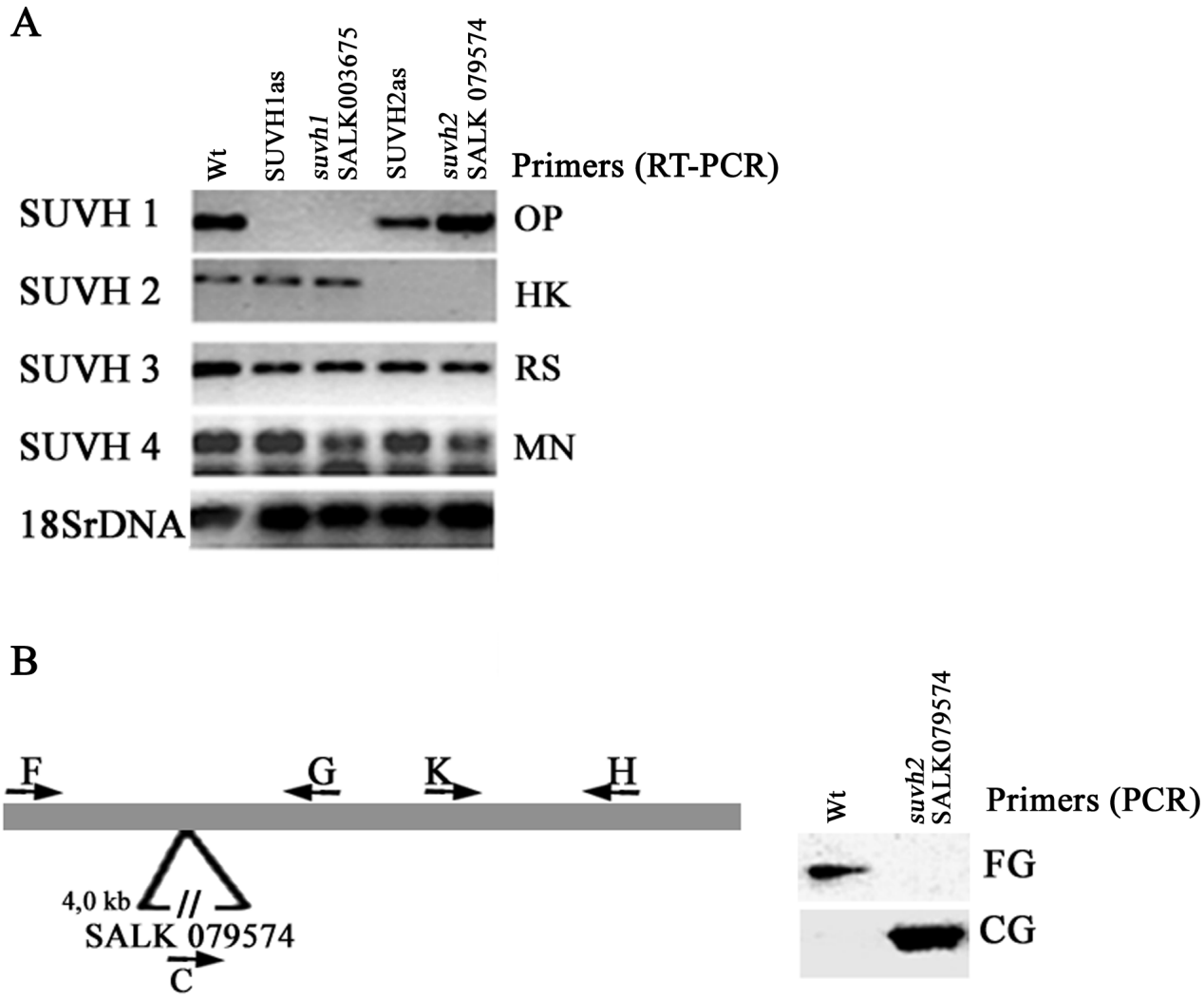
Supplementary Figure 2. Analysis of loss-of-function *suvh4* alleles and of the *SUVH4 kyp-2* mutation. (**A**) PCR and RT-PCR analysis of the T-DNA insertional *suvh4* mutations SALK 105816 and 130630. Primer pairs AB and DE do not amplify *SUVH4* specific genomic fragments in SALK 105816 and SALK 130630 homozygous mutant lines, respectively. By RT-PCR using primer pair MN no SUVH4 transcripts are detected in the SALK 105816 and 130630 T-DNA insertional mutations. (**B**) Western analysis of *suvh4* SALK 105816 homozygous plants with α-mono- and α-dimethyl H3K9. In *suvh4* homozygous mutant plants significant reduction of dimethylated H3K9 is found. (**C**) Immunostaining of *suvh4* and *kyp-2* mutant plants for heterochromatin specific histone methylation marks and 5-methylcytosine does not reveal any significant differences between wild-type and homozygous mutants. (**D**) Immunostaining for euchromatic histone methylation marks in wild-type and *suvh4* mutant plants.

Supplementary Figure 3 Quantification of DNA methylation by bisulfite sequence analysis. (**A**) DNA methylation at Athila transposon sequences in *suvh2* mutant and *SUVH2* overexpression line *35*::SUVH2#5*. The *suvh2* null mutant significantly reduces the DNA methylation while *SUVH2* overexpression leads to hypermethylation. Most significant effects are seen at non-symmetric cytosines. (**B**) DNA methylation at Athila transposon sequence in three *SUVH2* transgene mutant lines. Only the YDG domain mutant *35*::SUVH2#5-2* showed a significant reduction of DNA hypermethylation. In contrast the preSET and SET domain mutants (*35*::SUVH2#5-4 and #5-6*) do not reduce DNA hypermethylation although relaxation of Athila silencing is found (cf. Figure 5). (**C**) *GUS27* transgene silencing and its DNA methylation analysis in *35*::SUVH2#5* overexpression and *35*::SUVH2as#11*

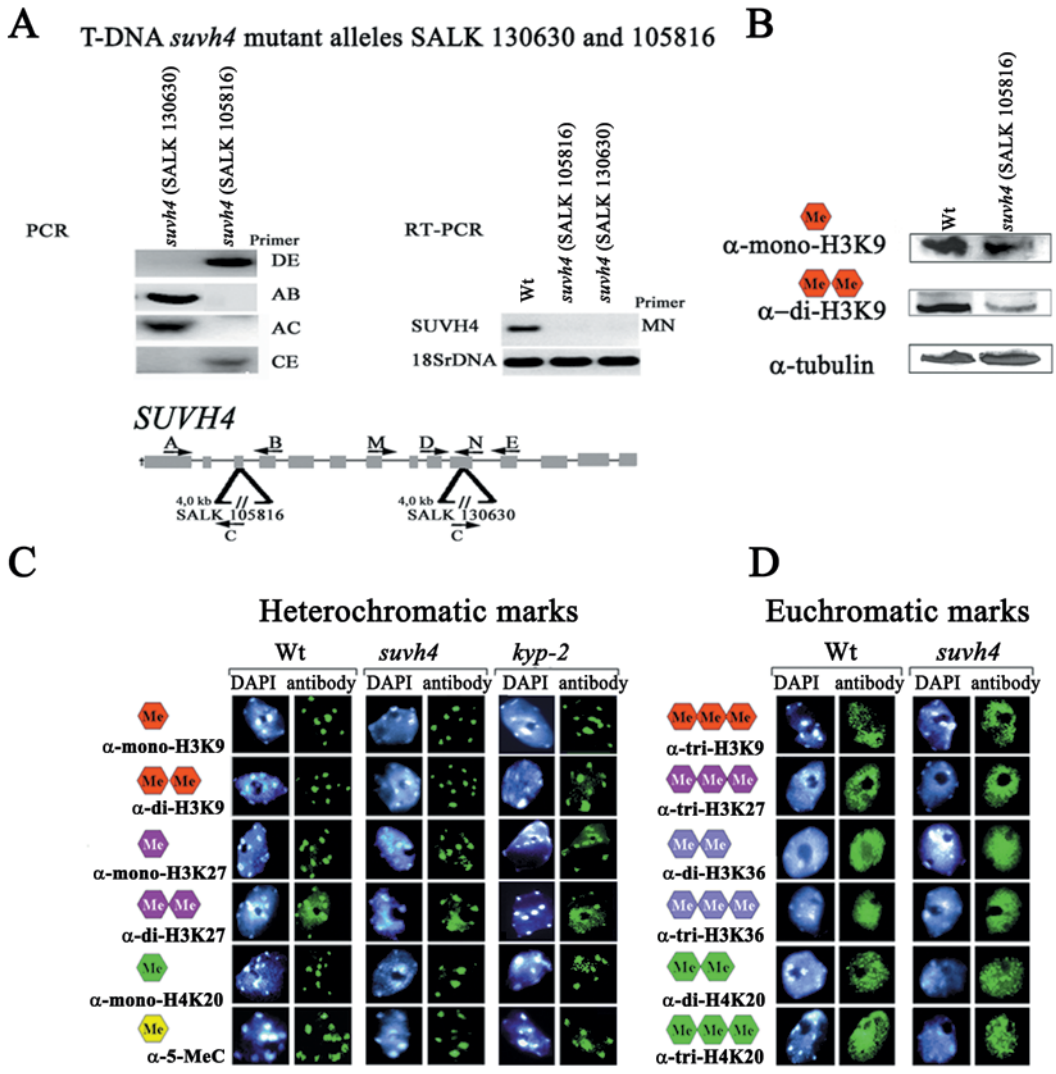
antisense plants. The *GUS27* transgene consist of three tandemly repeated copies of *GUS*, and significant enhancement of transgene silencing is observed in *SUVH2* overexpression plants. In *35*::SUVH2#5* plants DNA hypermethylation of *GUS27* transgene is found whereas significant reduction is found in *35*::SUVH2as#11* antisense lines in comparison to wild-type. The most significant effects are seen on non-symmetric cytosines.

Individual 5-methylcytosines are indicated by a line at the corresponding sequence position differentiating between the CpG (red), CpNpG (green) and CpNpN (blue) sequence context. Total amount of symmetric CpG (red bars) and CpNpG (green bars) and non-symmetric (blue bars) methylation is calculated for each genotype in the histogramme. We further differientated between CpA, CpC and CpT non-symmetric DNA methylation (differentially blue coloured bars).

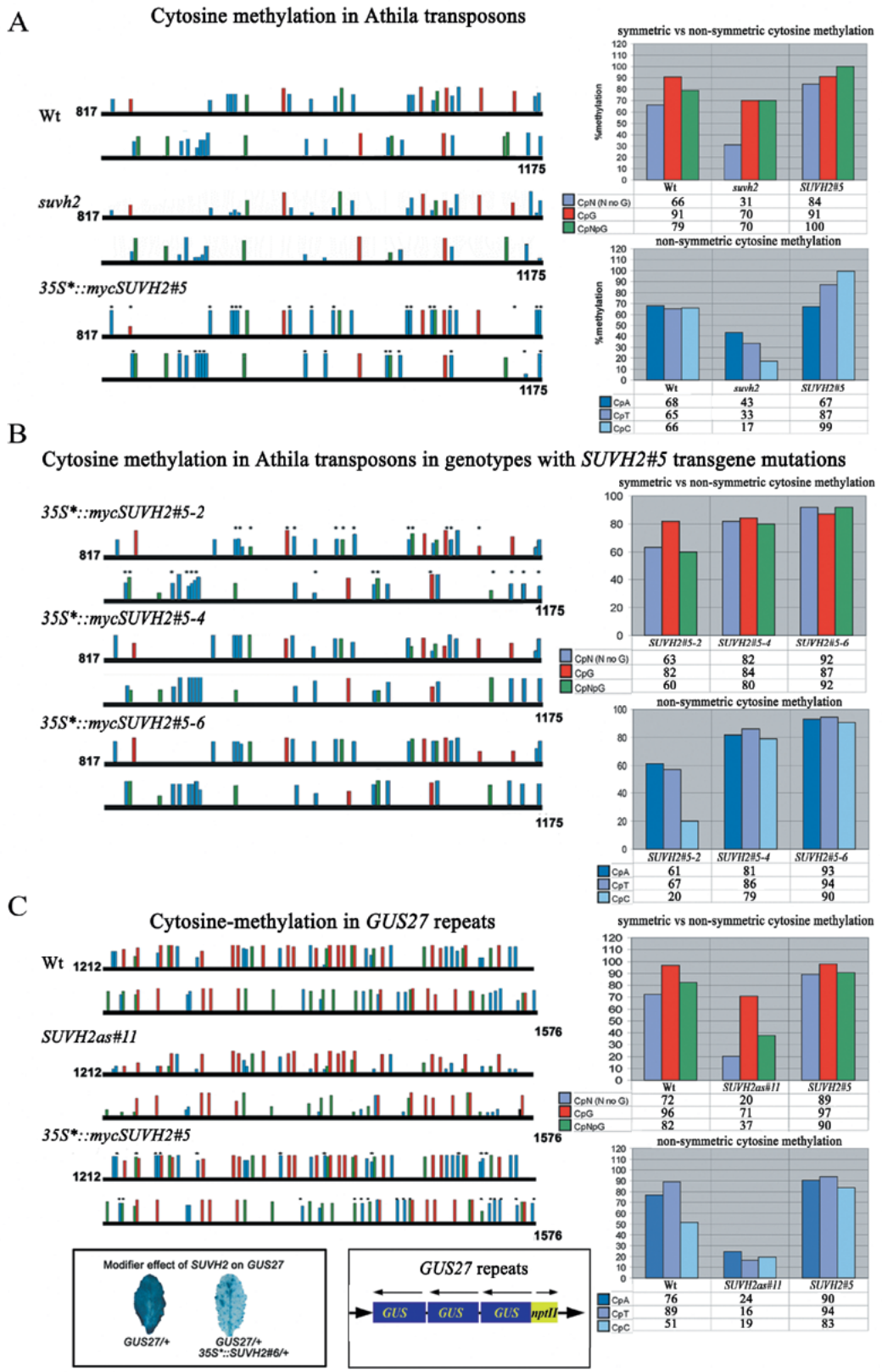
T-DNA mutant alleles and antisense lines of *SUVH1* and *SUVH2*



Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3