Supplementary Materials

Legends for Supplementary Figures

Figure S1. Comparison of HEN1 homologues

(A) Alignment of the conserved domain of HEN1 homologues. The methyltransferase domain is indicated by a black bar. Amino acids that are identical are highlighted in black, while similar amino acids are shaded in grey. Tt = *Tetrahymena thermophila*; Dm = *Drosophila melanogaster*; Mm = *Mus musculus*; At = *Arabidopsis thaliana*. (**B)** Schematic drawings of HEN1 homologues. Identity (I) and similarity (S) of the methyltransferase domains of HEN1 homologues when compared to the founding member of the HEN1 family, At HEN1 (gaps were omitted). Percentages of identical (I) and similar (S) residues are indicated. dsRNA indicates the location of the double-stranded RNA binding domain specific for plant HEN1.

Figure S2. Sequences of synthetic RNAs used for *in vitro* methylation assays

Figure S3. Methylation occurs on single-stranded scnRNA *in vivo*

Small RNAs from Twi1p slicer-dead mutant cells extracted at 4 h post-mixing were combined with synthetic, unmodified RNA of 21 nt and treated with (+) or without (−) periodate oxidation/β-elimination. The reactions were then mixed with synthetic RNA of 32 nt and analyzed by denaturing gel electrophoresis, followed by nucleic acid-specific fluorescent dye staining (left). Densitometric analysis of the fluorescent signal is shown on the right. The gel mobility of scnRNAs from Twi1p slicer-dead mutant cells increased by nearly 1.5 nt after periodate oxidation/β-elimination,

indicating that these scnRNAs bear 2′- and 3′-hydroxyl groups at their terminal nucleotides.

Figure S4. *HEN1* mRNA expression in wild-type (wt) cells

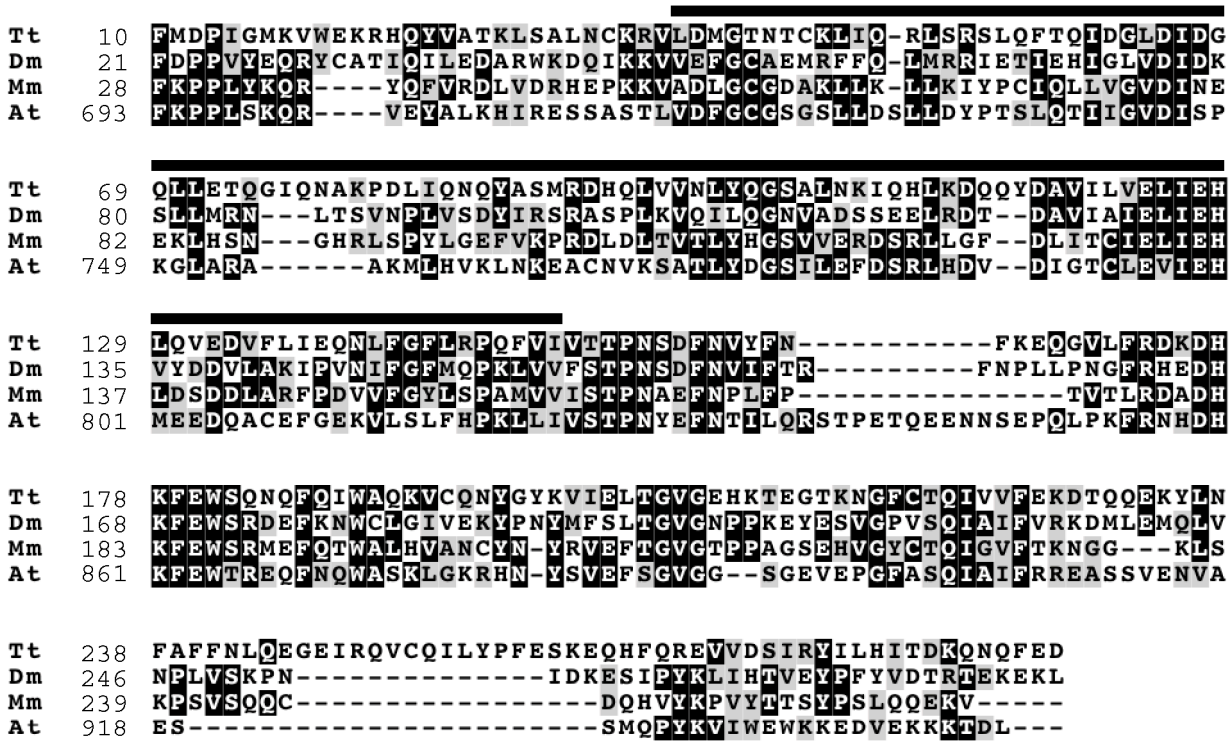
HEN1 mRNA expression in wt cells. Total RNA extracted from log-phase, vegetative, and mating cells was used for RT-PCR.

Figure S5. Construction and analysis of *HEN1* knockout cells

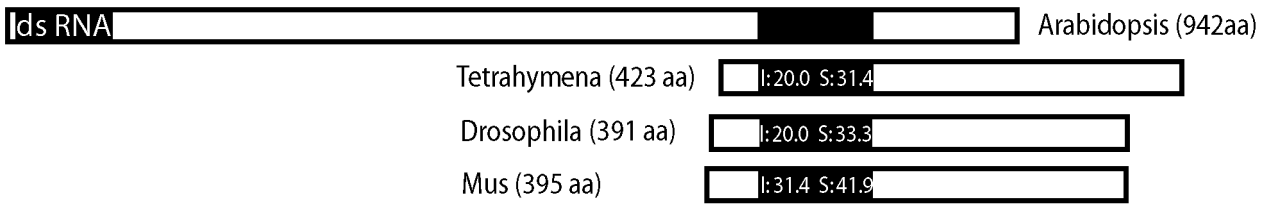
(A) Schematic drawing of the *HEN1* knockout strategy. The entire coding region was replaced with a drug resistance marker. The knockout construct was introduced into the *HEN1* locus by homologous recombination. **(B)** Confirmation of the complete replacement of the endogenous locus. Total DNA isolated from wild-type (wt) and Δ*HEN1* strains was digested with *Bgl*II (b) and analyzed by Southern hybridization using the radiolabelled probe shown in B. **(C)** Δ*HEN1* strains show a defect in viable progeny production. Single mating pairs were placed into drops of medium and were incubated for 60 h at 30 °C. Completion of mating was confirmed by testing the expression of a drug resistance marker that is active only in sexual progeny.

Figure S1

A


Tt 10 FMDPIGMKVWEKRHQYVATKLSALNCKRVLDMGTNTCKLIQ-RLSRSLQFTQIDGLDIDG
Dm 21 FDPPVYEQRYCATIQILEDARWKDQIKKVVEFGCAEMRFFQ-LMRRIETIEHIGLVDIDK
Mm 28 FKPPLYKQR----YQFVRDLVDRHEPKKVADLGCGDAKLLK-LLKIYPCIQLLVGVDINE
At 693 FKPPLSKQR----VEYALKHIRESSASTLVDFGCGSGSLLDSLLDYPTSLQTIIGVDISP
Tt 69 QLLETQGIQNAKPDLIQNQYASMRDHQLVVNLYQGSALNKIQHLKDQQYDAVILVELIEH
Dm 80 SLLMRN---LTSVNPLVSDYIRSRASPLKVQILQGNVADSSEELRDT--DAVIAIELIEH
Mm 82 EKLHSN---GHRLSPYLGEFVKPRDLDLTVTLYHGSVVERDSRLLGF--DLITCIELIEH
At 749 KGLARA------AKMLHVKLNKEACNVKSATLYDGSILEFDSRLHDV--DIGTCLEVIEH
Tt 129 LQVEDVFLIEQNLFGFLRPQFVIVTTPNSDFNVYFN----------FKEQGVLFRDKDH
Dm 135 VYDDVLAKIPVNIFGFMQPKLVVFSTPNSDFNVIFTR---------FNPLLPNGFRHEDH
Mm 137 LDSDDLARFPDVVFGYLSPAMVVISTPNAEFNPLFP---------TVTLRDADH
At 801 MEEDQACEFGEKVLSLFHPKLLIVSTPNYEFNTILQRSTPETQEENNSEPQLPKFRNHDH
Tt 178 KFEWSQNQFQIWAQKVCQNYGYKVIELTGVGEHKTEGTKNGFCTQIVVFEKDTQQEKYLN
Dm 168 KFEWSRDEFKNWCLGIVEKYPNYMFSLTGVGNPPKEYESVGPVSQIAIFVRKDMLEMQLV
Mm 183 KFEWSRMEFQTWALHVANCYN-YRVEFTGVGTPPAGSEHVGYCTQIGVFTKNGG---KLS
At 861 KFEWTREQFNQWASKLGKRHN-YSVEFSGVGG--SGEVEPGFASQIAIFRREASSVENVA
Tt 238 FAFFNLQEGEIRQVCQILYPFESKEQHFQREVVDSIRYILHITDKQNQFED
Dm 246 NPLVSKPN----------IDKESIPYKLIHTVEYPFYVDTRTEKEKL
Mm 239 KPSVSQQC----------DQHVYKPVYTTSYPSLQQEKV-----
At 918 ES----------SMQPYKVIWEWKKEDVEKKKTDL---

B

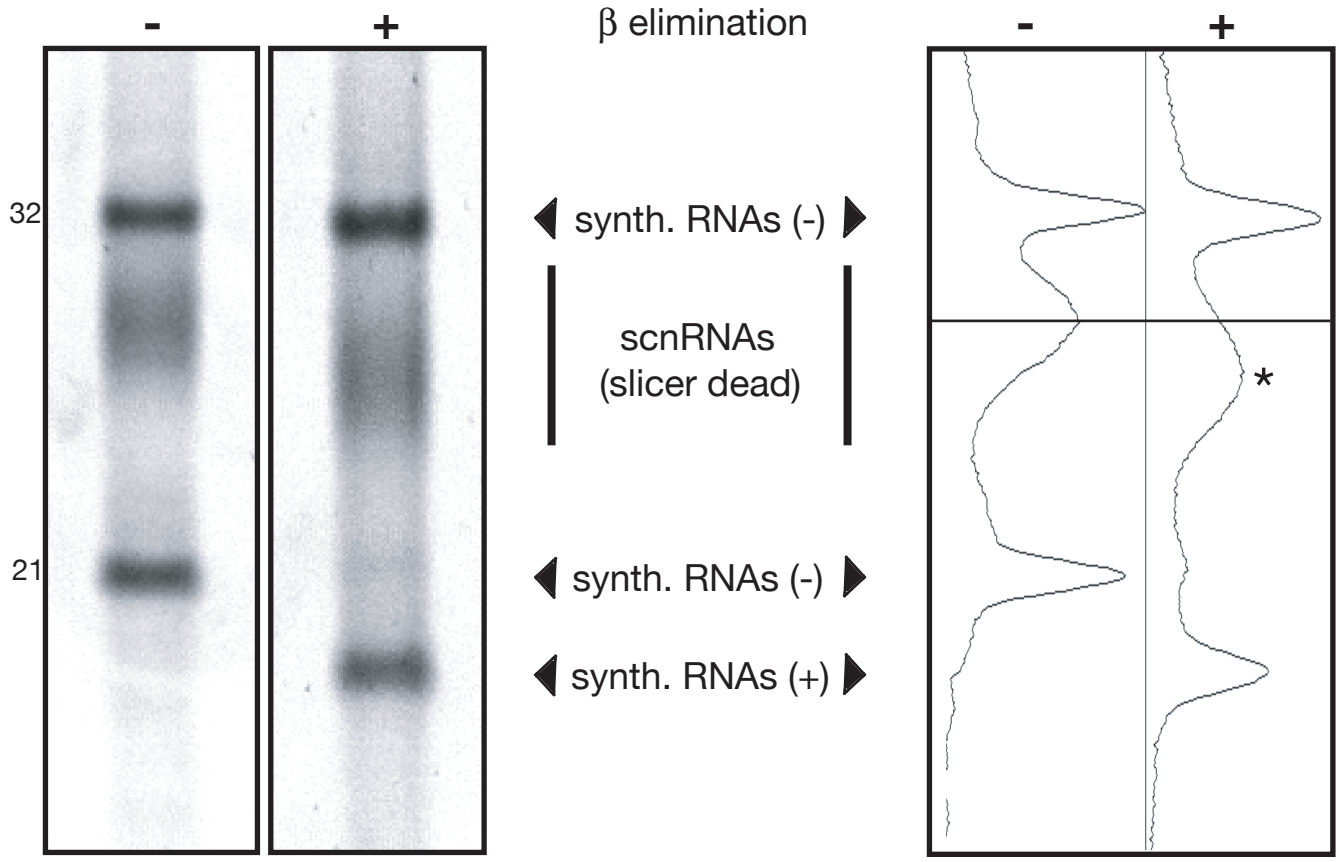

dsRNA
Arabidopsis (942aa)
Tetrahymena (423 aa)
I:20.0 S:31.4
Drosophila (391 aa)
I:20.0 S:33.3
Mus (395 aa)
I:31.4 S:41.9

Supplementary Figure S2

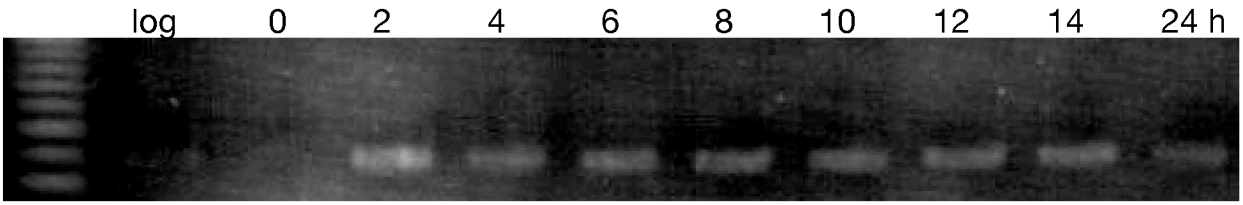
Synthetic RNAs:

I)	29 nt:	UCGAGUCGAGUUGAUCUUAGUUUCUUUUA
II)	29 nt me:	UCGAGUCGAGUUGAUCUUAGUUUCUUUUmA
III)	ds blunt:	UCGAGUCGAGUUGAUCUUAGUUUCUUUUA AGCUCAGCUCAACUAGAAUCAAAGAAAAU
IV)	ds overhang:	UCGAGUCGAGUUGAUCUUAGUUUCUUUUA ACAGCUCAGCUCAACUAGAAUCAAAGAAA
V)	32 nt	UCGAGUCGAGUUGAUCUUUAGUUUCUUUUAGC
	28 nt	UUCGAGUUGAUCUUUAGUUUCUUUUAGC
	24 nt	UGUUGAUCUUUAGUUUCUUUUAGC
	20 nt	UAUCUUUAGUUUCUUUUAGC
	16 nt	UUUAGUUUCUUUUAGC

Supplementary Figure 3

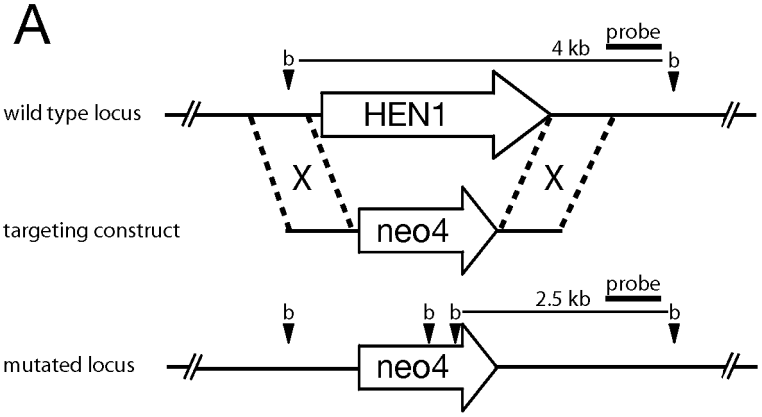


Supplementary Figure 4

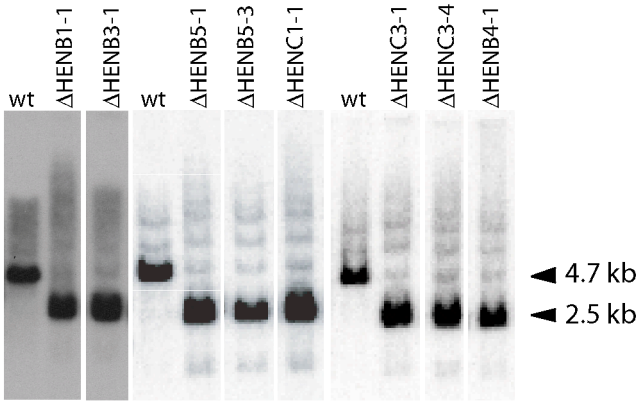


Supplementary Figure 5

A



B



C

Strains mated	Number of pairs examined	Number of pairs that gave rise to viable progeny	
ΔHEN1-B31 x ΔHEN1-C31	101	5	(5.0%)
ΔHEN1-B51 x ΔHEN1-C41	123	1	(0.8%)
ΔHEN1-B53 x ΔHEN1-C34	99	2	(2.0%)
ΔHEN1-B51 x ΔHEN1-C34	103	3	(2.9%)
B2086 x CU428	200	80	(40%)