

SUPPLEMENTARY INFORMATION

Arabidopsis *HUA ENHANCER 4* delays flowering by upregulating the MADS-box repressor genes *FLC* and *MAF4*

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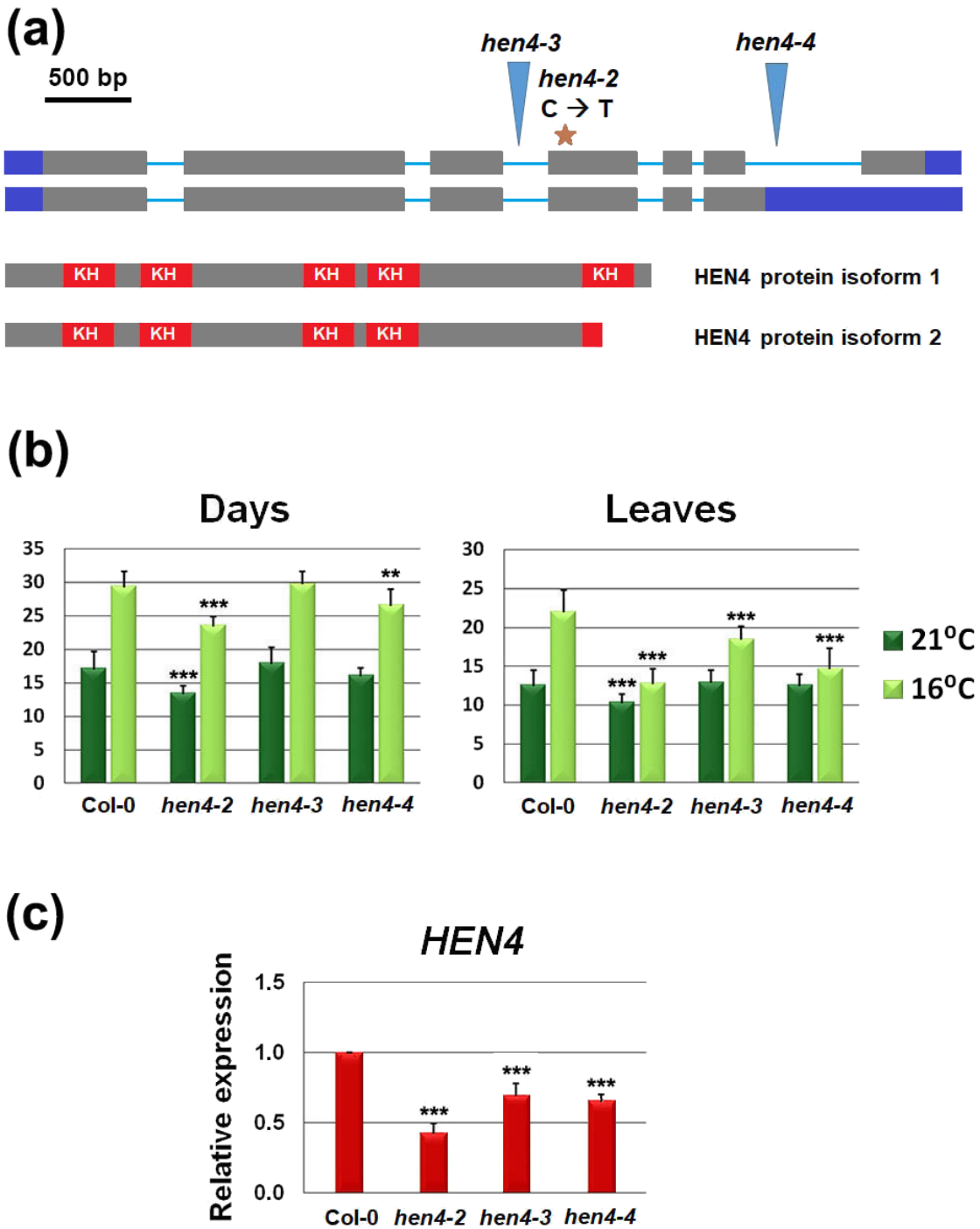
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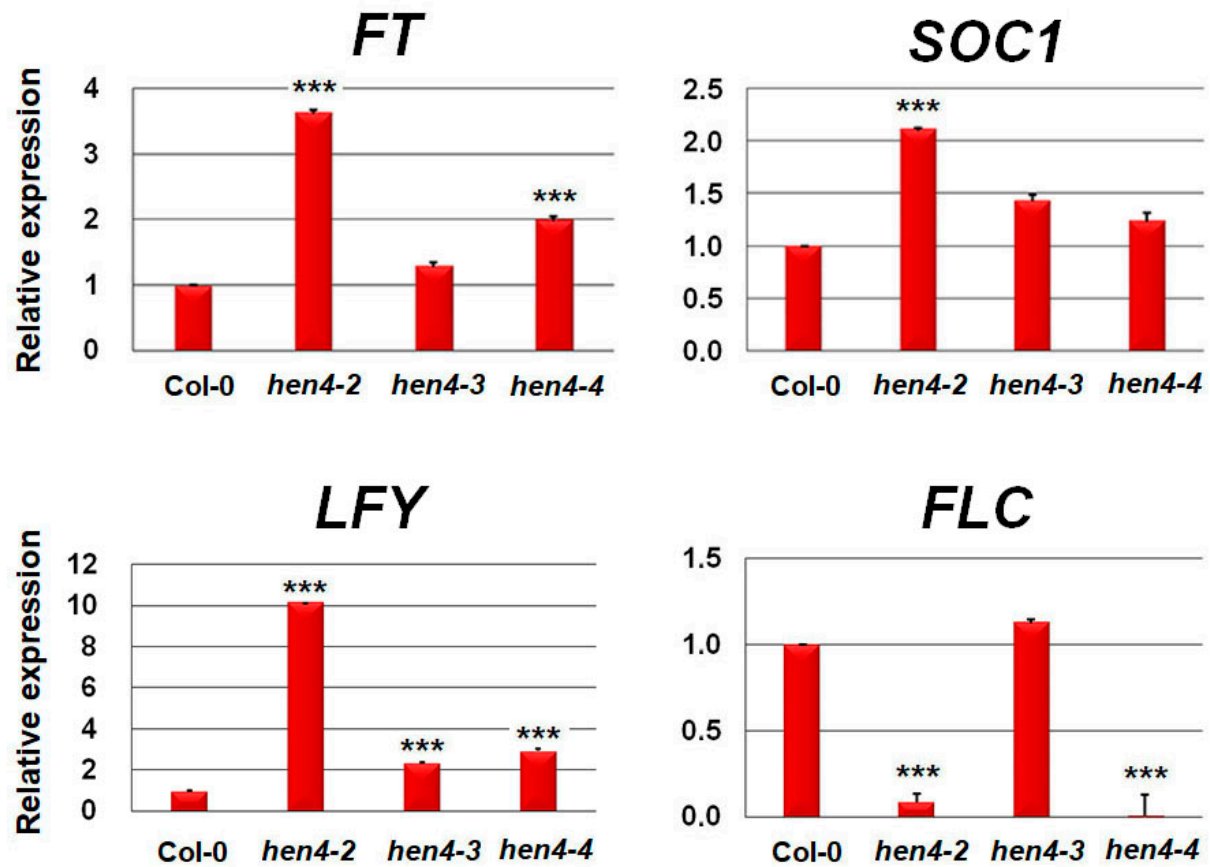
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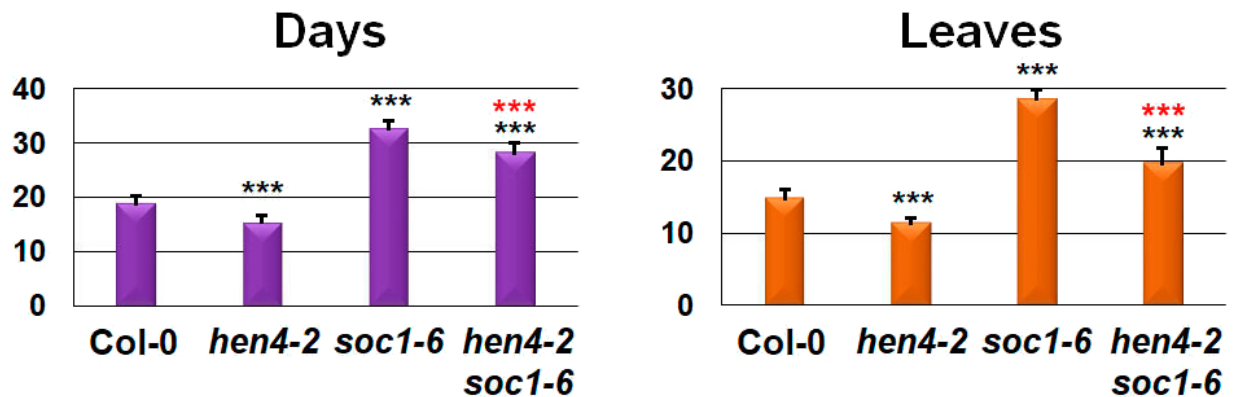
Supplementary Figure S1. *HEN4* gene structure and alleles employed. (a) The upper schematic view of the *HEN4* gene structure shows the two RNA isoforms described³¹ (TAIR10, <http://www.arabidopsis.org/>). The star indicates the relative position of the *hen4-2* point mutation³¹. T-DNA insertions corresponding to the *hen4-3* and *hen4-4*

alleles are marked by triangles. Thick bars denote exons, and introns are shown as thin blue lines. Grey-shaded areas correspond to coding sequences. Deep-blue areas are non-coding transcribed sequences. The two protein isoforms are represented below. Sequences corresponding to KH domains are red in color. (b) Flowering time at 21°C and 16°C of wild-type Col-0 strain and *hen4* mutants, measured as the number of days (left) or rosette leaves at bolting (right). Bars indicate means $\pm$ SD where n=21 plants per genotype. (c) Determination by qPCR of *HEN4* relative expression in *hen4* mutants grown at 21°C. Bars indicate means $\pm$ SD. In panels (b) and (c), significant differences with respect to Col-0 plants at the corresponding temperature are indicated: **, $P < 0.01$; ***, $P < 0.001$. ANOVA (b) and Student's *t*-test (c).



Supplementary Figure S2. Expression of *FT*, *SOC1*, *LFY* and *FLC* in different *hen4* mutants. Relative expression (qPCR) of relevant regulators of flowering time was measured in Col-0 and *hen4* mutant plants grown at 21°C. Bars indicate means $\pm$ SD. Significant differences with respect to Col-0 plants are indicated: ***, $P < 0.001$. Student's *t*-test.

(a)



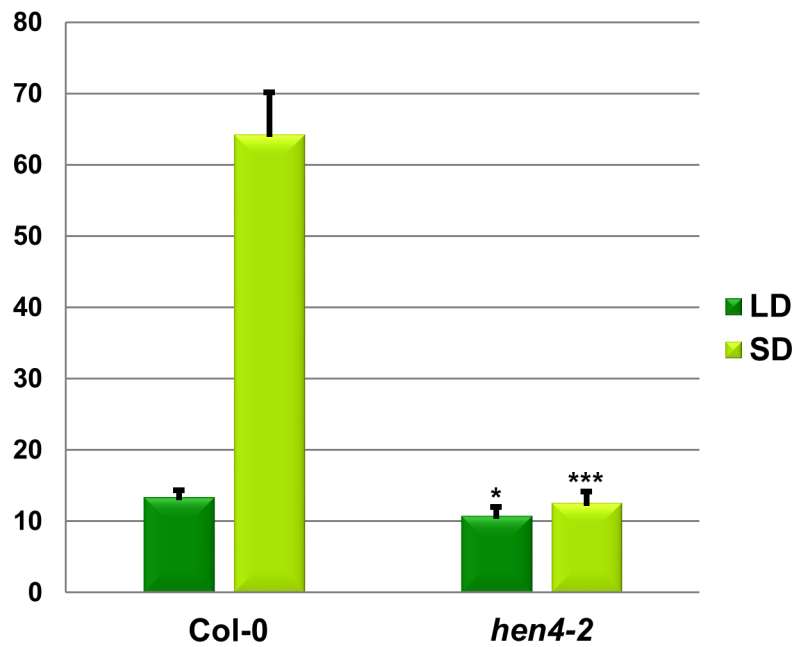
(b)



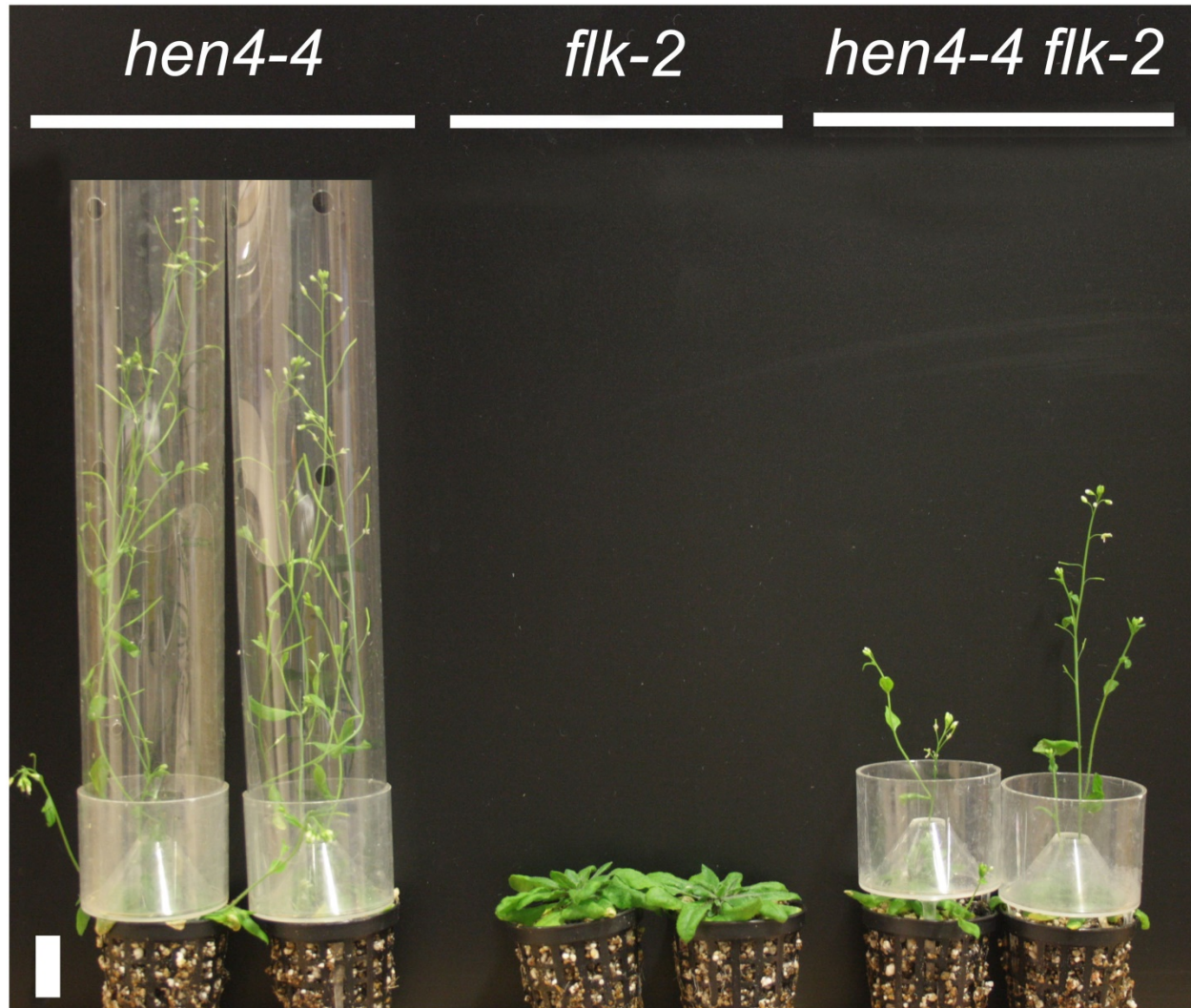
Supplementary Figure S3. Partial rescue of *soc1* late-flowering by *hen4*. (a) Flowering time measurements in terms of days (left) or rosette leaves at bolting (right) in the Col-0 wild type and diverse mutant strains. Bars indicate means $\pm$ SD where $n=21$ plants per genotype. Black asterisks indicate significant differences with respect to Col-0 whereas red asterisks denote significant variation with respect to *soc1-6* plants (***, $P < 0.001$, ANOVA). (b) Representative 28-day-old plants of the genotypes shown in (a) grown at 21°C. Scale bar: 2 cm.



Supplementary Figure S4. *hen4-2* rescues the *flk-2* late-flowering phenotype at 16°C. Representative 54-day-old *flk-2* and *hen4-2 flk-2* plants grown at 16°C. Scale bar: 2 cm.

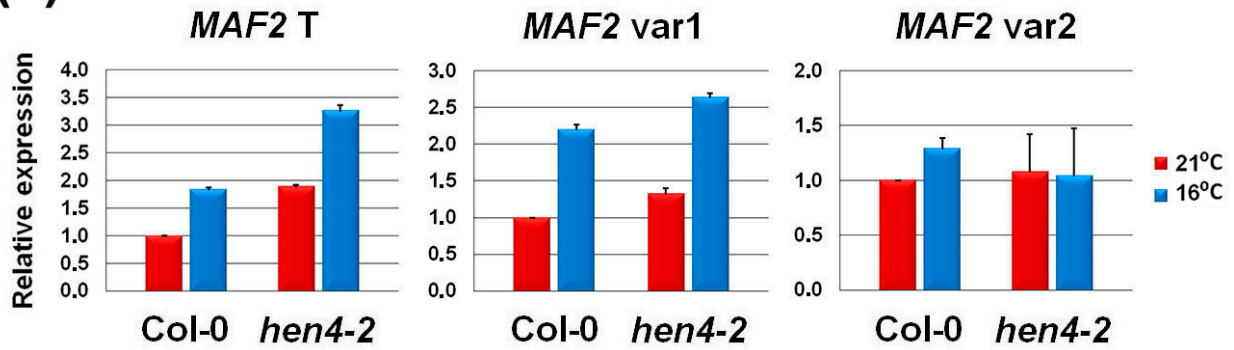


Supplementary Figure S5. Flowering time of Col-0 and *hen4-2* plants under short-day conditions. Flowering time was measured as the number of rosette leaves at bolting at 21°C under long-day (LD) or short-day (SD) light regimes. Bars indicate means $\pm$ SD where $n=21$ plants per genotype. Significant differences with respect to Col-0 plants under the same light regime are indicated: *, $P < 0.05$; ***, $P < 0.001$ (ANOVA).

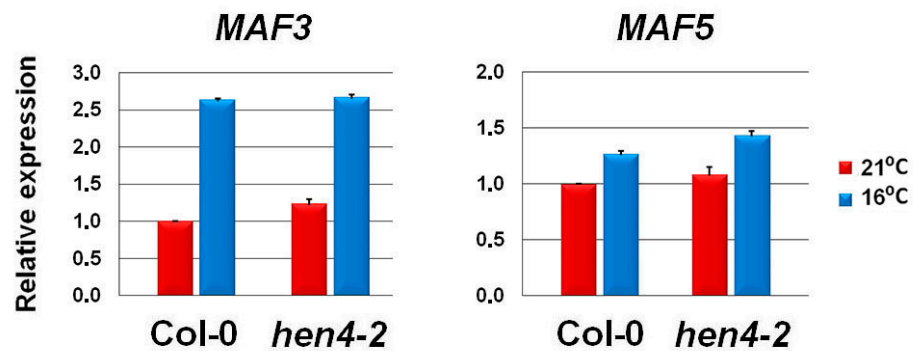


Supplementary Figure S6. Partial rescue of *flk-2* late-flowering by the *hen4-4* mutation. Representative 30-day-old *hen4-4*, *flk-2* and *hen4-4 flk-2* plants grown at 21°C. Scale bar: 2 cm.

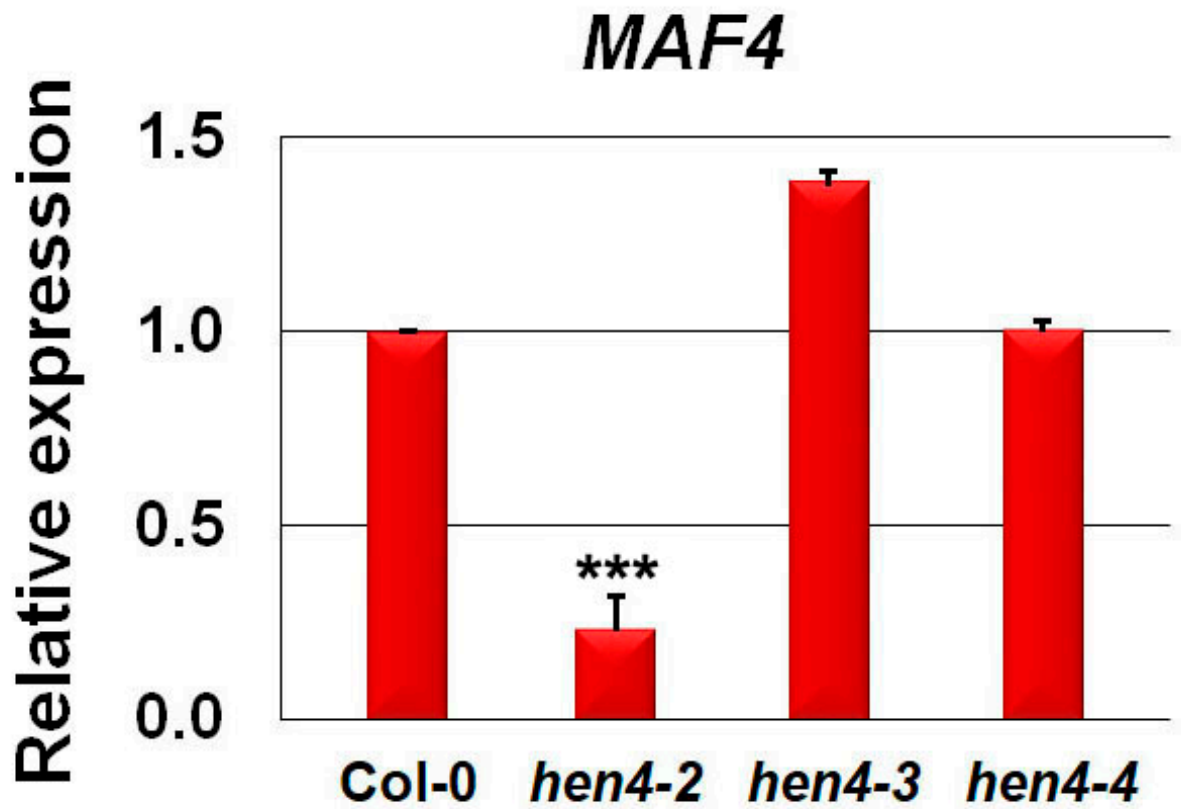
(a)



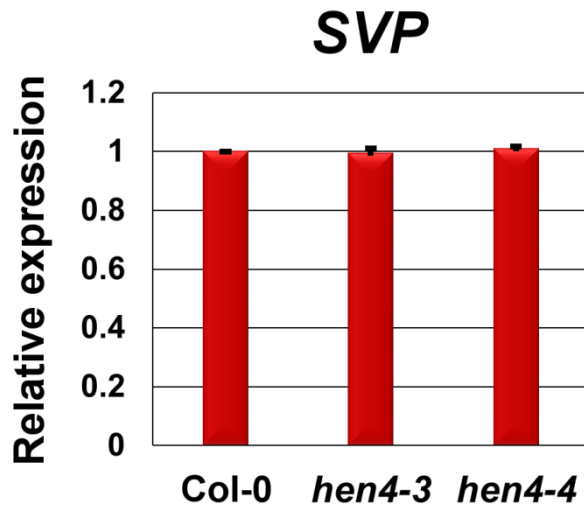
(b)



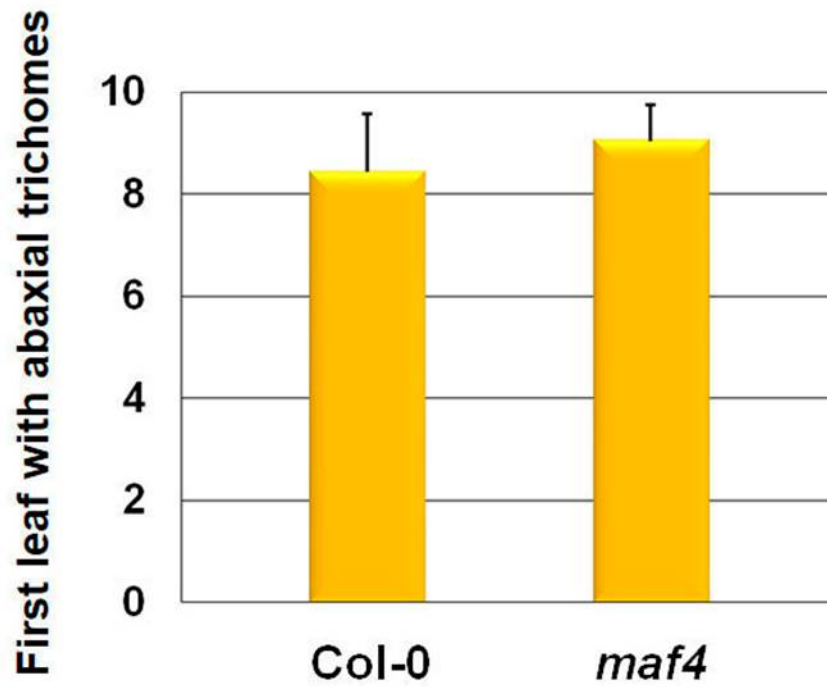
Supplementary Figure S7. Expression of *MAF2*, *MAF3* and *MAF5* in the *hen4-2* mutant background. (a) Relative expression (qPCR) of total and specific *MAF2* RNA splicing variants, *var1* and *var2*²⁸, at 21°C and 16°C, as monitored by qPCR. (b) Relative expression (qPCR) of *MAF3* (left) and *MAF5* (right) genes at 21°C and 16°C. Bars indicate means $\pm$ SD.



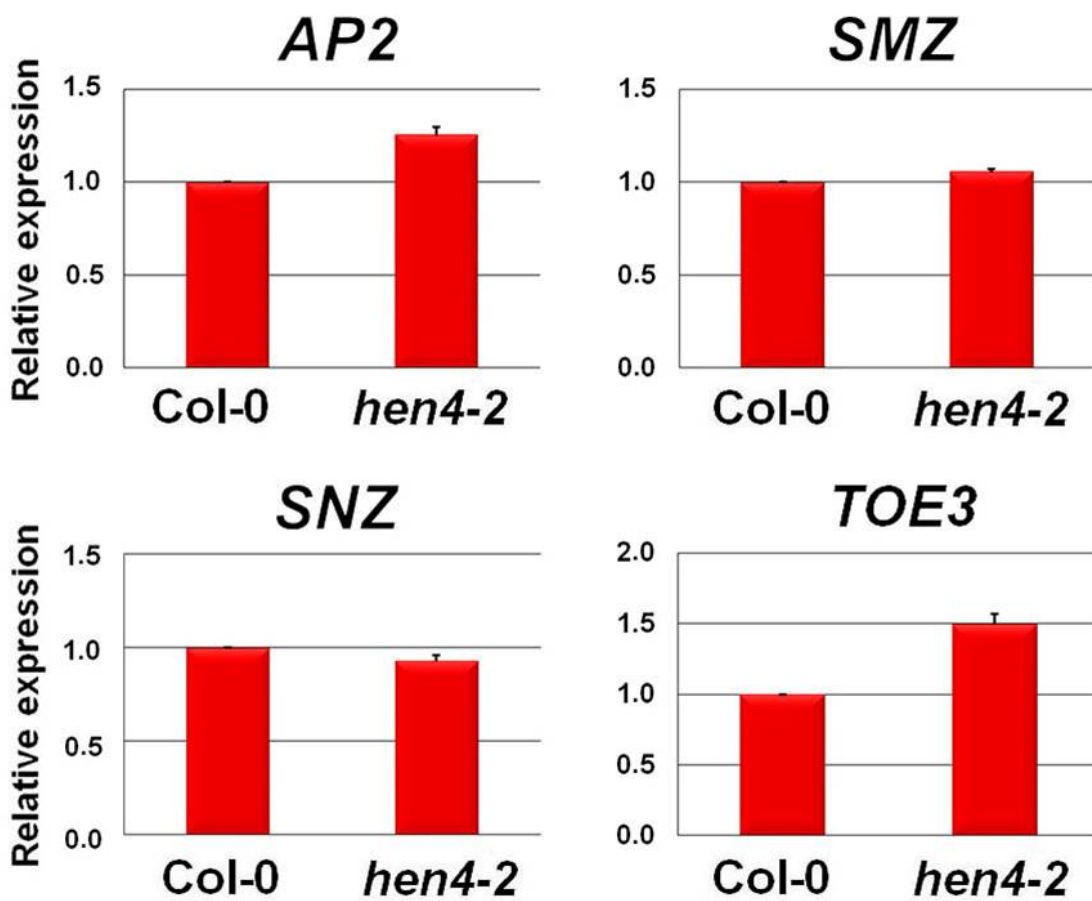
Supplementary Figure S8. *MAF4* expression in diverse *hen4* mutants. Relative *MAF4* expression (qPCR) at 21°C in the wild-type Col-0 and diverse *hen4* mutants. Bars indicate means $\pm$ SD and asterisks indicate significant differences with the wild type. ***, $P < 0.001$. Student's *t*-test.



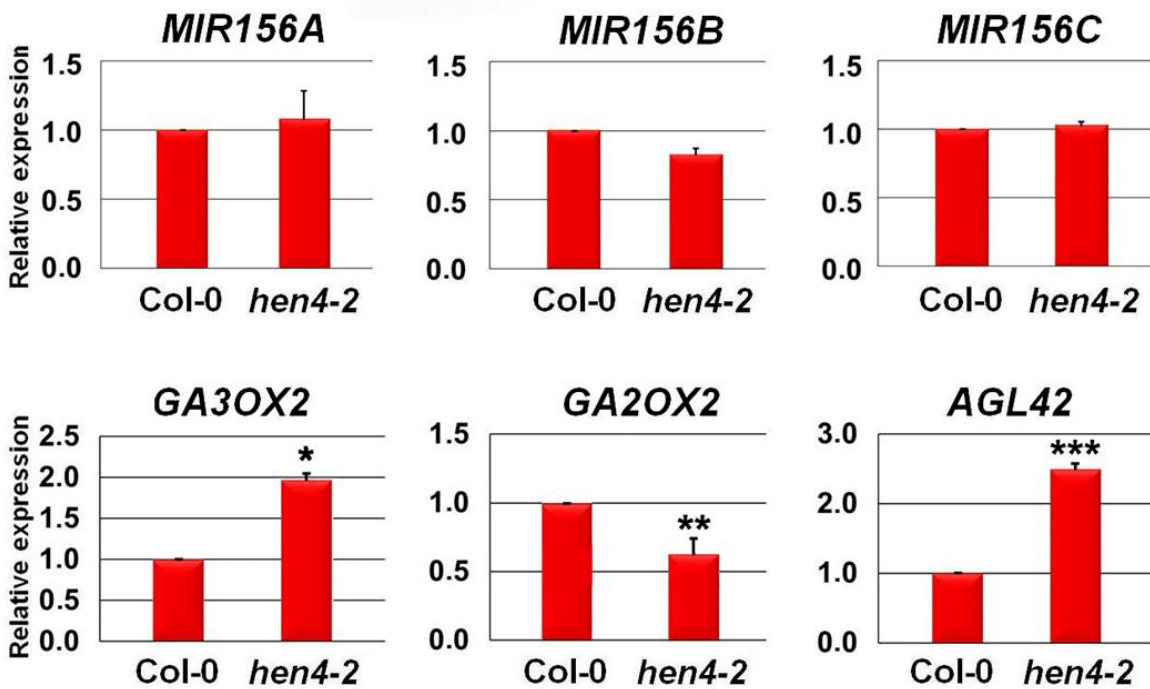
Supplementary Figure S9. *SVP* RNA levels were unaltered in the weaker *hen4-3* and *hen4-4* mutants. Relative expression of *SVP* in Col-0 wild type, and *hen4-3* and *hen4-4* mutant plants grown at 21°C as monitored by qPCR. Bars represent means $\pm$ SD. No significant differences were observed.



Supplementary Figure S10. Onset of abaxial trichomes in *maf4*. Appearance of leaf abaxial trichomes in wild-type Col-0 and *maf4* mutant plants grown at 21°C. Bars represent means $\pm$ SD where n=21 plants per genotype.



Supplementary Figure S11. Expression of *AP2*, *SMZ*, *SNZ* and *TOE3* in *hen4-2*. Relative expression at 21°C was monitored by qPCR. Bars represent means $\pm$ SD.



Supplementary Figure S12. Expression of *MIR156* and GA-related genes. Relative expression at 21°C was monitored by qPCR. Bars represent means $\pm$ SD. Asterisks indicate significant differences with Col-0. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Student's *t*-test.

Supplementary Table S1. Genotyping procedures.

Purpose	Name	Oligonucleotide sequence (5'-3')	PCR products size in base pairs
<i>flm-3</i> genotyping	SJ1-31	CGGAGAAACCTCAATGTTTTG	400 wild-type 450 mutant
	SJ1-32	GGTTTTGTGGAGTAATTGGTTG	
	LBa1	TGGTTCACGTAGTGGGCCATCG	
<i>hen4-3</i> genotyping	hen4-3 F1	GATGCTTCATAGCGAAACAGG	1187 wild-type ---- mutant
	hen4-3 R1	GTTTACCATGGACCACAGT	
	LBTM3	TAGCATCTGAATTTTCATAACCAATCTCGATACAC	
<i>hen4-4</i> genotyping	hen4-4 F1	ATCAAATCTCAGTTCCATGCG	1000 wild-type 600 mutant
	hen4-4 R1	AGTGGACACAAATCTGCCATC	
	LBTM3	TAGCATCTGAATTTTCATAACCAATCTCGATACAC	
<i>maf4</i> genotyping	SJ1-70	ACTGAGGATCTTGGCCATG	900 wild-type 700 mutant
	SJ1-112	TGAGTTATTGGGTCTCATGGG	
	LBb1	AACCAGCGTGGACCGCTTGCTG	
<i>soc1-6</i> genotyping	SJ1-118	AAGGATGAGGTTTCAAGCGTC	1112 wild-type 600 mutant
	SJ1-119	TGGCGAATTCATAAAGTTTGC	
	LBb1.3	ATTTTGCCGATTTTCGGAAC	
<i>svp-32</i> genotyping	SVP-32 F	TCATCCATATCTTGCAATGCC	1100 wild-type 800 mutant
	SVP-32 R	TCAGCGAACTTCAGAAAAAGG	
	LBb1.3	ATTTTGCCGATTTTCGGAAC	
In <i>hen4-3</i> genotyping “----” indicates absence of any band			

Additional genotyping procedures were as previously described:

- *flc-3* and *flk-2*³⁷
- *hen4-2*³³

Supplementary Table S2. Oligonucleotides for qPCR.

Gene	Name	Oligonucleotide sequence (5'-3')
<i>AGL42</i>	SJ1-43	GCAACGAGTAGACAAGTGAC
	SJ1-44	TTGTGAGTCGTGATTGCTG
<i>AP2</i>	AP2 qF	CGACGCACCACACCAAACACAA
	AP2 qR	CACGCCGACTCTTTTCAACGG
<i>FLM</i> β	FLMb qPCR 1F	CAACATGCTGATGAACTTAGAGCCTT
	FLM qPCR 1R	CATCTGTTGCCAGCAACGTAT
<i>FLM</i> δ	FLMd qPCR 1F	GCTGTTCAAGCCGGAGAAACCT
	FLM qPCR 1R	CATCTGTTGCCAGCAACGTAT
<i>GA2OX2</i>	SJ1-104	CGTACAAGGTGTTGGAGATGGTTGC
	SJ1-105	TCATCTTGCTCAGGGACAAGGCATG
<i>GA3OX2</i>	SJ1-106	CTGCTTGGGTTCCTTGAAAGTCTGAA
	SJ1-107	CGAAGGTTTCACCGTTATTGGCTCTC
<i>GIS</i>	SJ1-98	GGTAACTGTAAGGGTTATTGTCAGGATG
	SJ1-99	CCTAAACCCATTCTGTCTTCGGAA
<i>HEN4</i>	SJ1-29	TGGACACAAATCTGCCATCG
	SJ1-30	CAATGAGGTTTCACCTGTAAGG
<i>MAF2 T</i>	MAF23-qPCR-1F	CATTGTGGGTCTCCGGTG
	MAF2-QRT-1R	TTCTTTTCGCATGAGCTTCTGTTTTA
<i>MAF2 var1</i>	MAF23 qPCR 3F	GAGTTACTAGAAATAGTCCAAAGCAAGCTTGAA
	MAF2-QRT-1R	TTCTTTTCGCATGAGCTTCTGTTTTA
<i>MAF2 var2</i>	MAF2v2 qPCR 2F	AGCACAAAGACACTTTTATCTCCCTCT
	MAF2-QRT-1R	TTCTTTTCGCATGAGCTTCTGTTTTA
<i>MAF3</i>	SJ1- 126	ACAGAACTAATGATGGAGGATATGAA
	MAF3-qPCR-1R	ACTCTGATATTTGTCTACTAAGGT
<i>MAF4</i>	MAF4-QRT-1F	CACAATCAAATTAGGTCAGAAGAA
	SJ1- 70	ACTGAGGATCTTGGCCATG
<i>MAF5</i>	SJ1-96	TCGGAAGAGTGAAGCCATGGGAAGAA
	SJ1-116	CTTATCCTGAAGGTTCTTCACAAGCTCCATC

Gene	Name	Oligonucleotide sequence (5'-3')
<i>MIR156A</i>	SJ1-79	CTGACAGAAGAGAGTGAGCACACAAAG
	SJ1-81	AGTGAGCACGCAAGAGAAGCAAG
<i>MIR156B</i>	SJ1-68	GCTAGAAGAGGGAGAGATGGTGATTGAG
	SJ1-69	GTGAGCACGCACACGCAAAGTTATAGAC
<i>MIR156C</i>	SJ1-79	CTGACAGAAGAGAGTGAGCACACAAAG
	SJ1-80	CAGATAGAGCAGTGAGCACGCAAG
<i>MIR172B</i>	oJJR173q	TTATACAAGTTGTGCGCGGATCCATG
	oJJR174q	CGATCCAGACTTCAATCAATATCTTCAAG
<i>MIR172E</i>	oJJR185	TATCAGCCAGTAGTCGCAGATGCAG
	oJJR186	GACAAGAGTAGCCATGTATTTGCTG
<i>SMZ</i>	SJ1-120	CCCATCGTGGACCGATTGATACCTTATAGTA
	SJ1-121	ATCAAATTCCGTGGTCTCGATGCAGACATCAA
<i>SNZ</i>	SJ1-122	AAGATATAAGTCACATAATCATTCCGTCATTC
	SJ1-123	GATATTAAGTTGGGTGCCCATAGT
<i>SPL3</i>	SJ1-142	CGGATATGAGCAAAGCCAAACAG
	SJ1-143	CCTTCTCTCGTTGTGTCCAGC
<i>SPL9</i>	SJ1-86	CGAGACACCGAGTTTGTGGA
	SJ1-87	GCTAACACAGAGAGAGACGC
<i>SVP</i>	SVP qF	TTCGAAACGAAGAAGAGGGCT
	SVP qR	AGTCCTTGAAGTTCCTCTCCT
<i>TEM1</i>	SJ1-94	GAGCGACGTCGGGAAGCTGAACCGTTTA
	SJ1-95	TTTAACGAACCGGCTCCAGCCCTTGGT
<i>TEM2</i>	SJ1-84	CTTCAGAATCTTTCTCCGCC
	SJ1-85	TTTCGTGACTCCGTCTCTAG
<i>TOE3</i>	SJ1- 127	CGATTGCTGCTTTTCCGATCTGAC
	SJ1- 128	GACGACGAGGAACGGTCATAATCTT

Additional PCR reactions were as previously described:

FLM, *SPL8*, *SPL15*³³; *FT*³⁷; *LFY*, *SOC1*, *TSF*⁷⁰; *OTC*³²