miR396-targeted AtGRF transcription factors are required for coordination of cell division and differentiation during leaf development in *Arabidopsis*

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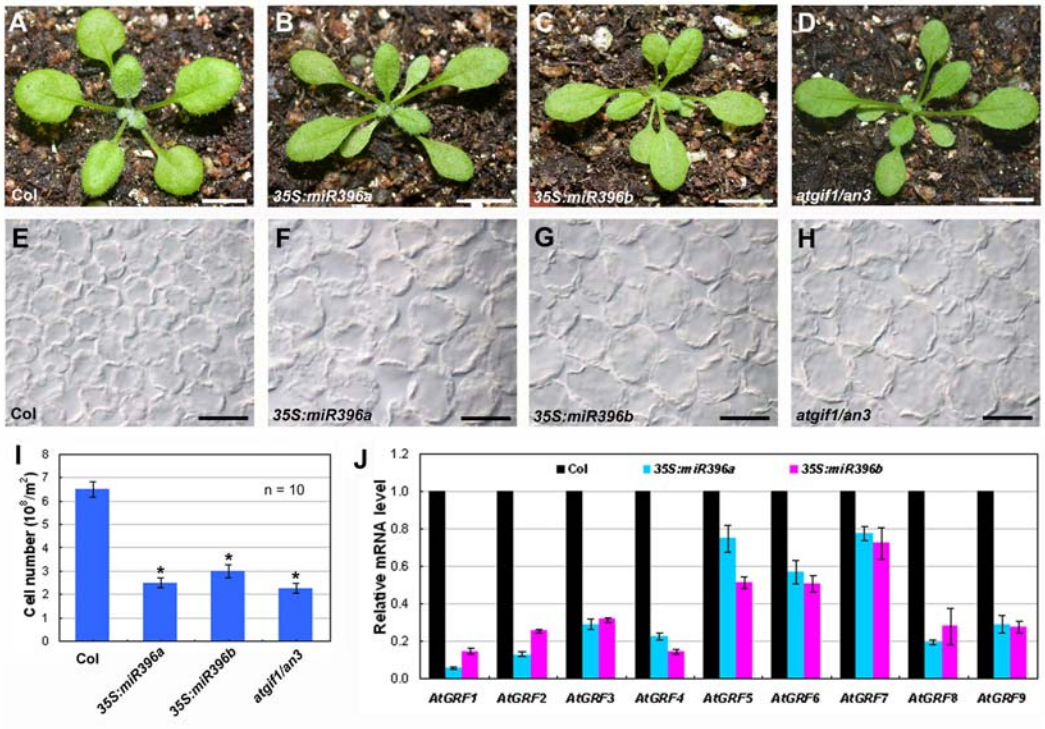
Supplemental Data

The following materials are available in the online version of this article.

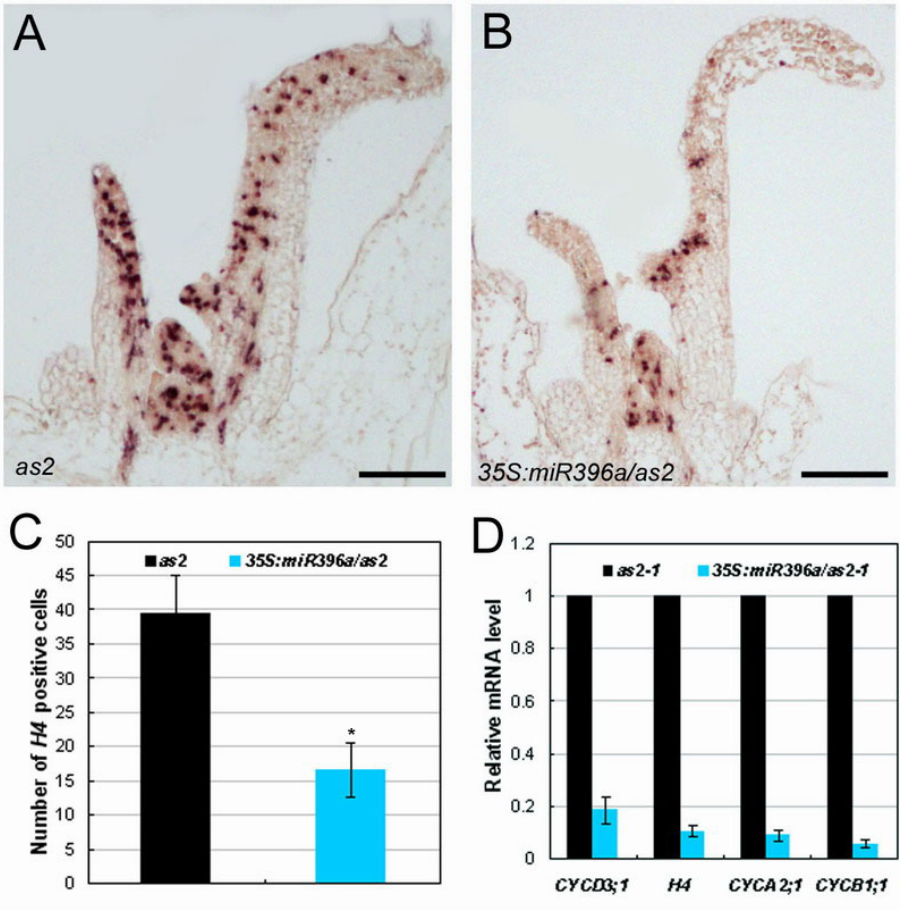
Supplemental Table S1. The sequences of primers used in this study.

Name	Gene	Sequence (5'-3')	Purpose
AtGRF1-F	*AtGRF1*	GCCAATGTCCCTGTTCCATC	qPCR
AtGRF1-R	*AtGRF1*	CTGTAAGTTCATCGTGGCAGG	qPCR
AtGRF2-F1	*AtGRF2*	GCATTCAGGGAATCACAATC	qPCR
AtGRF2-R1	*AtGRF2*	TCAGGTTGTGTAATGAAAGTAATC	qPCR
AtGRF3-F	*AtGRF3*	CTGAACTTCTCCACCTTAGTC	qPCR
AtGRF3-R	*AtGRF3*	CACTGGTCAATGAAAGGCTTG	qPCR
AtGRF4-F	*AtGRF4*	TGTTCCATGGACTTGCAACTG	qPCR
AtGRF4-R	*AtGRF4*	GCCGTCAGTTCTCTTACACCT	qPCR
AtGRF5-F	*AtGRF5*	GTTGTCTGATTCTGATCAAGAG	qPCR
AtGRF5-R	*AtGRF5*	GAGAATAAGATGATGAGTCT	qPCR
AtGRF6-F	*AtGRF6*	CTGATGGCAAGAAATGGAG	qPCR
AtGRF6-R	*AtGRF6*	TCAAATGAAGAGTGAAGTAG	qPCR
AtGRF7-F	*AtGRF7*	CTCAAACTATAGAGACTCC	qPCR
AtGRF7-R	*AtGRF7*	CTAAACCTGGCTGCTTTCG	qPCR
AtGRF8-F	*AtGRF8*	GCCTCCTCTCCATATGATC	qPCR
AtGRF8-R	*AtGRF8*	TTAGCTTGAGCTTCTGCTGCAGC	qPCR
AtGRF9-F1	*AtGRF9*	CTCAAGCAACAGGAAGAATG	qPCR
AtGRF9-R1	*AtGRF9*	AACACCTGGTGAAAACAAAGAC	qPCR
AtGRF2-F2	*AtGRF2*	CCTGTTGTCGCTGGTAATAC	probe
AtGRF2-R2	*AtGRF2*	GATTGGTATCCAAGTATTCACC	probe
AtGRF9-F	*AtGRF9*	AGATCTATGCAGAGCCCTAAAATGGAGC	clone
AtGRF9-R	*AtGRF9*	ACTTCTAGACTAAACACCTGGTGAAAAC	clone
rAtGRF9-F1	*AtGRF9*	CAGCCGCAAATTGGTAGAGTCTTCTTCTGAG GTTGC	clone

rAtGRF9-R1	*AtGRF9*	CTCTACCAATTTGCGGCTGCGTTTACGACCT CTATG	clone
p396A-F	*miR396a*	CACCGATGAGGACATGAGCTCTTTCGAGC	promoter
p396A-R	*miR396a*	GAATACAGAGAGGGTCATGTAGAG	promoter
p396B-F	*miR396b*	CACCCCGATTGTCCGTGGTAAACCGCG	promoter
p396B-R	*miR396b*	AAGTATGACCAGGATCTTCATC	promoter
H4-F	*Histone H4*	ACACGG ATC GAT ATC TCA GCC	probe
H4-R	*Histone H4*	CAC CGA ATC CGT AAA GAG TCC	probe
pAS1-F	*AS1*	AAGCTTACGGAGGGTGTGAGTGAGTAGT	promoter
pAS1-R	*AS1*	GAGCTCCTCCTACTCCTCCTGACATCAC	promoter
pSTM-F	*STM*	AAGCTTAGAAATGGCAGTGAAGGCAGTG	promoter
pSTM-R	*STM*	GAGCTCACTAGTATTATTATTCACTTTGG	promoter
pAN3-F	*AN3*	AAGCTTTCGGATCCATTTTTGGTACCAAC	promoter
pAN3-R	*AN3*	GAGCTCTTCTTTTGCTATTTTATATAAAACCT G	promoter
pREV-F	*REV*	ATCAAGCTTGAATTCAATTGTAACTACTAAC	promoter
pREV-R	*REV*	ATCGAGCTCTCGACCCTCAAAAAAAGTCTC	promoter
An3-F	*AN3*	ATCACTAGTGAATTCATGCAACAGCACCTGA TGCAG	T-DNA, probe
An3-R	*AN3*	ATCGGATCCATTCCCATCATCTGATGATTTC	T-DNA, probe
ACTIN-F	*ACTIN*	TGGCATCA(T/C)ACTTTCTACAA	qPCR
ACTIN-R	*ACTIN*	CCACCACT(G/A/T)AGCACAATGTT	qPCR
FIL-F	*FIL*	CTTACTTCAATCCCCAGG	probe
FIL-R	*FIL*	CTTTTGGACATGATAAACCC	probe

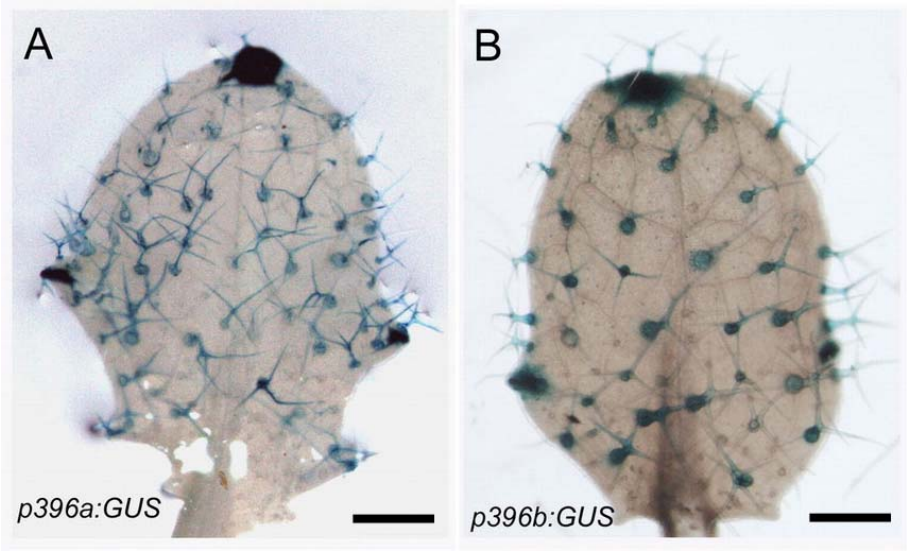


Supplemental Fig. S1 miR396 negatively regulates cell proliferation by repressing *AtGRFs* expression in the leaves. (A-D) Morphological observations of leaf development of wild-type Col (A), *35S:miR396a* (B), *35S:miR396b* (C) and *atgif1/an3* (D) plants. (E-H) Palisade cells of the first appearing rosette leaves of 3-week-old wild-type (E) and *ae7* (D,G) *35S:miR396a* (F), *35S:miR396b* (G) and *atgif1/an3* (H) seedlings, showing that the cell size in F,G and H is enlarged. (I) Compared with the wild-type plant, the number of palisade cells in the first rosette leaves of *35S:miR396a*, *35S:miR396b* and *atgif1/an3* plants is reduced. * Significant statistical differences by t-test ($P < 0.05$). (J) qRT-PCR to analyze the transcript levels of *AtGRFs* in 14-day-old plants of wild type, *35S:miR396a*, and *35S:miR396b* plants. Error bars in I and J indicate SE. Bars =1 cm in A-D, and 100 μm in E-H.



Supplemental Fig. S2 *In situ* hybridization and qRT-PCR analyses of cell cycle-related genes in *as2* and *35S:miR396a/as2* transgenic plants.

(A-B) *In situ* hybridization analysis of histone *H4* expression in shoot apices of 13-day-old *as2* (A) and *35S:miR396a/as2* (B) seedlings. (C) Statistical analysis of the number of histone *H4* positive cells in leaves of the 13-day-old *as2* and *35S:miR396a/as2* seedlings. * Significant statistical differences by t-test ($P < 0.05$). (D) Expression of cell cycle-related genes in 13-day-old *as2* and *35S:miR396a/as2* seedlings. Bars = 100 μm in A and B.



Supplemental Fig. S3 Expression of *p396a:GUS* and *p396b:GUS* in leaf trichomes.

(A-B) GUS staining of *p396a:GUS* (A)and *p396b:GUS* (B) transgenic leaves showing the strong signals in leaf trichomes. Bars = 1 mm in A and B.