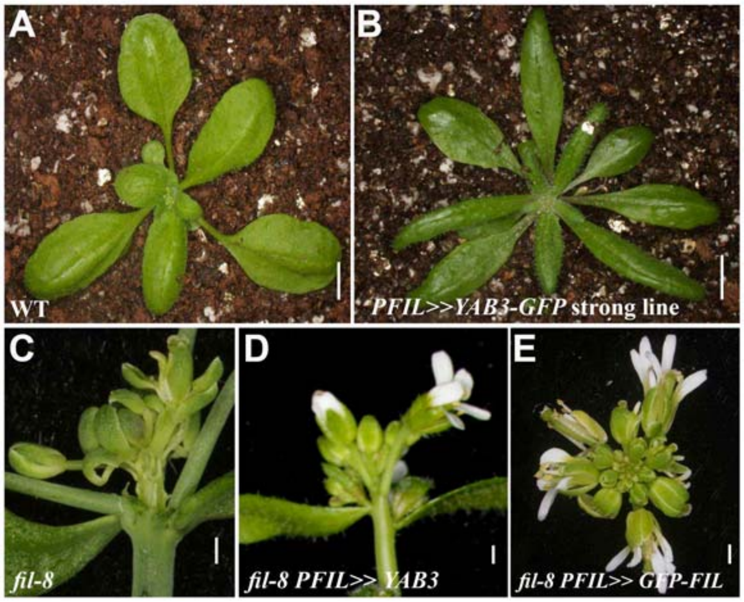
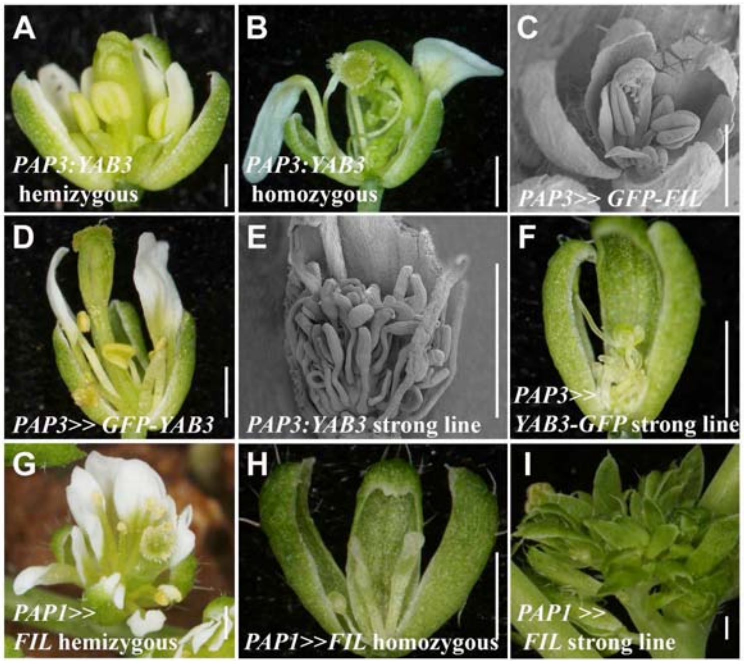


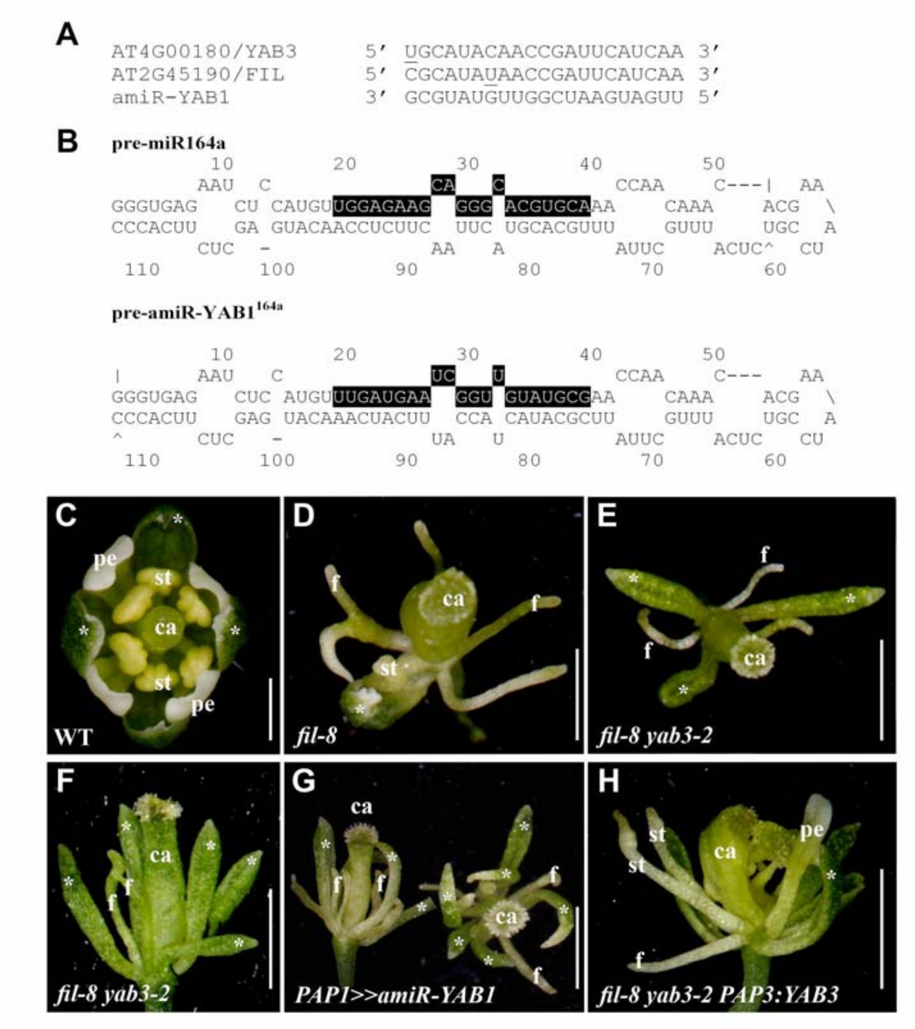
Supplemental Figure 1. Equivalence of FIL and YAB3 proteins.

A-B: Narrow leaves are induced when promoter *FIL* drives expression of a strong *OP:YAB3–GFP* line in a wild-type background. **C-E**: Complementation of the *fil-8* mutant (**C**) by a weak *OP:YAB3* responder line (**D**) or an *OP:GFP-FIL* intermediate line (**E**) trans activated by the promoter *FIL* driver. Bars are 1cm in (**A-B**) and 1mm in (**C-E**).



Supplemental Figure 2. Phenotypic range of effects induced by ectopic expression of various forms of YAB1.

A-F: Side view of *Arabidopsis* flowers with ectopic expression of YAB1 constructs driven by the *APETALA3* (*AP3*) promoter. Hemizygous (**A**) and homozygous (**B**) flowers from a weak *PAP3:YAB3* direct fusion line. Flowers with trans-activation of intermediate *OP:GFP–FIL* (**C**) and intermediate *OP:GFP-YAB3* (**D**) responder lines. Flowers of strong *PAP3:YAB3* (**E**) and *PAP3>>YAB3-GFP* lines (**F**). **G-I:** Side view of flowers with ectopic expression of YAB1 constructs mediated by the *APETALA1* (*AP1*) promoter. Flowers from plants expressing a weak *OP:FIL* line trans-activated by the *PAP1:LhG4* driver promoter in a hemizygous (**G**) or homozygous (**H**) state. Side-view of an inflorescence of a strong *PAP1>>FIL* line (**I**).



```
A
AT4G00180/YAB3     5' UGCAUACAACCGAUUCAUCAA 3'
AT2G45190/FIL      5' CGCAUAUAACCGAUUCAUCAA 3'
amiR-YAB1          3' GCGUAUGUUGGCUAAGUAGUU 5'
```

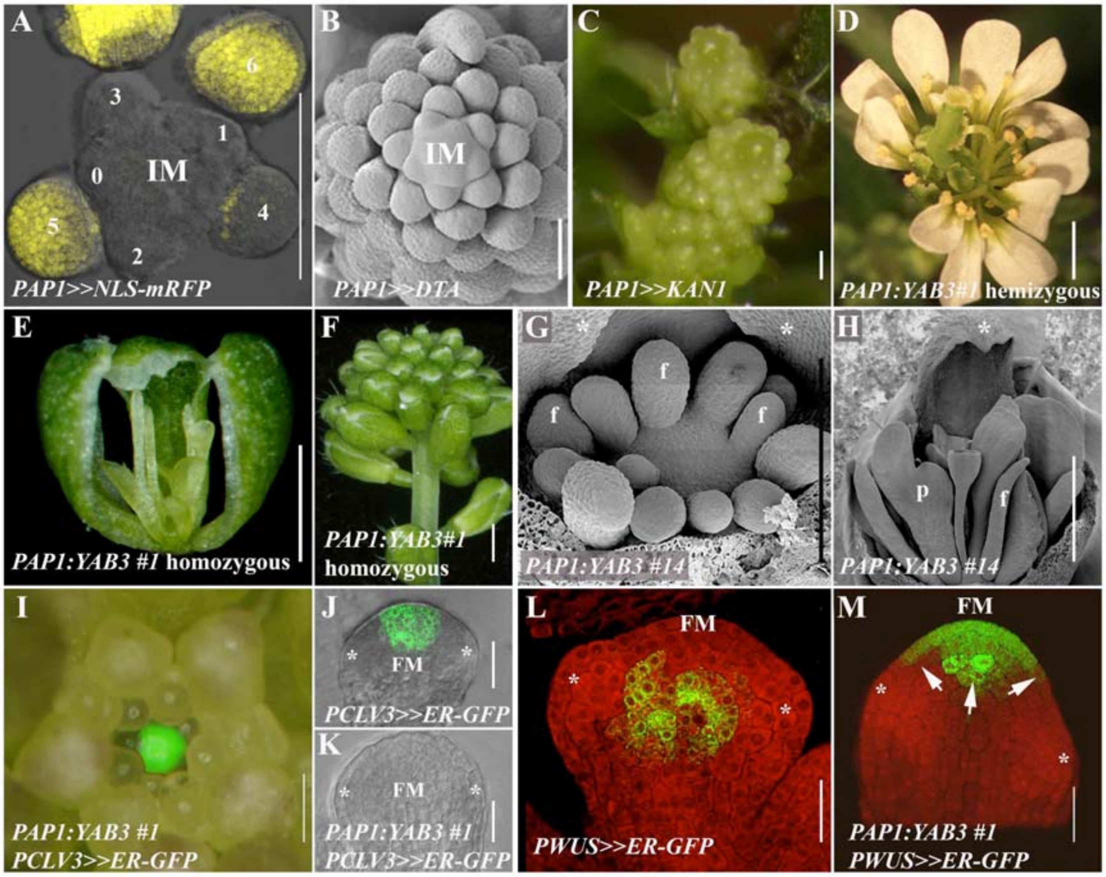
```
B pre-miR164a
        10        20        30        40        50
       AAU  C             CA   C         CCAA    C---|  AA
GGGUGAG   CU CAUGUUGGAGAAG  GGG ACGUGCAAA    CAAA    ACG  \
CCCACUU   GA GUACAACCUCUUC  UUC UGCACGUUU    GUUU    UGC  A
       CUC  -             AA   A         AUUC    ACUC^  CU
 110       100        90        80        70        60
```

```
pre-amiR-YAB1^164a

        10        20        30        40        50
|      AAU  C             UC   U         CCAA    C---   AA
GGGUGAG   CUC AUGUUUGAUGAA  GGU GUAUGCGAA    CAAA    ACG  \
CCCACUU   GAG UACAAACUACUU  CCA CAUACGCUU    GUUU    UGC  A
^      CUC   -            UA   U         AUUC    ACUC   CU
 110       100        90        80        70        60
```

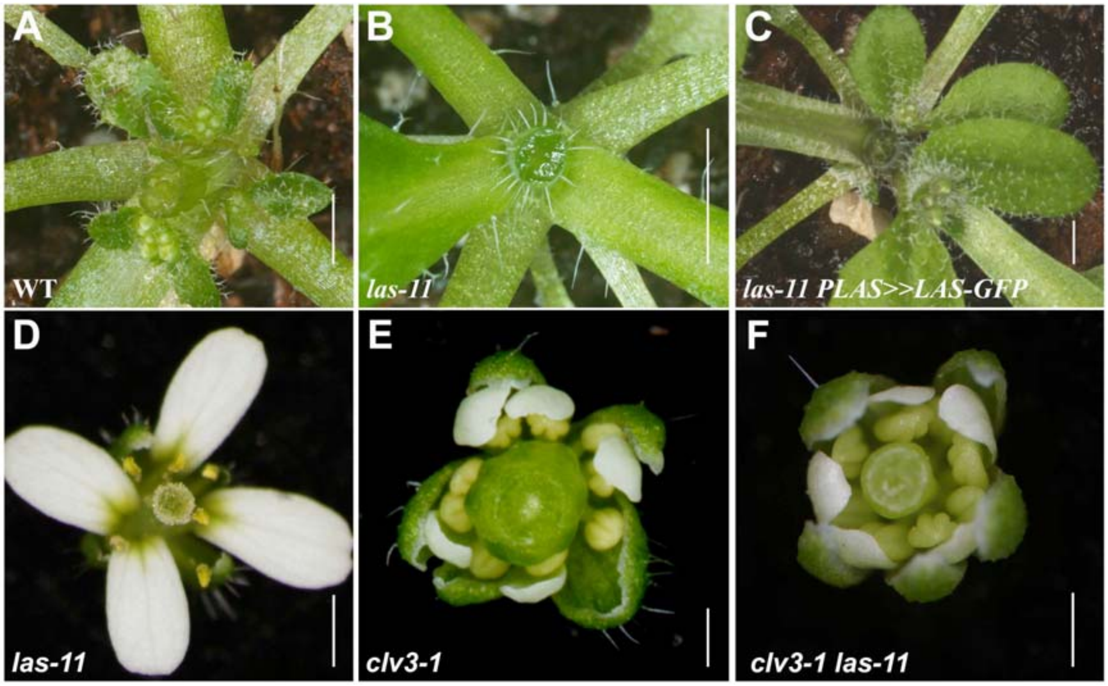
Supplemental Figure 3. Sequential effects of YAB1 revealed by the use of a synthetic miRNA targeting the YAB1 genes.

A-B: Conceptual design of the *amiR-YAB1*. (**A**) RNA alignment of *amiR-YAB1* and the complementary regions of *FIL* and *YAB3*. G-U wobbles between the *amiR-YAB1* and the target sequences are denoted by underlining the relevant nucleotide in the target sequence. (**B**) Theoretical representations of the pre-miR164a and pre-amiR-YAB1^{164a} secondary structures. The miRNA sequence is highlighted. **C-H:** Effects of altered YAB1 levels on 2nd, 3rd and 4th whorl development. **(C)** Stereotypic organization of wild-type flower. Disrupted phylotactic patterns and formation of filamentous or chimeric organs characterize mature *fil-8* (**D**) and *fil-8 yab3-2* (**E**) flowers. **(F)** Side view of a mature *fil-8 yab3* mutant flower. Only 2 filamentous structures have developed in 2nd and 3rd whorls. (**G**) Side and aerial view of *PAP1>>amiR-YAB1* flowers. Only filamentous structures are present in the 2nd and 3rd whorls. (**H**) Side view of a *fil-8 yab3* mutant flower with ectopic expression of YAB3 in the 2nd and 3rd whorls. Carpel development is abnormal while stamen morphology is partially complemented. Petal development and number are only partially complemented. Bars are 1mm. *- sepals, f- filaments, st- stamens, pe- petals, ca- carpels.



Supplemental Figure 4. Unique responses of flower meristems to ectopic YAB1 expression.

A: Transverse section of an inflorescence showing the onset of *PAP1>>NLS-mRFP* expression. **B:** Top view of an *PAP1>>DTA* inflorescence. Note the ordered phyllotaxis of the aborted flower primordia. **C:** Aerial view of *PAP1>>KAN1* inflorescences. **D:** Flower of a weak *PAP1:YAB3* hemizygous line with numerous petals and stamens and malformed, but fertile, carpels. **E-H:** Strong *PAP1:YAB3* lines. Flower (**E**; one sepal removed) and inflorescence (**F**) of a homozygous progeny of the weak line in (**D**). Mildly affected sepals are followed by retarded, filamentous petals. (**G**) A SEM micrograph of a strong *PAP1:YAB3* line flower. Numerous petals are produced that are arrested in development as is the central flower meristem. (**H**) Older flower (with three sepals removed) of the same shoot from which **G** was obtained. The expanding petals display variable shapes and sizes and are positioned in a disorganized fashion. **I-K:** Expression of *PCLV3>>ER-GFP*. Apices are of wild-type (**J**) and *PAP1:YAB3* plants (**I**, **K**). Note the absence of *PCLV3>>ER-GFP* expression in the *PAP1:YAB3* floral meristems. **L,M:** Expression of *PWUS>>ER-GFP* in wild-type (**L**) and *PAP1:YAB3* (**M**) flowers. Note the expansion (indicated by the arrows) of *PWUS>>ER-GFP* expression in *PAP1:YAB3* flowers. Bars are 100µm in A to C, F and I, 1mm in D and E and 50µm in J to M. Numbers represent the order of flower initiation. IM- inflorescence meristem, FM- floral meristem, * - sepals, pe - petals, f – filaments.



Supplemental Figure 5. Additional analyses of *lateral suppressor.*

A-C: Complementation of *las* by *PLAS>>LAS-GFP*. Axillary shoots are initiated in the axils of wild type rosettes (**A**), are missing from axils of *las-11* mutant leaves (**B**), and are restored in axils of *las-11 PLAS>>LAS-GFP* leaves (**C**). The primary stems were removed prior to photography. **D-F:** Additive interaction between *las* and *clv3*. **D:** Top view of a *clv3-1* mutant flower. **E**: Top view of a *las-11* mutant flower, indistinguishable from wild type. **F**: Top view of a *clv3-1 las-11* double mutant flower, indistinguishable from *clv3-1.* Bars are 1cm in **A-C** and 1mm in **D -F.**

Supplemental Table 1: Mean number [a] of floral organs in plants with altered *YAB1* expression.

Genotype	Sepal # ± SD	Petal # ± SD	Stamen # ± SD	Filament [b] # ± SD	Carpel # ± SD
wild type (Ler)	4 ± 0	4 ± 0	5.95 ± 0.2	0	2 ± 0
PFIL>>YAB3-GFP	4 ± 0	3.96 ± 0.2	5.91 ± 0.5	0	2 ± 0
PFIL>> GFP-FIL	4 ± 0	4 ± 0	5.91 ± 0.2	0	2 ± 0
fil-8	4.6 ± 0.8	1 ± 0.9	1.91 ± 1.2	2.2 ± 1.7	2.7 ± 0.8
fil-8 yab3-2	3.8 ± 0.7	0	0	1.45 ± 0.97	2 ± 0
fil-8 PFIL>>YAB3-GFP	4.3± 0.58	1.9± 1.58	5.8± 0.8	0.13±0.43	2 ± 0
PAP1:Y3 hemizygous	4 ± 0	7.2 ± 1.38	15.3 ± 1.8	0	3.54 ± 1.1
PAP3:Y3 hemizygous	4 ± 0	4 ± 0.6	5 ± 1.66	2 ± 1.38	2.12 ± 0.3
PAP3>>GFP-FIL	4 ± 0	3.6 ± 1.35	3 ± 1.3	2.17 ± 1.5	2.4 ± 0.71
PANT>>amiR-YAB1	4.2± 0.7	0	0	3.9 ± 1	2 ± 0.2
PAP1>>amiR-YAB1	4.13 ± 0.81	0	0	4.6 ± 1.5	2± 0.26
PAP3>>amiR-YAB1	4 ± 0	4 ± 0	5.56 ± 0.5	0	2 ± 0

[a] For each genotype, 5 flowers at positions ranging from 6th to 15th of the main shoot, from 6 different plants (30 flowers in total) were counted.

[b] Filamentous organs initiated at the 2nd or 3rd floral whorls.

Supplemental Table 2: *Arabidopsis* mutant and transgenic lines used in this study.

Genotype	GB accession or AGI numbers	Source/ reference
clv3-1	At2g27250	Eliot Meyerowitz (CalTech)
las-11	At1g55580	Present study
fil-8	At2g45190	Kumaran et al., 2002
yab3-2	At4g00180	Kumaran et al., 2002
PAP1:LhG4	At1g69120	Emery et al., 2003
PAP3:LhG4	At3g54340	Alvarez et al., 2006
PANT:LhG4	At4g37750	Schoof et al., 2000
PCLV3:LhG4	At2g27250	Present study
PCRC:LhG4	At1g69180	Pekker et al., 2005
PFIL:LhG4	At2g45190	Present study
PLAS:LhG4	At1g55580	Present study
PRBE:LhG4	At5g06070	Present study
PWUS:LhG4	At2g17950	Gross-Hardt et al., 2002
OP: ER-GFP	U87974	Friedrich Kragler (VU)
OP:NLS(LHP1)-GFP	At5g17690	Gideon Grafi (BGU)
OP:NLS (VirE2)- mRFP1	AAF77177 DD220331	Patricia Zambryski (UCB) Roger Tsien (UCSD)
OP:DTA	AY820132	Chuck Gasser (UCD)
OP:KAN1	At5g16560	Pekker et al., 2005
OP:miR165b	At4g00885	Alvarez et al., 2006
OP:LAS-GFP	At1g55580	Present study
OP:YAB3; OP:GFP-YAB3; OP:YAB3-GFP	At4g00180	Present study
OP: FIL; OP:GFP-FIL; OP:FIL-GFP	At2g45190	Present study
OP:amiR-YAB1	Supplemental Figure 3	Present study
PAP1:YAB3	At1g69120 /At4g00180	Present study
PAP3:YAB3	At3g54340/ At4g00180	Present study

Supplemental Table 3: Primers used for construction of responder and driver lines.

Name and source	Forward	Reverse
PRBE (1500bp 5’ upstream region)	5’AAACTGCAGTTTCAAGCAGTCTGATCACG	5’AAAGGATCCCAGTAGAAGAAGTTAAGGTG
PFIL (4000bp 5’ upstream region)	5’AAACCTAGGTGCCAAGTAGCAAGCATAGGGC3’	5’AAACTCGAGGATCCAAGCTTATCATGGTGATG
PLAS (3000bp 5’ upstream region)	5’AAACTGCAGGCCACCTAATGAAGTGTAC	5’AAACCTAGGAGATGAAGTTGGACCGACCAA
PAP1 (1800bp 5’ upstream region)	5’AAAGAATTCCCCGGATCTCCATAT	5’AAAGGATCCGATCCCCAGCTTCTG
PAP3 (500bp 5’ upstream region)	5’AAAGGTACCCCTTTGACGTTTACAAAC	5’AAAAAGCTTTATCGATTTCGAACCCATT
NLS (LHP1)	5’AAAGTCGACATCCATGGGAAAAAAACAGAATG	5’AAACTCGAGGATTGAGCTTCATCTTCTGGA
NLS (VirE2)	5’AAAGTCGACATGGAGCAGAAGCTGAT	5’AAACTCGAGCGAATTCATCTCTCTCGCGT
mRFP-1	5’AAAGGATCCATGGCCTCCTCCGAGGA	5’AAAAGATCTTTAGGCGCCGGTGGA
Diphtheria toxin A	5’AAAAACTCGAGATGGAAAACTTTTCTTCG	5’AAAAAGCTTTCATCGCCTGACACGATT
***LAS* for N terminal GFP fusion**	5’AAACTCGAGATGCTTACTTCCTTCAA	5’AAAGGATCCTCATTTCCACGACGAAACG
***LAS* for C terminal GFP fusion**	5‘AAAGGATCCGCTTTCCACGACGAAACGGAGA	5’AAACTCGAGTCAAATGCTTACTTCCTTCAAAT
YAB3	5’AAACTCGAGAATGTCGAGCATGTCCAT	5’AAAGGATCCGCGTTATGGGCCAC
***YAB3* for N terminal GFP fusion**	5’AAACTCGAGAATGTCGAGCATGTCCAT	5’AAAGGATCCGCGTTATGGGCCACCCC
***YAB3* for C terminal GFP fusion**	5’AAACTCGAGATGTCGAGCATGTCCA	5’AAAGGATCCTGGGCCACCCCAACGTTGG
FIL	5’AAACTCGAGATGTCTATGTCGTCTATGTC	5’AAACCATGGTACCTTAATAAGGAGTCACACCA
***FIL* for N terminal GFP fusion**	5’AAACTCGAGATGTCTATGTCGTCTATG	5’AAAGGATCCTTAATAAGGAGTCACACC
***FIL* for C terminal GFP fusion**	5’AAACTCGAGATGTCTATGTCGTCTATG	5’AAAGGATCCGCATAAGGAGTCACACCAAC

References:

Alvarez, J.P., Pekker, I., Goldshmidt, A., Blum, E., Amsellem, Z., and Eshed, Y. (2006). Endogenous and synthetic microRNAs stimulate simultaneous, efficient, and localized regulation of multiple targets in diverse species. Plant Cell **18**: 1134 -1151.

Emery, J.F., Floyd, S.K., Alvarez, J., Eshed, Y., Hawker, N.P., Izhaki, A., Baum, S.F., and Bowman, J.L. (2003). Radial patterning of Arabidopsis shoots by class III HD-ZIP and KANADI genes. Curr. Biol. **13:** 1768–1774.

Gross-Hardt, R., Lenhard, M., and Laux, T. (2002). WUSCHEL signaling functions in interregional communication during Arabidopsis ovule development. Genes Dev. **16:** 1129–1138.

Kumaran, M. K., Bowman, J.L., and Sundaresan, V. (2002). YABBY genes mediate down-regulation of KNOTTED-like genes during leaf development in *Arabidopsis thaliana.* Plant Cell **14:**2761-2770.

Pekker, I., Alvarez, J.P. and Eshed, Y. (2005). Auxin response factors mediate *Arabidopsis* organ asymmetry via modulation of KANADI activity. Plant Cell **17:** 2899–2910.

Schoof, H., Lenhard, M., Haecker, A., Mayer, K.F., Jürgens G., and Laux T. (2000). The stem cell population of *Arabidopsis* shoot meristems in maintained by a regulatory loop between the CLAVATA and WUSCHEL genes. Cell. **100:** 635-644.