Auxin Response Factors promote organogenesis by chromatin-mediated repression of the pluripotency gene SHOOTMERISTEMLESS

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Specification of new organs from transit amplifying cells is critical for higher eukaryote development. In plants, a central stem cell pool maintained by the pluripotency factor SHOOTMERISTEMLESS (STM), is surrounded by transit amplifying cells competent to respond to auxin hormone maxima by giving rise to new organs. Auxin triggers flower initiation through Auxin Response Factor (ARF) MONOPTEROS (MP) and recruitment of chromatin remodelers to activate genes promoting floral fate. The contribution of gene repression to reproductive primordium initiation is poorly understood. Here we show that downregulation of the STM pluripotency gene promotes initiation of flowers and uncover the mechanism for STM silencing. The ARFs ETTIN (ETT) and ARF4 promote organogenesis at the reproductive shoot apex in parallel with MP via histone-deacetylation mediated transcriptional silencing of STM. ETT and ARF4 directly repress STM, while MP acts indirectly, through its target FILAMENTOUS FLOWER (FIL). Our data suggest that – as in animals – downregulation of the pluripotency program is important for organogenesis in plants.
Plants give rise to new organs continuously throughout their life. During reproduction, primordia that give rise to flowers and cryptic bracts (henceforth termed reproductive primordia), arise from a population of transit amplifying cells at the flanks of the inflorescence shoot apex\(^1\). Flowers are plant organs important for reproductive success and yield\(^2\). Perception of a local auxin maximum triggers transcriptional responses that enable specification of reproductive primordium founder cells\(^3\). When auxin levels are low, small nuclear Aux/IAA proteins bind to MONOPTEROS/Auxin Response Factor 5 (MP; ARF5) and recruit co-repressor complexes to prevent activation of auxin responsive genes\(^4,5\). Increased auxin levels lead to ubiquitin-mediated degradation of Aux/IAA proteins\(^6\), releasing the corepressors and allowing MP to recruit the SWI/SNF family chromatin remodelers BRAHMA and SPLAYED (SYD)\(^8\). In this fashion, MP directly upregulates genes important for flower development, such as those encoding the transcription factors FILAMENTOUS FLOWER (FIL) and LEAFY (LFY)\(^9,10\). MP also directly modulates expression of genes that control hormone accumulation and response\(^11\–\(^13\)

Auxin Response Factors are divided into three evolutionarily conserved classes, A, B and C\(^14\). Class A ARFs are classified as transcriptional activators, while class B and C ARFs are classified as transcriptional repressors\(^15,16\). MP is a class A ARF with an unstructured glutamine-rich central domain that can interact with SWI/SNF family chromatin remodelers\(^9\). Besides MP, only two other ARFs are strongly expressed in founder cells of the reproductive primordia: ETTIN (ETT; also called ARF3) and ARF4\(^17\). Both ETT and ARF4 are ‘repressive’ class B ARFs\(^15,16\). ETT and ARF4 have partly redundant roles in several developmental processes in Arabidopsis, including lateral root initiation and leaf polarity\(^18\–\(^20\).

A key pluripotency gene in plants is the class I KNOX homeobox transcription factor SHOOTMERISTEMLESS (STM)\(^21\). STM promotes meristematic fate in part by upregulating biosynthesis of the hormone cytokinin, which acts in a positive feedback loop to promote stem cell fate\(^22,23\). STM frequently acts in concert with other class I KNOX genes such as BREVIPEDELICUS (BP) and the activity of the class I KNOX proteins is modulated by a different class of homeodomain proteins, the BEL-like proteins (see\(^24\) for review). STM expression is high throughout the shoot apical meristem, but is downregulated at sites of auxin maxima in transit amplifying cells\(^25,26\). Neither the functional significance nor the mechanism for STM downregulation in primordium initials are currently understood.

We show here that STM repression promotes initiation of reproductive primordia. We further demonstrate that the class B ARFs ETT and ARF4, which are expressed in incipient reproductive primordia, act in parallel with MP to downregulate STM as well as BP. We implicate the MP target FIL in direct repression of the class I KNOX genes together with ETT and ARF4. Finally, we reveal that silencing of the pluripotency gene STM and BP by FIL and ETT/ARF4 is mediated by histone deacetylation.

**Results**

**ETT and ARF4 promote flower initiation with MP.** To probe whether ETT and ARF4 contribute to flower initiation, we performed a genetic enhancer test using the hypomorphic mp allele mp-S319\(^27\), mp-S319 has a T-DNA insertion in the second to last exon of Mp\(^28\) and displays weaker phenotypes than mp null mutants, including partially compromised flower initiation (Fig. 1a, b)\(^10\). Loss of ETT and ARF4 activity in this background caused formation of naked inflorescence ‘pills’ that lack flowers (Fig. 1a, b). We also generated triple mutants between the MP interacting chromatin remodeler SYD and ETT/ARF4. Like mp-S319 ett arf4, syd-5 ett arf4 mutants formed naked inflorescence pins (Supplementary Fig. 1a, b). These data suggest that the class B ARFs ETT and ARF4 promote flower initiation.

Stipules formed on syd-5 ett arf4 inflorescence pins, pointing to increased meristematic activity\(^29,30\). Indeed, molecular characterization of the flower initiation defects of syd-5 ett arf4 and mp-S319 ett arf4 revealed a striking expansion of the STM expression into incipient reproductive primordia (Supplementary Fig. 1c, Fig. 1c). The expression domain of the shoot apical
meristem stem cell marker CLAVATA3 (CLV3) was not dramatically increased, suggesting that the expansion of the STM expression domain is not accompanied by an increase in the size of the CLV3-expressing stem cell pool. (Fig. 1c, Supplementary Fig. 1c). As in the wild type (WT)23,26, STM was downregulated at the flanks of the shoot apex of mp-S319 and ett arf4 mutants (Fig. 1c, Supplementary Fig. 2). Slightly elevated expression of STM was also detected by quantitative reverse transcriptase PCR (qRT-PCR) in entire mp-S319 ett arf4 or syd-5 ett arf4 inflorescences (Fig. 1d, Supplementary Fig. 1d). The observed increase in STM levels in mp-S319 ett arf4 is probably an underestimate, because mp-S319 ett arf4 mutants lack STM expressing flower meristems, in contrast to the other genotypes tested. We propose that the overexpression of STM in mp-S319 ett arf4 is likely to be functionally important, because it triggered a marked increase in the expression of the STM target IPT7, a cytokinin hormone biosynthesis gene23 (Fig. 1d).

To probe whether ETT and ARF4 act in parallel with MP, we generated triple mutants between the mp-12 null mutant31 and ett arf4. While most of the plant body is elaborated post embryogenesis, the root, hypocotyl and embryonic leaves (cotyledons) are formed during embryo development32,33. mp-12 ett arf4 mutants, like mp-12 mutants, did not form roots (Fig. 2a, b). In addition, mp-12 ett arf4 seedlings exhibited severe defects in cotyledon initiation and had enlarged shoot apical meristems (Fig. 2a–d). In situ hybridization in mp-12 ett arf4 seedlings demonstrated an expansion of the STM expression domain into the region from where primordia initiate (Fig. 2e). qRT-PCR confirmed strongly increased expression levels of STM and IPT7 in the triple mutant relative to the parental lines (Fig. 2f). An expanded STM expression domain was also apparent in mature embryos segregating in mp-12/+ ett/+ arf4/- plants that expressed a pSTM:GUS reporter34 (Fig. 2g). Based on these results, we conclude that ETT and ARF4 act in parallel with MP to promote organogenesis by repressing STM expression.

**STM downregulation is important for flower initiation.** To directly test for a biological role of STM in flower initiation, we used a steroid inducible version of STM, p35S:STM-GR25 (Supplementary Fig. 3a) to conditionally elevate STM levels in mp-S319 mutant inflorescence meristems. Increased STM accumulation significantly enhanced the mp-S319 flower initiation defect, causing formation of naked inflorescence pins (Fig. 3a, b). In agreement with the known positive feedback loop between STM and cytokinin22,23, treatment with cytokinin (zeatin) likewise enhanced the flower initiation defects of the hypomorph mp-S319 mutant (Supplementary Fig. 4). On the other hand, introduction of a previously characterized artificial microRNA that specifically targets STM35 into the mp-S319 ett arf4 triple mutant significantly rescued the floral initiation defect of the triple mutant (Fig. 3c, d) despite displaying only a partial decrease in STM accumulation (Supplementary Fig. 3b). The combined data suggest that downregulation of the STM pluripotency gene promotes reproductive primordium initiation.

**ETT directly and MP indirectly repress STM.** The effect of ETT/ARF4 or MP on STM expression could be direct or indirect. To distinguish between these possibilities, we employed plant lines expressing biologically active genomic constructs for ETT-GFP26 or MP-6xHA9 (Supplementary Fig. 3c) to conduct chromatin immunoprecipitation (ChIP) followed by qPCR. We assayed binding to five evolutionarily conserved regions of the STM locus (Fig. 4a). ETT-GFP bound strongly and specifically to STM, with the strongest binding observed in the second intron, which has many putative ETT binding sites (Fig. 4a, b). MP did not associate with any of the regions of the STM locus tested (Fig. 4a, c), but did bind the previously identified direct target FIL10. Although our genetic analyses suggest that MP acts in parallel with ETT and ARF4 to repress STM expression, the chromatin immunoprecipitation data point to a possible indirect role of MP in this process. This conclusion was supported by examination of a second class I KNOX gene, BREVIPEDICELLUS (BP), whose expression is also elevated in mp-S319 ett arf5 and mp-12 ett arf4

![Fig. 2](image-url) ETT and ARF4 act in a pathway parallel to MP. **a** Phenotypes of 10-day-old wild type (WT), ett arf4, mp-12 null mutant and mp-12 ett arf4 seedlings. Scale bar = 1 mm. Two phenotypic classes of mp-12 seedlings are indicated (I, II). **b** Top: Close-up view of mp-12 ett arf4 seedlings shown in a. Scale bar = 500 μm. Two phenotypic classes of mp-12 ett arf4 seedlings are indicated (III, IV). Bottom: Meristem of a class IV mp-12 ett arf4 seedling viewed from above (Scale bar = 200 μm). **c** Number of mp-12 or mp-12 ett arf4 seedlings belonging to the phenotypic classes (I–IV) shown in a and b. The error bars are proportional to the standard error of the pooled percentage computed using binomial distribution. **d** Scanning electron microscopy images of representative 10-day-old WT, mp-12 and class III mp-12 ett arf4 seedlings. Scale bar = 200 μm. **e** STM expression patterns by in situ hybridization in 7-day-old seedlings. Scale bar = 50 μm. **f** Relative expression of STM and the STM target IPT7 in 10-day-old seedlings normalized over that of UBQ10. Shown are mean ± SEM of three experiments. Source data are provided as a Source Data file. **g** Expression of pSTM:GUS in mature WT and mp-12 ett arf4 embryos. Scale bar = 50 μm.
escences displayed defects that ranged from initiation of primordia initiate9. FIL has been shown to suppress class I KNOX gene transcription factor FIL, a direct MP target with a role in leaf development.

The MP target FIL represses STM together with ETT/ARF4.

We next asked whether one or several MP targets might act in concert with ETT and ARF4 to directly repress class I KNOX gene expression. One obvious candidate is the YABBY transcription factor FIL, a direct MP target with a role in flower initiation9. FIL has been shown to suppress class I KNOX gene expression in leaves37. In addition to its expression in the abaxial domain of developing leaves and flowers, FIL is expressed at the flanks of the shoot apex in the primordium founder cells from where primordia initiate38–40. We compared the accumulation of FIL mRNA with that of ETT, ARF4 and MP in the reproductive shoot apex by situ hybridization and confirmed that all four genes are expressed in the primordium founder cells (Supplementary Fig. 6), the region where STM is depleted26. In agreement with a role for FIL in flower initiation, fil ett arf4 triple mutant inflorescences displayed defects that ranged from initiation of filamentous structures in lieu of flowers to pins in older fil ett arf4 inflorescences (Fig. 5a, b). Treatment with low doses of an auxin transport inhibitor caused formation of naked inflorescence pins in fil ett arf4 plants, but not in any of the control genotypes (Fig. 5a, b, Supplementary Fig. 7). Furthermore, fil ett arf4 mutant inflorescences also displayed increased expression of STM, IPT7 and BP relative to the parental lines (Fig. 5c, Supplementary Fig. 5c). To test whether FIL directly regulates STM expression, we generated a biologically active, 6xHA-tagged genomic construct for FIL (Supplementary Fig. 3d). ChIP qPCR revealed that FIL bound to the STM locus, with the strongest binding observed at the proximal STM promoter (Fig. 5d), a conserved region with a FIL binding motif31. FIL also bound to the BP class I KNOX gene locus (Supplementary Fig. 5i). These data suggest that FIL directly represses class I KNOX genes including STM downstream of MP.

A role for FIL and ETT/ARF4 in STM repression is further supported by the finding that fil ett arf4 mutants also displayed leaf phenotypes characteristic of class I KNOX overexpressing plants30. These phenotypes were already apparent in fil+/ ett arf4
mutants. Instead of the simple leaf typical of wild-type *Arabidopsis* plants, *fil/+; ett arf4* leaves were lobed and divided (Fig. 6a, b). The leaf phenotype of the *fil ett arf4* triple mutants was even more severe, with deep lobes and divisions forming in *fil ett arf4* mutants. We further show that this *STM* downregulation is important for primordium founder fate and that the ‘repressive’ class B ARFs cooperate with the ‘activating’ class A ARF MP to silence *STM* together with HDA19 through a YABBY/ARF co-repressor complex (Fig. 7f).

### Discussion

Organogenesis is critical to establish the body plan of higher eukaryotes. Here we investigate the initiation of reproductive primordia that give rise to flowers, plant organs critical for reproductive success. Prior studies uncovered the gene expression programs activated by MP and the mechanism for this activation in response to auxin accumulation in the founder cells of reproductive primordia.*9,10,25* Downregulation of expression of the pluripotency gene *STM* has been observed in the primordium initials, transit amplifying cells that have perceived an auxin signal. Interestingly, the double mutants of *STM* and *BP* showed enhanced expression levels in different plant tissues compared to parental lines (Supplementary Fig. 10). The combined data point to a role of FIL and ARF4 in STPM and BP silencing by generating a less-repressive chromatin state via histone deacetylation. They further reveal that *STM* downregulation is important for primordium founder fate and that the ‘repressive’ class B ARFs jointly recruit the histone deacetylase HDA19 via histone deacetylation in the context of chromatin. ETT, ARF4 and FIL interact with different transcriptional co-repressor complexes that all recruit the histone deacetylase HDA19. Combined yeast-two-hybrid, in planta biculture molecular complementation, and co-immunoprecipitation analyses confirmed an association of HDA19 with the ETT/ARF4 FIL complex (Supplementary Fig. 8). We therefore tested for a biological role of HDA19 in initiation of reproductive primordia. *mp-s319 hda19* double mutants had significantly enhanced flower initiation defects relative to the parental lines (Fig. 7a, b). The naked pin inflorescence phenotype of *mp-s319 hda19* was accompanied by increased expression of *STM*, *IPT7* and *BP* (Fig. 7c, Supplementary Fig. 5e). Treatment with histone deacetylase inhibitors also significantly enhanced the flower initiation defect of *mp-s319* (Supplementary Fig. 9). To further probe the biological role of HDA19 in initiation of reproductive primordia, we generated double mutants between HDA19 and the SWI/SNF chromatin remodeler SYD, which acts together with MP to upregulate genes that promote flower initiation. Like *mp-s319 hda19*, *syd-5 hda19* inflorescences formed inflorescence pins and initiated significantly fewer flowers than the parental lines (Supplementary Fig. 1). In addition, *syd-5 hda19* inflorescences displayed elevated *STM* and *IPT7* expression (Supplementary Fig. 1).

To assess whether the histone deacetylase HDA19 binds to class I KNOX loci, we performed ChIP-qPCR using pHDA19: HDA19-GFP plants. HDA19 associated strongly with multiple regions of the *STM* and *BP* loci (Fig. 7d, Supplementary Fig. 5). If FIL and ETT/ARF4 repression of *STM* is mediated by histone deacetylation, the triple mutant should display increased histone acetylation at the class I KNOX gene loci. We therefore tested for an increase in H3K27 acetylation in *fil ett arf4* mutants compared to parental lines. H3K27ac accumulation relative to that of *H3* was elevated at both the *STM* and *BP* loci in *fil ett arf4* mutants (Fig. 7e, Supplementary Fig. 5). H3K27ac/H3 accumulation correlates with *STM* expression levels in different plant tissues (Supplementary Fig. 10). The combined data point to a role of FIL, ETT and ARF4 in *STM* and *BP* silencing by generating a repressive chromatin state via histone deacetylation. They further reveal that *STM* downregulation is important for primordium founder fate and that the ‘repressive’ class B ARFs cooperate with the ‘activating’ class A ARF MP to silence *STM* together with HDA19 through a YABBY/ARF co-repressor complex (Fig. 7f).

YABBY/ARF complex silences *STM* via histone deacetylation.

Finally, we investigated how the YABBY/ARF complex represses *STM*. Histone deacetylation leads to repression of gene expression in the context of chromatin. ETT, ARF4 and FIL interact with different transcriptional co-repressor complexes that all recruit the histone deacetylase HDA19. Combined yeast-two-hybrid, in planta biculture molecular complementation, and co-immunoprecipitation analyses confirmed an association of HDA19 with the ETT/ARF4 FIL complex (Supplementary Fig. 8). We therefore tested for a biological role of HDA19 in initiation of reproductive primordia. *mp-s319 hda19* double mutants had significantly enhanced flower initiation defects relative to the parental lines (Fig. 7a, b). The naked pin inflorescence phenotype of *mp-s319 hda19* was accompanied by increased expression of *STM*, *IPT7* and *BP* (Fig. 7c, Supplementary Fig. 5e). Treatment with histone deacetylase inhibitors also significantly enhanced the flower initiation defect of *mp-s319* (Supplementary Fig. 9). To further probe the biological role of HDA19 in initiation of reproductive primordia, we generated double mutants between HDA19 and the SWI/SNF chromatin remodeler SYD, which acts together with MP to upregulate genes that promote flower initiation. Like *mp-s319 hda19*, *syd-5 hda19* inflorescences formed inflorescence pins and initiated significantly fewer flowers than the parental lines (Supplementary Fig. 1). In addition, *syd-5 hda19* inflorescences displayed elevated *STM* and *IPT7* expression (Supplementary Fig. 1).
gene, BP, is regulated in much the same way as STM (Fig. S5). On the basis of prior studies, BP downregulation also contributes to reproductive primordium initiation.

STM is known to be a target of Polycomb Repressive Complex 2 (PRC2)52–54. In our genetic enhancer tests, we did not observe enhanced flower initiation defects when we combined mp-S319 with a PRC2 mutant (Supplementary Fig. 11). Polycomb repression frequently drives long-term silencing and down-regulation of STM in the reproductive primordium is not permanent. Once flowers have reached stage 2 of development, STM is upregulated when the floral meristem forms.

It has been proposed that class B ARFs may inhibit activity of class A ARFs by competing for binding sites at the same target genes. We show here that class B and class A ARFs can act cooperatively in the same pathway by directly repressing and activating different classes of genes (this study and references9–13). Evidence is accumulating for unique DNA sequence binding preferences of ETT and MP41,57–59. It is likely that multiple different types of interactions between the evolutionarily conserved classes of ARFs enable unique transcriptional and developmental outcomes in response to a single hormonal cue, auxin. Additional specificity is provided by ARF interactions with other classes of transcription factors, such as the YABBY transcription factor FIL we identify here.

Auxin promotes FIL accumulation in incipient reproductive primordia by triggering ubiquitin-mediated degradation of Aux/IAA proteins complexed with MP, thus enabling MP to recruit chromatin remodelers to activate FIL expression. How auxin modulates ETT/ARF4 accumulation or activity to promote flower initiation is not clear. In a pathway parallel to MP, auxin may regulate ETT/ARF4 accumulation or activity to promote flower initiation. In a pathway parallel to MP, auxin may regulate ETT/ARF4 accumulation or activity to promote flower initiation. In a pathway parallel to MP, auxin may regulate ETT/ARF4 accumulation or activity to promote flower initiation. In a pathway parallel to MP, auxin may regulate ETT/ARF4 accumulation or activity to promote flower initiation. In a pathway parallel to MP, auxin may regulate ETT/ARF4 accumulation or activity to promote flower initiation.
in auxin concentration36,59, auxin might instead control ETT interaction with FIL and/or HDAC-containing co-repressor complexes.

In summary, our study implicates epigenetic silencing of a pluripotency factor in initiation of reproductive primordia, and elucidates the mechanism for this silencing. Our findings moreover suggest that in plants, like in animals62, organogenesis requires both activation of lineage-specific gene expression programs and silencing of pluripotency genes.

Methods

Plant materials and treatments. Plants were grown at 22 °C in long-day conditions (16 h day/8 h dark) in soil or on ½ Murashige and Skoog (MS) plates63. All mutations were in the Columbia accession unless otherwise indicated. Mutant alleles were described before including ett-h3, ett-42 (SALK_070506)30, mp-STM12, hda19-l44, mp-12 (SALK_049553)31, clf-285, eTT:ETT-GFP ett-3, syd-506 and pHDA19:HDA19-GFP35. fil-8 is in the Ler background and was outcrossed to Columbia more than three times. fil-049 (WiscDsLox367E6_049) was obtained from the Arabidopsis Biological Resource Center.

For activation of p35S:STM-GR in mp-STM1, plants were treated with 1 µM dexamethasone (DEX, Sigma) plus 0.015% Silwet from 12 days of age onwards, the synthetic steroid rapidly decays in plants69. For expression studies, 9-day-old MS cultures were treated with 1 µM cytokinin

Suberoylanilide hydroxamic acid (SAHA, Sigma) plus 0.015% Silwet every 3 days from 11 days of age onwards for a total of six treatments. After manifestation of pin inflorescence phenotypes, spraying was resumed once weekly for 2 weeks. Mock treatment consisted of 0.1% DMSO plus 0.015% Silwet. For NPA treatment, fl/– ett/– arf4 inflorescences were treated with 500 nM of NPA plus 0.015% Silwet every 2 days on from 11 days of age onwards for a total of eight treatments. Mock treatment consisted of 0.05% DMSO plus 0.015% Silwet. Because treated plants are less vigorous and make fewer flowers than untreated plants (compare WT in Fig. 1b to Fig. 3b), phenotypes of treated plants were always compared to treated control plants. The number of flowers formed was counted in 55–65-days-old plants, once development had ceased, to ensure an accurate count of all flowers or filamentous structures initiated.

Transgenic plants. p35S:STM-GR78 was transformed into mp-STM1/+ . In the T1 generation, plants heterozygous for mp-STM1 and hemizygous for STM-GR were selected by genotyping and growth on selective medium, respectively. In the T2 generation, seedlings were germinated on selective medium to recover plants hemizygous for amiRSTM. In the T2 generation, plants carrying the transgene (amiRSTM) were identified by genotyping. Steroid treatment and phenotypic analyses were conducted in the T2 generation on WT and mp-STM1 siblings.

p3SSmIR STM35 was transformed into mp-STM1/+ :ett-7/+ arf4-2. In the T1 generation, plants carrying the transgene (amiRSTM) were identified by BASTA treatment and plants heterozygous for mp and ett by genotyping. In the T2 generation, T1 progeny was sprayed with BASTA to identify plants hemi or homozygous for the transgene and transferred to soil. Plants homozygous for WT or mp were identified by genotyping. Phenotypic analyses were conducted in the T2 generation on WT and mp-STM1 siblings.

Generation of ETTr, the tasi-RNA target sites of ETT19 were mutagenised by site-directed mutagenesis using primer sets previously described34. The ETTr fragment was amplified by PCR and cloned into pENTR/D Topo (Thermo Fisher Scientific). To generate ARF4*, the full length ARF4 coding sequence was amplified and

![Fig. 6 Leaf phenotypes of plants with reduced FIL, ETT and ARF4 activity. a Representative images of leaf phenotypes of wild type (WT), fil-8, ett arf4, fil/+ ett arf4 and fil-8 ett arf4. I–III indicate the different phenotypic classes observed. I simple leaves; II lobed leaves; III divided leaves and leaves bearing ectopic meristems (inset). Scale bars: 1 cm and 1 mm (inset). b Quantification of number of leaves in each phenotype class shown in a. The error bars are proportional to the standard error of the pooled percentage computed using binomial distribution. *** P < 0.0001, two-tailed Mann–Whitney U test relative to ett arf4. n = 87 and 81 for WT and fil-8. n = 104, 148 and 64 for ett arf4, fil/+ ett arf4 and fil ett arf4. Source data are provided as a Source Data file. c Relative expression of STM and IPT7 in fully expanded adult leaves normalized over that of UBQ10. Shown are mean ± SEM of three experiments. Source data are provided as a Source Data file.

Fig. 6 Leaf phenotypes of plants with reduced FIL, ETT and ARF4 activity. a Representative images of leaf phenotypes of wild type (WT), fil-8, ett arf4, fil/+ ett arf4 and fil-8 ett arf4. I–III indicate the different phenotypic classes observed. I simple leaves; II lobed leaves; III divided leaves and leaves bearing ectopic meristems (inset). Scale bars: 1 cm and 1 mm (inset). b Quantification of number of leaves in each phenotype class shown in a. The error bars are proportional to the standard error of the pooled percentage computed using binomial distribution. *** P < 0.0001, two-tailed Mann–Whitney U test relative to ett arf4. n = 87 and 81 for WT and fil-8. n = 104, 148 and 64 for ett arf4, fil/+ ett arf4 and fil ett arf4. Source data are provided as a Source Data file. c Relative expression of STM and IPT7 in fully expanded adult leaves normalized over that of UBQ10. Shown are mean ± SEM of three experiments. Source data are provided as a Source Data file.
cloned into pENTR/D TOPO, 266 base pairs including the sequence with the mutated tasi-RNA target sites in ARF4 was synthesized as a gBlock Gene Fragment (Integrated DNA Technologies). The endogenous sequence was replaced by the synthesized gBlock in pENTR/D TOPO using Gibson assembly with the Gibson assembly master mix. For BiFC assays, ETTR, ARF4, as well as FIL and HDA19 in pENTR/D TOPO (see Supplemental Table 1 for primer information) were recombined to pUC-SPV-NEGW and pUC-SPV-ARFr, as well as FIL and HDA19 in pENTR/D TOPO (see Supplemental Table 1 for primer information) were recombined to pENTR/D TOPO using Gibson assembly with the synthesized gBlock in pENTR/D TOPO using Gibson assembly with the Gibson assembly master mix.

Expression analysis. In situ hybridization of STM, CLV3, MP and ETT was performed as described in our published protocol with minor modifications. The antisense probes for STM, FIL and ARF4 were generated using in vitro transcription from the full-length STM (1149 base pairs), FIL (990 base pairs) and ARF4 (2367 base pairs) coding sequences cloned into pGEM-T (Promega). The full-length (516 base pair) CLV3 coding sequence and 1273 base pair of the ETT coding sequence (551-1824 base pairs) were cloned into pGEM-T easy for probe synthesis. The MP probe has been described. Inflorescence tissue was harvested at 21-28 days of age for wild-type and ett etf arf4 and at 28 days of age for genotypes showing pin phenotypes (mp-S319 and mp-S319 etf arf4). Seedling tissue was grown on MS agar medium and harvested at 10 days of age. Pictures were taken of 8 µ sections using a brightfield microscope (Olympus BX51).

For qRT-PCR expression analysis from 1-5 cm bolt inflorescences and fully expanded adult leaves, RNA was extracted by TRIzol (Thermo Fisher Scientific) and further purified using the RNeasy mini kit (Qiagen). First strand cDNA was synthesized using the superscript III kit (Thermo Fisher Scientific) from 1 µg of RNA. Quantitative real time PCR was performed using Power SYBR Green master mix (Thermo Fisher Scientific). The expression value was determined using a cDNA standard curve and normalized over that of the housekeeping gene UBQ10 (AT4G05320).

Phylogenetic shadowing. Genomic sequence covering from AT1G62370 to the STM 3'UTR was used as a query to find sequences of closely related species. The genomic sequences of Brassicaceae species were obtained by running BLASTn discontinuous megablast. The mVISTA program was used to identify regions of conservation. Clustalomega (https://www.ebi.ac.uk/Tools/msa/clustalo/) was used to identify conserved cis motifs in the conserved regions of the STM locus.

Chromatin immunoprecipitation. ChIP was performed as described in our published protocol. For inflorescence ChIP, ~0.5 g of young inflorescences from plants with 0-5 cm bolts were harvested. All flowers from stage 6 onwards were removed. Antibody quality was validated by confirming published ChIP data for LFY-GFP and EIE-HA. In addition, Anti-H3K27ac antibodies (Abcam, ab4729, lot GR3219673-1, 1:500 dilution), anti-H3K9ac antibodies (Active Motif, 39157, lot GR319255-1, 1:500 dilution) were used for IP. The antibodies were validated by the manufacturers. For ChIP in wild-type leaves and ap1 cal inflorescences, ~2 g of fully expanded Columbia leaves and 0.5 g of ap1-1 cal-174 inflorescence tissue was collected and fixed for analysis. Antibody amounts were 5 µg for anti GFP and HA, and 2 µg for anti H3, H3K27ac and H3K9ac. The TA3 retrotransposon (Al1g37110) served as negative control locus (NC)3. The LFT locus region (LFT)20 served as positive control for MP ChIP and as a negative control for ETT ChIP. Throughout, ChIP was also performed using the same antibodies in the WT to control for nonspecific DNA enrichment. To estimate the DNA enrichment, the qPCR value of the ChIP product was normalized over input DNA. Primer sequences are listed in Supplementary Table 1.

Protein interaction. For test of interaction between ETT, ARF4, FIL and HDA19 in yeast, full length clones for all four proteins, as well as ETT-N (amino acids 1-389) and ETT-C (amino acids 390-608) were cloned into pENTR/D TOPO and recombined into pDEST22 and pDEST22. These constructs were co-transformed into competent AH109 yeast cells using the Frozen-EZ Yeast Transformation II Kit (Zymo research). To examine yeast growth, serially-diluted yeast cells were spotted on media lacking Leucine and Tryptophan. To test for interactions, yeast cells were
Data availability

The authors declare that the data supporting the findings of this study are available within the paper and its supplementary information files. The data underlying Fig. 2c, Fig. 6b and Supplemental Fig. 8c as well as P-values for all figures provided as a source data file.

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**Author contributions**

Y.C. conducted most genetic and phenotypic analyses, plant line generation, interaction studies and expression studies; Y.Z. performed all ChIP tests; M-F.W. contributed to BiFC analysis. D.W. and Y.C. conceived of the study and D.W. wrote the manuscript with help from S.G. Y.C. and all authors.
