STENOFOlia Recruits TOPELESS to Repress ASYMMETRIC LEAVES2 at the Leaf Margin and Promote Leaf Blade Outgrowth in Medicago truncatula

Fei Zhang, a Yewei Wang, a Guifen Li, b Yuhong Tang, b Elena M. Kramer, c and Million Tadege a,1

a Department of Plant and Soil Sciences, Institute for Agricultural Biosciences, Oklahoma State University, Ardmore, Oklahoma 73401
b Plant Biology Division, The Samuel Roberts Noble Foundation, Ardmore, Oklahoma 73401
c Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, Massachusetts 02138

The Medicago truncatula WUSCHEL-related homeobox (WOX) gene, STENOFOlia (STF), plays a key role in leaf blade outgrowth by promoting cell proliferation at the adaxial-abaxial junction. STF functions primarily as a transcriptional repressor, but the underlying molecular mechanism is unknown. Here, we report the identification of a protein interaction partner and a direct target, shedding light on the mechanism of STF function. Two highly conserved motifs in the C-terminal domain of STF, the WUSCHEL (WUS) box and the STF box, cooperatively recruit TOPLESS (Mt-TPL) family corepressors, and this recruitment is required for STF function, as deletion of these two domains (STFdel) impaired blade outgrowth whereas fusing Mt-TPL to STFdel restored function. The homeodomain motif is required for direct repression of ASYMMETRIC LEAVES2 (Mt-AS2), silencing of which partially rescues the stf mutant phenotype. STF and LAMINALESS1 (LAM1) are functional orthologs. A single amino acid (Asn to Ile) substitution in the homeodomain abolished the repression of Mt-AS2 and STF’s ability to complement the lam1 mutant of Nicotiana sylvestris. Our data together support a model in which STF recruits corepressors to transcriptionally repress its targets during leaf blade morphogenesis. We propose that recruitment of TPL/TPL-related proteins may be a common mechanism in the repressive function of modern/WUS clade WOX genes.

INTRODUCTION

Leaf primordia are determinate lateral organs initiated from a small group of pluripotent stem cells in the shoot apical meristem. After primordium initials are recruited from the shoot apical meristem, the primordium organizes itself into defined cell layers through highly regulated cell division and cell differentiation patterns, forming a flat lamina (blade) that develops along three distinct axes: proximal-distal, medial-lateral, and adaxial-abaxial. Despite tremendous progress, how such a highly organized structure develops from undifferentiated stem cells remains a fundamental question in plant developmental biology.

Polarity patterning along the adaxial-abaxial axis of the leaf blade is required for blade outgrowth (Waites and Hudson, 1995; McConnell and Barton, 1998; Bowman et al., 2002; Efroni et al., 2010; Byrne, 2012), and the genetic determinants of polarity have been well defined in the model plant Arabidopsis thaliana (Waites et al., 1998; Byrne et al., 2000; Kerstetter et al., 2001; McConnell et al., 2001; Emery et al., 2003; Juarez et al., 2004). It is well established that adaxial and abaxial polarity factors interact in a mutually antagonistic manner to maintain domain specificity and allow cell differentiation in their respective domains (Bowman et al., 2002; Tsukaya, 2006; Husbands et al., 2009; Efroni et al., 2010). The adaxial polarity factor ASYMMETRIC LEAVES2 (AS2) is proposed to prevent cell proliferation in the adaxial domain (Iwakawa et al., 2007) in addition to maintaining adaxial polarity by regulating the patterns of miR165/166 distribution (Jeno et al., 2007). AS2 forms a repressive complex with AS1 and prevents meristematic activity in leaves by directly repressing the transcription of KNOTTED1-like homeobox (KNOX) genes (Guo et al., 2008; Luo et al., 2012). The AS1/AS2 complex has also recently been shown to directly or indirectly repress the abaxial factors ETTN/AUXIN RESPONSE FACTOR3 (ARF3) and ARF4 (Iwasaki et al., 2013). The repression of KNOX genes in leaves by the AS1/AS2 complex is required not only for proper differentiation of leaf cells but also for the commitment of leaf founder initial cells to form a leaf primordium, where the down-regulation KNOX genes at the incipient primordium site is required for leaf initiation (Hake et al., 2004; Moon and Hake, 2011). The AS1/AS2 complex interacts with the histone chaperone HIRA (Phelps-Durr et al., 2005) and the Polycomb-repressive complex2 for the repression of KNOX genes (Lodha et al., 2013). These observations indicate that leaf primordium initiation and elaboration involves stable repression of shoot apical meristem programs by the AS1/AS2 complex and chromatin-remodeling factors maintaining a transcriptionally repressed chromatin state. However, how preventing cell proliferation alone or in combination with the promotion of cell differentiation by the AS1/AS2 complex or other polarity factors mediates blade outgrowth is unclear. Moreover, as the primordium grows away from the meristem, physical recruitment of meristematic cells from the shoot apical meristem becomes unfeasible, and the leaf founder initial cells could become limiting for leaf growth. This

1 Address correspondence to million.tadege@okstate.edu.

The author responsible for distribution of materials integral to the findings presented in this article in accordance with the policy described in the Instructions for Authors (www.plantcell.org) is: Million Tadege (million.tadege@okstate.edu).

Some figures in this article are displayed in color online but in black and white in the print edition.

Online version contains Web-only data.

www.plantcell.org/cgi/doi/10.1105/tpc.113.121947
suggests that there may be a need for the maintenance of undifferentiated cells in the determinate leaf primordium during leaf morphogenesis and elaboration. At the adaxial-abaxial boundary of the leaf primordium, the marginal blastozone (Hagemann and Gleissberg, 1996), previously termed the marginal meristem, would be a potential site for such undifferentiated cells, but it is difficult to imagine how such meristematic cells could be maintained while class I KNOX genes, including SHOOT MERISTEMLESS, BREVI-PEDICELLUS (KNAT1), KNAT2, and KNAT6, are stably repressed.

Recently, a third domain, located at the adaxial-abaxial junction of the lamina, was found to be required for cell proliferation–mediated blade outgrowth (Tadege et al., 2011a; Nakata et al., 2012). The identity of this inner (middle) domain is regulated by the WUSCHEL-related homeobox (WOX) family homeodomain (HD) transcription factor STENOPOLIA (STF) in Medicago truncatula and its orthologs in other dicots, including MAEWEST in Petunia × hybridra (Vandenbussche et al., 2009), LAM1 in Nicotiana sylvestris (Tadege et al., 2011a), WOX1 and PRESSED FLOWER (FRS) in Arabidopsis (Vandenbussche et al., 2009; Nakata et al., 2012), and LATHYROIDES in pea (Pisum sativum) (Zhuang et al., 2012). The diagnostic phenotype of stf and lam1 mutants is dramatic reduction in leaf width with no significant effect on leaf length. This defect results from reduced cell proliferation in the lateral direction (Tadege et al., 2011a; Nakata et al., 2012) and is most dramatically exhibited in the lam1 mutant of N. sylvestris (McHale, 1992), where the phenotype is so severe that, essentially, the main visible structure of the leaf is the midrib with vestigial blade strips. The involvement of WOX genes in leaf blade outgrowth has been demonstrated in maize (Zea mays), where two duplicate WOX genes, narrow sheath1 (ns1) and ns2, were shown to redundantly regulate blade outgrowth (Nardmann et al., 2004). Similarly, in rice (Oryza sativa), the orthologs of ns1 and ns2 are shown to be involved in leaf blade outgrowth (Cho et al., 2013), suggesting that the requirement for WOX function in leaf blade development is conserved in both dicots and monocots. However, the dicot genes STF/WOX1 and STF/WOX are not necessarily the orthologs of ns1 and ns2, as these are represented by PRS/WOX3 in dicots, and the STF/WOX1 lineage appears to have no homologs in monocots (Tadege et al., 2011a, 2011b).

STF acts mainly as a transcriptional repressor, and the evolutionarily conserved WUS box, which is specific to the modern WUS clade WOX genes, partially contributes to this repressive activity (Lin et al., 2013). These findings established that the STF-mediated transcriptional repression is central to cell proliferation at the adaxial-abaxial junction that regulates blade outgrowth and showed that this activity may be shared by other WUS clade WOX genes involved in cell proliferation, including WUS and WOX5 (Lin et al., 2013). This interpretation is consistent with the reports of Kieffer et al. (2006) and Ikeda et al. (2009), who proposed that WUS mainly acts as a repressor in meristem stem cell maintenance. However, the molecular mechanism underlying the repressive activity of STF in leaf development is unknown. Neither the targets of STF repression nor the interacting partners that are required for STF activity have been identified.

In this study, we show that M. truncatula STF physically interacts with the corepressor TOPLESS (Mt-TPL) by its WUS box and STF box and that this interaction is required for both leaf blade development and the repression of Mt-AS2 at the leaf margin, suggesting that the STF-TPL repressor complex promotes cell proliferation by preventing the activity of AS2.

**RESULTS**

The HD and the C-Terminal Domain of STF Are Essential for Leaf Blade Outgrowth Function

To explore how the M. truncatula STF protein functions as a transcription factor, we performed amino acid sequence alignment of STF proteins from different species. Based on the conserved domain analysis, we divided the STF protein into four major parts: the N-terminal domain (NTD; amino acids 1 to 90), HD (amino acids 91 to 163), middle domain (MD; amino acids 164 to 300), and C-terminal domain (CTD; amino acids 301 to 358), which contains two highly conserved motifs, the WUS box (amino acids 309 to 318) and the STF box (amino acids 349 to 358) (Figure 1A; Supplemental Figure 1).

To evaluate the functions of these domains, we performed individual and combined domain deletions and introduced the truncated forms, driven by a 2.6 kb STF promoter (Tadege et al., 2011a), into the lam1 mutant of N. sylvestris, an invaluable genetic tool for functional analysis (Figure 1B). Compared with the wild-type STF gene, which fully complemented the lam1 mutation (Figure 1C), truncation of the NTD or MD resulted in a partial reduction of the ability of STF to rescue lam1, leading to slightly narrowed leaves with wavy margins (Figures 1D and 1E). Deletion of both the NTD and MD resulted in weaker lam1 complementation with more pronounced leaf narrowing and increased waviness of the margins (Figure 1F), indicating that the NTD and MD may have redundant functions in leaf development. However, deletion of both of these domains fails to fully abolish STF function, suggesting that their roles are not critical. By contrast, deletion of either the HD (Figure 1G) or the CTD (Figure 1H) completely abolished the ability of STF to complement lam1, indicating that DNA binding ability and interactions via the CTD are indispensable to STF function.

Both the WUS Box and the STF Box of the CTD Are Required for STF-Repressive Function

Since truncated STF lacking the CTD completely failed to rescue lam1, we compared the individual contributions of the WUS box and STF box to STF activity. We first examined the transcriptional activity of STF and its CTD-deleted mutant (STFdel) by transient luciferase expression protoplast assays (Figures 2A to 2C). While expression of the intact STF protein repressed the expression of the reporter gene construct by more than 2-fold compared with the empty effector plasmid, STFdel showed little, if any, repressive activity (Figure 2C). We next examined whether the conserved WUS box and STF box in the CTD contribute to the repressive activity by substitution of Leu with Ala in the individual WUS box and STF box motifs (Figure 2B). Consistent with our previous report (Lin et al., 2013), mutation of the WUS box (m1) partially relieved the repression of reporter gene expression by STF. Likewise, mutation of the STF box (m2) also resulted in the partial loss of reporter gene repression. However, mutations in both WUS box and STF box (m1m2) almost fully abolished STF repression, similar
to STFdel (Figure 2C). These results indicate that the WUS box and STF box may act as partially redundant repression motifs and that their combined activity is required for full STF-mediated transcriptional repression.

The STFdel failure to complement lam1 may be due to the loss of repressive activity. Consistent with this, fusion of the exogenous ethylene-responsive element binding factor–associated amphiphilic repression (EAR) motif repression domain SRDX to STFdel (STF:SRDX-STFdel) complemented lam1 (Figure 2E), indicating that repressive activity is critical for STF function. To ascertain the individual contributions of the WUS box and STF box to STF function in planta, we performed lam1 complementation by introducing mutant constructs (m1 and m2) individually or in combination under the control of the STF promoter (Figure 2D). Mutation in the WUS box, STF:STFm1 (Lin et al., 2013), only partially complemented the lam1 mutant, with plants showing wavy leaf margins (Figure 2F). Likewise, mutation in the STF box, STF:STFm2, partially complemented lam1, with even narrower leaf blades and more wavy margins compared with STF:STFm1 transgenic plants (Figure 2G; Supplemental Table 1).

The combined WUS box and STF box mutant (m1m2), on the other hand, completely failed to complement lam1 (Figure 2H). Similar results were obtained for the complementation of the M. truncatula stf mutant by STFm1m2 (Supplemental Figure 2). These results demonstrate that the STF box and WUS box together account for the STF-repressive activity and are additively required for STF function in promoting leaf blade outgrowth.

The STF Box Is Conserved Only in Dicots and May Play a Stronger Role Than the WUS Box in Blade Outgrowth

To directly compare the ability of the WUS box and STF box in promoting leaf blade outgrowth, we fused either the WUS box or the STF box to STFdel driven by the STF promoter and introduced the constructs into lam1 (Figure 3A). Both the STF:STFdel-WUS-box and STF:STFdel-STF-box constructs partially rescued lam1 (Figures 3D and 3E), but the STF:STFdel-STF-box construct showed more complete rescue than did the STF:STFdel-WUS-box construct (Supplemental Table 1), suggesting that the STF box may be more important than the WUS box in promoting leaf blade outgrowth.

A BLAST search of sequenced genomes and ESTs revealed that the intact 10-amino acid STF box (QIFIEFLPLKN) is conserved in dicots and the early-diverging angiosperm Amborella trichopoda but not in grasses, gymnosperms, or nonvascular plants. Amino acid alignment of the STF box in different dicot species showed that there is an LPL core motif, which is similar to the LQL core of the WUS box. However, unlike the WUS box LQL signature, the second Leu of the STF box LPL is not conserved in Arabidopsis lyrata WOX1 and Mirulinus guttatus STL1 (Figure 3B). In Aquilegia coerulea, a eudicot phylogenetically located at the midpoint between grasses and core eudicots (Kramer, 2009), the STF-like1 (Aq STL1) protein sequence ends at the first Leu of the STF box (Figure 3B). This variability prompted us to test whether these two Leu residues have different significance for STF box function. We mutated either the first Leu (m2L1A) or the second Leu (m2L2A) to Ala in the presence of the m1 mutation and introduced the mutants into lam1 plants (Figure 3C). Mutation of the first Leu (STFm1-m2L1A) completely abolished lam1 complementation (Figure 3F), indicating that this amino acid is critical for STF box function. Mutation of the second Leu (STFm1-m2L2A), on the other hand, led to a small degree of rescue (Figure 3G), indicating that the second Leu of the STF box contributes to repressive function to a lesser extent than the first Leu. By contrast, both Leu residues in the LQL core sequence of the WUS box were conserved among WUS clade WOX genes, and mutation of either Leu of the WUS box in the presence of the m2 mutation (Figure 3C) completely abolished lam1 complementation (Figures 3H and 3I), indicating that both Leu residues of the WUS box are equally important. From these observations, we concluded that the WUS box and STF box are repressive motifs additively required for STF-mediated leaf blade outgrowth, but the
The STF-TPL Complex Represses AS2

The STF-TPL Complex Represses AS2

**Figure 2.** Both the WUS Box and STF Box in the CTD Are Required for STF Function.

(A) Schematic representation of reporter and effector constructs used in the luciferase assay. Red and blue lines represent the m1 and m2 mutations, respectively, shown in (B).

(B) Leu (L) to Ala (A) substitution mutations in the CTD, yielding m1 in the WUS box (WB) and m2 in the STF box (SB).

(C) Relative luciferase activities using STF, STFm1, STFm2, STFm1m2, or STFdel as effector compared with the GAL4-DB control. Error bars indicate SD (n = 3). **P < 0.01 (t test).

(D) Constructs used for complementation of the lam1 mutant. Red and blue lines represent m1 and m2 mutations, respectively.

(E) to (H) lam1 mutant plants and leaves complemented with STF:SRDX-STFdel (E), STF:STFm1 (F), STF:STFm2 (G), and STF:STFm1m2 (H). Bars = 5 cm.

**STF Interacts with the Corepressor TPL via Its STF Box and WUS Box**

To understand the molecular mechanisms underlying STF-mediated transcriptional repression in blade outgrowth, we performed yeast two-hybrid (Y2H) screening for STF partners using the Y2H Gold system. An *M. truncatula* protein with 83% amino acid identity to the *Arabidopsis* TPL protein was identified and designated Mt-TPL (Supplemental Figure 3). STF and Mt-TPL proteins interacted in Y2H assay under stringent conditions (Figure 4A). STF also interacted in Y2H with three of the four TPL-related proteins tested (Supplemental Figure 4). The interaction between STF and Mt-TPL was confirmed in planta by bimolecular fluorescence complementation (BiFC) assay using split YFP (Figure 4B; Supplemental Figure 5), while no YFP fluorescence was detected in negative controls where the split YFP was fused to either STF or Mt-TPL alone (Supplemental Figure 5). This interaction was further verified by a pull-down assay using plant-expressed protein (Szemenyei et al., 2008). Both maltose binding protein (MBP) and MBP-STF fusion protein were expressed in *Escherichia coli* and purified by binding to amylose resin. Equal amounts of MBP or MBP-STF proteins were immobilized with plant protein extract containing Mt-TPL-GFP fusion protein transiently expressed in tobacco (*Nicotiana benthamiana*) leaf cells. The MBP-STF fusion protein, but not the MBP control protein, was able to pull down the Mt-TPL-GFP fusion protein that was detected by anti-GFP antibody (Figure 4C), confirming the STF-MtTPL-specific interaction.

We next asked which domains of STF are responsible for this interaction and whether the Mt-TPL and STF interaction has biological relevance to leaf blade outgrowth. To answer these questions, we performed detailed Y2H assays combined with BiFC and transgenic analysis. STF deletion or substitution mutants were fused to GAL4-DBD and Mt-TPL was fused to

**dicot-specific STF box may play a slightly stronger role in this function.**

**STF interacts with the corepressor TPL via its STF box and WUS box**

To understand the molecular mechanisms underlying STF-mediated transcription in blade outgrowth, we performed yeast two-hybrid (Y2H) screening for STF partners using the Y2H Gold system. An *M. truncatula* protein with 83% amino acid identity to the *Arabidopsis* TPL protein was identified and designated Mt-TPL (Supplemental Figure 3). STF and Mt-TPL proteins interacted in Y2H assay under stringent conditions (Figure 4A). STF also interacted in Y2H with three of the four TPL-related proteins tested (Supplemental Figure 4). The interaction between STF and Mt-TPL was confirmed in planta by bimolecular fluorescence complementation (BiFC) assay using split YFP (Figure 4B; Supplemental Figure 5), while no YFP fluorescence was detected in negative controls where the split YFP was fused to either STF or Mt-TPL alone (Supplemental Figure 5). This interaction was further verified by a pull-down assay using plant-expressed protein (Szemenyei et al., 2008). Both maltose binding protein (MBP) and MBP-STF fusion protein were expressed in *Escherichia coli* and purified by binding to amylose resin. Equal amounts of MBP or MBP-STF proteins were immobilized with plant protein extract containing Mt-TPL-GFP fusion protein transiently expressed in tobacco (*Nicotiana benthamiana*) leaf cells. The MBP-STF fusion protein, but not the MBP control protein, was able to pull down the Mt-TPL-GFP fusion protein that was detected by anti-GFP antibody (Figure 4C), confirming the STF-MtTPL-specific interaction.

We next asked which domains of STF are responsible for this interaction and whether the Mt-TPL and STF interaction has biological relevance to leaf blade outgrowth. To answer these questions, we performed detailed Y2H assays combined with BiFC and transgenic analysis. STF deletion or substitution mutants were fused to GAL4-DBD and Mt-TPL was fused to
GAL4-AD for Y2H analysis. Control assays were performed to confirm that these constructs did not show self-activation (Supplemental Figure 6). The interaction with Mt-TPL was completely abolished by deletion of the CTD (Figure 4D), indicating that the interaction with Mt-TPL is likely to be important for the STF-repressive activity. Whereas Mt-TPL was unable to interact with the CTD alone (Figure 4D), fusion of either the MD or HD to the CTD restored the interaction between the CTD and Mt-TPL (Figure 4D). This suggests that, while the STF CTD is essential for the interaction with Mt-TPL, additional sequences in the HD or MD may be required to stabilize this interaction.

Mutation of the two Leu residues in the WUS box somewhat reduced its interaction with Mt-TPL (Figure 4D), while this interaction was more strongly reduced by mutation of the STF box Leu residues (Figure 4D). Combined mutations in both the WUS box and STF box abolished the interaction with Mt-TPL (Figure 4D), consistent with the CTD deletion results. This finding was also verified by BiFC assay, in which the STFm1-m2L1A mutant showed no interaction with Mt-TPL (Figure 4D), while the STFm1-m2L2A mutant exhibited greatly reduced interaction (Figure 4D). Likewise, attachment of either the WUS box or STF box to the STFdel mutant restored limited interaction with TPL.

We also mapped the domains of Mt-TPL required for interaction with STF (Figure 4F). The N-terminal domain (amino acids 1 to 350; TPLdel), including the LiSH domain, CTLH domain, and Pro-rich domain (Szemenyei et al., 2008), was sufficient for interaction with STF (Figure 4G). Further truncation of part of the Pro-rich domain (amino acids 1 to 250; TPLdel2) showed no interaction with Mt-TPL (Figure 4D), while the STFm1-m2L2A mutant exhibited greatly reduced interaction (Figure 4D). Likewise, attachment of either the WUS box or STF box to the STFdel mutant restored limited interaction with TPL.

We also mapped the domains of Mt-TPL required for interaction with STF (Figure 4F). The N-terminal domain (amino acids 1 to 350; TPLdel), including the LiSH domain, CTLH domain, and Pro-rich domain (Szemenyei et al., 2008), was sufficient for interaction with STF (Figure 4G). Further truncation of part of the Pro-rich domain (amino acids 1 to 250; TPLdel2) showed no interaction with Mt-TPL (Figure 4D), while the STFm1-m2L2A mutant exhibited greatly reduced interaction (Figure 4D). Likewise, attachment of either the WUS box or STF box to the STFdel mutant restored limited interaction with TPL.

We also mapped the domains of Mt-TPL required for interaction with STF (Figure 4F). The N-terminal domain (amino acids 1 to 350; TPLdel), including the LiSH domain, CTLH domain, and Pro-rich domain (Szemenyei et al., 2008), was sufficient for interaction with STF (Figure 4G). Further truncation of part of the Pro-rich domain (amino acids 1 to 250; TPLdel2) showed no interaction with Mt-TPL (Figure 4D), while the STFm1-m2L2A mutant exhibited greatly reduced interaction (Figure 4D). Likewise, attachment of either the WUS box or STF box to the STFdel mutant restored limited interaction with TPL.
interaction (Figure 4G). These data together suggested that the whole Pro-rich domain may be important for the TPL–STF interaction. Truncation of the LiSH and CTLH domains (amino acids 93 to 1134; delTPL) showed interaction with STF, but with greatly reduced strength (Figure 4G), suggesting that the LiSH and CTLH domains are not indispensable but may help in the TPL–STF interaction.

To provide direct evidence that STF and Mt-TPL interaction is essential for STF function, we constructed a chimeric fusion protein in which Mt-TPL was fused in-frame to the STFdel mutant, forming STF:STFdel-TPL (Figure 4H), and transformed it into lam1 and stf mutants. Neither STFdel (Figure 4I) nor TPL (Figure 4K) alone rescued blade outgrowth in the mutant plants, but expression of the STFdel-TPL fusion complemented the mutant narrow leaf phenotypes with wavy margins (Figure 4J), demonstrating that the corepressor TPL is required for STF-mediated leaf blade outgrowth. This suggests that the primary function of the WUS box and STF box may be to recruit TPL to the STF repressor complex.

**STF Represses AS2 during Leaf Blade Development**

The fact that STF acts as a transcriptional repressor and the HD is essential for its function indicates that STF may repress its target(s) involved in leaf development directly. In support of this notion, an Ile substitution at a conserved amino acid, Asn-147
(N147I; STFm3), in the third helix of the HD (Figure 5A), which is suggested to contact DNA (Zhang et al., 2010), abolished the ability of STF to complement the lam1 mutant (Figure 5B).

In our previous genome-wide transcript profiling analysis of the stf mutant, several LOB domain proteins showed elevated expression (Tadege et al., 2011a). AS2 encodes a LOB domain adaxial factor involved in leaf blade development in Arabidopsis. Since Mt-AS2 was not represented by probes in the M. truncatula Affymetrix gene chip, we wondered whether Mt-AS2 was upregulated in the stf mutant just like other LOB domain genes. We identified a single AS2 homolog from the M. truncatula genome that showed 61% amino acid identity to Arabidopsis AS2 protein (Supplemental Figure 7A) and that complements the Arabidopsis as2 mutant leaf phenotype and interacts with Mt-AS1 (Supplemental Figures 8A to 8C). Expression analysis by quantitative RT-PCR showed that Mt-AS2 has 2-fold higher expression in the stf mutant and 5-fold lower expression in STF-overexpressing young leaves compared with wild-type leaves (Figure 5C), suggesting that STF may repress AS2 expression. To evaluate this, we performed in situ hybridization in M. truncatula wild-type genotype R108 and stf mutant leaf primordia. In 4-week-old wild-type leaf primordium (P6 stage), AS2 was expressed in the adaxial region but never reached the leaf margin or middle mesophyll, where STF was predominantly expressed (Figures 5D and 5G). In the stf mutant leaf primordium, AS2 expression was extended to the leaf margin (Figures 5E and 5G), while the sense control showed no signal (Figure 5F), indicating that STF represses Mt-AS2 activity at the leaf margin, where cell proliferation takes place.

We next examined whether STF directly binds to the Mt-AS2 promoter and represses its activity. A 3-kb Mt-AS2 promoter region immediately upstream of the translation start site was cloned and designated as proAS2. The proAS2:AS2 construct, when introduced into the Arabidopsis as2-1 mutant, rescued the asymmetric rosette leaf phenotype, suggesting that proAS2:AS2 is functional in transgenic Arabidopsis (Supplemental Figure 8A) and the 3-kb Mt-AS2 upstream promoter sequence is sufficient to drive Mt-AS2 function. We also transformed the Arabidopsis wox1 prs double mutant with the proSTF:STF-YFP construct, which fully rescued the narrow leaf phenotype (Supplemental Figure 8B), indicating that STF-YFP is also functional when introduced into Arabidopsis. To investigate if STF is able to bind the Mt-AS2 promoter in plant cells, we performed chromatin immunoprecipitation (ChIP) assays using Arabidopsis leaf protoplasts from proAS2:AS2/as2-1 transgenic lines, as described in Methods. PCR analysis revealed that several promoter fragments, P1, P2, P3, and P4, were enriched in the ChIP samples prepared with the anti-HA antibody compared with the anti-HA antibody negative control (Figures 6A and 6B). By contrast, no PCR product was amplified using primers designed for the Mt-AS2 coding region or the Arabidopsis ACTIN gene, indicating that these sequences were absent from the immunoprecipitated chromatin (Figure 6B). Taken together, the ChIP assay indicates that STF specifically binds the Mt-AS2 upstream promoter region in vivo.

To test whether STF protein can directly bind to the fragments recovered by the ChIP assays, we performed electrophoretic mobility shift assay (EMSA). The P2 and P3 fragments were amplified by PCR and then labeled by biotin at the 3’ end, while the unlabeled probes were used as competitors. EMSA results showed that the recombinant MBP-STF protein was able to bind to P2 and P3 fragments, while binding was not detected by the control MBP protein (Figures 6C and 6D). Furthermore, 50-fold excess unlabeled probes competed out the binding completely (P2 fragment) or mostly (P3 fragment). We also detected binding between STF protein and the P4 fragment (Figure 6F). The STF...
m3 mutation in the HD, as expected, abolished the STF–DNA interaction for P2, P3, or P4 (Figures 6E and 6F). These biochemical data together indicate that STF protein can directly bind to multiple sites in the AS2 promoter.

To further establish that STF can repress Mt-AS2 promoter activity in protoplasts, a dual luciferase assay was performed in Arabidopsis protoplasts. The 3-kb Mt-AS2 upstream promoter region was fused to mini 35S driving the luciferase reporter gene (Figure 7A). The addition of STF effector significantly repressed Mt-AS2 promoter activity compared with the GUS control, while STFdel or STFm3 failed to repress it (Figure 7B). This result indicates that both the HD and CTD are required for STF-mediated repression of Mt-AS2.

The CTD of STF is required for interaction with Mt-TPL as well as for STF repression of Mt-AS2, suggesting that the STF–MT-TPL complex may be involved in the repression of Mt-AS2. Since Mt-STF had repressive activity in Arabidopsis protoplasts, we hypothesized that in the Arabidopsis tpi-1 background, Mt-STF would not fully repress the transcription of Mt-AS2 if TPL is required for this repression. To test this hypothesis, we performed a dual luciferase assay in Arabidopsis tpi-1 mutant protoplasts using STF as effector. In Landsberg erecta (Ler) protoplasts, luciferase relative activity was reduced by more than half in the presence of the STF effector compared with the GUS effector control (Figure 7C). However, in the Arabidopsis tpi-1 mutant protoplasts, STF lost its repression activity and no significant difference was observed between GUS and STF effectors (Figure 7C), indicating that the STF repression of Mt-AS2 promoter activity requires the TPL protein.

As shown in Figure 4, the STFdel-TPL fusion can rescue the N. sylvestris lam1 leaf blade phenotype. To check if the STFdel-TPL fusion can repress Mt-AS2 expression in planta, we introduced STFdel and STFdel-TPL fusion constructs under the control of the STF promoter into the M. truncatula stf mutant. In agreement with the lam1 complementation, STFdel showed no sign of complementation, while the STFdel-TPL fusion showed partial rescue of the stf mutant leaf blade with enhanced margin serrations (Figure 7D). We then compared the expression of Mt-AS2 transcript by real-time PCR in these transgenic lines. Consistent with the transgenic phenotypes, in the STFdel-transformed stf mutant plants, Mt-AS2 expression was elevated by approximately 2-fold over the wild type, similar to the untransformed stf mutant, while in the STF-complemented stf mutant, the Mt-AS2 expression level was similar to that in the wild type (Figure 7E). Interestingly, the STFdel-TPL fusion–complemented lines showed reduced Mt-AS2 expression comparable to the STF-complemented lines (Figure 7E), indicating that the STF-TPL repressor complex represses Mt-AS2 in planta. Taken together, these data indicate that recruitment of the corepressor TPL by the CTD of STF is required for direct repression of AS2 at the leaf margin during leaf blade development.

Silencing of Mt-AS2 Promotes Leaf Blade Outgrowth, While Overexpression of Mt-AS2 in N. sylvestris Results in a Narrow Leaf Phenotype

To investigate the role of AS2 in M. truncatula leaf development, we generated AS2-downregulated M. truncatula plants via RNA interference (RNAi). Silencing of Mt-AS2 in the wild type or in the stf mutant caused epinastic leaves (Figure 8A; Supplemental Figure 9A). Silencing of AS2 in the wild type resulted in wider leaf blades (Figure 8A), while silencing of AS2 in the stf mutant partially rescued the narrow leaf blade and smooth leaf margin phenotypes of stf (Figure 8B; Supplemental Figure 9B), suggesting that ectopic expression of AS2 partly
Figure 7. The STF-TPL Complex Represses Mt-AS2 Promoter Activity.
(A) Schematic representation of reporter and effector constructs used in the luciferase assay. The 3-kb Mt-AS2 promoter region was fused to a mini 35S promoter to drive the expression of the luciferase reporter gene. Elements of the scheme are not drawn to scale.
(B) Relative luciferase activities in Arabidopsis protoplasts using STF, STFm3, or STFdel as effector compared with the GUS control. Error bars indicate SD (n = 3). **P < 0.01 (t test).
(C) Relative luciferase activities using Mt-STF as effector compared with the GUS control in Arabidopsis wild-type (Ler) or tlp-1 mutant protoplasts. Error bars indicate SD (n = 3). **P < 0.01 (t test).
(D) Complementation of M. truncatula stf mutant leaf blade phenotypes by STF:STFdel (1), STF:STF (2), and STF:STFdel-TPL (3) constructs.
(E) Relative expression of AS2 determined by quantitative RT-PCR in the stf mutant background complemented with STFdel (1), STF:STF (2), and STF:STFdel-TPL (3) compared with the wild type. The expression level of AS2 in the wild type was set to 1.0. Error bars indicate SD (n = 3). **P < 0.01 (t test).
[See online article for color version of this figure.]

accounts for the narrow blade phenotype of the stf mutant. To further evaluate the consequences of Mt-AS2 ectopic expression, we transformed Mt-AS2 under the control of the cauliflower mosaic virus 35S promoter into N. sylvestris wild-type and lam1 mutant plants. Compared with the broad and flat wild-type leaves (Figure 8C), the Mt-AS2–overexpressing plants showed a range of blade phenotypes from severe curling to needle-like leaves (Figures 8D to 8F; Supplemental Figure 10). Likewise, overexpression of Mt-AS2 in the lam1 background worsened the lam1 mutant phenotype, producing shorter and erect leaves (Figure 8G). These data collectively indicate that STF-mediated repression of AS2 is essential for proper leaf blade development.

DISCUSSION

The WUS Box and STF Box of STF Are Repressive Motifs and Cooperatively Recruit the Corepressor TPL

WUS clade WOX genes are essential players in several plant developmental programs, including stem cell maintenance, embryonic patterning, seed development, leaf development, and flower development (Schoof et al., 2000; Lohmann et al., 2001; Matsumoto and Okada, 2001; Nardmann et al., 2004; Sarkar et al., 2007; Breuninger et al., 2008; Shimizu et al., 2009; Vandenbussche et al., 2009; Jü et al., 2010; Tadege et al., 2011a; Nakata et al., 2012). A common mechanism of action in WUS-clade WOX genes is their ability to act as transcriptional repressors (Lin et al., 2013). Here, molecular dissection of the M. truncatula WUS clade protein STF into four domains, NTD, HD, MD, and CTD, enabled us to gain mechanistic insight into the complexity of this repression mechanism. Our work underlines that DNA binding ability, mediated by the HD, and recruitment of corepressors, mediated by the CTD motifs, are critical for STF function. A single amino acid substitution in the HD (m3) abolished both the DNA binding ability and biological function. Thus, direct contact with DNA is indispensable for STF to promote leaf blade outgrowth. We demonstrated that two conserved motifs at the CTD, the WUS box and the STF box, are additively required for mediating transcriptional repression. These two motifs cooperate to recruit the TPL family corepressors into the STF repressor complex, and this recruitment is essential for leaf blade morphogenesis. The requirement for both the WUS box and STF box to complement the lam1 mutant can be bypassed by directly fusing Mt-TPL to truncated STF lacking these two motifs (Figure 4J), indicating that TPL is an essential partner of STF in the regulation of leaf blade development. These observations are in agreement with previous reports from Arabidopsis, in which the WUS box was shown to be required for WUS-repressive activity (Kieffer et al., 2006; Ikeda et al., 2009a) and TPL was shown to interact with the EAR-like motif (Szemenyei et al., 2008; Causier et al., 2012; Krogan et al., 2012; Wang et al., 2013), which closely resembles the WUS box and the STF box.

The STF Box Is Specific to Dicots

The WUS box and STF box appear to have been acquired relatively late in the evolution of WOX genes, with the WUS box preceding the STF box. The intact WUS box is conserved in the WUS clade (WUS, WOX1 to WOX7) WOX members found in seed plants, while partial sequences of WUS box sequences exist in intermediate and ancient WOX clades found in earlier diverging taxa (Lin et al., 2013). The intact STF box, on the other hand, is detected so far only in nonmonocot angiosperms, including the early-diverging angiosperm A. trichopoda (Figure 3B). A. trichopoda is a fascinating plant from an evolutionary standpoint, as it is the only extant species of the family Amborellaceae (endemic to the island of New Caledonia), having diverged from the angiosperm lineage 130 million years ago (Solits et al., 2008). Interestingly, the vesselless A. trichopoda has a typical dicot leaf blade with reticulate venation. One possibility is that A. trichopoda, along with eudicots but not grasses, acquired the STF lineage with its
characteristic STF box. Another possibility is that the specialized grass leaves may have lost the STF box during evolution. Further research using fossils and extant plants will help test these hypotheses and shed light on the evolution of angiosperm leaf development.

**The WOX-TPL Repressor Complex May Have a Widely Conserved Function in Cell Proliferation**

WOX acts as a transcriptional repressor in stem cell maintenance in the shoot apical meristem in *Arabidopsis* (Kieffer et al., 2006;...
The STF-TPL Complex Represses AS2 at the Adaxial-Abaxial Junction of the Leaf Margin

The development of plant lateral organs requires the coordination of cell division and cell differentiation. The major defect of the stf mutant leaf is a drastic reduction in leaf cell number in the lateral direction, which accounts for most of the narrow blade phenotype (Tadege et al., 2011a, 2011b). STF is expressed in a narrow domain at the adaxial-abaxial junction of leaf primordium including both the leaf margin and the middle mesophyll (Tadege et al., 2011a). We identified Mt-AS2 as a direct target of STF based on several types of experimental evidence, including ChiP, EMSA, luciferase assay, and expression analysis. In the ChiP analysis, STF associated with several regions in the Mt-AS2 promoter, indicating that STF may bind multiple sites in the AS2 promoter. This is similar to the direct regulation of AR7 by WUS in Arabidopsis, which was found to be associated with 11 regions spreading over the 2-kb AR7 promoter in the ChiP assay (Leibfried et al., 2005). The identification of Mt-AS2 as one of the STF direct targets explains some of the observed leaf blade phenotypes of the stf mutant. In Arabidopsis, AS2 is an adaxial polarity factor that, together with AS1, promotes cell differentiation and adaxial identity. The expression of AS2 is repressed directly by KANADI (KAN) in the abaxial domain. A G-tos-tA mutation in the KAN binding sites results in ectopic expression of AS2 in the abaxial domain and leads to adaxialized leaves of relatively normal size (Wu et al., 2008). It is possible that AS2 expression is still excluded from the WOX1/PRS region because of the repression activity of WOX1/PRS, and cell proliferation may not be affected substantially. Consistent with this, the wox1 prs kan2 quadruple mutant shows a very narrow leaf blade (Nakata et al., 2012). In M. truncatula, AS2 expression is restricted to the adaxial domain of the leaf primordium and is excluded from the STF expression domains in the leaf margin and middle mesophyll. In the stf mutant, the AS2 expression domain is expanded to the leaf margin (Figure 5), suggesting that it could repress cell proliferation at the margin by promoting premature differentiation of margin cells. In support of this, we found that silencing of M. truncatula AS2 broadens the leaf blade and partially complements the stf mutant, while its ectopic expression in N. sylvestris severely compromises leaf blade outgrowth (Figure 8). It is likely that STF coordinates cell proliferation, at least in part, by preventing the expression of cell differentiation polarity factors, including AS2, in the MD. Whether STF also directly represses abaxial polarity factors such as KAN, YABBY, ARF3, and ARF4, and whether this repression accounts for additional stf mutant phenotypes, remain to be shown. STF/LAM1 could establish and maintain a cell proliferation zone in the MD by repressing polarity factors, which may enable the achievement of a balance between cell proliferation and differentiation during leaf blade development. The requirement of Mt-TPL for both the repression of Mt-AS2 by STF and the STF-mediated regulation of blade outgrowth indicates that transcriptional repression of AS2 by the STF-TPL complex is essential for leaf blade development.

Taken together, our findings shed light on the mechanism of STF-mediated regulation of leaf blade outgrowth and suggest that the WOX-TPL complex may modulate crucial functions in plant development.

METHODS

Plant Materials and Growth Conditions

The Medicago truncatula genotype R108 and stf mutant, Nicotiana sylvestris wild type and lam1 mutant, and Arabidopsis thaliana eto1 mutant (Long et al., 2006) as well as Columbia and wox1 prs double mutant (Vandenbussche et al., 2009) were used in this research. Plants were grown at 24°C/16-h (day) and 20°C/8-h (night) photoperiods, 70 to 80% relative humidity, and 150 μmol m−2 s−1 light intensity in a greenhouse or growth chambers.

Sequence Alignment and Phylogenetic Analysis

Alignment of the amino acid sequences was performed using BioEdit software and the ClustalW program (http://bioedit.software.informer.com/). Phylogenetic analysis was performed using MEGAS2 (neighbor-joining method using default settings with 1000 bootstrap replications; http://www.megasoftware.net/). The alignment used to generate the tree is available as Supplemental Data Set 1.

Plasmid Construction and Plant Transformation

All the primers used in this study are listed in Supplemental Table 2. All lam1 or stf complementation assays utilize the pSTF-pMDC32 Gateway vector, in which the 2 × 35S promoter in pMDC32 was replaced by the STF promoter as described (Lin et al., 2013). The deletion mutations in STF (HD-MD-CTD, NTD-HD-CTD, HD-CTD, MD-CTD, and STFdel) were constructed using appropriate primers. The mutations in STF (STFm1, STFm2, STFm3, STFm1-m2L1A, STFm1-m2L2A, STFm1L1A-m2, STFm1L2A-m2, and STFm1m2) and TPLm1 were introduced using appropriate mutagenic primers; SDFX-STFdel was
constructed using synthetic sense primers with SRDX sequence. Fusion of WUS box or STF box to the STFdel mutant was performed using synthetic reverse primers containing either WUS box or STF box. To make the STFdel-TPL fusion protein, STFdel without a stop codon was cloned into the SacI and EcoRI sites of pRS300 vector (Schwab et al., 2006). Mt-TPL was then cloned in the EcoRI and NotI sites of pRS300-STFdel. STF forward primer and TPL reverse primer were used to amplify the STFdel-TPL fusion gene from pRS300-STFdel-TPL. All regions corresponding to the transgene were cloned into the pSTF-pMDC32 vector by using the Gateway system (Invitrogen). For Mt-AS2 constructs, the Mt-AS2 coding region (with or without stop codon) was amplified and cloned into pDONR207 vector and was transferred by LR reaction to other plasmids, including pMDC32 (overexpression) and pK7GWIWG2D(II) (for RNAi).

Constructs were introduced into Agrobacterium tumefaciens by freeze shock. Agrobacterium strain GV2260 was used for N. sylvestris transformation, strain GV3101 was used for Arabidopsis transformation, and strain AGL1 was used for M. truncatula transformation as described (Tadege et al., 2011a). Transgenic plants were transformed to yeast strain Y2H Gold (Clontech). Library screens were performed using the manufacturer's instructions (Clontech; Matchmaker Gold Yeast Two-Hybrid System User Manual). Over 1 million diploids were screened for interactions, and putative positives were isolated on minimal medium plates lacking His but containing 100 mg/mL aureobasidin A (for reporter AUR1-C). Interactions were further validated by the use of the ade2 (−ade) and/or MEL1 (K-/a-Gal) reporters. Positive interactor plasmids were isolated using the Easy Yeast Plasmid Isolation kit (Clontech), and each plasmid was individually retransformed into Y2H Gold with pGBK7T-STF for further confirmation. Plasmids that were confirmed to be positive with all the reporters (AUR1-C, HIS3, ADE2, and MEL1) were sequenced.

### Transient Expression Assays

Construction of the reporter GAL4-LUC plasmid was described previously (Ikeda et al., 2009a). For effector plasmids, the coding sequences of STF and mutant derivatives of STF (STFm1, STFm2, STFm1m2, and STFdel) were first cloned into pGBK7T. Then, the coding regions of the BD fusion were amplified using appropriate primers and cloned into p2GWL7 using the Gateway system (Invitrogen) to yield effector plasmids.

Construction of the reporter proAS2-mini-3SS-LUC plasmid was conducted as follows. First, a mini 3SS promoter (Lodha et al., 2013) was synthesized and annealed. Second, PCR was performed with proMAs2-F and proMAs2-R-3SS primers, which amplified the 3-kb Mt-AS2 promoter region with a mini 3SS extension. Third, the PCR product was mixed with the annealed 3SS promoter and used as the template to clone the proAS2-3SS-mini fusion, which was cloned into pCR8-D-TOPO vector and recombined with p2GWL7 using LR reaction to yield the reporter.

Transient expression assays were performed with Arabidopsis protoplasts as described (Asai et al., 2002). For each transformation, 5 mg of reporter plasmid and 4 mg of effector plasmid were used. For normalization of the activity of the reporter gene, 0.5 mg of plasmid pRLC was used as an internal control.

### Y2H Library Construction and Screening

An M. truncatula whole-plant Y2H library was prepared in the pGADT7-Rec plasmid (prey) and transformed into yeast strain Y187 (Clontech; Make Your Own “Mate and Plate” Library System). Full-length STF coding sequence was cloned into the EcoRI and BamHI sites of bait vector pGBK7T, which was transformed to yeast strain Y2H Gold (Clontech). Library screens were performed by yeast mating between the bait and prey strains according to the manufacturer’s instructions (Clontech; Matchmaker Gold Yeast Two-Hybrid System User Manual). Over 1 million diploids were screened for interactions, and putative positives were isolated on minimal medium plates lacking His but containing 100 mg/mL aureobasidin A (for reporter AUR1-C). Interactions were further validated by the use of the ade2 (−ade) and/or MEL1 (K-/a-Gal) reporters. Positive interactor plasmids were isolated using the Easy Yeast Plasmid Isolation kit (Clontech), and each plasmid was individually retransformed into Y2H Gold with pGBK7T-STF for further confirmation. Plasmids that were confirmed to be positive with all the reporters (AUR1-C, HIS3, ADE2, and MEL1) were sequenced.

### ChIP Assays

The ChIP assays were performed as described previously (Lee et al., 2007; Du et al., 2008; Xiong et al., 2013). Two million protoplasts from 14-d-old proMAs2:MAs2/As2-1 transgenic plant leaves were transformed with 60 mg of pro35S::STF-YFP DNA using the polyethylene glycol-mediated transformation method. Protoplasts were incubated at room temperature for 12 h under dark conditions. Protoplasts were cross-linked by 1% formaldehyde in W5 medium for 20 min and quenched with Gly (0.2 M) for 5 min. The protoplasts were then lysed, and the DNA was sheared on ice with sonication. The sheared chromatin was precleared by salmon sperm–sheared DNA/protein A agarose beads. Preceded chromatin (100 mL) was kept as input, while the remaining supernatant was divided into two equal volumes (1.4 mL each), with 5 mL of anti-GFP antibody (ab290) added to one and 5 mL of anti-HA antibody (ab6910) added to the other. After overnight incubation at 4°C, protein A agarose beads (40 mL) were added to each, and the samples were incubated at 4°C for 2 h to collect the immunoprecipitate. Beads were then washed five times, resuspended in elution buffer, and incubated at 65°C overnight to reverse the cross-link. DNA was purified and diluted 2-fold, and 2 mL of DNA was used as a template for each PCR amplification. The input DNA and HA antibody–precipitated DNA were used as PCR templates for the positive and negative controls, respectively. The PCR products were visualized after 35 cycles. Experiments were repeated three times with similar results. The primers used for the ChIP assays are described in Supplemental Table 2.

### EMSA

The Mt-AS2 promoter fragments (P2 to P4) were amplified by PCR using Fusion Taq and labeled by using the Biotin 3' End DNA Labeling kit (Pierce) according to the manufacturer’s manual. Fifty times molar unlabeled probes were used as competitors in the competing assays. Purified MBP, MBP-STF, and MBP-STFm3 were used in the EMSA. EMSA was performed as described previously (Zhang et al., 2010).

### Pull-Down Assays

Full-length STF CDS was amplified with appropriate primers, cloned to the Ncol and HindIII sites in pMBP-28b vector, and introduced to Escherichia coli BL21 (DE3) cells for expression. The expression of MBP-STF fusion protein was induced by 0.1 M isopropyl b-1-thiogalactopyranoside at 24°C for 9 h and purified using the amylose resin (New England Biolabs). Purified proteins were dialyzed with 1× PBS buffer with 10% (v/v) glycerol and quantified by Bradford kit (Bio-Rad). Equal amounts of MBP-STF or MBP proteins were immobilized to amine resin beads. An aliquot of 50 mL of MBP-STF beads or MBP beads was boiled in 50 mL of Laemmli buffer (4% SDS, 20% glycerol, 0.02% bromophenol blue, 10% b-mercaptoethanol, and 125 mM Tris-HCl pH 6.8). Ten microliters of supernatant was loaded onto an SDS-PAGE gel, stained with Coomassie Brilliant Blue R 250, and used as the bait control. Plant lysates were prepared from infiltrated tobacco (Nicotiana benthamiana) leaves using lysis buffer (50 mM PBS, 150 mM NaCl, 1% Triton X-100, 15% glycerol, and protease inhibitor cocktail 1:100 from Sigma-Aldrich). An aliquot of 1.2 mL of lysates was first precleared by incubating with 100 mL of amylose resin for 1 h. An aliquot of 0.5 mL of precleared lysate was incubated with 50 mL of MBP-STF beads or MBP beads for 2.5 h. The MBP-STF beads or MBP beads were centrifuged and washed five times with wash buffer (50 mM PBS, 150 mM NaCl, 1% Triton X-100, 10% glycerol, and proteinase inhibitor cocktail 1:100). After the final wash, beads were boiled in 50 mL of Laemmli buffer. Immunoblot analysis was performed using rabbit anti-GFP primary antibody (1:5000, Abcam) and goat anti-rabbit IgG secondary antibody (1:10,000; Abcam).

### Y2H Assays

The bait and prey clones used in Y2H assays were cloned into the Gateway version of pGBK7T–GW (bait) and pGADT7–GW (prey) vectors.
using the recombination-based Gateway cloning system (Invitrogen). Sets of constructs were cotransformed into Y2H Gold yeast strain (Clontech). Yeast transformants were selected on synthetic minimal double dropout medium deficient in Trp and Leu. Protein interaction tests were assessed on triple dropout medium deficient in His, Trp, and Leu or quadruple dropout medium deficient in His, Trp, Leu, and adenine with X-α-Gal. At least three clones were analyzed, and experiments were repeated three times with similar results.

BiFC Analysis and Confocal Microscopy

BiFC assays were conducted according to Lu et al. (2010). Briefly, STF, STFm1m2, and Mt-AS1 were cloned into pEARLEYGATE201-YN, while Mt-TPL and Mt-AS2 were cloned into pEARLEYGATE202-YC, by LR reaction. STF-YN, STFm1m2-YN, AS1-YN, TPL-YC, and AS2-YC were introduced into Agrobacterium by freeze shock. Pairs of combinations were coinfiltrated into 4-week-old N. benthamiana leaves. P19 was used to inhibit transgenic silencing. YFP signal was observed 48 to 60 h after infiltration by a TCS SP2 AOB5 confocal laser scanning microscope (Leica Microsystems). Results were verified in at least three repeats.

In Situ Hybridization

RNA in situ hybridization was performed on shoot apices of 4-week-old M. truncatula plants using AS2-specific probe. An Mt-AS2-specific fragment of 319 bp at the 3’ end of the CDS was amplified using primer pair ishMAS2-F and ishMAS2-R (Supplemental Table 2). The PCR product was labeled with digoxigenin (Digoxigenin-11-UTP; Roche Diagnostics) for both sense and antisense probes. The sections of shoot apices of the wild type or the stf mutant were processed and hybridized with digoxigenin-labeled probes as described previously (Zhou et al., 2011).

Gene Expression Analysis Assays

For quantitative RT-PCR, total RNAs from unexpanded young leaf tissues of genotype R108, stf mutant, and the various transgenic lines were extracted using TRIzol reagent (Invitrogen). Two micrograms of total RNA was reverse transcribed using TaqMan reverse transcription reagent (Invitrogen) in a reaction volume of 100 μL. Quantitative RT-PCR was performed with three biological repeats using SYBR Green real-time PCR Master Mix (Invitrogen). PCR was performed at 95°C for 10 min followed by 40 cycles of 95°C for 15s and 60°C for 1 min. The relative expression level of each gene was calculated using the ddCt method (Lin et al., 2013), by 40 cycles of 95°C for 15s and 60°C for 1 min. The relative expression level of each gene was calculated using the ddCt method (Lin et al., 2013), by 40 cycles of 95°C for 15s and 60°C for 1 min. The relative expression level of each gene was calculated using the ddCt method (Lin et al., 2013), by 40 cycles of 95°C for 15s and 60°C for 1 min. The relative expression level of each gene was calculated using the ddCt method (Lin et al., 2013), by 40 cycles of 95°C for 15s and 60°C for 1 min.

Accession Numbers

Sequence data from this article can be found in TAIR (http://www.arabidopsis.org), Medicago truncatula Information Resource (http://www.medicago.org), or GenBank/EMBL/Phytozone databases under the accession numbers listed in Supplemental Table 3.

Supplemental Data

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

**Supplemental Figure 1.** Sequence Alignment of STF with Its Orthologs.

**Supplemental Figure 2.** Concurrent Mutations in Both WUS Box and STF Box Abolished Complementation of the M. truncatula stf Mutant.

**Supplemental Figure 3.** Sequence Alignment and Phylogenetic Analysis of Mt-TPL and Other Members of the TPL Family Using Full-Length Amino Acid Sequences.

**Supplemental Figure 4.** STF Interacts with Other TPL Family Proteins from M. truncatula.

**Supplemental Figure 5.** STF Interacts with Mt-TPL and Localizes in the Nucleus.

**Supplemental Figure 6.** Negative Control for STF Interaction Domain Mapping.

**Supplemental Figure 7.** Identification of an M. truncatula AS2 and Interaction of Mt-AS2 with Mt-AS1.

**Supplemental Figure 8.** Complementation of Arabidopsis Mutants by M. truncatula Genes.

**Supplemental Figure 9.** Silencing of Mt-AS2 Promotes Leaf Laminar Growth and Partially Complements the stf Narrow Leaf Phenotype.

**Supplemental Figure 10.** Ectopic Expression of Mt-AS2 in Tobacco Resulted in Adaxialized Narrow Leaves.

**Supplemental Table 1.** Phenotype of Complementation Assays in N. sylvestris.

**Supplemental Table 2.** Sequences of Primers Used in This Study.

**Supplemental Table 3.** Accession Numbers or Gene Identifiers of Sequences Used for Multiple Sequence Alignment and Phylogenetic Tree Construction.

**Supplemental Data Set 1.** Text File of the Alignment Corresponding to Supplemental Figure 3.

ACKNOWLEDGMENTS

We thank Elison Blancaflor and Jin Nakashima for their help with confocal microscopy, Kranikumar Mysore for providing greenhouse space, Yuhai Cui for providing Gateway-compatible Y2H and BiFC vectors, Michiel Vandenbussche for providing wox1 prs seeds, Jennifer Fletcher for providing the as2-1 seeds, Jeffrey A. Long and TAIR for providing the tpl-1 seeds, Jen Sheen and Lei Li for sharing the protoplast-ChIP protocol, Neil McHale for stimulating discussions, and Randy Allen, Hao Lin, and Lifang Niu for critical reading of the manuscript and stimulating discussions. This work was supported by the Oklahoma Center for the Advancement of Science and Technology (Grant PBS11-002) and the National Science Foundation (Grants EPS-0814361, IOS-1354422, and DBI 0400580).

AUTHOR CONTRIBUTIONS

F.Z. designed the research, performed research, analyzed data, and wrote the article. Y.W., G.L., and Y.T. performed research, E.M.K. analyzed data and edited the article. M.T. designed the research, analyzed data, and wrote the article.

Received December 14, 2013; revised January 28, 2014; accepted February 3, 2014; published February 28, 2014.

REFERENCES


Supplemental Figure 1. Sequence alignment of STF with its orthologs.

Homeodomain (HD), teal; C terminal domain (CTD), pink, includes WUS-box, red; STF-box, blue. NTD is before HD while MD is between HD and CTD.


Supplemental Figure 2. Concurrent mutation in both WUS-box and STF-box abolished complementation of *M. truncatula* stf mutant. (A) *stf* mutant (left), *stf* mutant complemented with proSTF::STF construct (middle), and *stf* mutant transformed with proSTF::STFm1m2 constructs (right). (B) Close-up view of leaves corresponding to the mutant and transgenic plants in (A).
Supplemental Figure 3. Sequence alignment and phylogenetic analysis of Mt-TPL and other members of the TPL family using full-length amino acid sequences.

(A) Sequence alignment between Mt-TPL and At-TPL. The identical residues were boxed.

(B) Phylogenetic analysis of Medicago and Arabidopsis TPL family members.

Red star highlights the Mt-TPL used in this study. Bootstrap values from 1,000 trials were shown.
Supplemental Figure 4. STF Interacts with Other TPL Family Proteins from *M. truncatula*

Empty BD vector was used as a negative control for all AD-TPRs. The interaction between STF and TPR proteins was examined by the growth on TDO plates. STFdel which lacks the CTD showed no interaction to any TPR proteins examined supported that the CTD was essential for STF-TPR proteins interaction.
**Supplemental Figure 5.** STF Interacts with Mt-TPL and localizes in the Nucleus.

(A) Empty YN vector with empty YC vector.
(B) STF-YN vector with empty YC vector.
(C) Empty YN vector with Mt-TPL-YC vector.
(D) STF-YN and Mt-TPL-YC, Arrows point to the nucleus signals which were magnified in (E) to (G).
(E) STF-YN and Mt-TPL-YC, fluorescence channel.
(F) STF-YN and Mt-TPL-YC, corresponding differential interference contrast (DIC) image of (E) shows the nucleus pointed by arrows.
(G) STF-YN and Mt-TPL-YC, merged image of (E) and (F). FL, Fluorescence channel. BR, Bright field. Merged, fluorescence channel merged with bright field.
Supplemental Figure 6. Negative control for STF interaction domain mapping.
Different STF mutants were fused to GAL4-DBD domain and co-transformed with empty AD vector. Different point mutations in CTD are showed by lines with different colors. Red, blue, light blue or pink lines represent m1 mutation in WUS-box, m2 mutation in STF-box, m2-L1A mutation in STF-box, or m2-L2A mutation in STF-box, respectively. WB, WUS-box; SB, STF-box, respectively. EV stands for empty vector. Representative data are shown from three repeats.
Supplemental Figure 7. Identification of a *M. truncatula* AS2 and Interaction of Mt-AS2 with Mt-AS1.

(A) Protein sequence alignment of AS2 proteins from *Arabidopsis thaliana* (At-AS2) and *M. truncatula* (Mt-AS2).

(B) Yeast two-hybrid showed that Mt-AS2 interacts with Mt-AS1.

(C) BiFC assay showed that Mt-AS2 interacts with Mt-AS1 in plant cells. Bars=50 μm.
Supplemental Figure 8. Complementation of *Arabidopsis thaliana* mutants by *M. truncatula* genes

(A) *Arabidopsis as2-1* asymmetric leaf phenotype (left, asymmetric leaves indicated by white arrows) was rescued by the expression of MtAS2 protein under Medicago *AS2* promoter (*proAS2:AS2*, right)

(B) *Arabidopsis wox1 prs* mutant (left) was rescued by the expression of STF-YFP fusion protein under *STF* promoter (*proSTF:STF-YFP*, right)
Supplemental Figure 9. Silencing of Mt-AS2 promotes leaf laminar growth and partially rescued stf narrow leaf phenotype

(A) Control plant for R108 (Plants genotyped as negative from the same stage of transgenic plants were used as CK)
(B) Mt-AS2-RNAi line 1, Leaves showed down-curling and wider blade than control plants.
(C) Control plant for stf (Plants genotyped as negative from the same stage of transgenic plants were used as CK)
(D) One representative Mt-AS2-RNAi plant in stf background, Leaves showed down-curling, more serrations and also partial rescue of stf narrow leaf phenotypes.
(E) Real-time PCR analysis of Mt-AS2 expression level in CK and Mt-AS2-RNAi-1 plants.
(F) Real-time PCR analysis of Mt-AS2 expression level in CK and Mt-AS2-RNAi/stf plants.
Bars=5 cm.
Supplemental Figure 10  Ectopic expression of Mt-AS2 in tobacco resulted in adaxialized narrow leaves
Supplemental Table 1. Phenotype of complementation assays in *Nicotiana sylvestris*

<table>
<thead>
<tr>
<th>STF proteins</th>
<th>width of the biggest leaves (cm)</th>
<th>wavy leaf margin</th>
<th>plants examined</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&gt;6</td>
<td>4-6</td>
<td>2-4</td>
</tr>
<tr>
<td><strong>STF</strong></td>
<td>22</td>
<td>18</td>
<td>2</td>
</tr>
<tr>
<td><strong>HD-MD-CTD</strong></td>
<td>3</td>
<td>17</td>
<td>14</td>
</tr>
<tr>
<td><strong>NTD-HD-CTD</strong></td>
<td>0</td>
<td>24</td>
<td>12</td>
</tr>
<tr>
<td><strong>STF HD-CTD</strong></td>
<td>0</td>
<td>5</td>
<td>26</td>
</tr>
<tr>
<td><strong>STF MD-CTD</strong></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>STFdel</strong></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>SRDX-STFdel</strong></td>
<td>4</td>
<td>20</td>
<td>12</td>
</tr>
<tr>
<td><strong>STFm1</strong></td>
<td>0</td>
<td>15</td>
<td>18</td>
</tr>
<tr>
<td><strong>STFm2</strong></td>
<td>0</td>
<td>8</td>
<td>21</td>
</tr>
<tr>
<td><strong>STFm1m2</strong></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>STFdel-WB</strong></td>
<td>0</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td><strong>STFdel-SB</strong></td>
<td>0</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td><strong>STFm1-m2L1A</strong></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>STFm1-m2L2A</strong></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>STFm1L1A-m2</strong></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>STFm1L2A-m2</strong></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>STFm3</strong></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>STFdel-TPL</strong></td>
<td>3</td>
<td>12</td>
<td>19</td>
</tr>
<tr>
<td><strong>TPL</strong></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Plants were measured after 6 wk of growth in the greenhouse. The biggest leaf of each plant was measured for its width and the smoothness of leaf margin.
### Supplemental Table 2. Sequences of primers used in this study.

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Deletion assay</strong></td>
<td></td>
</tr>
<tr>
<td>STF-attB1</td>
<td>GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGTGGATGGTGTTACAAT</td>
</tr>
<tr>
<td>STF-91-attB1</td>
<td>GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGTTGTGGTGAGTTCAAGATGG</td>
</tr>
<tr>
<td>STF-164-attB1</td>
<td>GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGCTGCTGAGTTTGATTCTGC</td>
</tr>
<tr>
<td>STF-301-attB1</td>
<td>GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGTTGTGGTGAGTTCAAGATGG</td>
</tr>
<tr>
<td>STF-163-attB2</td>
<td>GGGGACCACTTTGTACAAAAAAGCAGGCTTCATGGTTGTGGTGAGTTCAAGATGG</td>
</tr>
<tr>
<td>STF-300-attB2</td>
<td>GGGGACCACTTTGTACAAAAAAGCAGGCTTCATGGTTGTGGTGAGTTCAAGATGG</td>
</tr>
<tr>
<td>STF-stop-attB2</td>
<td>GGGGACCACTTTGTACAAAAAAGCAGGCTTCATGGTTGTGGTGAGTTCAAGATGG</td>
</tr>
<tr>
<td>163-301-F</td>
<td>GCCTCAAAATGGAATCAGAATCTCATCATAAAATGAATCTGATC</td>
</tr>
<tr>
<td>163-301-R</td>
<td>AGATTCATTGTGAGTACTTGCTGATTCATTTGACGC</td>
</tr>
<tr>
<td>MtTPL-350-R</td>
<td>GGGGACCACTTTGTACAAAAAAGCAGGCTTCATGGTTGTGGTGAGTTCAAGATGG</td>
</tr>
<tr>
<td>MtTPL-93-F</td>
<td>GCCTCAAAATGGAATCAGAATCTCATCATAAAATGAATCTGATC</td>
</tr>
<tr>
<td><strong>Point mutation</strong></td>
<td>mutated sites are indicated by red and lowercase</td>
</tr>
<tr>
<td>STFm1-F</td>
<td>AATCTGATCAAACCGGCTAACGAAAGTACATTTGAGCTGAGGCAATGGC</td>
</tr>
<tr>
<td>STFm1-R</td>
<td>CATTCCTTAATGGGAAAAGTACATTTGAGCTGAGGCAATGGC</td>
</tr>
<tr>
<td>STFm1L1-F</td>
<td>AATCTGATCAAACCGGCTAACGAAAGTACATTTGAGCTGAGGCAATGGC</td>
</tr>
<tr>
<td>STFm1L1-R</td>
<td>CATTCCTTAATGGGAAAAGTACATTTGAGCTGAGGCAATGGC</td>
</tr>
<tr>
<td>STFm1L2-F</td>
<td>AATCTGATCAAACCGGCTAACGAAAGTACATTTGAGCTGAGGCAATGGC</td>
</tr>
<tr>
<td>STFm1L2-R</td>
<td>CATTCCTTAATGGGAAAAGTACATTTGAGCTGAGGCAATGGC</td>
</tr>
<tr>
<td>STFm2-R1</td>
<td>TCAGTTTGTGCGGCGAACACCAAAATCAAA</td>
</tr>
<tr>
<td>STFm2-R2-attB2</td>
<td>GGGGACCACTTTGTACAAAAAAGCAGGCTTCATGGTTGTGGTGAGTTCAAGATGG</td>
</tr>
<tr>
<td>STFm2L2A-attB2</td>
<td>GGGGACCACTTTGTACAAAAAAGCAGGCTTCATGGTTGTGGTGAGTTCAAGATGG</td>
</tr>
<tr>
<td>STFm3-F</td>
<td>TCTATTGGTTTCAGATTCAAGGAAAGTACATTTGAGCTGAGGCAATGGC</td>
</tr>
<tr>
<td>STFm3-R</td>
<td>GCCTCAAAATGGAATCAGAATCTCATCATAAAATGAATCTGATC</td>
</tr>
<tr>
<td>MtTPL-m1-F</td>
<td>CTTAAAAACTCAAGGTTAAGGACACTGAATACATTTGAGCTGAGGCAATGGC</td>
</tr>
<tr>
<td>MtTPL-m1-R</td>
<td>TAGTTGAGTCTGCAATATCTATTTGAGCTGAGGCAATGGC</td>
</tr>
<tr>
<td><strong>Protein Fusion</strong></td>
<td></td>
</tr>
<tr>
<td>SRDX-STF-F</td>
<td>ATGGATCTGGATCTAGAAAAACTGAGGTGAAAGTACATTTGAGCTGAGGCAATGGC</td>
</tr>
<tr>
<td>SRDX-attB1</td>
<td>GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGTTGTGGTGAGTTCAAGATGG</td>
</tr>
<tr>
<td>300-WUS-box-R</td>
<td>ATTCCTTAATGGGAAAAGTACATTTGAGCTGAGGCAATGGC</td>
</tr>
<tr>
<td>WUS-box-attB2</td>
<td>GGGGACCACTTTGTACAAAAAAGCAGGCTTCATGGTTGTGGTGAGTTCAAGATGG</td>
</tr>
<tr>
<td>300-STF-box-R</td>
<td>GTTTTTCAAGGAAAGAAGGACACTCAATTTGAGCTGAGGCAATGGC</td>
</tr>
<tr>
<td>STF-SalI-F</td>
<td>GAGAATCTGGATCTAGAAAAACTGAGGTGAAAGTACATTTGAGCTGAGGCAATGGC</td>
</tr>
<tr>
<td>STF-300-EcoRI-no stop</td>
<td>GGATCTGGATCTAGAAAAACTGAGGTGAAAGTACATTTGAGCTGAGGCAATGGC</td>
</tr>
<tr>
<td>MtTPL-EcoRI-F</td>
<td>GAATTCAAGGCAATCATTCTTTGAGCTGAGGCAATGGC</td>
</tr>
<tr>
<td>MtTPL-NolI-R</td>
<td>GCCTCAAAATGGAATCAGAATCTCATCATAAAATGAATCTGATC</td>
</tr>
<tr>
<td>MtTPL-attB2</td>
<td>GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGTTGTGGTGAGTTCAAGATGG</td>
</tr>
<tr>
<td>MtTPL-attB1</td>
<td>GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGTTGTGGTGAGTTCAAGATGG</td>
</tr>
<tr>
<td>MtTPL-no stop</td>
<td>GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGTTGTGGTGAGTTCAAGATGG</td>
</tr>
<tr>
<td>STF-no stop-attB2</td>
<td>GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGTTGTGGTGAGTTCAAGATGG</td>
</tr>
<tr>
<td>STF-m2-no stop</td>
<td>GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGTTGTGGTGAGTTCAAGATGG</td>
</tr>
<tr>
<td>MtAS2-no stop</td>
<td>GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGTTGTGGTGAGTTCAAGATGG</td>
</tr>
</tbody>
</table>
MtAS1-no stop  GGGGACCATTGTTGATCAAGAAAGCTGGGTCCTTTCCATTTGATTCAGCATG
Cloning
MtTPR3-F  GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGACTTCGTTGAGTAGAGAATTGG
MtTPR3-R  GGGGACCACTTTGTACAAAAAAGCAGGCTTCTTTTGAGTAGAGAATTGG
MtTPR4-F  GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGACTTTGAGCAGAGAATTGG
MtTPR4-R  GGGGACCACTTTGTACAAAAAAGCAGGCTTCTTTGTGTCAGGGATCGGGTG
MtAS2-F  GGGGACCAAGTTTGTACAAAAAAGCAGGCTTCATGACTTCGTTGAGTAGAGAATTGG
MtAS2-R  GGGGACCACTTTGTACAAAAAAGCAGGCTTCTTTTGAGTAGAGAATTGG
ProMtAS2-F  GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGACTTCGTTGAGTAGAGAATTGG
ProMtAS2-R  GGGGACCACTTTGTACAAAAAAGCAGGCTTCTTTTGAGTAGAGAATTGG
MtAS1-F  GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGACTTCGTTGAGTAGAGAATTGG
MtAS1-R  GGGGACCACTTTGTACAAAAAAGCAGGCTTCTTTTGAGTAGAGAATTGG
AtAS2-F  GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGACTTCGTTGAGTAGAGAATTGG
AtAS2-R  GGGGACCACTTTGTACAAAAAAGCAGGCTTCTTTTGAGTAGAGAATTGG
ProWUS-F  aaggtaccGTGTGTTTGATTCGACTTTTGTTCAC
ProWUS-R  aaggtaccGTGTGTTTGATTCGACTTTTGTTCAC
EMSA and ChIP assays
ProMtAS2-p1-F  GTTGGATTGTGTTCAATAAGTCTAC
ProMtAS2-p1-R  TCTCTTCCCAAATTGTCAATTCT
ProMtAS2-p2-F  ACCAAACAAATTGGAATGATGTTATG
ProMtAS2-p2-R  GTCGTTGAACTTGAATGATGTTATG
ProMtAS2-p3-F  TTGGATTGTGTTCAATAAGTCTAC
ProMtAS2-p3-R  CAAAATATACGGAAGGTTATG
ProMtAS2-p4-F  GAAAGGAAAAAGTGTGTTGTTA
ProMtAS2-p4-R  GGTGGATTGTGTTCAATAAGTCTAC
MtAS2-CDS-F  TGGGAATGGAAGGTTATG
MtAS2-CDS-R  TAAACATCAAGGACTCGT
AtActin2-F  CTCACACCGTCACCAATCT
AtActin2-R  CCAAAAAATGAACCAAGGACCC
Realtime PCR
qMtAS2-F  TGGAATGGAAGGACTCGT
qMtAS2-R  TAATGGAAGGACTCGT
qActin-F  TCAATGGAAGGACTCGT
qActin-R  ACTCAACCGTCACCAATCT
In situ
IshMtAS2-F  AAAATGGAAGGACTCGT
IshMtAS2-R  ACCCAACAGACGAAATTG
T7-sense  GCCGAATGGAAGGACTCGT
T7-antisense  GCCGAATGGAAGGACTCGT
ProMtAS2-mini35SSS
35S-F  CGGAAGGACTCGT
35S-R  CAACAGACGAAATTG
ProMtAS2-35-R  TACGAACGTTCTCCTCTCAAG
35S-R-final  CACGAACGTTCTCCTCTCAAG
**Supplemental Table 3.** Accession numbers or gene identifiers of sequences used for multiple sequence alignment and phylogenetic tree construction.

<table>
<thead>
<tr>
<th></th>
<th>Accession Number</th>
<th>Accession Number</th>
<th>Accession Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mt-STF</td>
<td>JF276252</td>
<td>Mt-TPL</td>
<td>KC525957</td>
</tr>
<tr>
<td>Ms-STL1</td>
<td>AEL30894.1</td>
<td>Mt-TPR1</td>
<td>Medtr2g104140.1</td>
</tr>
<tr>
<td>Ms-STL2</td>
<td>AEL30895.1</td>
<td>Mt-TPR2</td>
<td>Medtr3g043840.1</td>
</tr>
<tr>
<td>Gm-STL1</td>
<td>XP_003530958</td>
<td>Mt-TPR3</td>
<td>Medtr1g083700.1</td>
</tr>
<tr>
<td>Gm-STL2</td>
<td>XP_003525189</td>
<td>Mt-TPR4</td>
<td>Medtr7g112460.1</td>
</tr>
<tr>
<td>Ps-LATH</td>
<td>AFQ69082</td>
<td>Mt-TPR5</td>
<td>Medtr4g120900.1</td>
</tr>
<tr>
<td>Lj-NAO1</td>
<td>AFQ69083</td>
<td>Mt-TPR6</td>
<td>Medtr4g114980.1</td>
</tr>
<tr>
<td>Csi-STL1</td>
<td>orange1.1g039144m.g</td>
<td>Mt-AS1</td>
<td>Medtr7g061550.1</td>
</tr>
<tr>
<td>Vv-STL1</td>
<td>XP_002278336</td>
<td>Mt-AS2</td>
<td>KF150768</td>
</tr>
<tr>
<td>Vv-STL2</td>
<td>CBI15564</td>
<td>At-TPL</td>
<td>At1g15750</td>
</tr>
<tr>
<td>Rc-STL1</td>
<td>XP_002532093</td>
<td>At-TPR1</td>
<td>At1g80490</td>
</tr>
<tr>
<td>Ns-LAM1</td>
<td>AEL30893</td>
<td>At-TPR2</td>
<td>At3g16830</td>
</tr>
<tr>
<td>Pxl-MAW</td>
<td>ACA64093</td>
<td>At-TPR3</td>
<td>At5g27030</td>
</tr>
<tr>
<td>St-STL1</td>
<td>XP_006341531</td>
<td>At-TPR4</td>
<td>At3g15880</td>
</tr>
<tr>
<td>Sl-STL1</td>
<td>XP_004235799</td>
<td>At-AS2</td>
<td>AT1G65620</td>
</tr>
<tr>
<td>Pt-STL1</td>
<td>XP_002322100</td>
<td>Mt-AS2 promoter</td>
<td>KF150770</td>
</tr>
<tr>
<td>Pt-STL2</td>
<td>XP_002317877</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aq-STL1</td>
<td>Aquca_091_00003.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mg-STL1</td>
<td>mgv1a021220m</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Al-WOX1</td>
<td>XP_002862807</td>
<td></td>
<td></td>
</tr>
<tr>
<td>At-WOX1</td>
<td>AT3G18010</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amt-STL1</td>
<td>ERM94101</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
STENOFOLIA Recruits TOPELESS to Repress ASYMMETRICH LEAVES2 at the Leaf Margin and Promote Leaf Blade Outgrowth in *Medicago truncatula*

Fei Zhang, Yewei Wang, Guifen Li, Yuhong Tang, Elena M. Kramer and Million Tadege

*Plant Cell* 2014;26;650-664; originally published online February 28, 2014;
DOI 10.1105/tpc.113.121947

This information is current as of May 22, 2015