BRI1 controls vascular cell fate in the *Arabidopsis* root through RLP44 and phytosulfokine signaling

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Multicellularity arose independently in plants and animals, but invari-
ably requires a robust determination and maintenance of cell fate that is adaptive to the environment. This is exemplified by the highly specialized water- and nutrient-conducting cells of the plant vascu-
lature, the organization of which is already prepatterned close to the stem-cell niche, but can be modified according to extrinsic cues. Here, we show that the hormone receptor BRASSINOSTEROID INSENSITIVE 1 (BRI1) is required for root vascular cell-fate maintenance, as BRI1 mutants show ectopic xylem in procambial position. However, this phenotype seems unrelated to canonical brassinosteroid signaling outputs. Instead, BRI1 is required for the expression and function of its interacting partner RECEPTOR-LIKE PROTEIN 44 (RLP44), which, in turn, associates with the receptor for the peptide hormone phyto-
sulfokine (PSK). We show that PSK signaling is required for the maintenance of procambial cell identity and quantitatively controlled by RLP44, which promotes complex formation between the PSK receptor and its coreceptor. Mimicking the loss of RLP44, PSK-related mutants show ectopic xylem in the position of the procambium, whereas *rlp44* is rescued by exogenous PSK. Based on these findings, we propose that RLP44 controls cell fate by connecting BRI1 and PSK signaling, providing a mechanistic framework for the dynamic bal-
ancing of signaling mediated by the plethora of plant receptor-like kinases at the plasma membrane.

Significance

Cell-fate determination and cellular behavior in plants rely mainly on positional information and intercellular communication. A plethora of cues are perceived by surface receptors and inte-
grated into an adequate cellular output. Here, we show that the small receptor-like protein RLP44 acts as an intermediary to connect the receptors for two well-known signaling molecules, brassinosteroid and phytosulfokine, to control cell fate in the root vascu-
lature. Furthermore, we show that the brassinosteroid re-
ceptor has functions that are independent from the responses to its hormone ligands and reveal that phytosulfokine signaling promotes procambial cell identity. These results provide a mech-

A key function of signaling networks in multicellular organ-
isms is to ensure robust determination and maintenance of cell fate. In plants, extreme specialization is displayed by the cells of the vascular tissues, which are vital for the distribution of water, nutrients, and signaling molecules. Xylem tracheary elements are characterized by lignified secondary cell-wall thickenings that protect against collapse and provide mechanical support for vertical growth. Positioned between xylem and the nutrient-transporting phloem are the cells of the procambium, which give rise to the lateral meristems during secondary growth (1). In *Arabidopsis*, root vascular tissue patterning is set up in the embryo by mutual an-
tagonism of auxin and cytokinin signaling domains (2–5), but can adapt to environmental conditions later in development (6). After xylem precursor cells are displaced from the root meristem, an in-
tricate gene-regulatory network connected to patterning mecha-



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RLP44 is genetically required for the BR-mediated response to cell-wall modification and is sufficient to elevate BR signaling when overexpressed. RLP44 was shown to be in a complex with BRI1 and BAK1. Thus, we hypothesized that RLP44 modulates BR signaling strength in response to cues from the cell wall (35). However, it is not clear whether the RLP44-BR-signaling module plays additional roles in plant physiology (36). Here, we show that RLP44 is required for the maintenance of cell fate in the root vasculature by connecting components of the BR and PSK signaling pathways. RLP44 controls xylem differentiation in a BRI1-dependent manner by directly interacting with PSKR1 and promoting its interaction with BAK1. In addition, the rlp4 phenotype can be rescued by application of PSK peptide, and mutants affected in PSK signaling show an rlp4-like xylem phenotype, suggesting that RLP44 has a positive effect on PSK signaling, which, in turn, promotes procambial identity.

Results

RLP44 Is Expressed in the Developing Root Vasculature. We previously demonstrated that RLP44 is present in a complex with BRI1 and BAK1 and is able to promote BR signaling upon cues from the cell wall or when overexpressed (35). To study the function of RLP44 in more depth, we generated transgenic plants expressing a translational GFP fusion of RLP44 under the control of the RLP44 promoter (pRLP44:RLP44-GFP). These plants displayed elongated, narrow leaf blades and elongated petioles, reminiscent of BRI1-overexpressing plants (Fig. 1 A and B) (37), as previously observed for RLP44 overexpression (35). We crossed a pRLP44:RLP44-GFP line with the RLP44 loss-of-function mutant rlp4–null (35), resulting in plants with a wild-type–like appearance (Fig. 1C), demonstrating that the fusion protein is functional and confirming that the transgenic RLP44 expression (SI Appendix, Fig. S1A) is causative for the observed morphological effects. In the root apical meristem of pRLP44:RLP44-GFP and pRLP44:RLP44-GFP (rlp4–null) fusion protein fluorescence was markedly enriched in the stele toward the more mature part of the root (Fig. 1 D–G and SI Appendix, Fig. S1 B–E) in accordance with previously published transcriptomic data (37) and β-glucuronidase reporter activity under control of the RLP44 promoter (SI Appendix, Fig. S1 F and G). In the differentiating part of the root stele, RLP44-GFP fluorescence was present in all cell types, including the undifferentiated procambial cells (Fig. 1 H and I and SI Appendix, Fig. S1 C and D).

RLP44 Controls Xylem Cell Fate. Because our reporter lines suggested expression of RLP44 in the stele, we assessed the role of RLP44 in vascular development. We visualized lignified secondary cell walls in rlp4–null loss-of-function mutants through basic fuchsin staining. Strikingly, we observed supernumerary metaxylem cells, frequently outside the primary xylem axis in the position of the procambium (Fig. 2 A and B and SI Appendix, Fig. S2A), a phenotype we never observed in wild-type roots. Quantification of metaxylem cells in seedling roots of both rlp4–null and the T-DNA insertion line rlp4–3 d 6 after germination showed a significant increase (Fig. 2C), suggesting that RLP44 controls xylem cell fate. Expression of RLP44 under control of its own promoter complemented this phenotype (SI Appendix, Fig. S2B). Since we had previously identified RLP44 as an activator of BR signaling, we analyzed the root xylem of a number of BR-related mutants spanning a broad range of growth phenotypes. Hypomorphic bri1 mutants such as bri1–null (38), bri1–301 (20), and bri1–5 (39), the more severe signaling mutant bri1–2 (20), as well as the BR-deficient biosynthetic mutants constitutive photomorphogenic dwarf (cpd) (40) and dwarf4–102 (41) did not show a pronounced increase in xylem cell number (Fig. 2 D and SI Appendix, Fig. S2 C and D). In sharp contrast, bri1 null alleles such as a previously characterized T-DNA mutant (termed bri1–null) (42) and the bri1 bri3 bri3 triple mutant (called bri–triple from hereon) (43) displayed a marked increase in the number of differentiated xylem cells and cells in the procambium (Fig. 2E), whereas expression of BRI1 under the control of its own promoter in bri1–null restored wild-type–like xylem (Fig. 2F). Taken together, our results show that the xylem differentiation phenotype does not correlate with the severity of BR-deficiency–related growth phenotypes (SI Appendix, Fig. S2E). This is exemplified by the comparison between cpd and bri1–null, with cpd displaying wild-type–like xylem cell numbers, despite exhibiting a bri1–null–like growth phenotype. Thus, the control of xylem cell number requires the presence of both BRI1 and RLP44. To test whether increased levels of BRs in BRI1 loss-of-function mutants (39) contribute to the xylem phenotype, we depleted endogenous BRs in the wild type and bri–triple plants with the BR biosynthesis inhibitor propiconazole (PPZ) (44), rendering wild-type plants indistinguishable from the mutant (SI Appendix, Fig. S4A). However, metaxylem cell number was not significantly affected in either genotype by PPZ treatment (SI Appendix, Fig. S4B), despite a slightly elevated number of metaxylem cells in wild type. However, PPZ treatment occasionally led to gaps in the protoxylem (SI Appendix, Fig. S4C), a phenotype also found in bri–triple (Fig. 2D and SI Appendix, Fig. S3) and in dnf4–102 (SI Appendix, Fig. S4D), but not in any other mutant (SI Appendix, Fig. S4 D–F), suggesting that BR signaling has a role in the maintenance of protodermal cells that the cpd mutant is not strictly equivalent to dnf4–102 for unknown reasons. Conversely, neither root-growth–promoting nor root-growth–inhibiting doses of brassinolide (BL) (SI Appendix, Fig. S4G) affected xylem cell numbers (SI Appendix, Fig. S4H). Activating BR signaling downstream of BRI1 by inhibiting BIN2 and other GSK3-like kinases through bikinin treatment (45) partially rescued the short-root phenotype of bri–triple (SI Appendix, Fig. S5A), but did not significantly alter the metaxylem cell number in either mutant or wild type (SI Appendix, Fig. S5B). This indicates that BR1, rather than canonical downstream BR-signaling components, is critical for normal xylem cell fate. To assess whether BRI1 kinase activity is required for the control of cell fate, we analyzed bri1–1 (46, 47), which harbors a point mutation in the kinase domain (A909T) and is expected to prevent adenine nucleotide binding and thus to render the protein kinase dead (48). The bri1–1 mutant, which is morphologically indistinguishable from the transcriptional knockout bri1–null, showed supernumerary xylem cells despite the presence of BRI1 protein (SI Appendix, Fig. S5C), suggesting that BRI1 kinase activity is required for the control of xylem cell fate.

BRI1 Is Required for Normal RLP44 Expression. To analyze how BRI1 and RLP44 could be linked in the control of xylem cell fate, we investigated RLP44 expression in BR-related mutants. Interestingly, the expression of RLP44 was reduced in bri1–null, suggesting that
RLP44 and BRI1 are required for the control of xylem cell fate. (A) Overview of xylem differentiation in the Arabidopsis root and schematic representation of the stele. Gray square in root schematic indicates point of xylem observation. (B) Basic fuchsin staining of 6-d-old Arabidopsis root. DIC image shows secondary cell-wall thickenings of metaxylem and metaxylem (Left), and basic fuchsin labels lignified secondary cell walls (Middle). Confocal stacks allow xylem number quantification of the indicated genotypes in orthogonal view (Right). Note ectopic metaxylem in procambial position (arrow). (Left) A median plane image. (Middle) A maximum projection. (Scale bar: 50 µM.) (C and D) Frequency of roots with the indicated number of metaxylem cells in rlp4 and BR-related mutants. Right in D shows orthogonal view and maximum projection of br-triple root. Note ectopic metaxylem (arrow) and disrupted protostele (arrowhead). Asterisks indicate statistically significant difference from Col-0 based on Dunn’s post hoc test with Benjamini–Hochberg correction after Kruskal–Wallis modified U test (*P < 0.05; **P < 0.001). (E) Transgenic expression of BRI1 under control of its own regulatory 5′ sequence rescues the ectopic xylem phenotype of brl-null.

Fig. 2. RLP44 and BRI1 are required for the control of xylem cell fate. (A) Overview of xylem differentiation in the Arabidopsis root and schematic representation of the stele. Gray square in root schematic indicates point of xylem observation. (B) Basic fuchsin staining of 6-d-old Arabidopsis root. DIC image shows secondary cell-wall thickenings of metaxylem and metaxylem (Left), and basic fuchsin labels lignified secondary cell walls (Middle). Confocal stacks allow xylem number quantification of the indicated genotypes in orthogonal view (Right). Note ectopic metaxylem in procambial position (arrow). (Left) A median plane image. (Middle) A maximum projection. (Scale bar: 50 µM.) (C and D) Frequency of roots with the indicated number of metaxylem cells in rlp4 and BR-related mutants. Right in D shows orthogonal view and maximum projection of br-triple root. Note ectopic metaxylem (arrow) and disrupted protostele (arrowhead). Asterisks indicate statistically significant difference from Col-0 based on Dunn’s post hoc test with Benjamini–Hochberg correction after Kruskal–Wallis modified U test (*P < 0.05; **P < 0.001). (E) Transgenic expression of BRI1 under control of its own regulatory 5′ sequence rescues the ectopic xylem phenotype of brl-null.

reduced RLP44 levels could at least partially explain the xylem phenotype of this mutant (SI Appendix, Fig. S6 A and B). Consistent with this notion, uncoupling RLP44 transcription from BR11 control through driving the expression of an RLP44 transgene by the 35S promoter could alleviate the brl-null xylem phenotype (SI Appendix, Fig. S6C), but not its growth defects (SI Appendix, Fig. S7), suggesting that BR11 and RLP44 indeed act in the same pathway regulating xylem cell fate. As RLP44 expression was only mildly affected in brl hypomorphs, cpd, dafw-102, bin2-1 or by BR depletion (SI Appendix, Figs. S6A and B and S8A–C), BR signaling output-independenent control of RLP44 expression by BR11 may explain the presence and absence of vascular cell-fate defects in the various BR-related mutants. Conversely, BL or bikinin treatment, as well as BR11-independent activation of BR-signaling outputs through hyperactive versions of the transcription factors BES1 and BZR1, did not alter RLP44 transcript levels in an appreciable manner (SI Appendix, Fig. S8A). These results corroborate previous genome-wide transcriptome analyses showing that expression of RLP44 is strongly reduced in the null mutant bni1-116, but in contrast to that of bona fide BR target genes, is not recovered in the bni1-116 bsr1-1D double mutant, which has constitutively activated BR-signaling outputs (24) (SI Appendix, Fig. S8D). In line with this, RLP44 is not among the experimentally defined targets of BZR1 or BES1 (24, 25). Finally, the limited effects of BR-signaling-related cues on RLP44 transcript levels are consistent with publicly available transcriptome data (49), SI Appendix, Fig. S9). Taken together, our findings indicate that the phenotype of bni1 loss-of-function mutants is at least partially independent from BR-signaling outputs and suggest that RLP44 exerts its function downstream of BR11 through other signaling components.

Vascular Cell-Fate Determination by RLP44 and BR11 Is Independent of BR-Signaling–Mediated Control of Cell Proliferation. We next asked whether the increase in xylem cell number observed in the rlp44 mutant could be caused by enhanced cell proliferation. In rlp44-3, vascular cell number was indistinguishable from wild type in the differentiation zone, suggesting normal meristematic activity (SI Appendix, Fig. S10A). The bni1null mutant, which did not display ectopic xylem cells, showed a significant increase in total vascular cell number (SI Appendix, Fig. S10A), consistent with the described role of BR signaling in controlling formative cell divisions (50). These results suggest that increased proliferation in the vasculature is not a prerequisite for an increase in metaxylem. In line with this, depletion of BRs by PPZ resulted in a pronounced increase of vascular cell number (SI Appendix, Fig. S10 B and C). When PPZ-treated roots were supplemented with 0.5 nM of BL, both root growth and vascular cell number were fully recovered (SI Appendix, Fig. S10 B and C). A higher dose of 5 nM BL suppressed root growth and led to a strongly decreased vascular cell number (SI Appendix, Fig. S10C). The rlp44null mutant displayed a wild-type–like response to the manipulation of BR levels in terms of cell number (SI Appendix, Fig. S10C), further supporting the independence of xylem cell fate from BR-signaling-mediated control of cell proliferation. Moreover, the expression domain of the xylem precursor marker pTMO3:NL5:3sGFP (51) was unaltered in rlp44null root meristems (SI Appendix, Fig. S11), suggesting that the acquisition of xylem cell fate in the mutant is a late event occurring outside of the meristem.

RLP44 Controls Xylem Cell Fate by Promoting PSK Signaling. The results described so far suggested that the maintenance of procambial cell identity in the root requires the presence of both BR11 and RLP44, with RLP44 acting downstream of BRI1. Thus, we speculated that, devoid of a kinase domain, RLP44 is likely required to interact with and influence the activity of another signaling component(s), which, in turn, control(s) xylem cell fate. Interestingly, in addition to BRI1 and its close homologs BRL1, BRL2, and BRL3, the LRR X clade of RLKs harbors the receptors for the peptide growth factor PSK, PSKR1, and -2 (17). As PSK signaling has also been implicated in promoting the
transdifferentiation of *Zinnia elegans* mesophyll cells into tracheary elements (52, 53), depends on functional BR signaling (29), and BRI1 (14), PSKR1 (54), PSK4, and PSK3 (SI Appendix, Fig. S12A) are coexpressed with RLP44 in the vasculature, we tested the association of RLP44 with PSKR1. Coinmunoprecipitation experiments in *Nicotiana benthamiana* showed that PSK1R1-GFP (34) was present in RLP44-RFP (35) immunoprecipitates (Fig. 3A). In addition, Forcser resonance energy transfer-fluorescence lifetime imaging microscopy (FRET-FLIM) analysis showed a pronounced reduction in fluorescence lifetime when PSKR1-GFP was coexpressed with RLP44-RFP, suggesting a direct interaction (Fig. 3B and SI Appendix, Fig. S12B), which was not affected by exogenous application of PSK peptide (SI Appendix, Fig. S12 C and D). Supporting a role of PSK signaling in the control of xylem cell fate, the pskr1-3 pskr2-1 double mutant (54) showed increased metaxylem cell numbers, reminiscent of rlp44 (Fig. 3C). A similar phenotype was observed in the *psl-1* mutant, which is impaired in the biosynthesis of PSK and other sulfated peptides (Fig. 3C) (30, 55). While exogenous PSK had no effect on wild-type xylem, it partially rescued metaxylem cell number in *psl-1* (SI Appendix, Fig. S12E) and reverted rlp44 xylem back to a wild-type pattern (Fig. 3D). Consistent with RLP44 acting through PSK signaling, the pskr1-3 pskr2-1 rlp44 Δ/Δ triple mutant did not show an enhanced phenotype compared with pskr1-3 pskr2-1 (SI Appendix, Fig. S12F). In addition, RLP44 overexpression, which was able to rescue the *brl1-null* metaxylem phenotype (SI Appendix, Fig. S6C), did not show that of pskr1-3 pskr2-1 (SI Appendix, Fig. S12G). In accordance with this, rlp44 Δ/Δ is quantitatively challenged in the root growth response to exogenous PSK (SI Appendix, Fig. S12H). Similar to RLP44 and in contrast to BR deficiency conditions, PSK-related mutants did not show gaps in the protoxylem (SI Appendix, Fig. S12I). Taken together, our results suggest that RLP44 acts through PSK receptors and is required to quantitatively control PSK-signaling strength.

**RLP44 Promotes the Association of PSKR1/BRI1 and Their Coreceptor.** To elucidate how RLP44 might promote PSK signaling, we assessed whether its presence affects the association between PSKR1 and its coreceptor BAK1, both of which also directly interact with RLP44 (Fig. 3) (35). Indeed, more BAK1 was detected in immunoprecipitates of PSKR1-GFP when RLP44-RFP was coexpressed (Fig. 4A), suggesting that RLP44 might act as a scaffold in the complex. Supporting this notion, BAK1 levels in immunoprecipitates of PSKR1-GFP when RLP44-RFP was coexpressed (Fig. 4B) (35). Indeed, more BAK1 was detected in immunoprecipitates of PSKR1-GFP when RLP44-RFP was coexpressed (Fig. 4B), suggesting that RLP44 might act as a scaffold in the complex. Supporting this notion, BAK1 levels in immunoprecipitates of PSKR1-GFP were reduced in the rlp44 Δ/Δ mutant (SI Appendix, Fig. S13A). Consistent with an essential role of BAK1/SERK3 and other SERKs as coreceptors in PSK signaling (33, 34), the serk1-3 serk2-1 serk4-1 triple mutant (56) showed increased metaxylem cell numbers (Fig. 4B). Because we had previously demonstrated that RLP44 can activate BR signaling upon cues from the cell wall (35), we assessed whether BR-signaling activation by RLP44 might occur through a similar mechanism. RLP44 and BRI1 showed direct interaction in yeast-mating-based split ubiquitin assays (SI Appendix, Fig. S13B) and FRET-FLIM analysis after transient expression in *N. benthamiana* (Fig. 4C and SI Appendix, Fig. S13C and D). Furthermore, endogenous BRI1 and BAK1 were detected in immunoprecipitates of RLP44-GFP expressed under the control of its own promoter in the rlp44 Δ/Δ mutant background (SI Appendix, Fig. S13E). Similar to what was observed for PSKR1, the presence of RLP44 increased the association of BRI1 with its coreceptor BAK1 (SI Appendix, Fig. S13F) in a line that expresses BRI1-mCitrine and BAK1-HA under control of their own promoters in the *bri1-null* background. In summary, our data suggest that RLP44 acts as a scaffold to stabilize the PSKR1-BAK1 and BRI1-BAK1 complexes, respectively. While the interaction between RLP44 and BRI1 might not play a role in the context of vascular cell-fate determination, RLP44 is controlled by BRI1 at the transcriptional level and is required to promote PSK signaling in the vasculature, which, in turn, suppresses the progression from procambial to xylem identity (Fig. 4D).

**Discussion**

**RLP44 Controls Vascular Cell Fate Through PSK Signaling.** The expanded family of plant RLK proteins and their ligands play central roles in intercellular communication, cell identity maintenance, and the regulation of cell expansion and proliferation (57). Currently, our view of these pathways is evolving to integrate the extensive cross-talk and interdependence of diverse signaling pathways (58). Here, we report that BR and PSK signaling are linked at the level of their plasma membrane receptors through RLP44 and that this signaling module is required to control xylem cell fate. Our genetic and biochemical data support a scenario where PSK-signaling strength is quantitatively controlled by RLP44, which itself is dependent on the presence of BRI1. While we cannot rule out posttranslational control of RLP44 by BRI1, for example, through phosphorylation of the cytoplasmic domain or through a role of BRI1 in the correct receptor complex assembly, this dependency is at least partially based on BRI1-mediated control of RLP44 expression. More work will be needed to understand how these BRI1-dependent, but apparently BR-signaling output-independent functions, such as the control of *RLP44* expression, are achieved at the molecular level, but the branching of signaling transduction pathways immediately downstream or even at the level of plasma membrane receptor complexes is emerging as a common feature of RLK-dependent signaling (58). BRI1 and PSKR1/2 share the requirement for interaction with SERK coreceptors to form an active, heteromeric signaling complex (33, 34, 59). Consistent with this, the
of RLP44 for the interaction between PSKR1 and its coreceptor BAK1, expanding the mechanistic diversity of RLPs.

The Role of BR Signaling in Vascular Development. It has been described that BR signaling plays an important role in the development of vascular tissue (14, 15). In addition, it has been reported that BR signaling is kept at low levels in procambial cells of leaf and hypocotyl to prevent their differentiation into xylem cells (66). Our results suggest that, in the primary xylem of the root, BR signaling plays only a minor role in controlling differentiation, in marked contrast to the strong patterning defects of BR signaling and biosynthetic mutants in the shoot (15). Conversely, at least in the root, the presence of BRI1 has a negative effect on xylem cell fate through RLP44- and PSK-signaling-mediated maintenance of procambial identity. Therefore, our results identify a role of BRI1 in root development that is independent of its role as a BR receptor.

PSK Signaling Likely Promotes Procambial Identity. Alongside classical plant hormones, signaling peptides play major roles in plant development and stress responses (67, 68). The sulfated peptide PSK has been implicated in diverse processes (26, 68). Here, we propose that PSK signaling controls xylem cell fate through promoting the maintenance of procambial identity. A number of observations support this hypothesis. First, PSK treatment rescued the ectopic xylem phenotype in rlp44 mutants. Second, PSK-related mutants showed increased xylem differentiation in procambial position, and PSK genes are coexpressed with RLP44 in procambial cells (37). Third, PSK expression is transiently increased before the acquisition of a procambial intermediate state by cells transdifferentiating into tracheary elements (52, 69), which could explain why PSK promotes tracheary element formation in Z. elegans only when applied early to the cell culture (52, 53). Finally, PSK signaling promotes callus growth and longevity, in line with a role in the maintenance of cell identity (29). However, it is unclear how PSK signaling affects cellular behavior, in part due to a lack of knowledge about potential downstream targets. To gain a deeper understanding of xylem differentiation, it will be important to unveil how the BRI1-RLP44-PSK-signaling module described here integrates with the fundamental patterning mechanisms and the gene regulatory networks controlling cell fate (2, 8).

Materials and Methods

The sources of mutants used in this study are described in SI Appendix, Table S1. Seeds were sterilized with 1:2% NaOCl in 70% ethanol and washed twice with absolute ethanol before being dried under the sterile hood. Plants were grown in 1/2 strength MS medium supplemented with 1% sucrose and 0.9% plant agar. If appropriate, 24 epi-βL, PPZ, or bikinin were added to the medium after sterilization at the indicated concentrations. After a 48- to 72-h incubation in the dark, plants were grown at 23 °C during a 16-h light period.

Details regarding the construction of plasmids and generation of transgenic plants, analysis of xylem and vascular cell number, immunoprecipitation, qPCR, interaction assays, and microscopy are provided in SI Appendix, Materials and Methods. Mutants and transgenic lines used in this study are listed in SI Appendix, Table S1.

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