An AINTEGUMENTA phospho-switch controls bilateral stem cell activity during secondary growth

Authors: Wei Xiao[1,2,3]†*, Ling Yang[4]†, David Molina[1,5], Houming Chen[6], Shan Yu[7], Yingjing Miao[6], Dagmar Ripper[1], Shulin Deng[4], Martin Bayer[1,6], Bert De Rybel[2,3], Laura Ragni[1,5]*

Affiliations:

[1]ZMBP-Center for Plant Molecular Biology, University of Tübingen, Auf der Morgenstelle 32, 72076 Tübingen, Germany.

[2]Ghent University, Department of Plant Biotechnology and Bioinformatics, Technologiepark 71, 9052 Ghent, Belgium

[3]VIB Centre for Plant Systems Biology, Technologiepark 71, 9052 Ghent, Belgium.

[4]State Key Laboratory of Plant Diversity and Specialty Crops & Guangdong Provincial Key Laboratory of Applied Botany, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China.

[5]Institute of Biology II, University of Freiburg, Schänzlerstr 1, 79104 Freiburg, Germany.

[6]Max Planck Institute for Biology, Department of Cell Biology, Max-Planck-Ring 5, 72076 Tübingen, Germany.

[7]State Key Laboratory of Biocontrol and Guangdong Key Laboratory of Plant Resources, School of Life Sciences, Sun Yat-sen University, Guangzhou, 510275, China.

†These authors contribute equally to this work.

*Corresponding author. Email: wei.xiao@psb.ugent.be (W.X.); laura.ragni@biologie.uni-freiburg.de (L.R.)

Abstract:

Plant stem cells have the remarkable ability to give rise to distinct tissues and organs throughout development. Two concentric cylinders of actively dividing stem cells are the main drivers of radial thickening during secondary growth, each producing distinct vascular cell types towards the inside and the outside. While the molecular mechanisms underlying their initiation have been well studied, it remains unclear how these stem cell layers determine which cell type is generated. Here, we demonstrate that the cross-talk between the ERECTA (ER) receptor pathway and auxin signalling controls the amount and choice of output tissue in these two stem cell regions. Mechanistically, we show that the ER pathway phosphorylates the transcription factor ANTEGUMENTA, thereby modulating bilateral stem cell activity and favouring the differentiation of only one type of cell. Our results thus show that the phosphorylation status of ANT regulates root girth and biomass accumulation by influencing bilateral stem cell production. This highlights the critical role of post-translational modifications in plant growth and development.

INTRODUCTION:

During secondary growth, plant organs undergo a tremendous increase in girth. This is required to provide sufficient mechanical strength during growth and simultaneously increases the capacity of the vascular tissues. In woody plants, most biomass is produced by organs undergoing secondary growth in the form of wood and cork[1,2]. The morphogenesis of secondary growth primarily relies on proliferation within two lateral stem cell zones called the vascular cambium and cork cambium[3-5]. In the Arabidopsis root, these stem cells are organized as two concentric cylinders of dividing cells. In the vascular cambium, the xylem is formed toward the inside and phloem towards the outside. In the cork cambium, the phelloderm forms on the inside and the cork on the outside (**FigS1A**)[6-8]. The relative amount of tissues produced toward the inside or outside varies in response to plant development or environmental cues[9,10]. However, the molecular mechanisms that control bilateral output production remain elusive.

It is, however, known that phytohormones regulate the self-renewing and differentiation of stem cells in plants[11-13]. For example, recent studies have shown that auxin maxima in proximity to the xylem vessels are required for the initiation and maintenance of vascular cambium activity[14]. Downstream of auxin, AUXIN RESPONSE FACTOR 5/ MONOPTEROS (ARF5/MP) activates the expression of *WUSCHEL-RELATED HOMEOBOX 4* (*WOX4*) and *CLASS III HOMEODOMAIN-LEUCINE ZIPPER* (*HD-ZIPIII*) genes, which regulate cell proliferation[14,15]. During periderm development, auxin accumulates in the cork cambium and controls its initiation by regulating the expression of *WOX4* and *BREVIPEDICELLUS* (*BP*)[7]. Furthermore, activation of *BP* and *WOX4* significantly enhances cambial proliferation and leads to an increase in the number of periderm layers[7,15]. Cytokinins (CK) also regulate secondary growth by promoting cell proliferation[16] via transcriptional regulators such as *LATERAL ORGAN BOUNDARIES DOMAIN 3* (*LBD3*), *LBD4*[17], and *AINTEGUMENTA* (*ANT*)[18]. The member of the AP2 transcription factor (TF) family, *ANT,* was first described to control flower organ initiation and maintenance of meristematic competence of plants[19-21]. Likewise, it works with cell-cycle player CYCD3;1 to promote cell divisions in the root vascular cambium[18]. Besides phytohormones, several peptides and their respective receptor kinases act as signalling hubs to modulate secondary growth. For example, the leucine-rich receptor-like kinases (LRR-RLK) of the ERECTA (ER) and PHLOEM INTERCALATED WITH XYLEM/TDIF RECEPTOR (PXY/TDR) families control procambium cell divisions[7,14,15,22-30]. Moreover, in hypocotyls, ER recognizes its ligands EPIDERMAL PATTERNING FACTOR (EPF)/EPFL-LIKE (EPFL4/6) and functions in xylem initiation[24].

In this study, we show that auxin signalling triggers ER-dependent phosphorylation of ANT. The phosphorylation status of ANT defines whether more cells are generated toward the inside or the outside of the vascular and cork cambium. This ANT-dependent phospho-switch thus serves as a mechanism to precisely control which conductive and protective tissue types are generated by the lateral stem cell niches during secondary growth.

RESULTS:

Auxin signalling controls the type of secondary tissues produced by lateral stem cell regions

To gain insights into the proliferation dynamics of the vascular and cork cambium stem cell regions involved in secondary growth, we initially performed a quantitative description of these stem cell regions during development (**Fig1A**). We first focussed our attention on the vascular cambium stem cell region by quantifying the number of xylem cells (representing the output toward the inside of the vascular cambium) and the total number of secondary vascular tissue cells (representing the combined bilateral output of the vascular cambium). We sampled the uppermost parts of roots (0.5 cm from hypocotyl) and quantified the aforementioned features before (6-day-old roots), during (8-day-old roots), and after (12-day-old roots) the onset of secondary growth. As an additional control, we included 20-day-old roots with prominent secondary growth (**Fig1A** and **FigS1B**). The number of secondary vascular cells and secondary xylem cells steadily and significantly increased during secondary growth (**Fig1A-B**), which led to a clear expansion of the root girth. The number of secondary xylem cells most prominently increased after day 12, confirming previous results in the hypocotyl region[31]. Although a 2-day treatment with the synthetic auxin 1-naphthaleneacetic acid (NAA) significantly increased the total number of secondary vascular tissue cells, there was no significant effect on the number of secondary xylem cells[7,14,15] (**Fig1C-D**). These results indicated that auxin treatment modified the normal bilateral vascular cambium output.

To consolidate these results, we next specifically impaired canonical auxin signalling in the vascular cambium only, and in an inducible manner using a p*PXY*::*shy2-2*-GR construct[7,32]. Repression of auxin signalling in the vascular cambium suppressed the number of secondary vascular tissue without a significant effect on the number of xylem cells (**Fig1E-F**). To investigate whether a similar response could be observed in the cork cambium stem cell region, we next quantified the number of cork cells, the total number of periderm cells and the number of periderm layers[7] during (12-day-old roots) and after (20-day-old roots) periderm formation (**FigS1B-E**). Although the number of periderm layers increased over time, the number of cork layers remained constant, with only a single cork layer (**FigS1C**). Exogenous auxin treatment significantly stimulated cork cambium cell proliferation but exhibited no discernible effect on cork cell formation (**FigS2A-D**). Next, we specifically impaired canonical auxin signalling in the cork cambium in an inducible manner using a p*PER15*::*slr*-GR construct[7,32]. Blocking auxin signalling overall resulted in fewer periderm layers, but an increase in the number of cork cells (**FigS2E-H**). Taken together these results suggest that auxin is not only required for

110 cell proliferation in the vascular and cork cambium[7,14] but also influences the relative amounts of secondary tissues that are formed from both stem cell regions.

ERECTA controls directional vascular cambium activity downstream of auxin signalling

To uncover how auxin signalling might influence vascular and cork cambium activities on a
115 molecular level, we performed bulk RNA sequencing (RNA-seq) on 12-day-old roots of the p*PXY*::*shy2-2*-GR and p*PER15*::*slr*-GR lines grown on mock or DEX (**FigS3A**). Among the subset of genes significantly repressed by DEX treatment in both lines, we found several known regulators, including *WOX4*, *BP*, *ER, LBD3*, *PIN4* and *ANT* (**FigS3B-C**). These have been demonstrated to promote vascular and or cork cambium activity[7,14,15,18,33]. In Arabidopsis, the
120 ER receptor kinase family comprises three genes: *ER, ER-LIKE1* (*ERL*1) and *ERL2*. They play a role in many aspects of plant development, including maintaining vascular cambium activity[27,29,30]. However, little is known about the underlying mechanism of ER-dependent signalling in this developmental context. Given our transcriptomic data suggested that auxin promotes expression of *ER*, we examined *ER* expression patterns (p*ER*::NLS-3xVenus) in roots
125 undergoing secondary growth (**FigS4A**)[7]. *ER* expression was found in procambium and pericycle cells, which resembles the expression pattern of auxin[7,14] (**Fig2A** and **FigS4A**). Moreover, a 2-day auxin treatment in an *er erl2* double mutant did not increase secondary vascular tissues and xylem cell numbers compared to a WT control (**FigS3D-E**), suggesting a connection between the ER pathway and auxin signalling. To further dissect a putative role of
130 ER in specifying the output of the vascular cambium, we took advantage of a previously published BAK1-INTERACTING RECEPTOR-LIKE KINASE3 (BIR3)-ER chimera protein[34]. This line allows the activation of the ER pathway in a ligand-independent way. Driving the chimeric BIR3-ER protein from a vascular cambium specific promoter (p*PXY*::BIR3-ER) triggered cell proliferation in secondary vascular tissues while the number
135 of secondary xylem cells was repressed (**Fig2B-C** and **FigS4B-C**). In addition, we observed a larger distribution of dividing vascular cambium cells in secondary vascular tissues and ectopic secondary xylem cells next to the phloem pool (**Fig2D** and **FigS4D-E**). To further explore the functions of ER during secondary growth, we investigated the effect of ER inactivation. The *er erl1 erl2* triple mutant is severely affected in primary growth[29], preventing the analysis of
140 secondary growth. Single and double mutants, however, show mild phenotypes under normal growth conditions (**FigS3D-E**)[24,27]. To overcome these limitations, we employed tissue-specific expression of an anti-GFP nanobody, which targets the ER-YPet fusion protein for

degradation. We introduced this construct in an *er erl1 erl2* triple mutant complemented with a p*ER*::ER-YPet construct[35]. Reducing expression levels of ER-YPet in the vascular cambium using this genetic tool led to a reduction in secondary vascular tissues without changing the number of secondary xylem cells (**FigS5A-B**). Collectively, our findings indicate that activation of ER signalling preferentially activates and directs the production of the vascular cambium toward the outside of the root, resulting in more secondary vascular cells.

ER phosphorylates ANT via the MPK6 kinase to determine vascular cambium activity

To explore the potential cross-talk between auxin signalling and ER pathway, we tested whether ER activates well-known auxin-responsive cambial regulators (**FigS3B-E** and **FigS5C**)[7,15]. *ANT* expression was detected in pericycle and vascular cambium cells (**Fig3A** and **FigS5D**)[15] and *ANT* levels were specifically increased in p*PXY*::BIR3-ER (**FigS5C**) roots, suggesting that ANT might be activated by the ER pathway.

Given that the ER pathway acts by phosphorylating downstream TF targets[36,37], we next explored whether this could occur via the conserved YODA-MKK4/5-MPK3/6 MAPK cascade. This pathway has been shown to function as an integral part of the ER signalling pathway in other developmental contexts, such as embryogenesis and stomatal development[29,30,35,38-41]. Thus, to understand whether MPK6 acts as a downstream component of ER in regulating root secondary growth, we generated lines expressing a dominant negative version of MPK6[42] in the vascular cambium (p*PXY*::MPK6-DN-GFP). Roots harbouring this construct showed a significant increase in the number of secondary xylem cells without impacting overall secondary vascular tissues (**Fig2E-F**). As inactivating the MPK6 pathway results in the opposite effect compared to constitutively activating ER (p*PXY*::BIR3-ER) (**Fig2B-C**), our results suggest that the ER pathway might activate MPK6 to favour directional vascular cambium activity towards secondary phloem cells.

To next explore whether ER might phosphorylate ANT via MPK6, we first conducted an *in silico* analysis to identify potential phosphorylation sites in ANT (**FigS6A**)[43-45]. We then transiently expressed HA-tagged ANT in *Nicotiana benthamiana* leaves in the presence of the Calf-intestinal alkaline phosphatase (CIP) and found that the phosphorylated form of ANT was reduced after CIP treatment (**Fig3B**), indicating that ANT is phosphorylated in *N. benthamiana* leaves. Phosphorylation of ANT was increased in the presence of MPK6 (**Fig3C**) and reduced when co-expressed with a dominant negative MPK6 version, suggesting that MPK6 can phosphorylate ANT *in planta*. To further support this result, we generated a phospho-inactive

version of ANT, in which three putative phospho-sites were mutated (S113A, S202A, and T496A, called ANT-3A). Phosphorylation of the mutated ANT-3A version was similar in the presence of MPK6 or MPK6-DN (**SFig6B**). Taken together, our data suggests that ER phosphorylates ANT via the MPK6 kinase pathway.

To next determine if ANT phosphorylation status plays a role in modulating vascular cambium activity, we first studied the effect of specifically inducing ANT or the ANT-3A in the vascular cambium (p*PXY*::ANT-GR or p*PXY*::ANT-3A-GR on DEX). *ANT* induction stimulated vascular cambium activity, resulting in more secondary vascular cells and fewer secondary xylem cells (**Fig4A-B** and **FigS6C-F**). This effect was reduced when inducing the phosphorylation mutated ANT-3A version (**FigS6C-F**), highlighting a role for ANT and its phosphorylation status in regulating directional vascular cambium activity. To further understand the functions of ANT, we generated an inducible chimeric repressor construct by fusing ANT with an EAR-repression domain (SRDX) (p*PXY*::ANT-SRDX-GR)[46]. Induction of ANT-SRDX (p*PXY*::ANT-SRDX-GR on DEX) in the vascular cambium triggered a reduction of secondary vascular tissues while xylem cell numbered was increased (**Fig4C-D**). These results are opposite to the effect of inducing ANT in the vascular cambium (**Fig4A-B**). Together, these results suggest that ER-MPK6-dependent phosphorylation of ANT controls directional vascular cambium activity and that ANT is an important factor controlling secondary xylem formation.

The ANT-dependent phospho-switch acts in both lateral cambia

Our results showed that both *ANT* and *ER* are expressed in pericycle and cork cambium cells (**Fig2A**, **Fig3A**, **FigS4A**, and **FigS5D**), and expression was downregulated by blocking auxin signalling in the cork cambium (**FigS3B-C**). Therefore, we hypothesized that a similar ER-ANT-dependent phospho-switch might be active in the periderm to control the directional output of the cork cambium. Indeed, activation of ER specifically in the periderm (p*PER15*::BIR3-ER) increased the number of the periderm cells and layers, while it showed no impact on the number of cork layers (**FigS7A-D**). Reducing expression levels of ER, specifically in the cork cambium using an anti-GFP nanobody (p*PER15*::NSlmb-vhhGFP4 in the *er erl1 erl2* triple mutant complemented with p*ER*::ER-YPet) decreased the number of periderm layers and number of periderm cells without affecting the number of cork layers or cork cells (**FigS7E-H**). Moreover, induction of *ANT,* specifically in the cork cambium (p*PER15*::ANT-GR on DEX), resulted in roots with an increased number of periderm layers

and cells without alteration of cork layers and cell numbers (**FigS8A-D**). These results thus suggest a similar role for ER in regulating ANT to control directional cork cambium activity. In summary, we proposed that both the vascular and cork cambium share a core regulatory mechanism based on an ER-dependent phospho-switch of ANT to control directional cambium activity (**Fig4E**).

DISCUSSION:

Two concentric cylinders of stem cells function in the root to accommodate the vast increase in girth during secondary growth: the vascular and cork cambium (**FigS1A**). Each of these stem cell regions can produce cells towards the inside or the outside, and the relative amounts depend on the phase of development, but can be influenced by environmental factors[9,10]. Our results shed light on the molecular regulation of cambium activity by showing that the phosphorylation status of ANT controls this differential output of both cambia (**Fig4E**). ANT is phosphorylated via the ER-MPK6 receptor pathway, and this is promoted by local auxin signalling. As such, this auxin-dependent ANT phospho-switch defines the directionality of the vascular and cork cambium activity and thus controls the relative proportions of cell types being made during secondary growth.

To date, during plant growth, the ERECTA pathway acts by phosphorylating WRKY TFs during embryogenesis[35,47,48], and *bHLH* TFs during stomata formation[36,40,49-51], and *AP2* factors during radial growth. This suggests that while the ER-MPK6 receptor pathway is conserved, developmental specificity is achieved at the level of the TF family that is phosphorylated. An alternative explanation is that only a few ER targets have been identified, and the activity of TFs such as *BABY BOOM* / *PLETHORA4* (*AP2* family), *TMO5*, *LHW*, and *T5Ls* (*bHLH* family), which are known to regulate cell proliferation during embryogenesis[52] and vascular development[53,54], respectively, is regulated by ER-mediated phosphorylation.

Interestingly, a recent study showed that the activation of the LRR-RLK receptor PXY by its ligand TDIF promotes the expression of *ANT* and *ANT-like* TFs in controlling vascular cambium proliferation[33], implying that an unknown regulator that promotes the expression of *ANT* and *ANT-like* is phosphorylated by PXY-TDIF signalling. Furthermore, it has been recently shown that ER forms a complex with PXY, which is needed for PXY-TDIF responses during xylem differentiation[55]. However, future research focusing on the interaction of ER with all members of the PXY family (PXY is not expressed in the cork cambium[7]) and whether activation of PXY signalling triggers ANT phosphorylation are necessary to dissect the

different contributions and specificity of LRR-RLK receptors in balancing cambia output by ANT phospho-switch.

Altogether, these results place ANT at the cross-talk of several pathways, highlighting the possibility that *ANT* transcript levels and phosphorylation status can be exploited to boost root radial growth and favour the formation of cell types that are relevant for biomass accumulation, such as xylem and cork (**FigS9A-C**). This will be valuable in tree breeding programs aiming to enforce biomass accumulation as wood[56] or in initiatives aiming to store more carbons in the cork and xylem of roots[1,2,57], which represent strategies to mitigate the effects of global warming.

Acknowledgments: We thank Ari Pekka Mähönen and Peter Etchells for sharing plant and materials. We thank Andrea Boch and Xudong Zhang for technical help. We thank Perrine Dalby for the initial experiment on the mutants of *ER*. We thank Shihao Su for critical reading of the manuscript. We thank Max Minne and Nataliia Konstantinova for their help with statistical analysis.

Funding: Research is supported by the German Science Foundation (Deutsche Forschungsgemeinschaft, DFG - grant RA-2590/1-3 to L.R., BA3356/3-1, BA3356/4-1, and SFB1101/B12 to M.B.), the Science and Technology Project in Guangzhou (E33309001-4 to S.D.), the European Research Council (ERC CoG PIPELINES; 101043257 to B.D.R), and DFG Major Research Instrumentation grants (INST 37/965-1 FUGG and INST 37/819-1 FUGG to the LSM880 and the Arabidopsis walk-in chamber used in this study were acquired).

Author contributions: W.X, and L.R, conceived the project. W.X, L.Y, M.B and L.R, designed the experiments. W.X, and L.Y, conducted the experiments, and analysed the data. W.X. performed RNA-seq, and W.X, and L.R, analysed the obtained data. W.X, H.C, D.M, and Y.M, generated the plant transgenic lines. W.X, and L.R, acquired the confocal images. W.X, performed the qRT-PCRs and plant embedding. W.X, L.Y, and S.Y, performed the phosphorylation experiments. W.X, and L.Y, performed the western blot. W.X, analysed data measurement. W.X., B.D.R. and LR wrote the paper with input from all authors.

Competing interests: Authors declare that they have no competing interests.

Data and materials availability:

Supplementary Materials:

Materials and Methods

Figs. S1 to S9

Tables S1 to S5

Data S1 to S2

References (1-18)

285

Figure Legends:

Fig. 1. Auxin signalling preferential controls the specific output of vascular cambium. (A), Secondary growth progression in the Arabidopsis root. Cross-sections of the most mature part of 6-day, 8-day, 12-day, and 20-day roots. Xylem cells are highlighted in blue **(B)**, Quantification of secondary vascular tissue and xylem cell numbers of the experiment shown in (Fig1A). **(C)**, Cross-sections (plastic embedding) of the uppermost part of Col0 roots. 6-day-old plants were transferred to either Mock (left panel) or 1μM NAA (right panel) plates for 2 days. **(D)**, Quantification of the number of secondary vascular tissues and xylem cells in (Fig1C). **(E)**, Cross-sections (plastic embedding) quantification of the uppermost part of 12-day-old p*PXY*::*shy2-2*-GR roots (p*MYB84*::NLS-3xGFP and W131Y background); 6-day-old plants were transferred to either Mock (left panel) or 10 μM DEX (right panel) plates for 2 days. **(F)**, Quantification of the number of secondary vascular tissues and xylem cells in (Fig1E). Black scale bars: 20μm. Count data was modelled with a generalized mixed model. Here the Poisson mix model or Poisson model was selected. P-values were adjusted using Holm-Bonferroni correction.

Fig. 2. The ERECTA and MPK6 kinase trigger the directional vascular cambium activity. (A), Vibratome-sections of the uppermost part of 18-day-old p*ER::*3xVenus-N7 root. The Red arrow indicates the cork cambium and the green arrow indicates the vascular cambium. **(B)**, Cross-sections (plastic embedding) of the uppermost part of 20-day-old Col0 (left panel) and p*PXY*::BIR3-ER (right panel) roots. **(C)**, Quantification of the number of secondary vascular tissues and xylem cells in (Fig2B). **(D)**, Edu staining of vibratome-sections of the uppermost part of 20-day-old Col0 (left panel) and p*PXY*::BIR3-ER (right panel) roots. **(E)**, Cross-sections (plastic embedding) of the uppermost part of 20-day-old Col0 (up-left panel) and p*PXY*::MPK6-DN-GFP (up-right panel) roots. **(F)**, Quantification of the number of secondary vascular tissues and xylem cells in (Fig2E). Black and white scale bars: 20μm. Count data was modelled with a generalized mixed model. Here the Poisson mix model was selected. P values were adjusted using Holm-Bonferroni correction.

Fig. 3. ER phosphorylates ANT via the MPK6 kinase. (A), Vibratome-sections of the uppermost part of 18-day-old p*ANT*::RFP roots. The Red arrow indicates the cork cambium and the green arrow indicates the vascular cambium. **(B)**, *In vivo* phosphorylation assays in *N. benthamiana.* Protein extracts, with or without treatment with Calf-intestinal alkaline (CIP), were analyzed on a Phos-tag SDS-PAGE gel and probed with an anti-HA antibody. **(C)**, *In vivo* phosphorylation assays in *N. benthamiana.* p*35S*::ANT-3xHA were transiently expressed

in leaves together with either p*35S*::MPK6-GFP or p*35S*::MPK6-DN-GFP (its dominant negative version). Protein extracts were analysed on a Phos-tag SDS-PAGE gel and probed with an anti-HA antibody. The concentration of phosphorylated ANT protein and unphosphorylated ANT protein was calculated using Fiji. White scale bars: 20μm.

Fig. 4. ANT determines vascular cambium activity by repressing xylem cell formation. (A), Cross-sections (plastic embedding) of the uppermost part of 12-day-old p*PXY*::ANT-GR roots; 7-day-old plants were transferred to either Mock (left panel) or 10 μM DEX (right panel) plates for 5 days. **(B)**, Quantification of the number of secondary vascular tissues and xylem cells shown in (Fig4A). **(C)**, Cross-sections (plastic embedding) of the uppermost part of 12-d-old p*PXY*::ANT-SRDX-GR roots; 7-day-old plants were transferred to either Mock (left panel) or 10 μM DEX (right panel) plates for 5 days. **(D)**, Quantification of the number of secondary vascular tissues and xylem cells shown in (Fig4C). **(E)**, Model explaining cambia output specificity. White scale bars: 20μm. Count data was modelled with a generalized mixed model. Here the Poisson mix model was selected. P values were adjusted using Holm-Bonferroni correction.

335 **References:**

1 Pan, Y. *et al.* A large and persistent carbon sink in the world's forests. *Science* **333**, 988-993, doi:10.1126/science.1201609 (2011).

2 Richardson, A. D. *et al.* Climate change, phenology, and phenological control of vegetation feedbacks to the climate system. *Agricultural and Forest Meteorology* **169**, 156-173, doi:10.1016/j.agrformet.2012.09.012 (2013).

3 Barra-Jimenez, A. & Ragni, L. Secondary development in the stem: when Arabidopsis and trees are closer than it seems. *Curr Opin Plant Biol* **35**, 145-151, doi:10.1016/j.pbi.2016.12.002 (2017).

4 Campilho, A., Nieminen, K. & Ragni, L. The development of the periderm: the final frontier between a plant and its environment. *Curr Opin Plant Biol* **53**, 10-14, doi:10.1016/j.pbi.2019.08.008 (2020).

5 Ragni, L. & Greb, T. Secondary growth as a determinant of plant shape and form. *Semin Cell Dev Biol* **79**, 58-67, doi:10.1016/j.semcdb.2017.08.050 (2018).

6 Wunderling, A. *et al.* A molecular framework to study periderm formation in Arabidopsis. *New Phytol* **219**, 216-229, doi:10.1111/nph.15128 (2018).

7 Xiao, W. *et al.* Pluripotent Pericycle Cells Trigger Different Growth Outputs by Integrating Developmental Cues into Distinct Regulatory Modules. *Curr Biol* **30**, 4384-4398 e4385, doi:10.1016/j.cub.2020.08.053 (2020).

8 Serra, O., Mahonen, A. P., Hetherington, A. J. & Ragni, L. The Making of Plant Armor: The Periderm. *Annu Rev Plant Biol* **73**, 405-432, doi:10.1146/annurev-arplant-102720-031405 (2022).

9 Agusti, J. & Greb, T. Going with the wind--adaptive dynamics of plant secondary meristems. *Mech Dev* **130**, 34-44, doi:10.1016/j.mod.2012.05.011 (2013).

10 Rowe, N. & Speck, T. Plant growth forms: an ecological and evolutionary perspective. *New Phytol* **166**, 61-72, doi:10.1111/j.1469-8137.2004.01309.x (2005).

11 Kende, H. & Zeevaart, J. The Five "Classical" Plant Hormones. *Plant Cell* **9**, 1197-1210, doi:10.1105/tpc.9.7.1197 (1997).

12 Gray, W. M. Hormonal regulation of plant growth and development. *PLoS Biol* **2**, E311, doi:10.1371/journal.pbio.0020311 (2004).

13 Hodge, A. Root decisions. *Plant Cell Environ* **32**, 628-640, doi:10.1111/j.1365-3040.2008.01891.x (2009).

14 Smetana, O. *et al.* High levels of auxin signalling define the stem-cell organizer of the vascular cambium. *Nature* **565**, 485-489, doi:10.1038/s41586-018-0837-0 (2019).

15 Zhang, J. *et al.* Transcriptional regulatory framework for vascular cambium development in Arabidopsis roots. *Nat Plants* **5**, 1033-1042, doi:10.1038/s41477-019-0522-9 (2019).

16 Mahonen, A. P. *et al.* Cytokinin signaling and its inhibitor AHP6 regulate cell fate during vascular development. *Science* **311**, 94-98, doi:10.1126/science.1118875 (2006).

17 Ye, L. *et al.* Cytokinins initiate secondary growth in the Arabidopsis root through a set of LBD genes. *Curr Biol* **31**, 3365-3373 e3367, doi:10.1016/j.cub.2021.05.036 (2021).

18 Randall, R. S. *et al.* AINTEGUMENTA and the D-type cyclin CYCD3;1 regulate root secondary growth and respond to cytokinins. *Biol Open* **4**, 1229-1236, doi:10.1242/bio.013128 (2015).

19 Elliott, R. C. *et al.* AINTEGUMENTA, an APETALA2-like gene of Arabidopsis with pleiotropic roles in ovule development and floral organ growth. *Plant Cell* **8**, 155-168, doi:10.1105/tpc.8.2.155 (1996).

20 Klucher, K. M., Chow, H., Reiser, L. & Fischer, R. L. The AINTEGUMENTA gene of Arabidopsis required for ovule and female gametophyte development is related to

the floral homeotic gene APETALA2. *Plant Cell* **8**, 137-153, doi:10.1105/tpc.8.2.137 (1996).
21 Mizukami, Y. & Fischer, R. L. Plant organ size control: AINTEGUMENTA regulates growth and cell numbers during organogenesis. *Proc Natl Acad Sci U S A* **97**, 942-947, doi:10.1073/pnas.97.2.942 (2000).
22 Etchells, J. P., Provost, C. M., Mishra, L. & Turner, S. R. WOX4 and WOX14 act downstream of the PXY receptor kinase to regulate plant vascular proliferation independently of any role in vascular organisation. *Development* **140**, 2224-2234, doi:10.1242/dev.091314 (2013).
23 Uchida, N. & Tasaka, M. Regulation of plant vascular stem cells by endodermis-derived EPFL-family peptide hormones and phloem-expressed ERECTA-family receptor kinases. *J Exp Bot* **64**, 5335-5343, doi:10.1093/jxb/ert196 (2013).
24 Wang, N. *et al.* Organ-specific genetic interactions between paralogues of the PXY and ER receptor kinases enforce radial patterning in Arabidopsis vascular tissue. *Development* **146**, doi:10.1242/dev.177105 (2019).
25 Hirakawa, Y., Kondo, Y. & Fukuda, H. TDIF peptide signaling regulates vascular stem cell proliferation via the WOX4 homeobox gene in Arabidopsis. *Plant Cell* **22**, 2618-2629, doi:10.1105/tpc.110.076083 (2010).
26 Etchells, J. P. & Turner, S. R. The PXY-CLE41 receptor ligand pair defines a multifunctional pathway that controls the rate and orientation of vascular cell division. *Development* **137**, 767-774, doi:10.1242/dev.044941 (2010).
27 Ikematsu, S., Tasaka, M., Torii, K. U. & Uchida, N. ERECTA-family receptor kinase genes redundantly prevent premature progression of secondary growth in the Arabidopsis hypocotyl. *New Phytol* **213**, 1697-1709, doi:10.1111/nph.14335 (2017).
28 Uchida, N. *et al.* Regulation of inflorescence architecture by intertissue layer ligand-receptor communication between endodermis and phloem. *Proceedings of the National Academy of Sciences of the United States of America* **109**, 6337-6342, doi:10.1073/pnas.1117537109 (2012).
29 Shpak, E. D., Berthiaume, C. T., Hill, E. J. & Torii, K. U. Synergistic interaction of three ERECTA-family receptor-like kinases controls Arabidopsis organ growth and flower development by promoting cell proliferation. *Development* **131**, 1491-1501, doi:10.1242/dev.01028 (2004).
30 Torii, K. U. *et al.* The Arabidopsis ERECTA gene encodes a putative receptor protein kinase with extracellular leucine-rich repeats. *Plant Cell* **8**, 735-746, doi:10.1105/tpc.8.4.735 (1996).
31 Sankar, M., Nieminen, K., Ragni, L., Xenarios, I. & Hardtke, C. S. Automated quantitative histology reveals vascular morphodynamics during Arabidopsis hypocotyl secondary growth. *Elife* **3**, e01567, doi:10.7554/eLife.01567 (2014).
32 Vermeer, J. E. *et al.* A spatial accommodation by neighboring cells is required for organ initiation in Arabidopsis. *Science* **343**, 178-183, doi:10.1126/science.1245871 (2014).
33 Eswaran, G. *et al.* Identification of cambium stem cell factors and their positioning mechanism. *bioRxiv*, 2023.2007.2020.549889, doi:10.1101/2023.07.20.549889 (2023).
34 Hohmann, U. *et al.* Constitutive Activation of Leucine-Rich Repeat Receptor Kinase Signaling Pathways by BAK1-INTERACTING RECEPTOR-LIKE KINASE3 Chimera. *Plant Cell* **32**, 3311-3323, doi:10.1105/tpc.20.00138 (2020).
35 Wang, K. *et al.* Independent parental contributions initiate zygote polarization in Arabidopsis thaliana. *Curr Biol* **31**, 4810-4816 e4815, doi:10.1016/j.cub.2021.08.033 (2021).

36 Pillitteri, L. J., Sloan, D. B., Bogenschutz, N. L. & Torii, K. U. Termination of asymmetric cell division and differentiation of stomata. *Nature* **445**, 501-505, doi:10.1038/nature05467 (2007).
37 Liu, T., Ohashi-Ito, K. & Bergmann, D. C. Orthologs of Arabidopsis thaliana stomatal bHLH genes and regulation of stomatal development in grasses. *Development* **136**, 2265-2276, doi:10.1242/dev.032938 (2009).
38 Bergmann, D. C., Lukowitz, W. & Somerville, C. R. Stomatal development and pattern controlled by a MAPKK kinase. *Science* **304**, 1494-1497, doi:10.1126/science.1096014 (2004).
39 Lukowitz, W., Roeder, A., Parmenter, D. & Somerville, C. A MAPKK kinase gene regulates extra-embryonic cell fate in Arabidopsis. *Cell* **116**, 109-119, doi:10.1016/s0092-8674(03)01067-5 (2004).
40 Meng, X. *et al.* A MAPK cascade downstream of ERECTA receptor-like protein kinase regulates Arabidopsis inflorescence architecture by promoting localized cell proliferation. *Plant Cell* **24**, 4948-4960, doi:10.1105/tpc.112.104695 (2012).
41 Shpak, E. D., McAbee, J. M., Pillitteri, L. J. & Torii, K. U. Stomatal patterning and differentiation by synergistic interactions of receptor kinases. *Science* **309**, 290-293, doi:10.1126/science.1109710 (2005).
42 Bush, S. M. & Krysan, P. J. Mutational evidence that the Arabidopsis MAP kinase MPK6 is involved in anther, inflorescence, and embryo development. *J Exp Bot* **58**, 2181-2191, doi:10.1093/jxb/erm092 (2007).
43 Zhang, M. & Zhang, S. Mitogen-activated protein kinase cascades in plant signaling. *J Integr Plant Biol* **64**, 301-341, doi:10.1111/jipb.13215 (2022).
44 Li, H. *et al.* MPK3- and MPK6-Mediated ICE1 Phosphorylation Negatively Regulates ICE1 Stability and Freezing Tolerance in Arabidopsis. *Dev Cell* **43**, 630-642 e634, doi:10.1016/j.devcel.2017.09.025 (2017).
45 Sorensson, C. *et al.* Determination of primary sequence specificity of Arabidopsis MAPKs MPK3 and MPK6 leads to identification of new substrates. *Biochem J* **446**, 271-278, doi:10.1042/BJ20111809 (2012).
46 Mahfouz, M. M. *et al.* Targeted transcriptional repression using a chimeric TALE-SRDX repressor protein. *Plant molecular biology* **78**, 311-321, doi:10.1007/s11103-011-9866-x (2012).
47 Ueda, M. *et al.* Transcriptional integration of paternal and maternal factors in the Arabidopsis zygote. *Genes Dev* **31**, 617-627, doi:10.1101/gad.292409.116 (2017).
48 Ueda, M., Zhang, Z. & Laux, T. Transcriptional activation of Arabidopsis axis patterning genes WOX8/9 links zygote polarity to embryo development. *Dev Cell* **20**, 264-270, doi:10.1016/j.devcel.2011.01.009 (2011).
49 Herrmann, A. & Torii, K. U. Shouting out loud: signaling modules in the regulation of stomatal development. *Plant Physiol* **185**, 765-780, doi:10.1093/plphys/kiaa061 (2021).
50 MacAlister, C. A., Ohashi-Ito, K. & Bergmann, D. C. Transcription factor control of asymmetric cell divisions that establish the stomatal lineage. *Nature* **445**, 537-540, doi:10.1038/nature05491 (2007).
51 Lampard, G. R., Macalister, C. A. & Bergmann, D. C. Arabidopsis stomatal initiation is controlled by MAPK-mediated regulation of the bHLH SPEECHLESS. *Science* **322**, 1113-1116, doi:10.1126/science.1162263 (2008).
52 Boutilier, K. *et al.* Ectopic expression of BABY BOOM triggers a conversion from vegetative to embryonic growth. *Plant Cell* **14**, 1737-1749, doi:10.1105/tpc.001941 (2002).

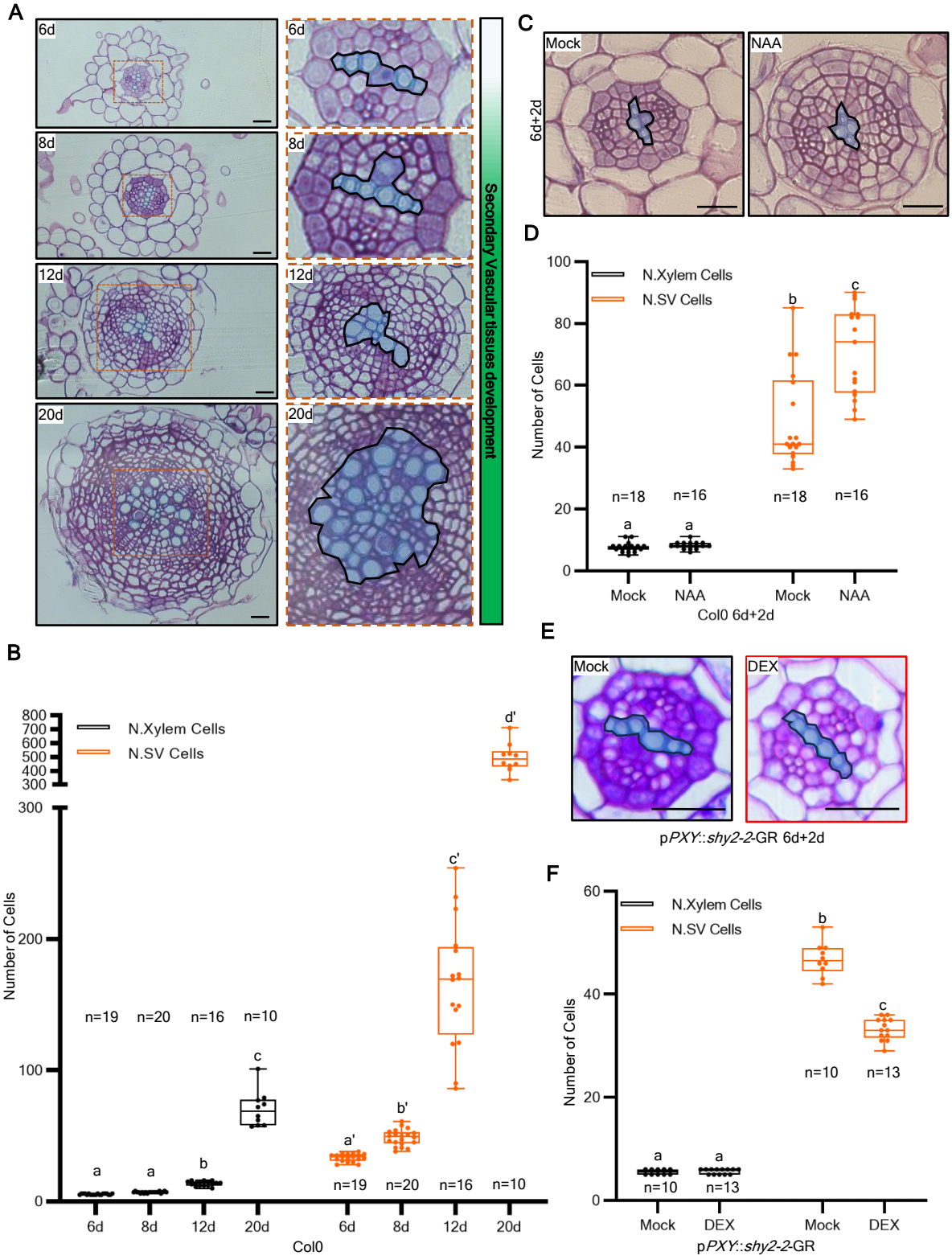
53 De Rybel, B. *et al.* A bHLH complex controls embryonic vascular tissue establishment and indeterminate growth in Arabidopsis. *Dev Cell* **24**, 426-437, doi:10.1016/j.devcel.2012.12.013 (2013).

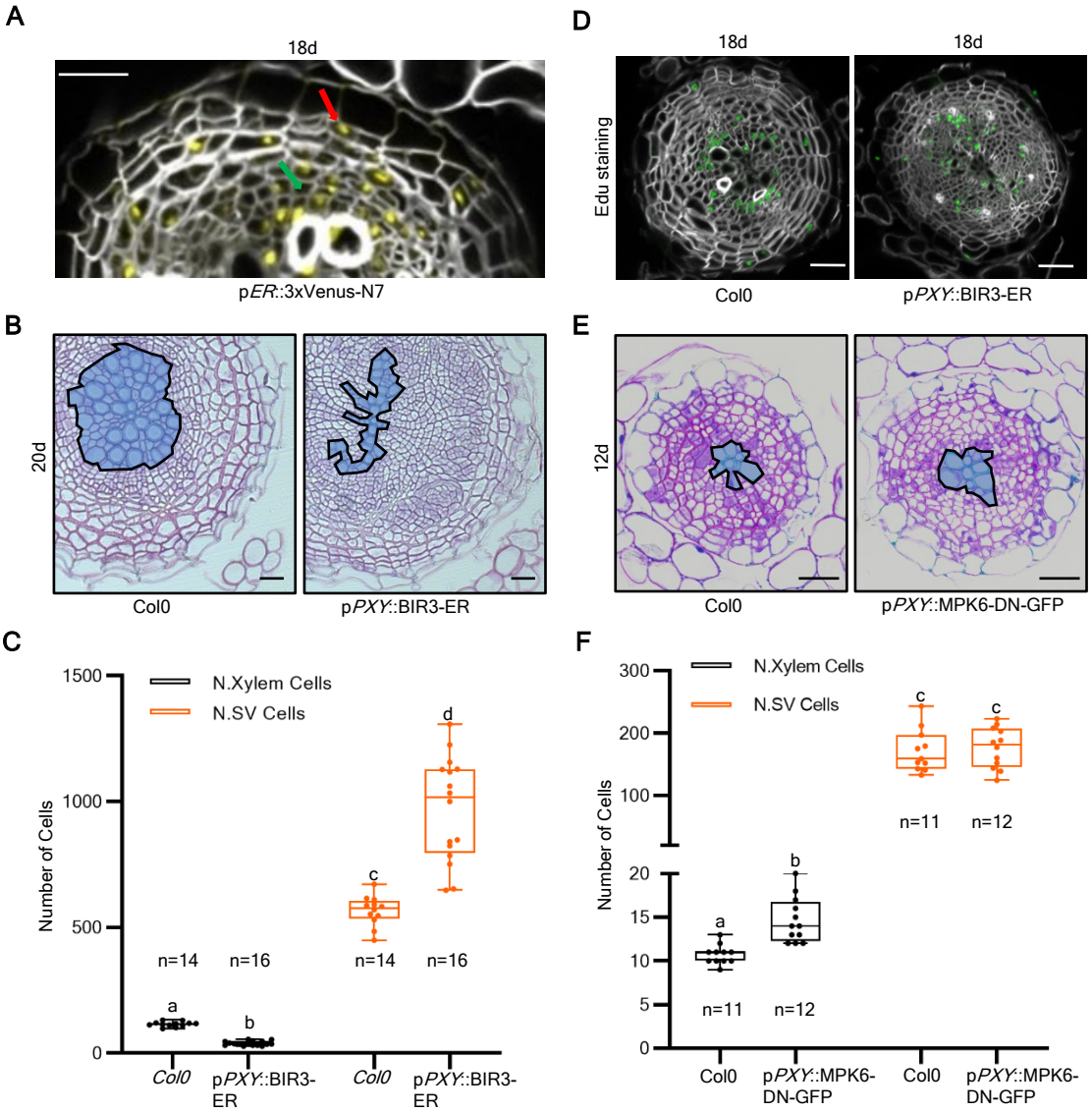
54 Mor, E. *et al.* bHLH heterodimer complex variations regulate cell proliferation activity in the meristems of Arabidopsis thaliana. *iScience* **25**, 105364, doi:10.1016/j.isci.2022.105364 (2022).

55 He, Q. *et al.* A receptor kinase complex refines cambium activity in Arabidopsis. *bioRxiv*, 2024.2005.2016.594489, doi:10.1101/2024.05.16.594489 (2024).

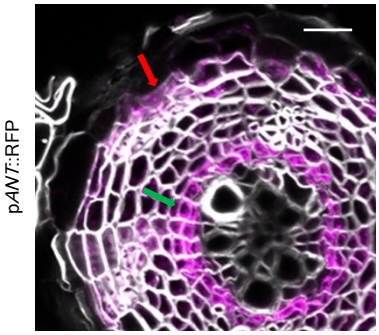
56 Koch, G. W., Sillett, S. C., Jennings, G. M. & Davis, S. D. The limits to tree height. *Nature* **428**, 851-854, doi:10.1038/nature02417 (2004).

57 *Harnessing Plants Initiative*, <https://www.salk.edu/harnessing-plants-initiative/>

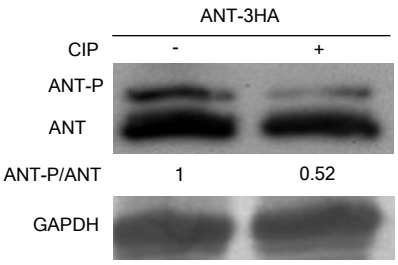

A
6d
8d
12d
20d
Secondary Vascular tissues development
C
Mock
NAA
6d+2d
D
N.Xylem Cells
N.SV Cells
Number of Cells
n=18
n=16
n=18
n=16
Mock
NAA
Mock
NAA
Col0 6d+2d
B
N.Xylem Cells
N.SV Cells
Number of Cells
n=19
n=20
n=16
n=10
n=19
n=20
n=16
n=10
6d
8d
12d
20d
6d
8d
12d
20d
Col0
E
Mock
DEX
pPXY::shy2-2-GR 6d+2d
F
N.Xylem Cells
N.SV Cells
Number of Cells
n=10
n=13
n=10
n=13
Mock
DEX
Mock
DEX
pPXY::shy2-2-GR


A
18d
pER::3xVenus-N7
B
20d
Col0
pPXY::BIR3-ER
C
N.Xylem Cells
N.SV Cells
Number of Cells
1500
1000
500
0
n=14
n=16
n=14
n=16
a
b
c
d
Col0
pPXY::BIR3-ER
Col0
pPXY::BIR3-ER
D
18d
18d
Edu staining
Col0
pPXY::BIR3-ER
E
12d
Col0
pPXY::MPK6-DN-GFP
F
N.Xylem Cells
N.SV Cells
Number of Cells
300
200
100
20
15
10
5
0
n=11
n=12
n=11
n=12
a
b
c
c
Col0
pPXY::MPK6-DN-GFP
Col0
pPXY::MPK6-DN-GFP

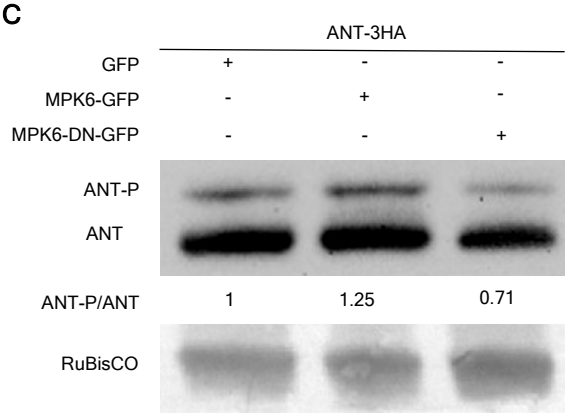
A

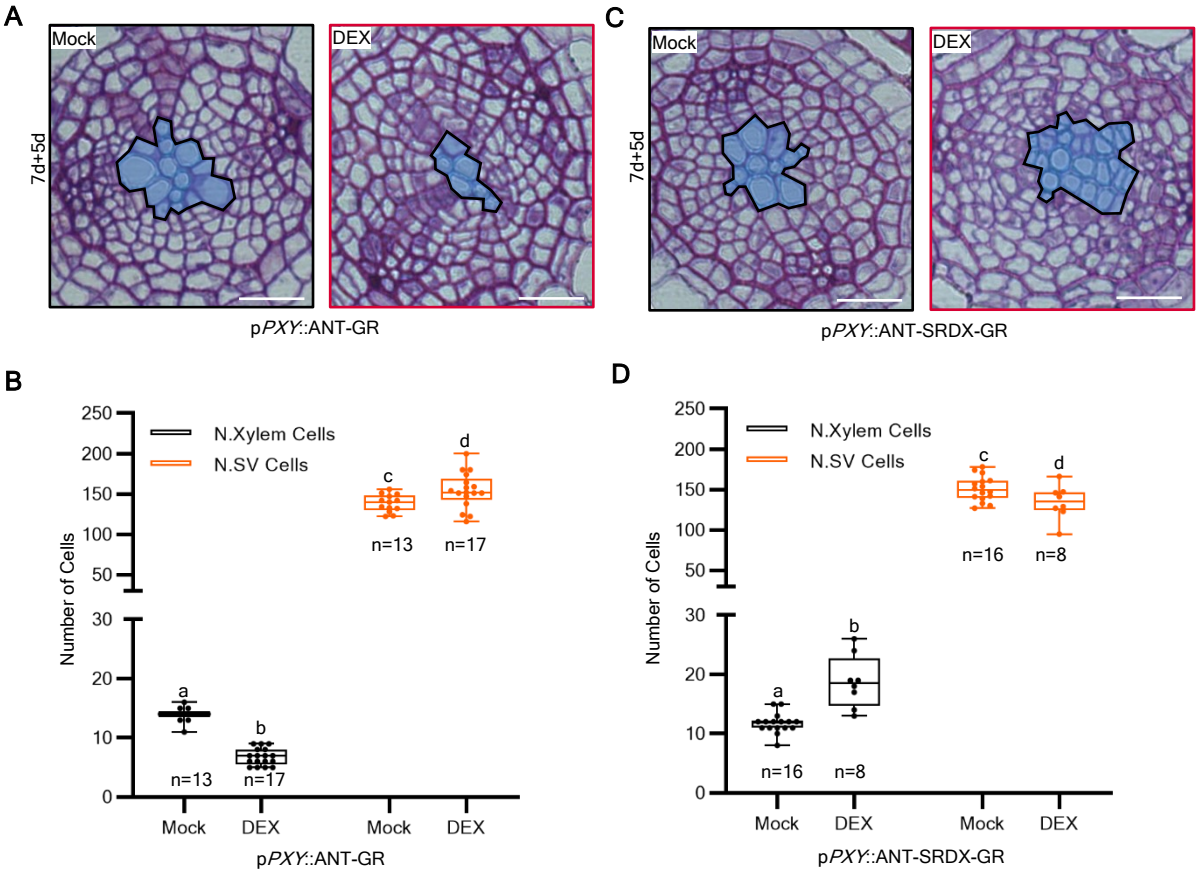

pAN7::RFP

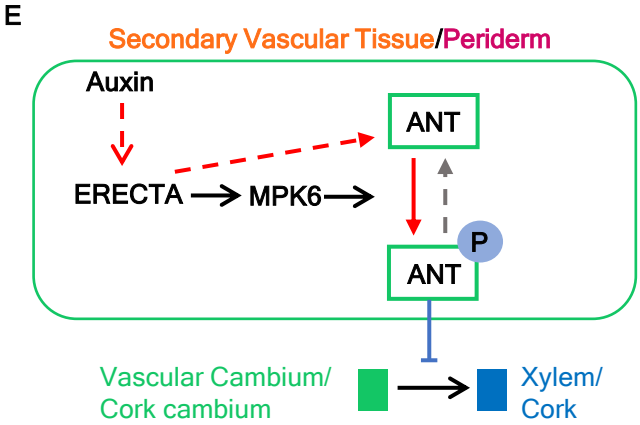
B


ANT-3HA
CIP
-
+
ANT-P
ANT
ANT-P/ANT
1
0.52
GAPDH

C


ANT-3HA
GFP
+
-
-
MPK6-GFP
-
+
-
MPK6-DN-GFP
-
-
+
ANT-P
ANT
ANT-P/ANT
1
1.25
0.71
RuBisCO


A
Mock
DEX
7d+5d
pPXY::ANT-GR
C
Mock
DEX
7d+5d
pPXY::ANT-SRDX-GR
B
N.Xylem Cells
N.SV Cells
Number of Cells
250
200
150
100
50
30
20
10
0
a
b
c
d
n=13
n=17
n=13
n=17
Mock
DEX
Mock
DEX
pPXY::ANT-GR
D
N.Xylem Cells
N.SV Cells
Number of Cells
a
b
c
d
n=16
n=8
n=16
n=8
Mock
DEX
Mock
DEX
pPXY::ANT-SRDX-GR


E
Secondary Vascular Tissue/Periderm
Auxin
ERECTA
MPK6
ANT
ANT
P
Vascular Cambium/
Cork cambium
Xylem/
Cork