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Supplementary Information for

The receptor-like kinases BAM1 and BAM2 are required for root xylem patterning

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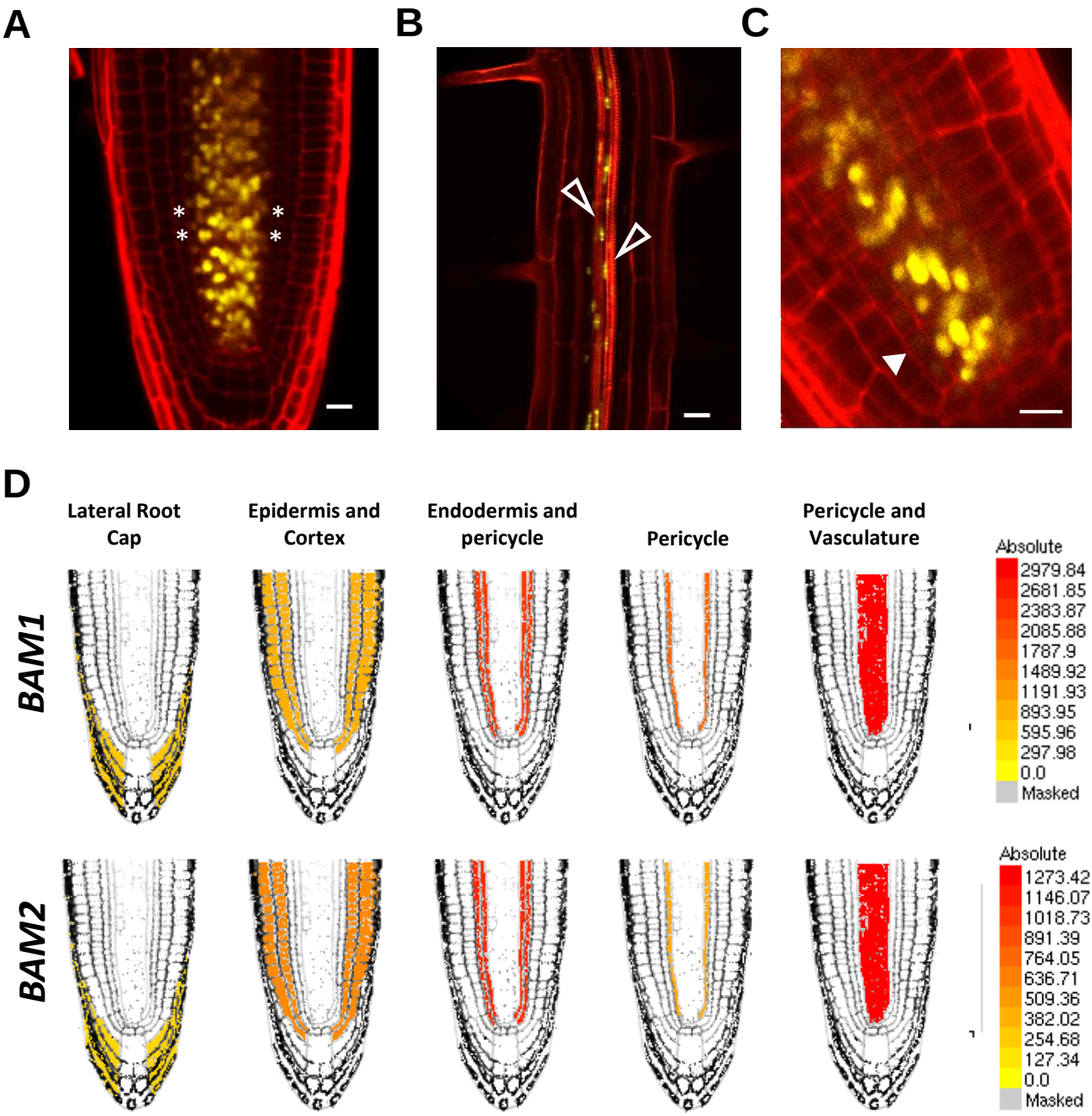
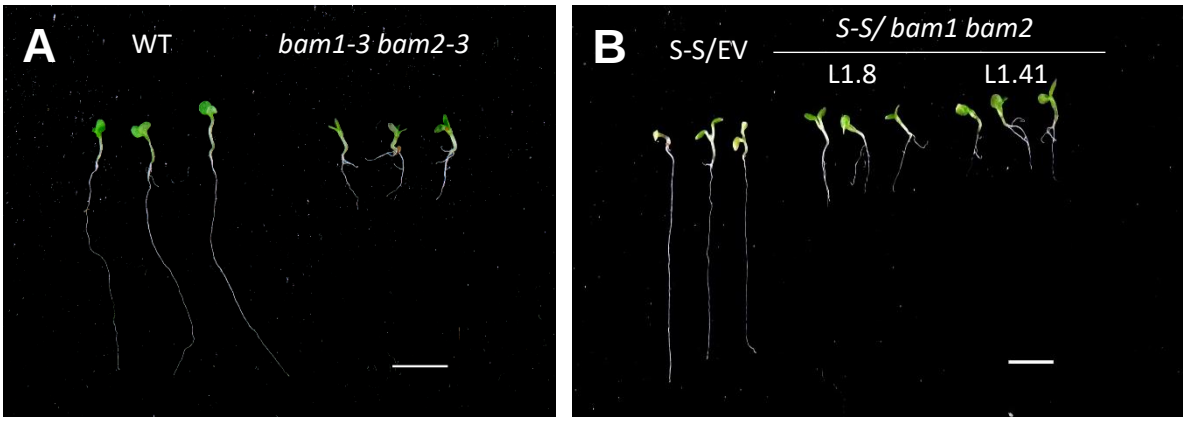
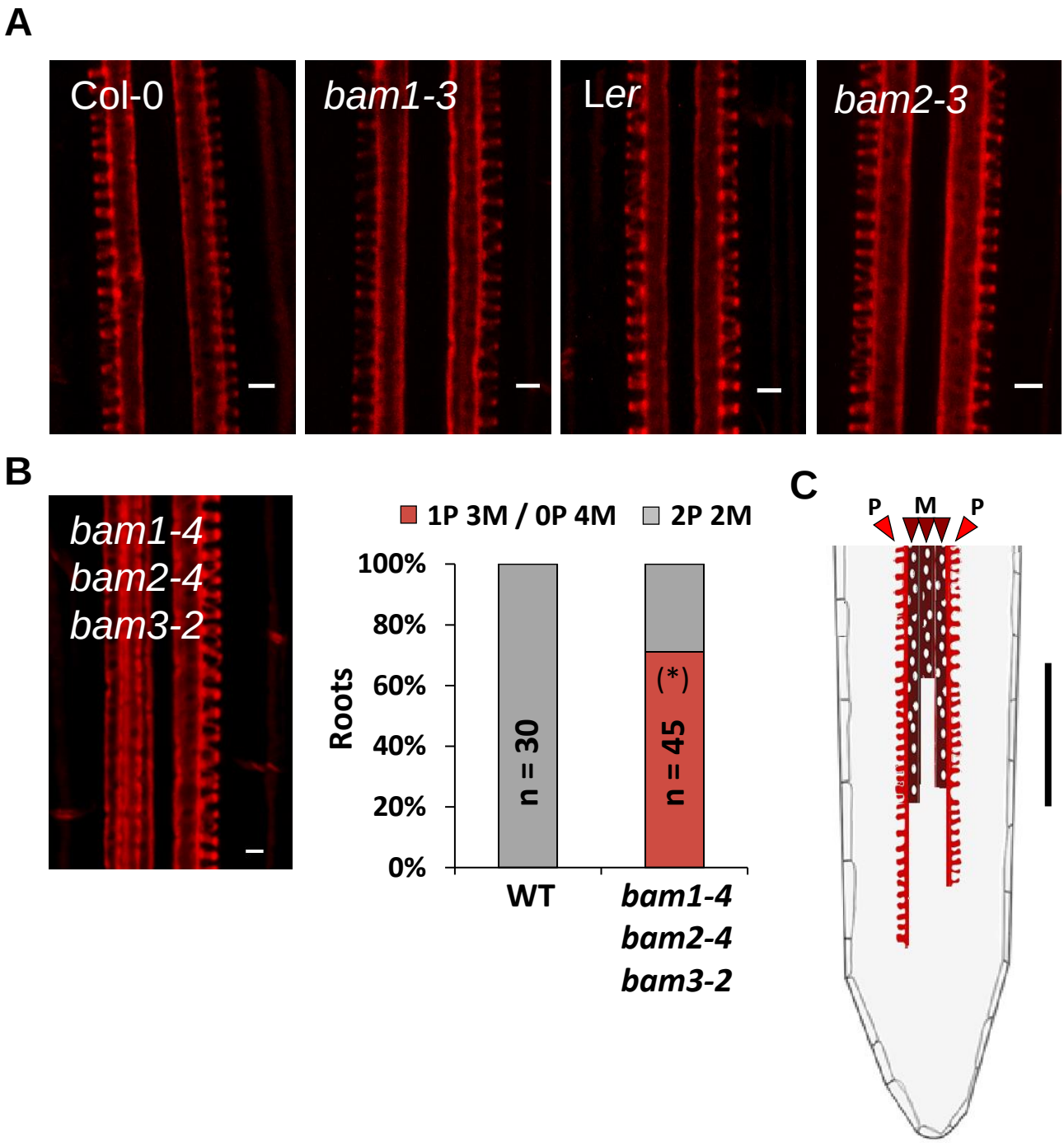
- **Supplementary videos 1-5**
- **Supplementary references**



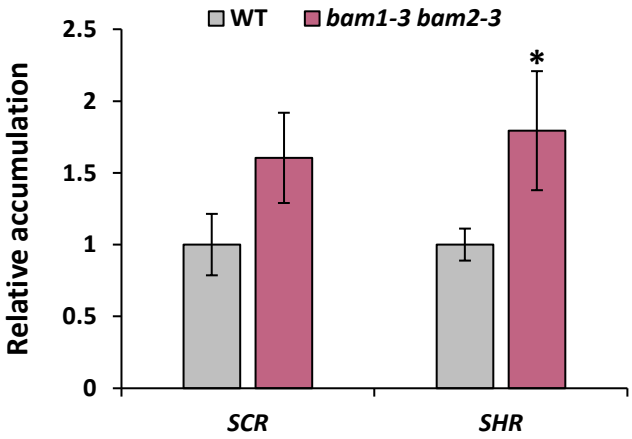
Supplementary figure 1. Expression pattern of *BAM1/2* in the root. A, B. Propidium iodide-stained root of a six-day-old transgenic *pBAM1*:*YFP-NLS* Arabidopsis seedlings. Scale bar = 10 µm (A), 20 µm (B). Asterisks indicate the position of the endodermis. Arrowheads indicate xylem cell files. **C.** Propidium iodide-stained root of a three-day-old transgenic *pBAM1*:*YFP-NLS* Arabidopsis seedlings. Scale bar = 10 µm. **D**. Tissue-specific expression of *BAM1* and *BAM2* in roots (images taken from the Arabidopsis eFP browser; data are from Gifford et al., 2008).



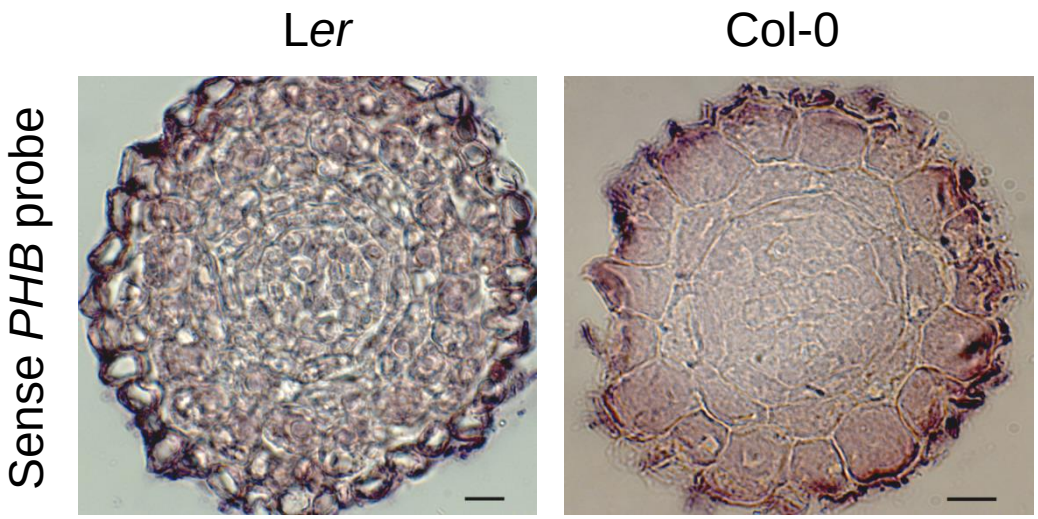
Supplementary figure 2. *bam1 bam2* double mutants display short roots. **A, B.** Six-day-old seedlings of *bam1-3 bam2-3* double mutants (**A**) or *SUC:SUL/bam1 bam2* (lines 1.8 and 1.41) (**B**) and their respective controls. WT: wild type (L*er*); S-S: *SUC:SUL*; EV: empty vector. Scale bar = 0.5 cm.



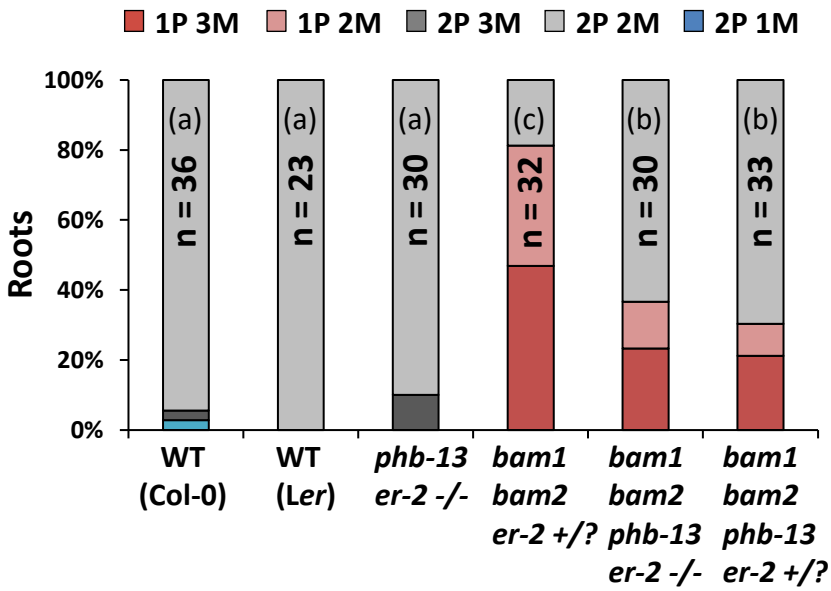
Supplementary figure 3. *bam1* and *bam2* single mutants, but not *bam1 bam2 bam3* triple mutants, have normal xylem. A, B. Basic fuchsin-stained xylem of six-day-old Col-0 WT, *bam1-3*, L*er* WT, and *bam2-3* (A) and *bam1-4 bam2-4 bam3-2* (B). Scale bar = 4µm. In B, right, quantification of xylem patterning phenotypes observed in the *bam1 bam2 bam3* triple mutant. Statistical differences in the distribution of % of xylem phenotypes (normal patterning = 2P vs. abnormal patterning < 2P) between the two genotypes were assessed by applying Fisher's Exact test; asterisks in brackets indicate significant differences at $P<0.05$. n = number of roots. **C**. Region of imaging for evaluation of xylem patterning. P: protoxylem; M: metaxylem.



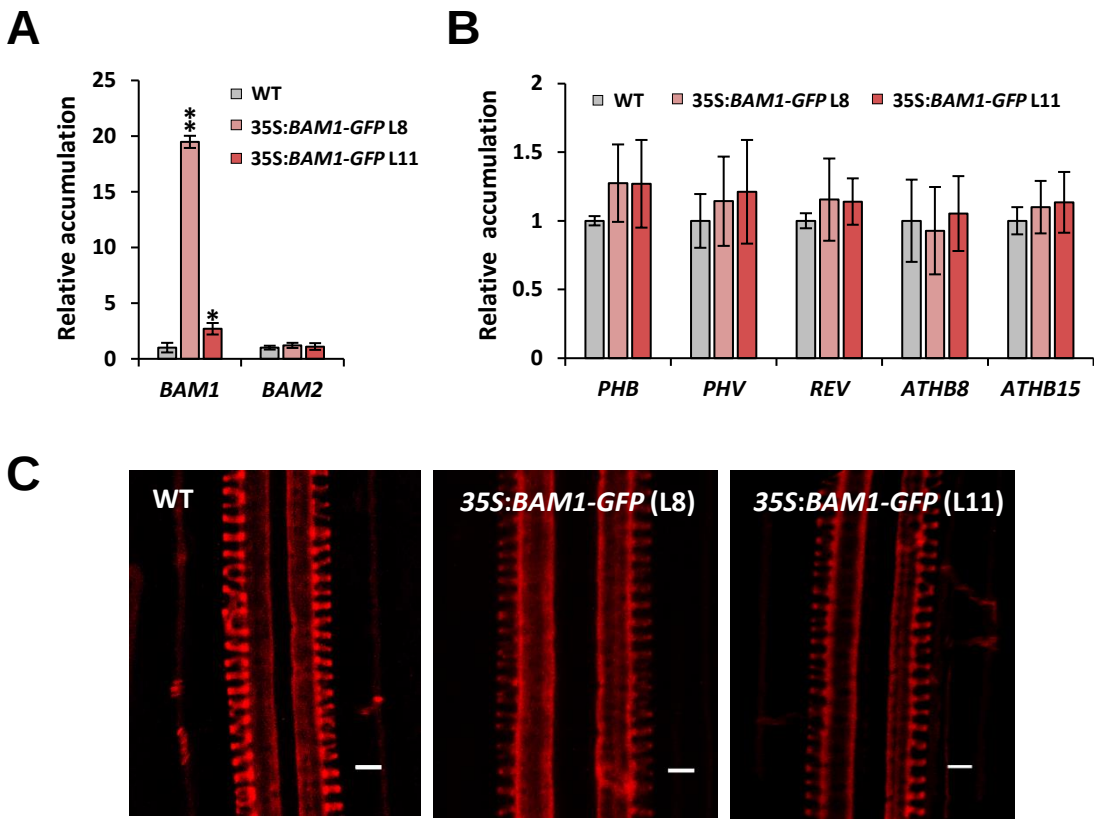
Supplementary figure 4. Expression of *SCR* and *SHR* is not reduced in the *bam1 bam2* double mutant. Accumulation of *SHR* and *SCR* transcripts in six-day-old *bam1-3 bam2-3* double mutant roots compared to the WT (L*er*) control, as measured by qRT-PCR. Results are the mean of three biological replicates; error bars indicate SD. Asterisks indicate significant differences compared to the control group (WT) according to Student's t-test with $P<0.05$ (*).



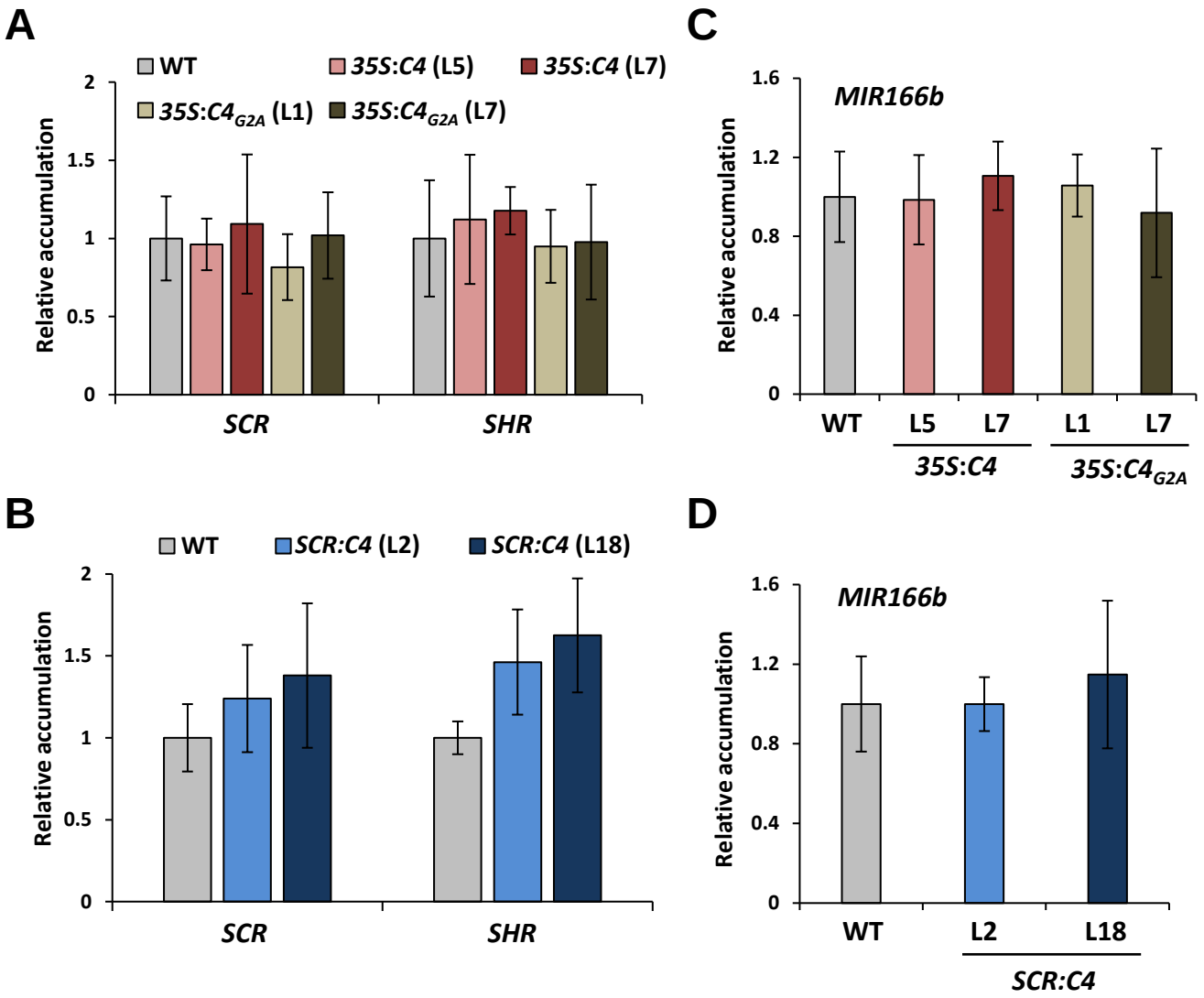
Supplementary figure 5. Negative control of the *in situ* hybridization experiment to detect the *PHB* transcript. WT L*er* and Col-0 samples are shown; the probe used is a sense *PHB* probe. Scale bar: 10 µm.



Supplementary figure 6. Normal xylem patterning is partially restored in the *bam1 bam2* double mutant when *PHABULOSA* (*PHB*) is knocked out. F3 plants deriving from crosses between *phb-13 er-2* x SUC:*SUL*/*CRISPR-Cas9 bam1 bam2 +/-* (line 1.41) plants were screened to isolate *phb-13 bam1 bam2* triple mutants. Six-day-old plants were then stained with basic fuchsin and xylem patterning phenotypes were assessed (P, protoxylem; M, metaxylem). Note that the segregation for the *er-2* knock out allele was not complete for some F3 (depicted as *er2 +/?* plants). Col-0 (functional *ER* gene) and L*er* WT (*er-1 -/-*) plants were included as controls. L*er* control is the same one already shown in Fig. 2F, which was grown in parallel with these plants. This graph represents the aggregate data obtained in two independent experiments, each of them with similar results. Statistical differences in the distribution of % of xylem phenotypes (normal patterning = 2P vs. abnormal patterning < 2P) between different genotypes were assessed by applying Fisher's Exact test; letters in brackets indicate significant differences at $P<0.003$ (Bonferroni's adjusted level for multiple comparisons). n = number of roots. *ER, ERECTA* (*QUANTITATIVE RESISTANCE TO PLECTOSPHAERELLA I*; At2g26330).

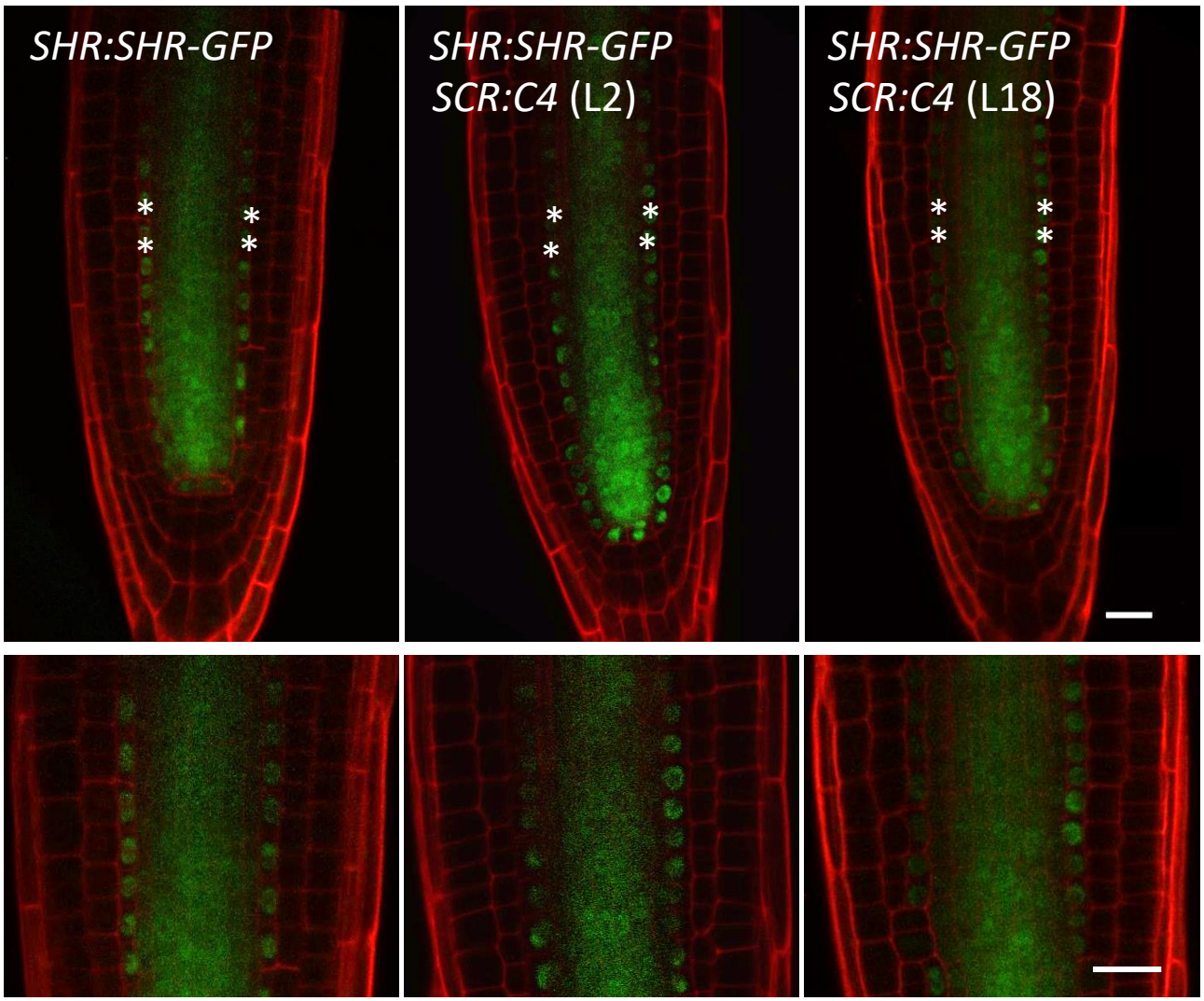


Supplementary figure 7. Overexpression of *BAM1* has no effect on xylem development. A, B. Accumulation of *BAM1* and *BAM2* (A) and *HD-ZIPIII* family genes (B) transcripts in roots of WT (Col-0) and *35S*:*BAM1-GFP* eleven-day-old seedlings, as measured by qRT-PCR. Results are the mean of three biological replicates; error bars represent SD. Asterisks indicate significant differences compared to the control group (WT) according to Dunnett's test with $P<0.05$ (*) and $P<0.01$ (**). **C.** Basic fuchsin-stained xylem of WT (Col-0) and *35S*:*BAM1-GFP* six-day-old roots. Scale bar = 4µm.

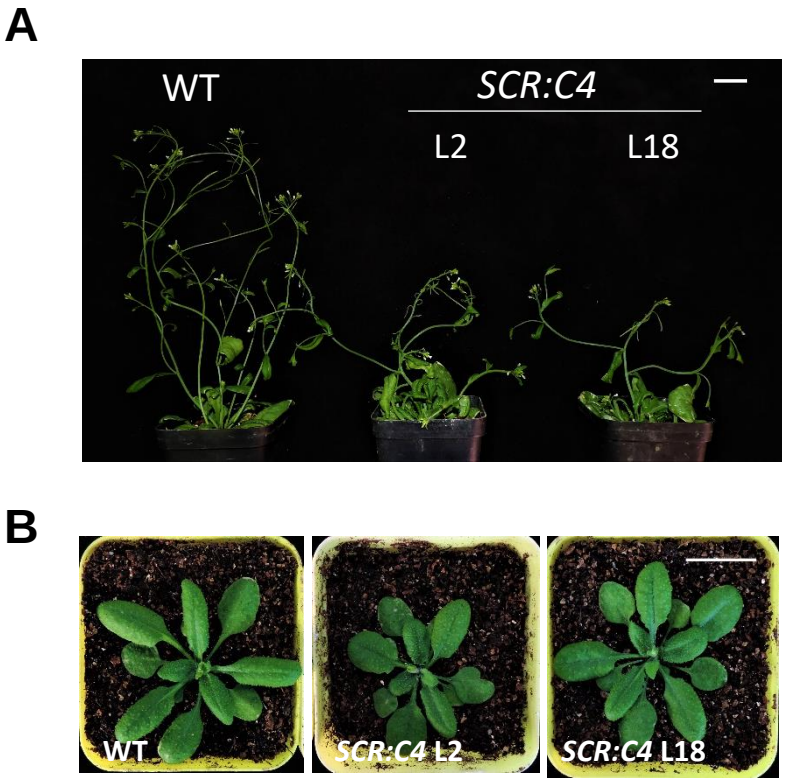


Supplementary figure 8. Expression of *SCR, SHR,* or *MIR166b* is not reduced in transgenic plants expressing C4. A, B. Accumulation of *SHR* and *SCR* transcripts in roots of eleven-day-old *35S:C4* and *35S:C4*$_{G2A}$ seedlings (A), or five-day-old *SCR:C4* seedlings (B) compared to the WT (Col-0) control as measured by qRT-PCR. Results are the mean of three biological replicates; error bars indicate SD. **C, D.** Accumulation of *MIR166b* transcripts in eleven-day-old *35S:C4* and *35S:C4*$_{G2A}$ roots (C), or in five-day-old *SCR:C4* seedlings (D) compared to the WT (Col-0) control as measured by qRT-PCR. Results are the mean of three biological replicates; error bars indicate SD. Statistical comparisons of means relative to the control group (WT) were made by Dunnett's test, with no significant differences at $P<0.05$. WT: wild type; MIR: pri-miRNA species.

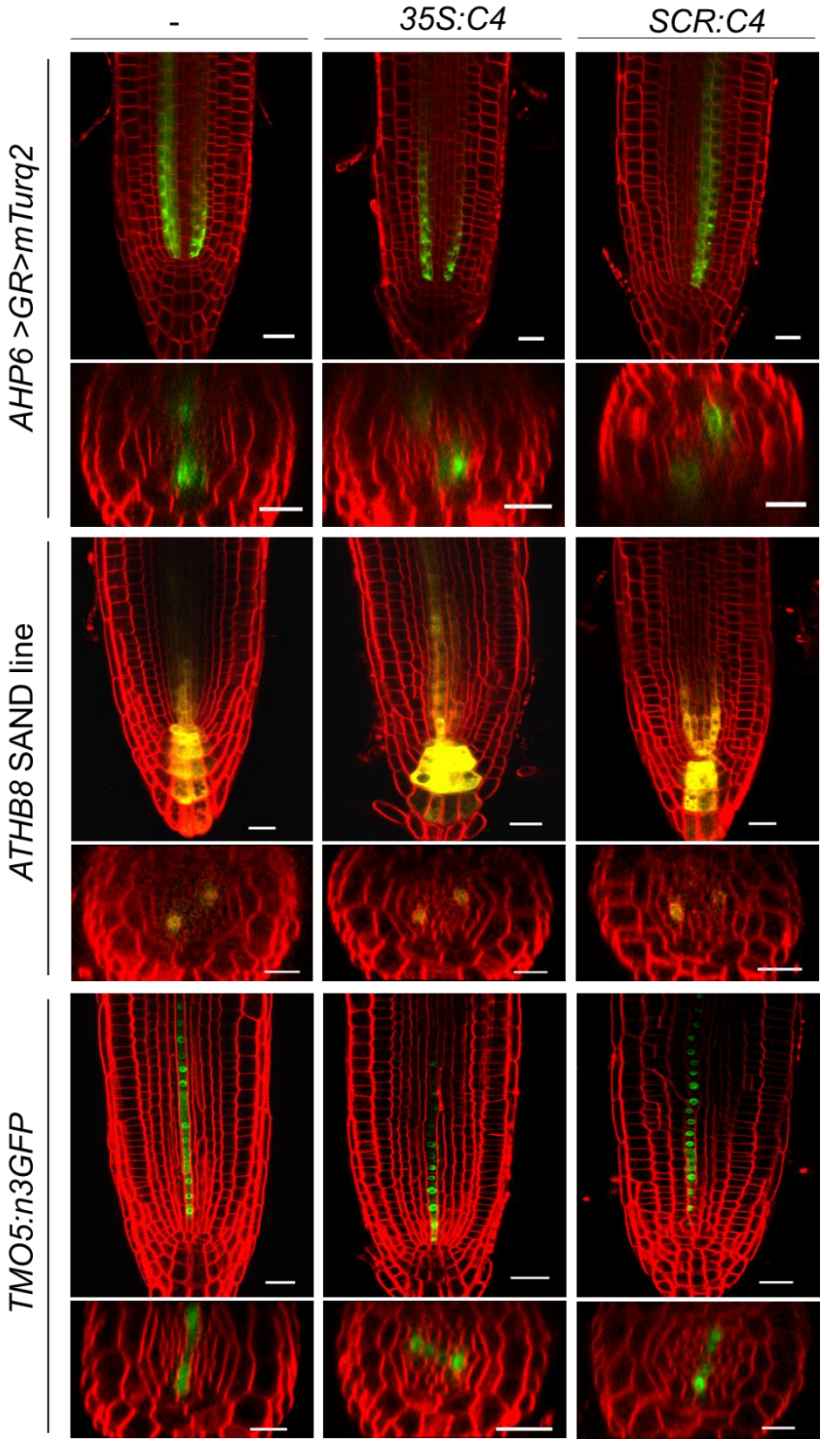
Supplementary figure 9. C4-expressing transgenic plants have roots of wild-type-like length. Six-day-old *35S:C4* (L5 and L7) , *SCR:C4* (L2 and L18) and WT control plants. WT: wild type; S-S: *SUC:SUL*; EV: empty vector. Scale bar = 0.5 cm.



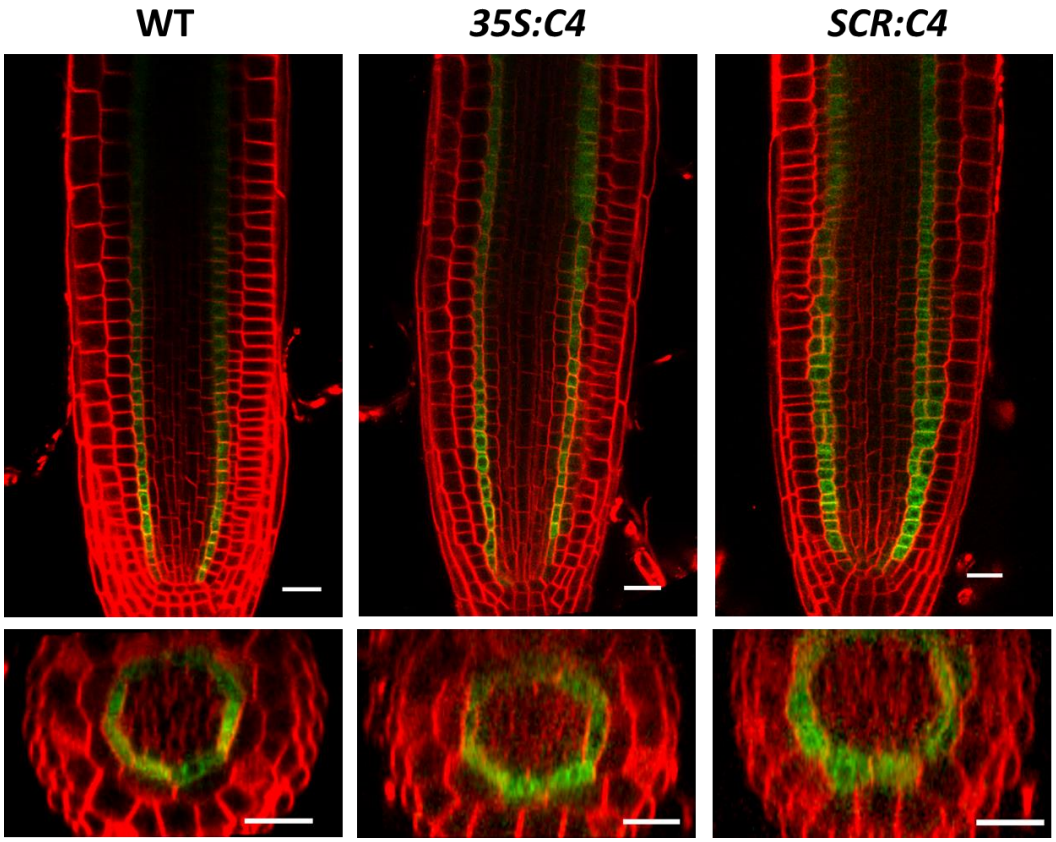
Supplementary figure 10. C4 does not affect SHR movement. Localization of SHR-GFP in transgenic *SHR:SHR-GFP* five-day-old roots in the absence (WT) or presence of *SCR:C4* (lines 2 and 18). Scale bar = 20 µm. Asterisks indicate the position of the endodermis.



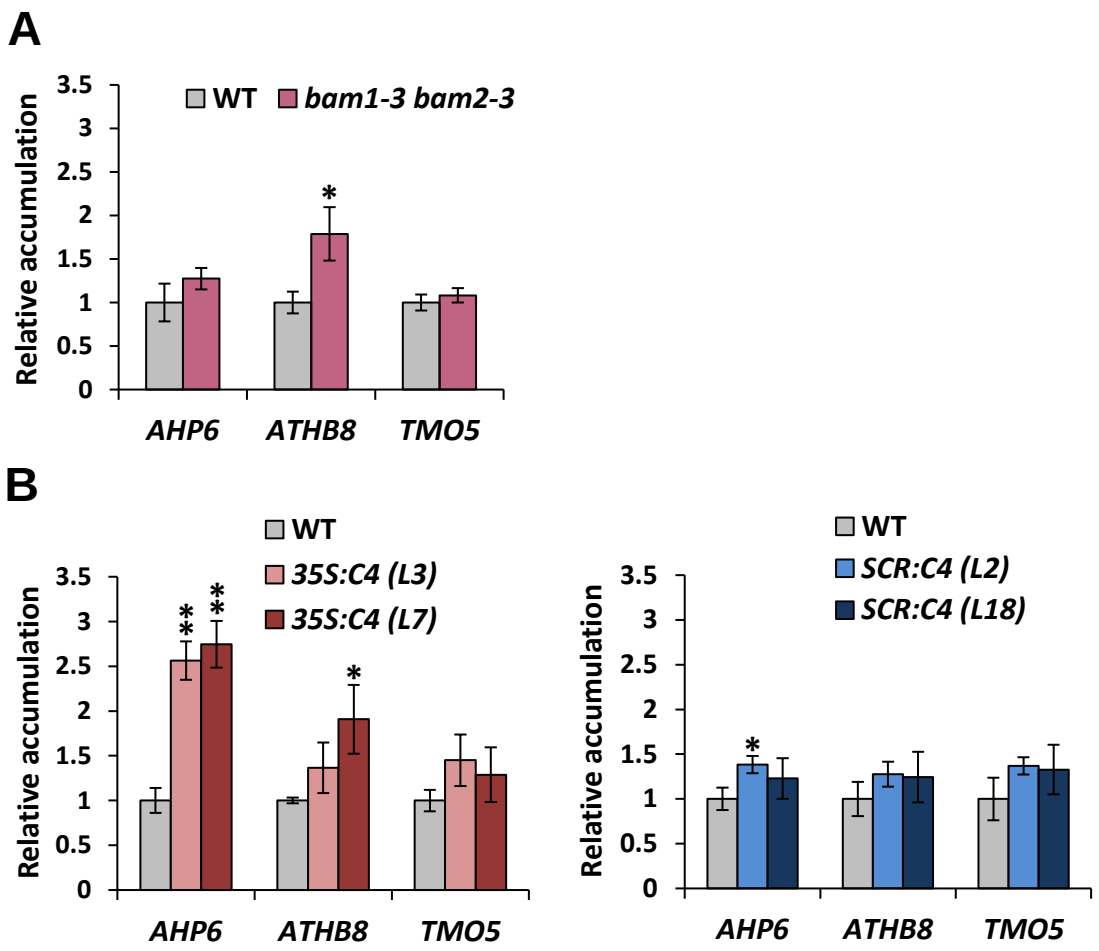
Supplementary figure 11. Developmental phenotypes of *SCR:C4* plants. A. Flowering six-week-old plants grown in long day conditions. **B.** Rosettes of four-week-old plants grown in long day conditions. Scale bar = 2 cm.



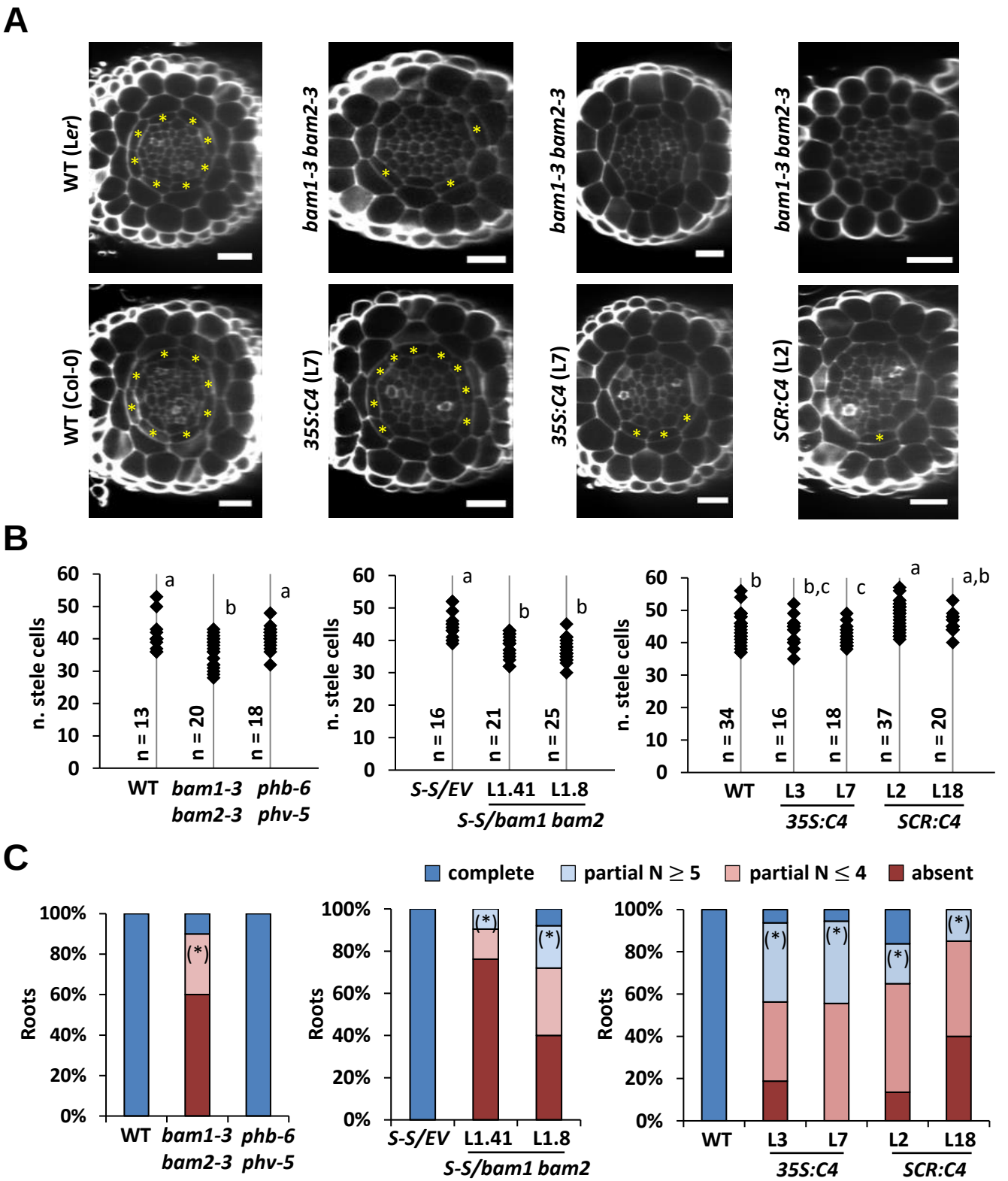
Supplementary figure 12. Expression patterns of the xylem markers *AHP6*, *ATHB8*, and *TMO5* in roots of transgenic *35S:C4* and *SCR:C4* plants. Scale bar = 20 µm. The cross-section images were captured 210 to 270 µm from the QC of six-day-old seedlings. This experiment was repeated twice with n≥10 plants per replicate with similar results; the figure shows representative images for each genotype. *35S:C4* L7 and *SCR:C4* L18 were used as parental lines in crosses with each marker line.



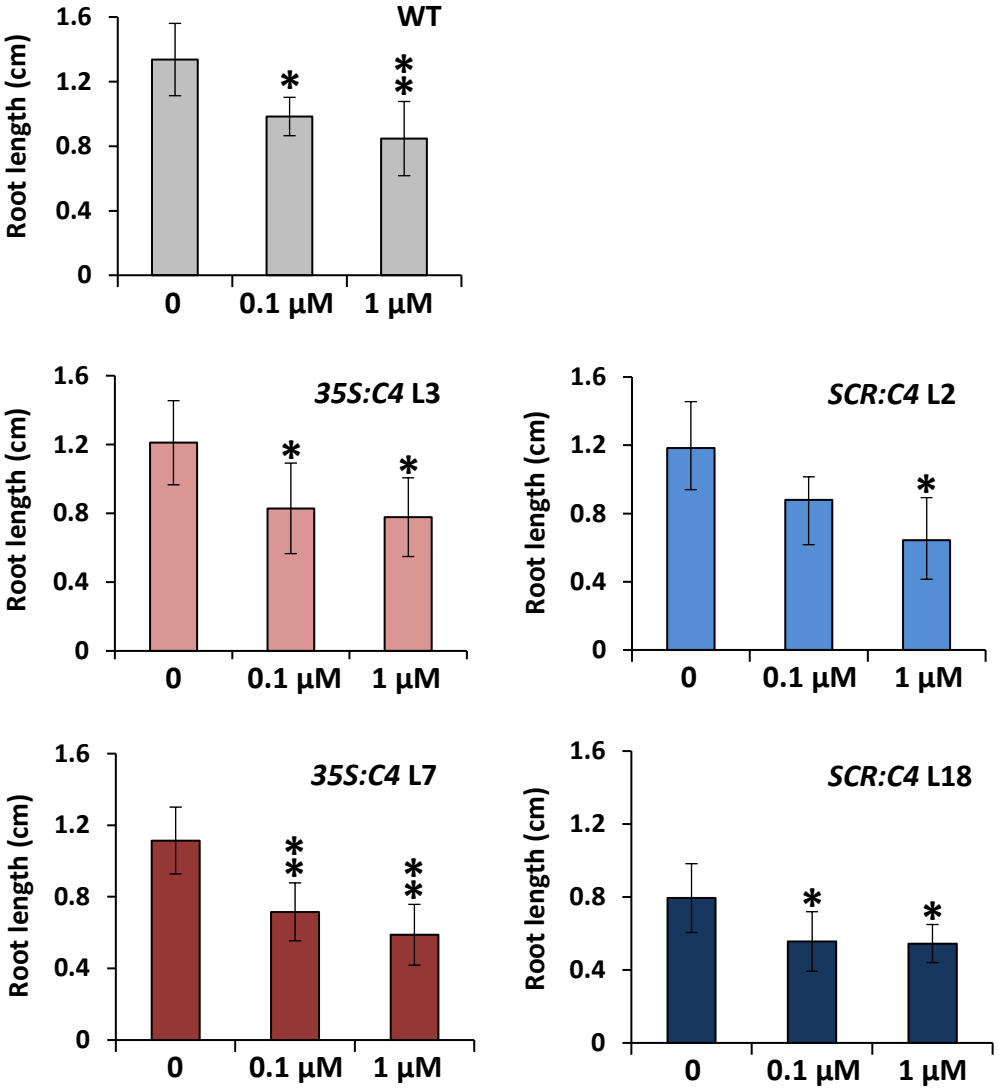
Supplementary figure 13. Expression pattern of *MIR165a* in transgenic *35S:C4* and *SCR:C4* plants. Scale bar = 20 µm. The cross-section images were captured 170 to 220 µm from the QC of six-day-old seedlings. This experiment was repeated twice with n≥10 plants per replicate with similar results; the figure shows representative images for each genotype. *35S:C4* L7 and *SCR:C4* L18 were used as parental lines in crosses with the marker line *pMIR165a:GFP*. MIR: pri-miRNA species.



Supplementary figure 14. Accumulation of the xylem markers *AHP6, ATHB8* and *TMO5* in roots of *bam1-3 bam2-3* mutants and C4-expressing plants. A,B. The relative accumulation was analyzed in roots of six-day-old *bam1-3 bam2-3* seedlings (A) and eleven-day-old C4-expressing transgenic plants (*35S:C4* L3 and L7; *SCR:C4* L2 and L18) (B), as measured by qRT-PCR. Results are the mean of three biological replicates; error bars represent SD. Asterisks indicate significant differences compared to the control group (WT) according to Student's t-test (A) or Dunnett's test (B) with $P<0.05$ (*) and $P<0.01$ (**). This experiment was repeated twice with similar results. WT: wild type (Col-0 or L*er*).


A
WT (Ler)
bam1-3 bam2-3
bam1-3 bam2-3
bam1-3 bam2-3
WT (Col-0)
35S:C4 (L7)
35S:C4 (L7)
SCR:C4 (L2)
B
n. stele cells
n = 13
n = 20
n = 18
WT
bam1-3 bam2-3
phb-6 phv-5
n = 16
n = 21
n = 25
S-S/EV
L1.41
L1.8
S-S/bam1 bam2
n = 34
n = 16
n = 18
n = 37
n = 20
WT
L3
L7
L2
L18
35S:C4
SCR:C4
C
complete
partial N ≥ 5
partial N ≤ 4
absent
Roots
100%
80%
60%
40%
20%
0%
(*)

Supplementary figure 15. Number of stele and endodermal cells in *bam1-3 bam2-3* and *phb-6 phv-5* mutants and C4-expressing plants. Six-day-old seedlings were treated with Clear See, stained with calcofluor-white and imaged under the confocal microscope. Reconstructed cross sections obtained at 150-200 nm from the QC were analyzed. **A.** Representative images are shown for each genotype; yellow asterisks indicate the position of endodermis (in WT plants, both Col-0 and L*er* ecotypes) or endodermal-like cells (*bam1 bam2* mutants and C4-expressing plants). Note that in most cases the endodermal ring is only partially complete in *bam1 bam2* mutants and C4-expressing plants, or not present. Scale bar = 20 nm. **B, C**. Stele cells count (B) and analysis of endodermal phenotypes (C), in *bam1-3 bam2-3* and *phb-6 phv-5* double mutants (L*er* WT is used as control), *S-S/bam1 bam2* L1.41 and L1.8 (S-S is used as control), and *35S:C4* L3 and L7 or *SCR:C4* L2 and L18 (Col-0 WT is used as control). Because the normal shape of the endodermis is altered in *bam1 bam2* mutants and C4-expressing plants, the cells localized in the third layer inward, when present, were considered as endodermal-like for quantification purposes. Endodermal phenotypes were assigned to one of four categories: i) complete ring of endodermal cells (complete), ii) endodermal ring lacking some cells in its usual location, containing up to four cells (partial, $N \leq 4$) or more than four cells (partial, $N \geq 5$), and iii) absence of endodermis (absent). These graphs represent the aggregate data obtained in two independent replicates, each of them with similar results. Statistical multiple comparisons between means (B) were made by employing Scheffé's multiple range test; letters indicate significant differences at $P < 0.05$. Statistical differences in the distribution of % of endodermal phenotypes (normal phenotype vs. abnormal) between each different genotype and its respective WT control (C) were assessed by applying Fisher's Exact test; asterisks in brackets indicate significant differences at $P < 0.05$. WT (wild type Col-0 or L*er*); *S-S*: *SUC:SUL*; *EV*: Empty vector; N: number of endodermal-like cells; n: number of roots.



Supplementary figure 16. Transgenic expression of C4 does not interfere with the response to CLE9/10 in the root. Root length of six-day-old WT, *35S:C4*, or *SCR:C4* Arabidopsis seedlings grown in the presence of CLE9/10 treatments (0.1 µM and 1 µM). Results are the average of the root length of 6-10 seedlings; error bars indicate SD. Asterisks indicate a significant difference compared to the control group (0) according to Dunnett's test with $P<0.05$ (*) and $P<0.001$ (**). This experiment was repeated three times with similar results, and representative results are shown.

SUPPLEMENTARY TABLES

Table S1. Plant material used in this study.

Name	Ecotype	Reference
***35S:C4* L5**	Col-0	(1)
***35S:C4* L7**	Col-0	(1)
***SCR:C4* L2**	Col-0	This study
***SCR:C4* L18**	Col-0	This study
bam1-3	Col-0	(2)
bam2-3	L*er*	(2)
bam1-3 bam2-3	Introgression of Col-0 into L*er*	(2)
S-S/CRISPR-CAS9 *bam1 bam2* L1.8	Col-0	(1)
S-S/CRISPR-CAS9 *bam1 bam2* L1.41	Col-0	(1)
pBAM1*:*YFP-NLS	Col-0	(1)
pSHR*:*SHR-GFP	Ws	(3)
pMIR165a:GFP	Col-0	(4)
pATHB8 SAND line	Col-0	(5)
pAHP6>GR>mTurquoise2	Col-0	(6)
pTMO5:n3GFP	Col-0	(7)
cle9/10	Col-0	(8)
phb-6 phv-5	Ler	(4, 9)
phb-6	Ler	This study
phv-5	Ler	This study
bam1-3 bam2-3 phb-6	Ler	This study
bam1-3 bam2-3 phv-5	Ler	This study
bam1-3 bam2-3 phb-6 phv-5	Ler	This study
phb-13 er-2	Col-0	(10)
bam1 bam2 er-2	Col-0	This study
bam1 bam2 phb-13 er-2	Col-0	This study

Table S2. Primers used in this study.

Name	Sequence (5'-3')	Reference	Purpose
qPHB-F	CTTTGGTAGTGGCGTGCTTT	This study	qPCR for *PHB*
qPHB-R	GCCCATTCAGATCGGTGTTC	This study	qPCR for *PHB*
qPHV-F	CCAAGATCATGCAGCAGGGA	This study	qPCR for *PHV*
qPHV-R	CGCTTGCTCATACGAAACCG	This study	qPCR for *PHV*
qREV-F	AGAGCATCGATCTGAGTGGG	This study	qPCR for *REV*
qREV-R	TTGTTGGTCTCATTCCCGGA	This study	qPCR for *REV*
qATHB15-F	AGAATGTTCCTCCGGCGATC	This study	qPCR for *ATHB15*
qATHB15-R	TGCCCTCCAAATCCTCCAAC	This study	qPCR for *ATHB15*
qATHB8-F	TGTTGCTCACTCAAGGCCTT	This study	qPCR for *ATHB8*
qATHB8-R	TCTTGAAGTGCCACCAACGT	This study	qPCR for *ATHB8*
qAHP6-F	GTGCTTGAGAGGACTGGAGG	(11)	qPCR for *AHP6*
qAHP6-R	TACATTGGATATCTGACTCCTG	(11)	qPCR for *AHP6*
qTMO5-F	TGAGTGCACAAGAAGTCATGGATGC	(12)	qPCR for *TMO5*
qTMO5-R	GAAGCTTTGTCCGTTTTGGTTGTGT	(12)	qPCR for *TMO5*
ACT2-F	CTAAGCTCTCAAGATCAAAGGCTTA	(13)	qPCR for *ACTIN*
ACT2-R	ACTAAAACGCAAAACGAAAGCGGTT	(13)	qPCR for *ACTIN*
qPri-MIR166b-F	TGTCTGGCTCGAGGACTCTT	This study	qPCR for pri-miR166b
qPri-MIR166b-R	TCCGACGACACTAAAACCCT	This study	qPCR for pri-miR166b
RT-primer	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCAC TGGATACGACGGGGAA	(14)	stem loop qPCR for miR166
qmiR166-F	TCGCTTCGGACCAGGCTTCA	(14)	stem loop qPCR for miR166
qmiR166-R	GTGCAGGGTCCGAGGT	(14)	stem loop qPCR for miR166
qSHR-F	GGCGGATGATGTCAGAGCTT	This study	qPCR for *SHR*
qSHR-R	CAAACCACCGGCTGATCTCT	This study	qPCR for *SHR*

qSCR-F	CAGCAGCACCAACAACAACA	This study	qPCR for *SCR*
qSCR-R	GGTGGTGCATCGGTAGAAGA	This study	qPCR for *SCR*
qBAM1-F	TCCAATAAGCTGACCGGAAC	This study	qPCR for *BAM1*
qBAM1-R	CCGGGTCAAAGACTCACATT	This study	qPCR for *BAM1*
qBAM2-F	ATGTTTACCGGCGAGATTCC	This study	qPCR for *BAM2*
qBAM2-R	GCACCGTAGAGCTTATTCCTGA	This study	qPCR for *BAM2*
pSCR:GW-F	GGAGATCGTGAAGACGATCAAG	This study	genotyping p*SCR:C4* transgenic lines
C4-S-R	TTAATATATTGAGGGCCTCGG	This study	genotyping p*SCR:C5* transgenic lines
BAM2 5´3	CAATTACCTTACCGGAGAGTTG	(2)	genotyping *bam2-3*
BAM2 3'10	GGACATTGTAGCCAATCGTTTG	(2)	genotyping *bam2-3*
Ds3-1	ACCCGACCGGATCGTATCGGT	(2)	genotyping *bam2-3*
			genotyping *phb6*
BAM1-F	CACCATGAAACTTTTTCTTCTCCT	(1)	genotyping *bam1bam2* mutant by CRISPR-Cas9
BAM1-R	GAAAGCGTTGTAGTAGCCGAT	(1)	genotyping *bam1bam2* mutant by CRISPR-Cas9
BAM2-F	CACCATGAAGCTTCTTCTTC	(1)	genotyping *bam1bam2* mutant by CRISPR-Cas9
BAM2-R	CGTTAGGTTTCCGATCTCCG	(1)	genotyping *bam1bam2* mutant by CRISPR-CAS9
PHB-F	ACAGAAATCTACTCCGAACGGTGC	(15)	synthesizing *PHB* probes for in situ hybridization
PHB-R	TGCCTGCTCGTAAGATACCATC	(15)	synthesizing *PHB* probes for in situ hybridization
***phb-13* LP**	TGTCTAAACCGGTTTGGTTTG	This study	genotyping *phb-13*
***phb-13* RP**	CAAGTCATTCTTCCTCTTGCG	This study	genotyping *phb-13*
LBb1.3	ATTTTGCCGATTTCGGAAC	SALK T-DNA primer design tool	genotyping *phb-13*
***phb-6* F2**	TCGAGATTGGCGTCTGAGATAAA	(4)	genotyping *phb-6*
***phb-6* R2**	TTGGAAACGCATTCAAAGACAAT	(4)	genotyping *phb-6*
***phv-5* F**	GTTCCTTGTCCTTTCTCTCTCAG	(4)	genotyping *phv-5*

***phv-5* R**	GTTTGATTAGCTGTCACTTTTCC	(4)	genotyping *phv-5*
SUC2 F	GACAGACACGTGTCACGAAG	(1)	detecting presence of the *SUC2:SUL* cassette
SUC2 R	CTTCCCACAATTCGTCGGCC	(1)	detecting presence of the *SUC2:SUL* cassette
Cas9 F	GACAAGAAGTACAGCATCGGC	(1)	detecting presence of the *Cas9* cassette
Cas9 R	CGTTGATGGGGTTTTCCTCG	(1)	Detecting presence of the *Cas9* cassette

SUPPLEMENTARY VIDEOS

Supplementary video 1. Reconstruction of the cross-view of a PI-stained root from z-stack images (Col-0 wild type).

Supplementary video 2. Reconstruction of the cross-view of a PI-stained root from z-stack images (L*er* wild type).

Supplementary video 3. Reconstruction of the cross-view of a PI-stained root from z-stack images (*bam1-3 bam2-3*).

Supplementary video 4. Reconstruction of the cross-view of a PI-stained root from z-stack images (*35S:C4* (L7)).

Supplementary video 5. Reconstruction of the cross-view of a PI-stained root from z-stack images (*SCR:C4* (L18)).

SUPPLEMENTARY REFERENCES

1. Rosas-Diaz T*, et al.* (2018) A virus-targeted plant receptor-like kinase promotes cell-to-cell spread of RNAi. *Proceedings of the National Academy of Sciences of the United States of America* 115(6):1388-1393.
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