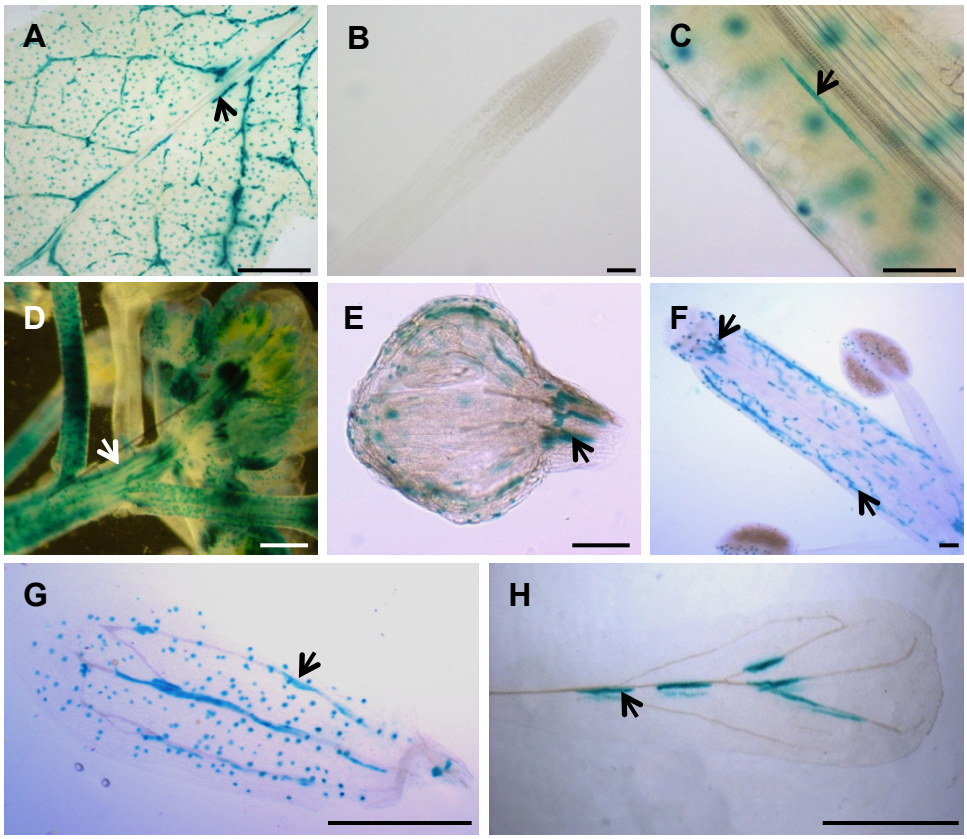


Supplemental Figure 1. A *ProFAMA:GUS* transcriptional fusion is expressed throughout the shoot in GCs and MIs.

(A) 14 DAG rosette leaf showing expression in MIs and in stomata.

(B) No *FAMA:GUS* expression was found in roots.

(C) *FAMA* expression in an MI (arrow), as well as in GCs.

(D) Inflorescence flower stalk. The large blocks of GUS staining in (D), as opposed to GCs and MIs, appear to be an artifact, presumably due to over-staining.

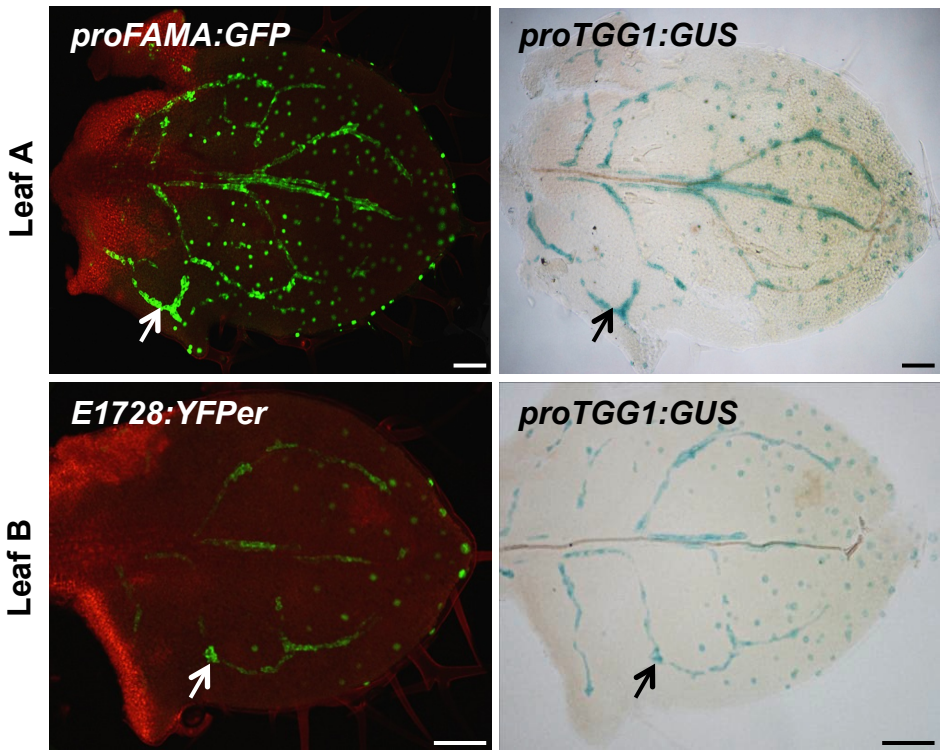
(E) Flower bud

(F) Carpel

(G) Sepal

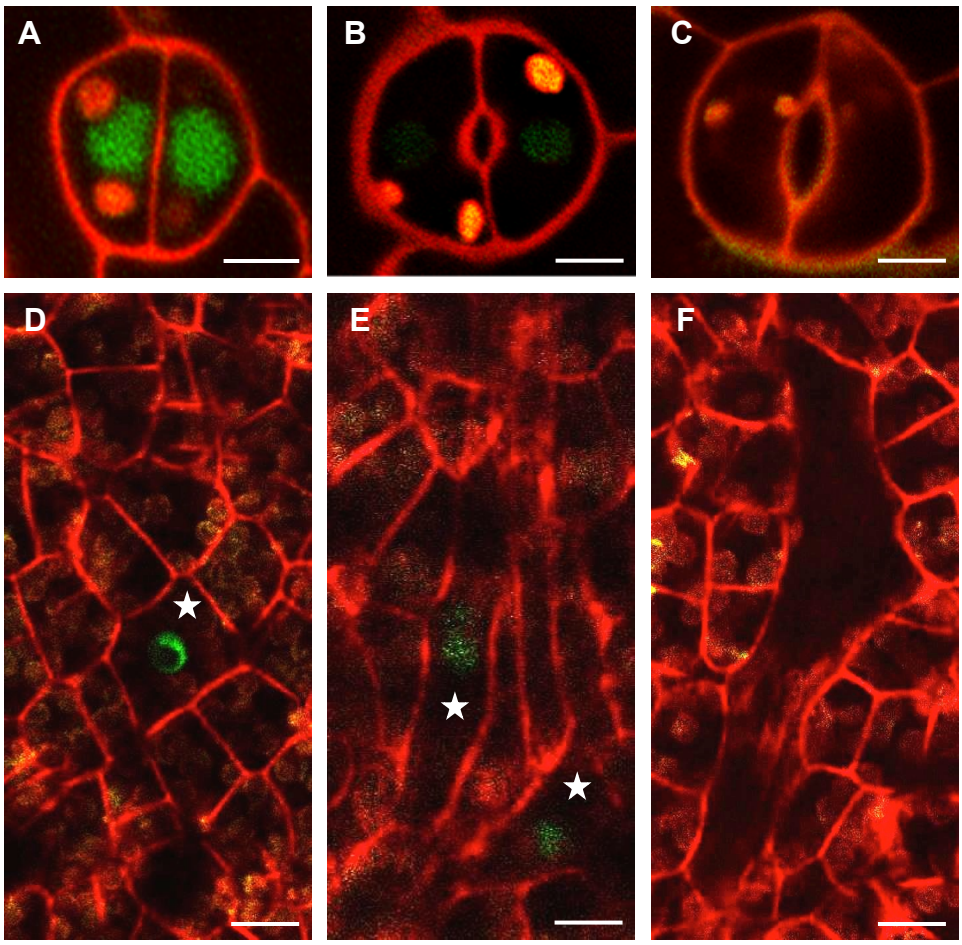
(H) Petal

Bright-field microscopy. All the samples were taken from 14 DAG to one week after bolting. MI cells are elongated and indicated with black arrows near the vasculature. GCs can be visualized as blue dots at low magnification. Scale bars = 1 mm in (A), (D), (G) and (H); 100 μm in (B) and (F); 50 μm in (C) and (F).



Supplemental Figure 2. Co-expression of *FAMA* and *E1728* with *TGG1* in MIs.

All samples are from 12 DAG 3rd leaves. Arrows indicate specific MIs that co-express fluorescence (from either *ProFAMA:GFP* or *E1728:YFPer*), as well as *ProTGG1:GUS*, in each of two different leaves ("A" and "B", 7-8 DAG first leaves). All scale bars = 0.1 mm.



Supplemental Figure 3. A *FAMA-GFP* translational fusion (*ProFAMA:FAMA-GFP)* is expressed in young but not mature GCs and MIs.

(A) Young GC just after symmetric division exhibits GFP fluorescence.

(B) Newly formed stoma shows only weak nuclear *FAMA* expression.

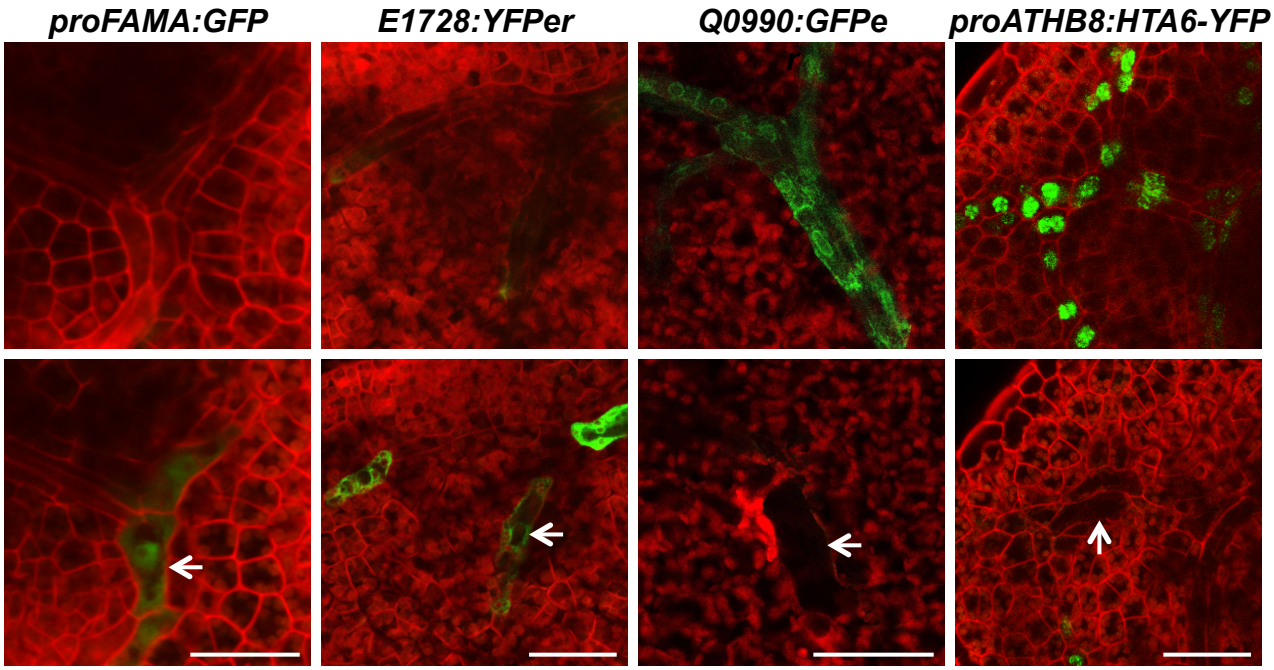
(C) Expression is absent from mature GCs.

(D) *FAMA* expression is present in the nucleus of a young (not yet elongated) MI (star).

(E) Expression is also present in two elongating MIs (stars).

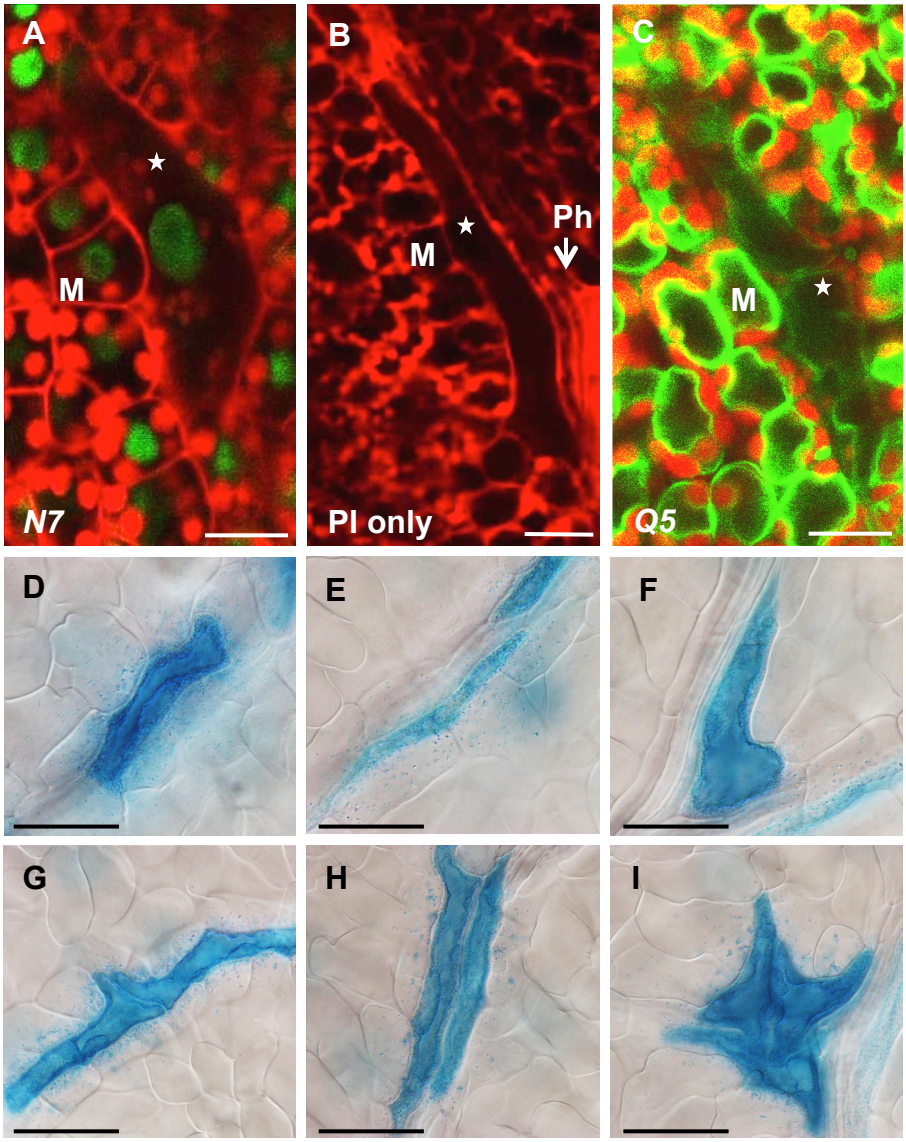
(F) No *FAMA* expression was detected in this older, larger, and more elongated MI.

Cell walls were visualized using Propidium Iodide (PI) staining. Scale bars = 5 μm in (A)-(C) and 10 μm in (D)-(F).



Supplemental Figure 4. The GFP expression patterns of procambial and MI markers.

Four columns showing vertically paired confocal images at two planes of focus. Each upper image is focused on procambial/provascular tissue, whereas the lower image is focused on the MI layer (white arrows). Each column shows GFP/YFP expression from a different marker. *Q0990:GFPer* marks the procambium, and at the sub-cellular level, localizes to the endoplasmic reticulum (ER) (images associated with SMovie 1). Fluorescence from *ProATHB8:HTA6-YFP* fusion labels the procambium and is localized to the nucleus (The frames from SMovie 7). PI labels the cell walls. Note fluorescence from the MI markers *ProFAMA:GFP* and *E1728* at lower left images. All scale bars = 50 μm.



Supplemental Figure 5. Visualization of MIs with different markers showing positional relationships between MIs and surrounding tissues.

(A) Fluorescence expression (related to auxin levels) from the *35S:3XVENUS-N7* fusion protein that marks nuclei, is expressed in an MI (*), as well as in the surrounding mesophyll (M).

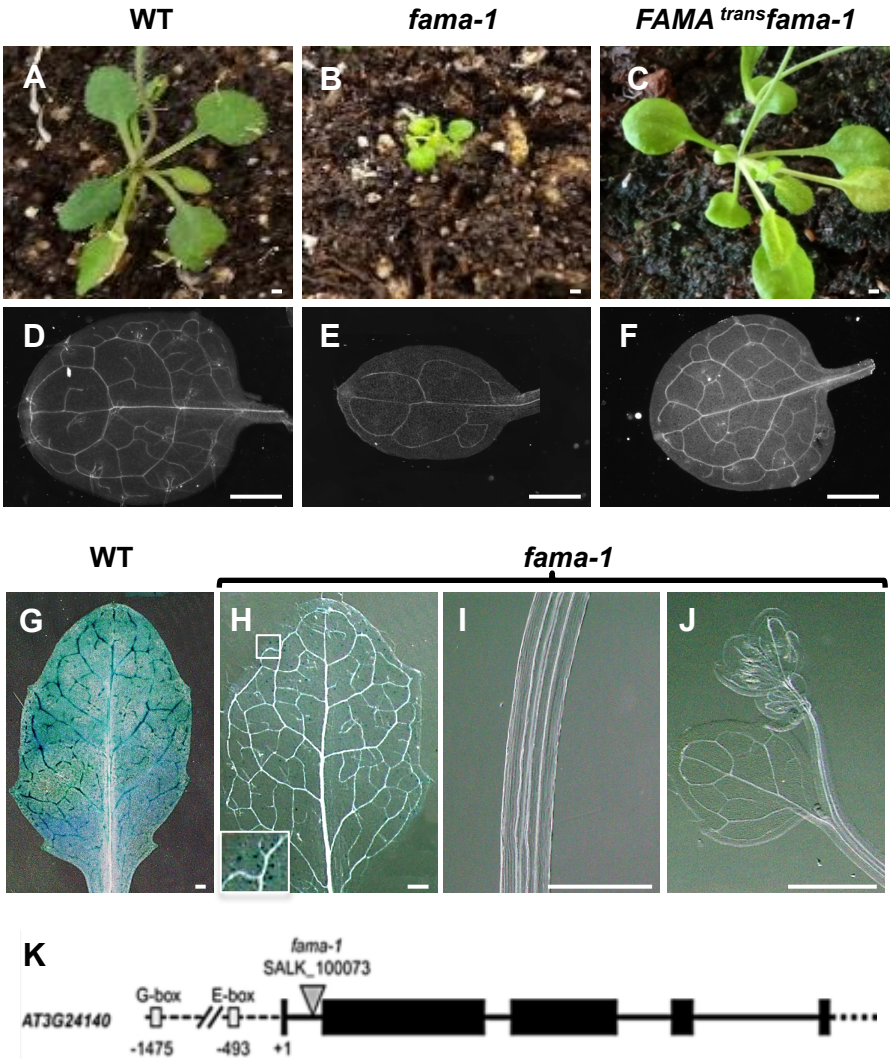
(B) MI (*). Cell walls were visualized using PI staining. “Ph” (center right) marks the location of the phloem, indicating the close proximity of MIs to the phloem. “M” denotes mesophyll tissue.

(C) Fluorescence from the *Q5* enhancer trap marks the vacuolar membrane. The * indicates an MI. “M” refers to a mesophyll cell.

(D) - (F) Many MIs are isolated/separated from each other.

(G) - (I) Other MIs normally form in contact *e.g.* in clusters of two (H) or three (I) MIs.

Images (A)-(C) are from confocal fluorescence microscopy; (D)-(I) are bright-filed. Scale bars = 20 μm in (A)-(C) and 50 μm in (D)-(I).



Supplemental Figure 6. Plant and vasculature phenotypes of three genotypes compared: wild type, *fama-1*, and *FAMA* ^{trans} *fama-1* plants.

(A) - (C) Flowering plants are diminutive in size in the *fama-1* mutant, but appear almost normal when complemented by the *ProFAMA:FAMA-GFP* gene fusion (*FAMA*^{trans}).

(D) - (F) Dark-field microscopy showing an apparently normal (complemented) vasculature compared with the highly reduced vasculature in the *fama-1* mutant.

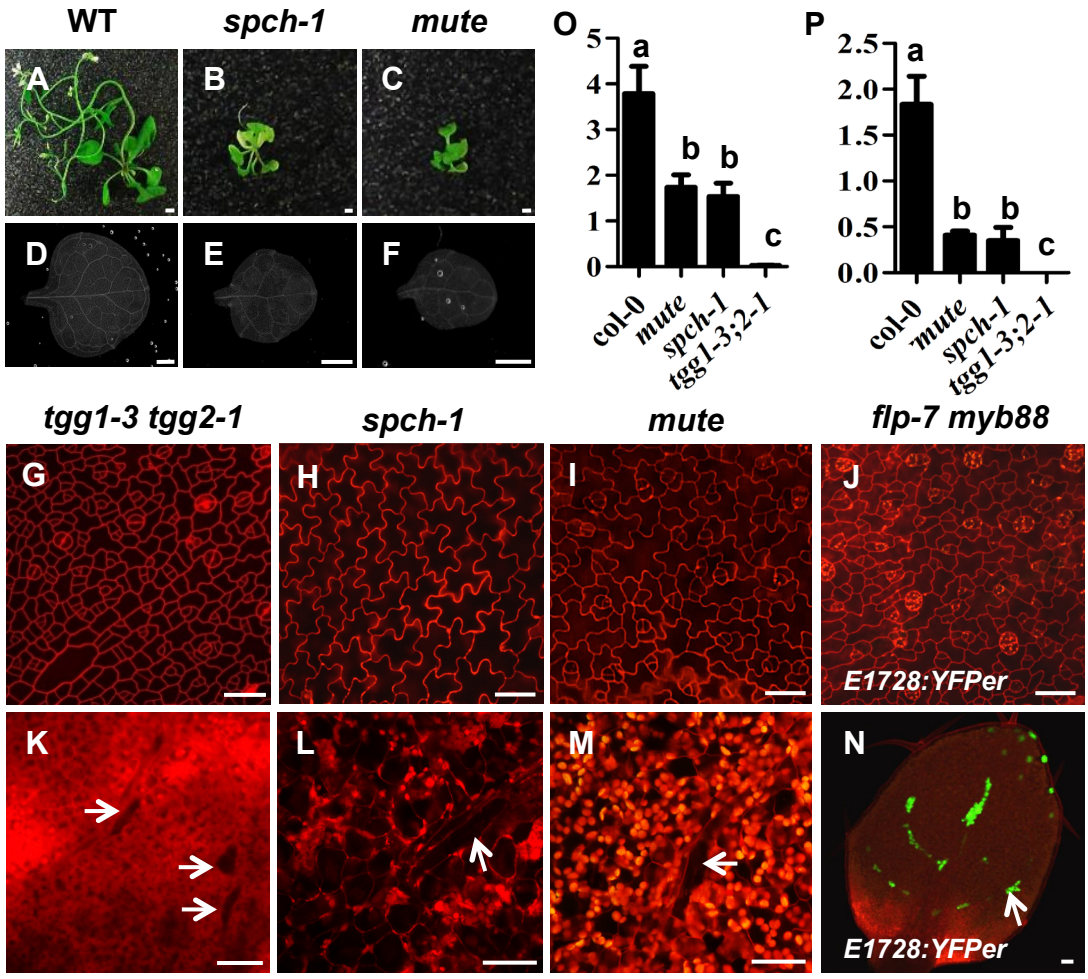
(G) wild type rosette leaf showing *ProFAMA:GUS* expression in stomata and in isolated MIs.

(H) The *fama-1* rosette leaf lacks MIs which normally express *ProFAMA:GUS* and instead GUS staining can only be visualized in clusters of Guard Mother Cells (blue dots). The image at low left show a higher magnification of white box.

(I)-(J) *ProFAMA:GUS* expression is absent from *fama-1* stems and infertile flowers that lack MIs.

(K) T-DNA insertion sites in *fama-1*. Black boxes show exons, and gray triangles indicate T-DNA insertions. Diagram reproduced from Ohashi-Ito and Bergmann, (2006).

Both wild type and *fama-1* plants are one to two weeks after bolting. All scale bars = 1 mm.



Supplemental Figure 7. MIs are still present in *spch-1, mute* and double mutants - *tgg1-3 tgg2-1* and *flp-7 myb88*.

(A) - (C) Plants one week after bolting.

(D) - (F) Dark-field microscopy showing an apparently wild type vasculature compared with the reduced vasculature in the *spch-1* and *mute* mutants. All the samples were taken from 21 DAG 2rd rosette leaves.

(G) and (K) *tgg1-3 tgg2-1* shows GCs and MIs. Arrows in (K) indicate MIs in focus.

(H) and (L) *spch-1* lacks stomatal lineage cells in the epidermis. MIs can be identified, in part, by their lack of chloroplasts and by their larger size compared to surrounding mesophyll cells (D).

(I) and (M) The loss of *MUTE* function abolishes the fate of Guard Mother Cells but not MIs.

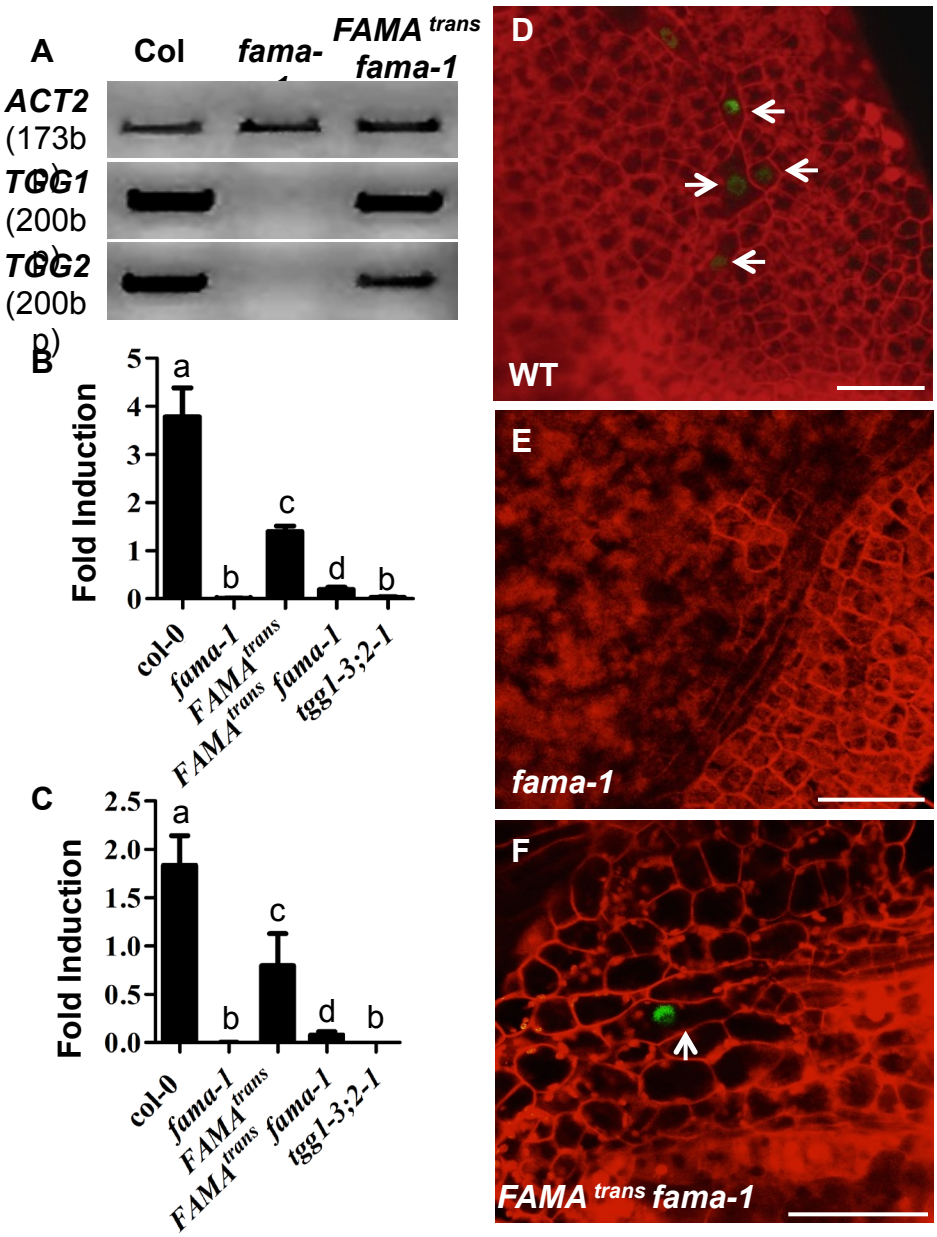
(J) *flp-7 myb88* epidermis exhibits excess clusters of GCs in contact that lack *E1728* expression.

(N) The distribution and development of MIs as marked by *E1728* is normal in *flp-7 myb88.*

All samples (A)-(N) from 10 DAG first rosette leaves with cell walls visualized using PI fluorescence. Arrows indicate examples of developing or mature MI cells. MIs can be identified, in part, by their lack of chloroplasts and by their larger size compared to surrounding mesophyll cells in these fields. Scale bars =2 mm in (A) ,1 mm in (B)-(F) and 50 µm in (G)-(N).

(O) Data from quantitative (q) RT-PCR showing the expression of *TGG1* in 20 DAG plant shoots in wild type, *spch-1, mute* and in the *tgg1-3 tgg2-1* double mutant. Y-axis showing Fold Induction. The expression of *TGG1* was normalized to that of *ACTIN2*. One-way ANOVA were used to analyze statistical differences. Bars represent means of three biological replicates ± SD.

(P) As in (G) except for *TGG2* transcript levels.



Supplemental Figure 8. Test for complementation as well as qRT-PCR analysis of the wild type, *fama-1*, and *FAMA trans fama-1* plants.

(A) RT-PCR showing *TGG1* and *TGG2* expression in wild type, *fama-1*, and in the *fama-1* mutant complemented with *FAMA trans*. *TGG1&2* expression was normalized to *ACTIN2* expression.

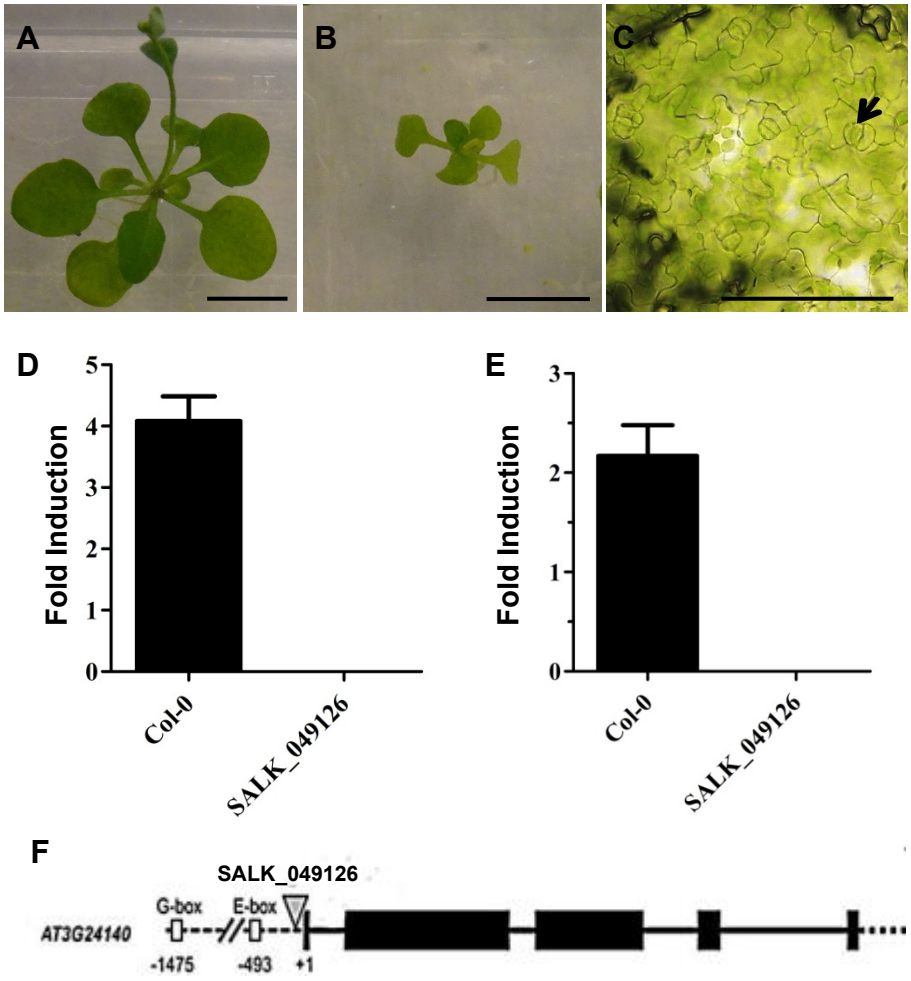
(B) Data from quantitative (q) RT-PCR showing *TGG1* transcription in the wild type, *fama-1*, *FAMA trans* , in the *tgg1-3; 2-1* double mutant, and in the *fama-1* mutant complemented with *FAMA trans*. The expression of *TGG1* and *TGG2* was normalized to that of *ACTIN2*. One-way ANOVA was used to analyze statistical differences. Bars represent means of three replicates ± SD.

(C) As in (B) except for *TGG2* transcript levels.

(D) *ProFAMA:FAMA-GFP* fluorescence in MIs in wild type (arrows). This image from SMovie 3.

(E) No expression from the transcriptional *ProFAMA:GFP* reporter was found near the phloem since MIs are absent from the *fama-1* mutant.

(F) Transformation of the *FAMA trans* construct into the *fama-1* mutant restores *FAMA-GFP* expression to MIs (arrow). All scale bars = 20 μm.



Supplemental Figure 9. Plant phenotypes in *fama* mutant allele SALK_049126.

(A) Four-week-old wild type plant grown on ½ MS plate. Bar = 1 cm.

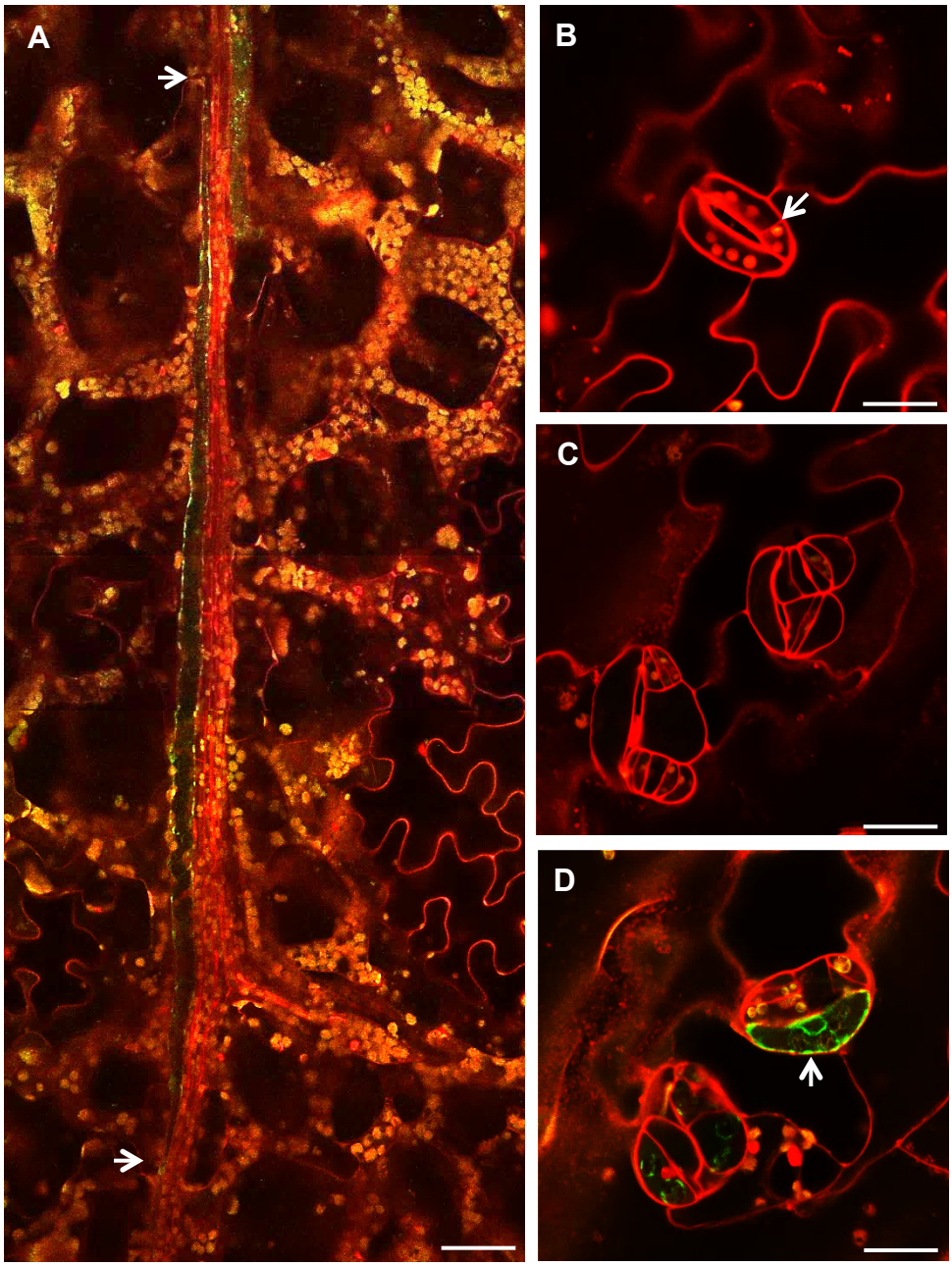
(B) Four-week-old *fama* mutant grown on ½ MS plate is much smaller than the wild type. Bar = 1 cm.

(C) *fama* mutant leaf epidermis. Arrow indicates a cluster of 3 Guard Mother Cells. Bar = 0.1 mm

(D) Data from quantitative (q) RT-PCR showing *TGG1* transcription in the wild type and *fama* mutant. The expression of *TGG1* and *TGG2* was normalized to that of *ACTIN2*. Unpaired t-tests were used to analyze statistical differences. Bars represent means of three replicates ± SD.

(E) As in (D) except for *TGG2* transcript levels.

(F) T-DNA insertion sites in *fama* allele SALK_049126. Black boxes show exons, and gray triangles indicate T-DNA insertions. Diagram modified from Ohashi-Ito and Bergmann (Ohashi-Ito, K., and Bergmann, D.C. (2006). *Arabidopsis* FAMA controls the final proliferation/differentiation switch during stomatal development. Plant Cell. **18**: 2493-505.).

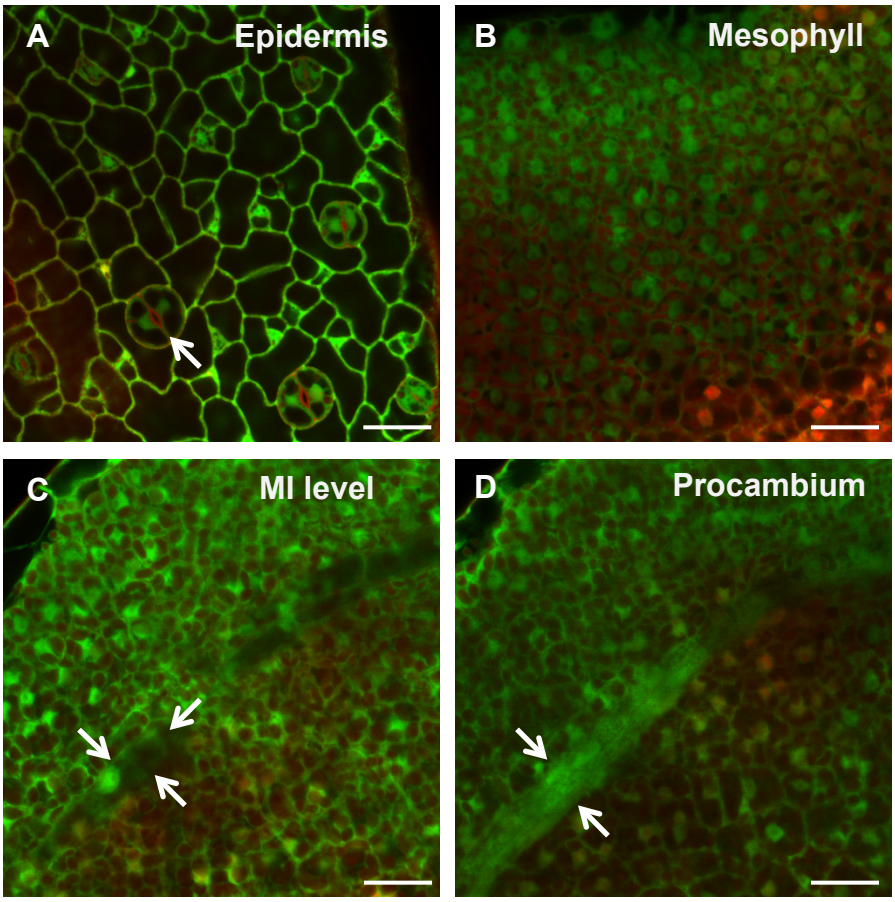


Supplemental Figure 10. Gain-of-function phenotypes in *FAMA* ^{trans} plants.

(A) Long and mature MI (arrow) in cotyledon from four-week-old plant is marked by *E1728:YFPer* expression. No MI divisions were found in 5 different samples from more that 50 cells. Bar = 50 µm.

(B) Four-week-old mature first leaf showing an asymmetric division (arrow) in one of two mature guard cells in the epidermis. Bar = 20 µm

(C) and (D) a 'Stoma in Stoma' (SIS) excess divisions in four-week-old cotyledon. *E1728:YFPer* is only expressed in a undivided guard cell (arrow in D).



Supplemental Figure 11. Myrosinase Idioblasts are located between the procambium and the mesophyll as shown in one of four different focal planes using ubiquitous *35S:GFP* expression

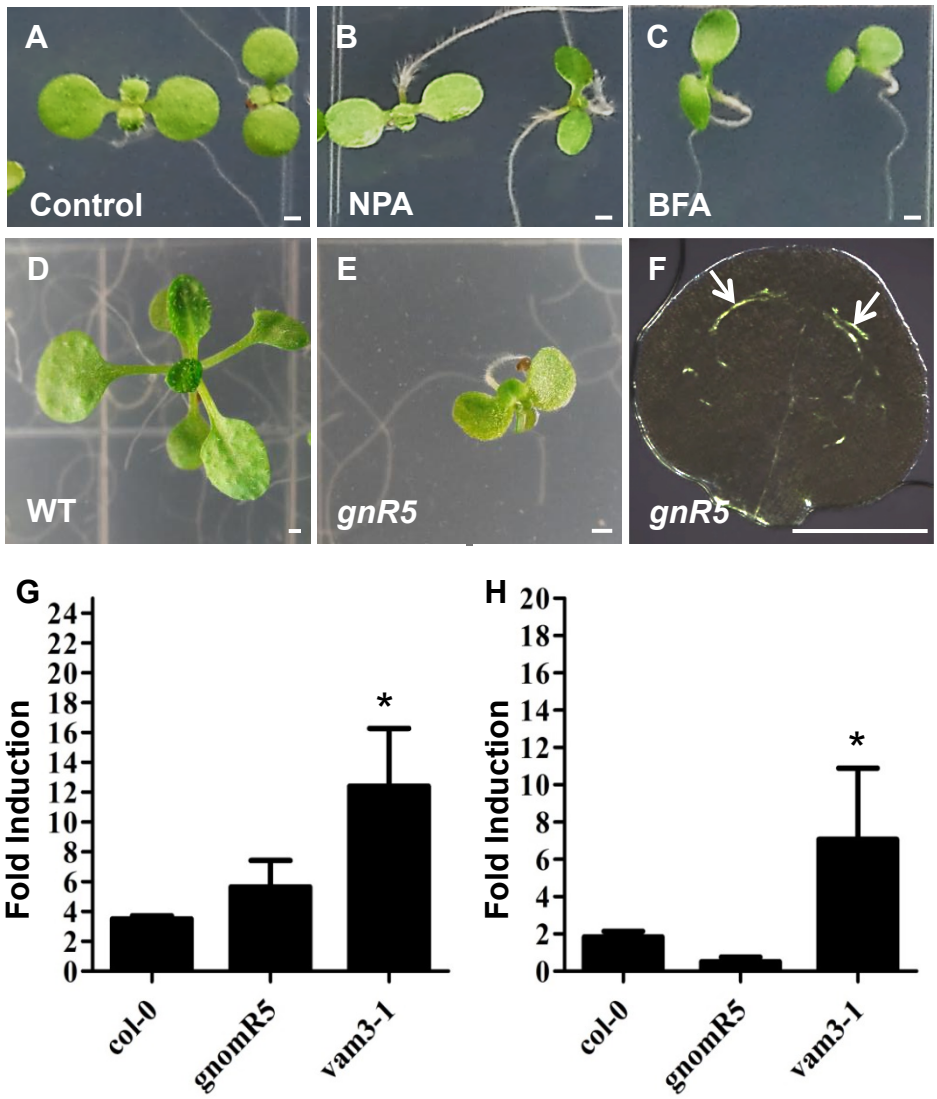
(A) Lower epidermis. Arrow indicates mature GCs.

(B) Optical section of spongy mesophyll shows neither GCs nor MI cells.

(C) The MI layer is located between the spongy mesophyll and the procambium. The arrow marks an elongating MI which is larger than surrounding mesophyll cells. The MI is wider than the procambium. C and D show the same fields but at different planes of focus.

(D) Arrows mark a procambial strand.

All figures from 12 DAG first rosette leaves. Cell walls visualized with propidium iodide (PI). All scale bars = 10 μm.



Supplemental Figure 12. Plant phenotypes of wild type, chemical-treated and *gnom*^{R5} seedlings.

(A) - (C) Seedlings of 8 DAG grown with or without 10 μM NPA or 20 μM BFA. Both chemicals slow down seedling growth and development.

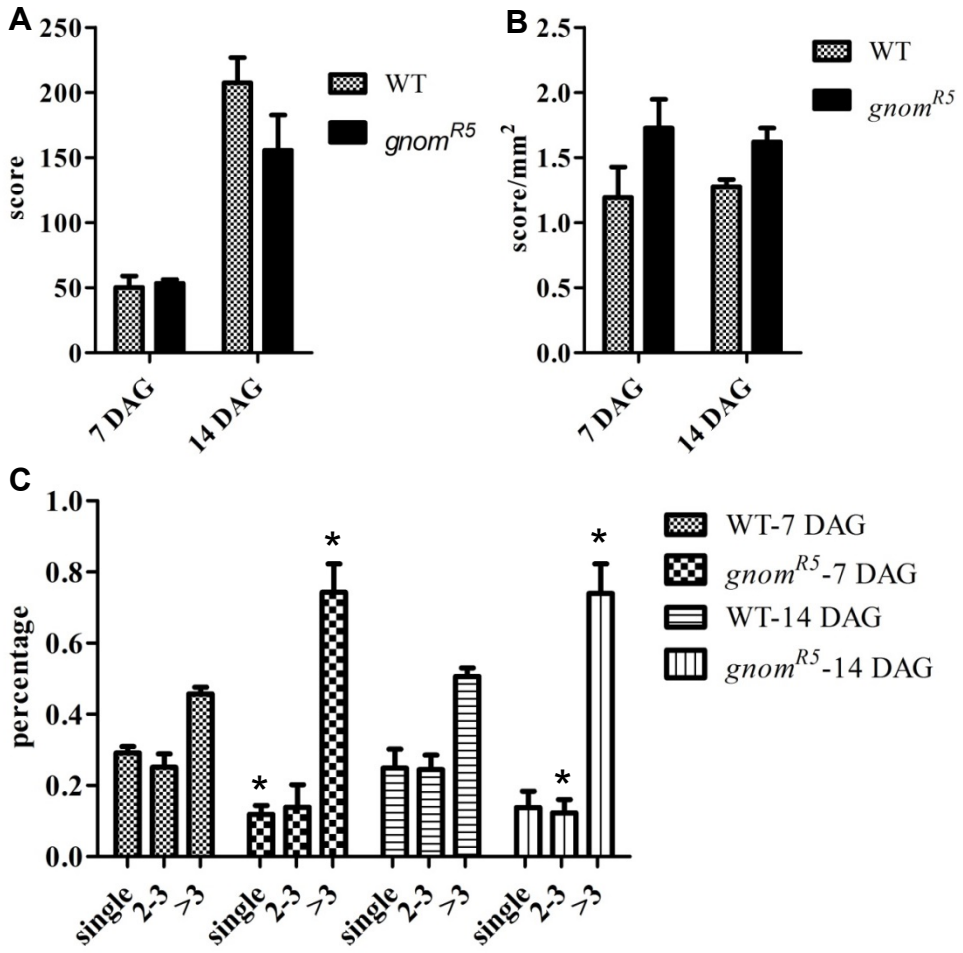
(D) and (E) wild type and stunted *gnom*^{R5} mutants. 14 DAG seedlings under bright-field image.

(F) Dark-field image of *gnom*^{R5} leaf. Arrows indicate short vascular strands.

Scale bars = 1 mm in (A)-(C) and 0.5 mm in (D)-(F).

(D) Data from quantitative (q) RT-PCR showing that *TGG1* expression from 20 DAG plant shoots in the wild type, *gnom*^{R5}, and *vam3-1.* The expression of *TGG1* and *TGG2* was normalized to that of *ACTIN2*. One-way ANOVA was used to analyze statistical differences ($P < 0.05$). Stars (*) indicate the significant difference compared with the wild type. Bars represent means of three biological replicates ± SD.

(E) As in (B) except for *TGG2* transcript levels.



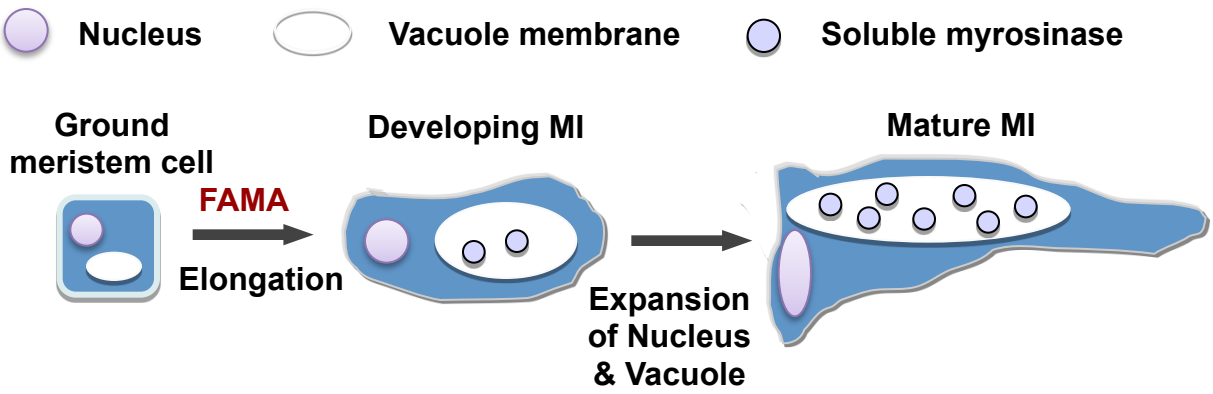
Supplemental Figure 13. Quantification of MI number in wild type and in *gnom^{R5}* mutant backgrounds.

(A) Total numbers of MIs scored from 7 DAG and 14 DAG first leaves.

(B) Quantification of MI density in 7 DAG and 14 DAG first leaves. Y-axis indicates the number of MI's scored per leaf area (1 mm^2).

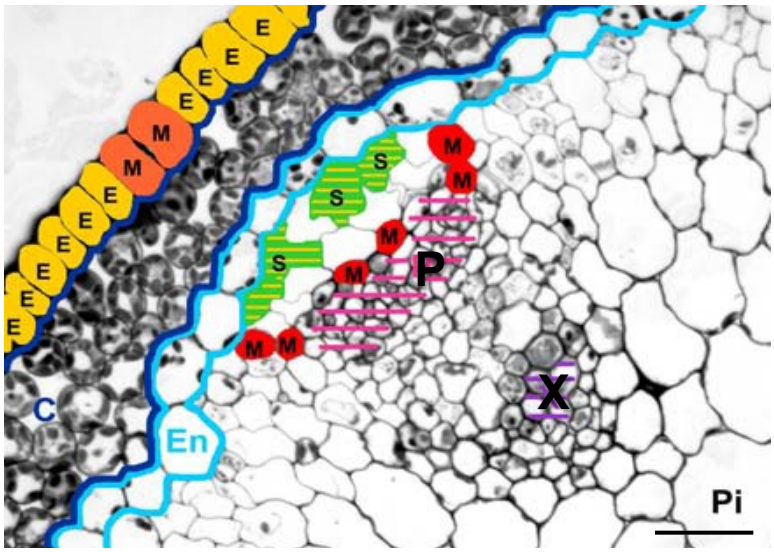
(C) Extent of single MIs compared to those with two-three or more than three MIs in direct contact in first leaves from 7 DAG and 14 DAG plants

Each value is the mean calculated from five leaves in different plants and counted using *ProFAMA:GFP* as an MI marker in confocal microscopy. Bars represent means of three replicates ± SD. Stars (*) indicates the significant difference compared with the wild type as analyzed by 2-way ANOVA and unpaired t-test (p<0.05).



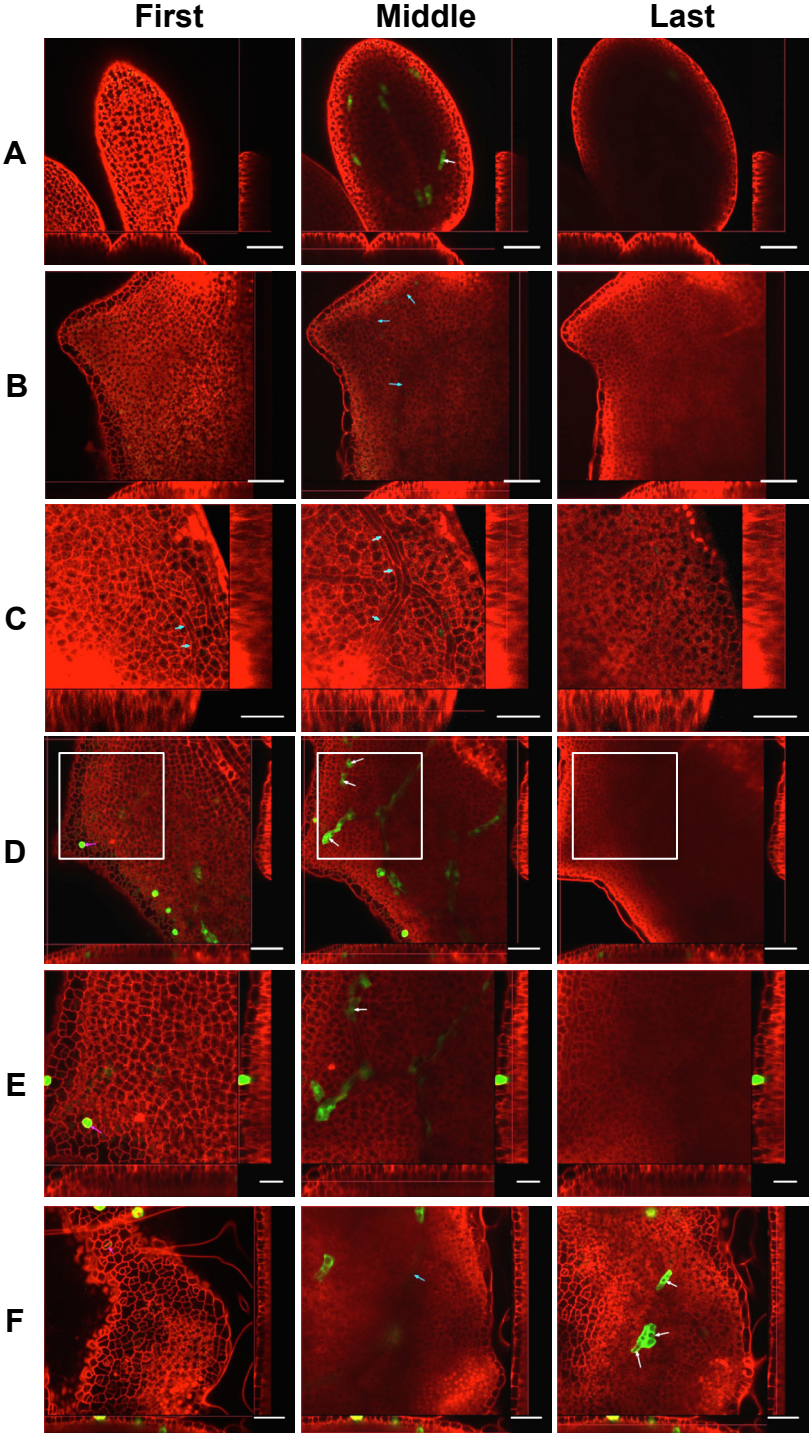
Supplemental Figure 14. Diagram showing developmental model of a single Myrosin Idioblast.

Square-shaped ground meristem cells acquire an MI cell fate upon *FAMA* expression. The MI then elongates and expands as do the nucleus and vacuole. No *FAMA* expression (from the translational fusion) was detected in mature MI's. TGG1 & 2 are stored in MI vacuoles. Mature MI's are irregularly-shaped, harbor spindle-shaped nuclei, and a large central vacuole.



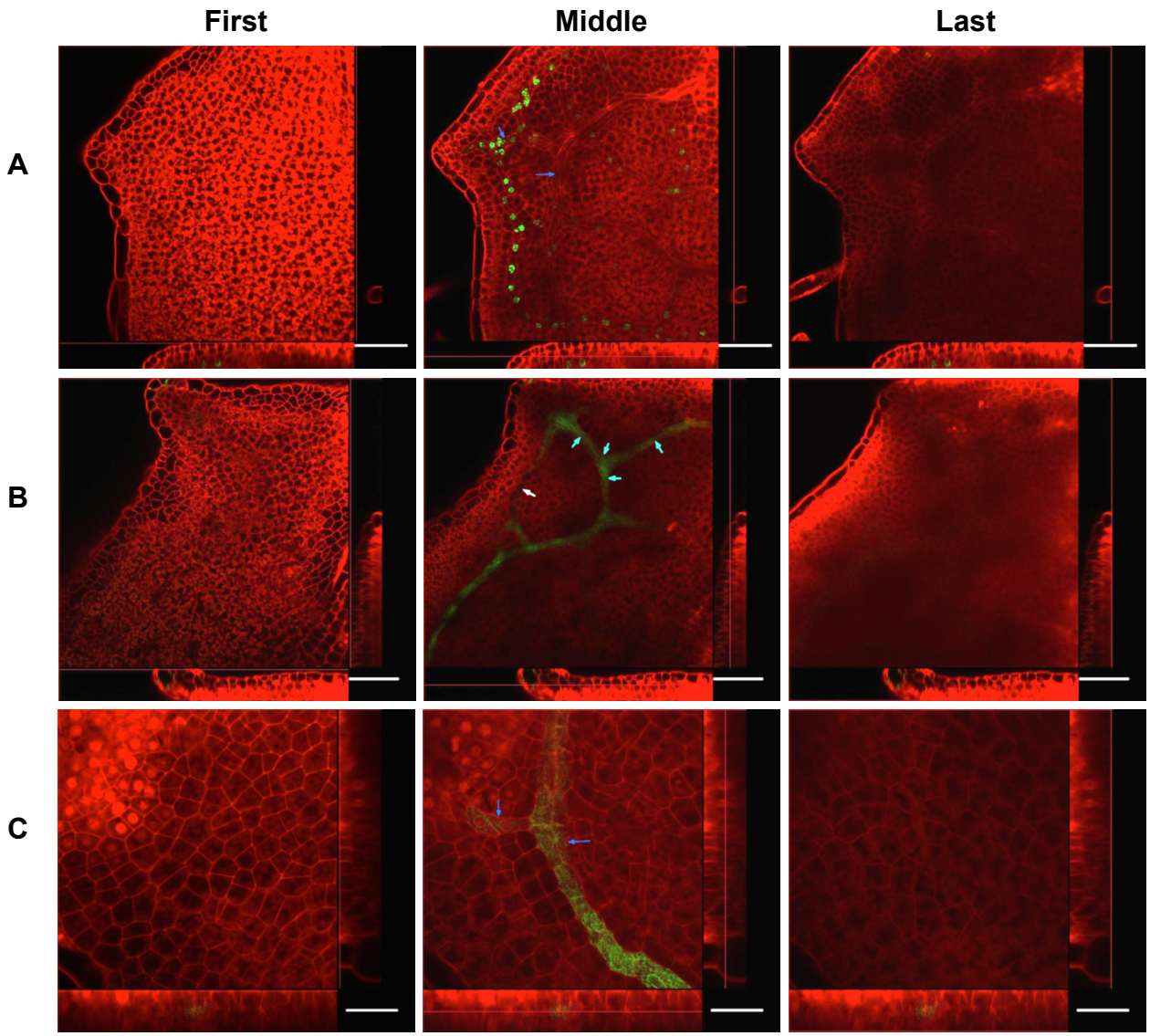
Supplemental Figure 15. Diagram showing the distribution of myrosin cells and glucosinolate-rich/S-cells in a partial cross-section of an *Arabidopsis* stem.

Pi, Pith; X, Xylem; P, Phloem; M on red background, Myrosin Idioblast, MI; S on green/yellow background, S-cell; M on orange, Myrosinase containing Guard Cell; E, Epidermis. Image reproduced from Kissen, R., Rossiter, J.T., and Bones, A.M. (2009). The “mustard oil bomb”: not so easy to assemble?! Localization, expression and distribution of the components of the myrosinase enzyme system, Phytochem. Rev. **8**: 69–86. Scale bar = 50 µm.



Supplemental Figure 16. Each row (A-F) shows three frames from Z-stacks of GCs (green dots) and MI's (more elongated) from supplemental movies, with expression from *FAMA* (A-E) and (F) *E1728.*

(A) SMovie 1. *ProFAMA:GFP* expression, embryonic cotyledon.
(B) SMovie 2. *ProFAMA:cFAMA-GFP*, differentiating hydathode region.
(C) SMovie 3. *ProFAMA:cFAMA-GFP,* young leaf.
(D) SMovie 4. *ProFAMA:GFP,* differentiating hydathode region. White boxes magnified in SMovie 5.
(E) SMovie 5. Higher magnification from white boxes of SMovie 4*, ProFAMA:GFP.*
(F) SMovie 6. *E1728* expression in differentiating hydathode region of a 7 DAG first leaf.
All images from 12 DAG first leaves except (F). Cell walls visualized using PI staining. Pink arrow, Guard Cell; white arrows, MIs; blue Arrows, procambium or developing vascular. Scale bars = 50 μm in (A), (B), (D) and (F); 20 μm in (C) and (E).



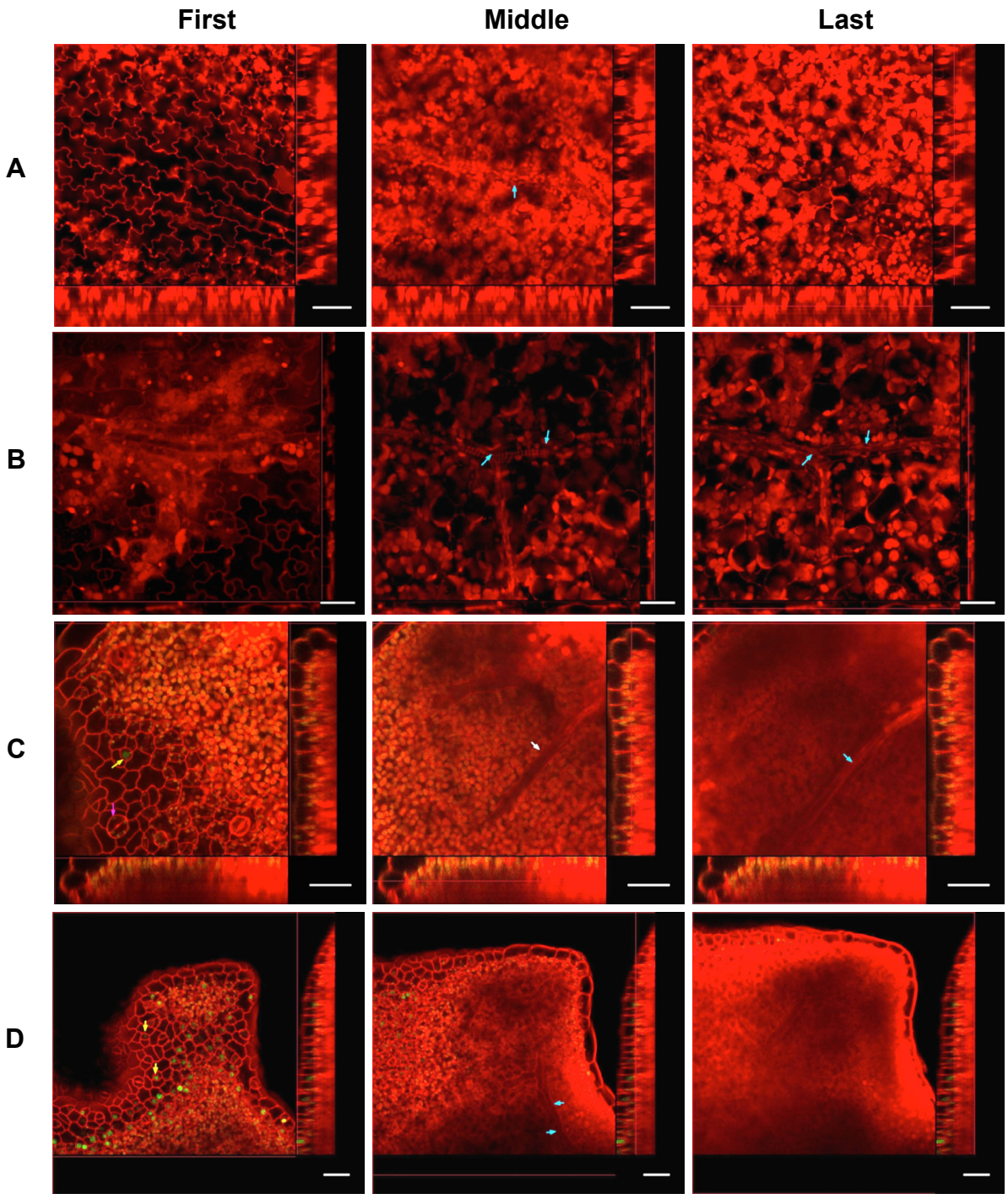
Supplemental Figure 17. Each row (A-C) shows three frames from Supplemental movies from Z-stacks of procambial expression markers *At-HB8* and *Q0990*.

(A) Frames from SMovie 7. *ProATHB8:HTA6-YFP* expression in differentiating hydathode region.

(B) Frames from SMovie 8. *Q0990:GFPer* expression in differentiating hydathode region.

(C) Frames from SMovie 9 of *Q0990:GFPer* expression in 8 DAG young leaf.

All images from 12 DAG first leaves except (C). Cell walls visualized using PI staining. White arrow, MI; blue arrows, procambium or developing vascular tissue. Scale bars = 50 μm in (A) -(B) and 20 μm in (C).



Supplemental Figure 18. Three frames (first, middle & last) from Supplemental Movies of *spch-1* and *mute* mutants as well as *SPCH* and *MUTE* expression.

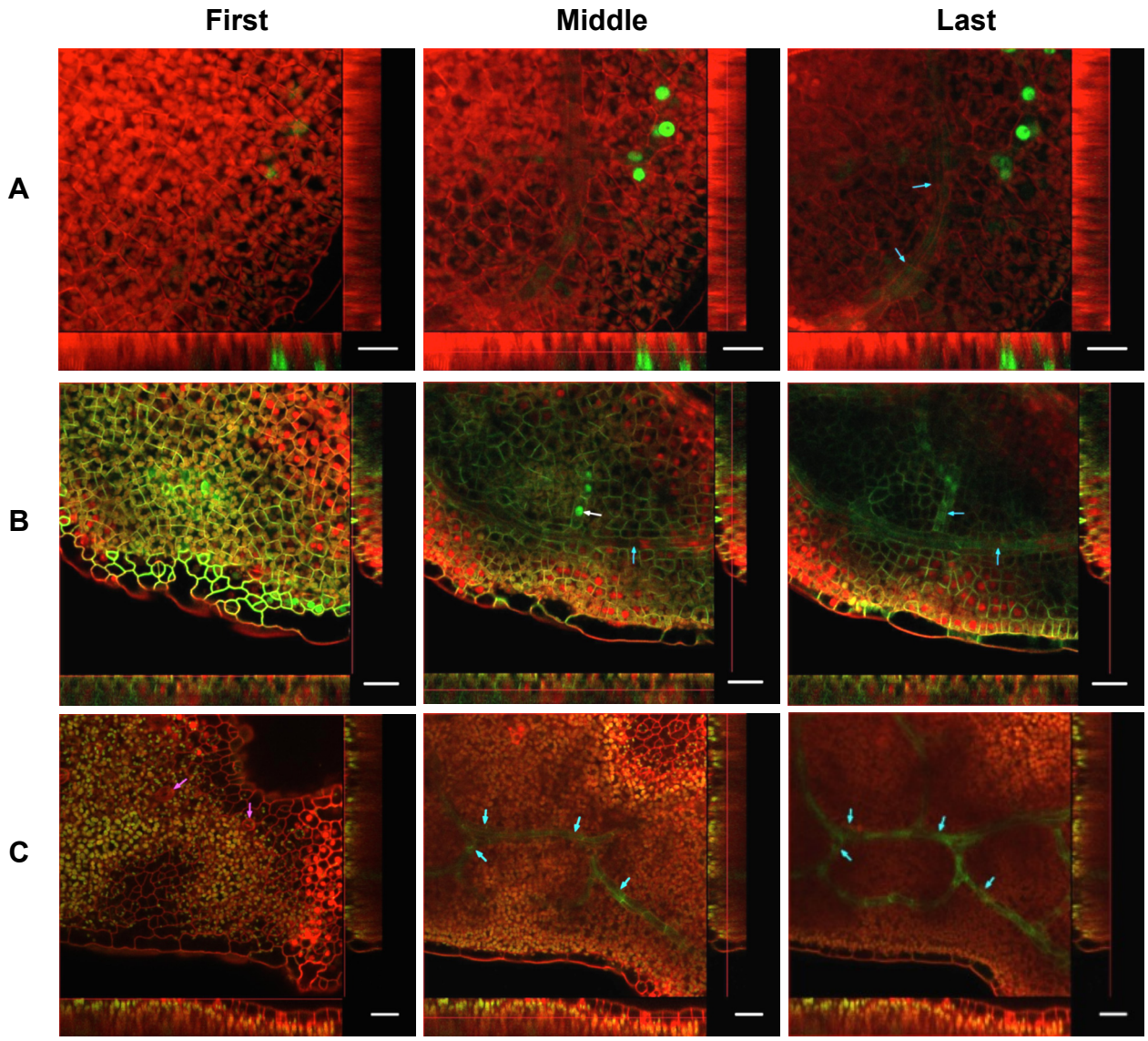
(A) SMovie 10. *spch-1* mutant leaf.

(B) SMovie 11. *mute* mutant leaf.

(C) SMovie 12. *ProSPCH:SPCH-GFP* expression in differentiating hydathode region.

(D) SMovie 13. *ProMUTE:MUTE-GFP* expression in differentiating hydathode region.

All images from 12 DAG first leaves. Cell walls visualized with PI staining. Yellow arrows, young Guard Mother Cells; pink arrow, Guard Cell; white arrows, MIs; blue Arrows, procambium or developing vascular tissue. Scale bars = 50 μm in (A)-(B), and 20 μm in (C)-(D).



Supplemental Figure 19. Three frames (first, middle & last) from Supplemental Movies showing expression from the auxin activity markers *DR5, PIN1,* and *PIN3.*

(A) SMovie 14. *ProDR5:3XVENUS-N7* expression near differentiating procambium.

(B) SMovie 15. *ProDR5:3XVENUS-N7* and *ProPIN3:PIN3-GFP* expression near differentiating hydathode region.

(C) SMovie 16. *ProPIN1:PIN1-GFP* expression near differentiating hydathode region.

All images from 12 DAG first leaves. Cell walls visualized using PI staining. Pink arrows, Guard Cells; blue arrows, procambium or developing vasculature. Scale bars = 10 μm in (A) and 20 μm in (B)-(C).

Supplemental Table1. List of primers and DNA sequences used for genotype analysis.

Name of Primer	DNA sequence (5'-to-3')
FAMA-LP	TCATTCATTTGCTTCCTACGG
FAMA-RP	CAATACAAAAAGCTCCCCTCAC
VAM3-LP	TTTGAGCTCAAAGGGTGAATC
VAM3-RP	TGCTGCTGAAAGAGAAACCAC
LBa1	TGGTTCACGTAGTGGGCCATCG
gnomR5-F(Ddel)	GAAAGTGAAAGTAAGAGGCAAACTAA
gnomR5-F(Ddel)	ATTCTTGATCAGGGAATAGCTCC

Supplemental Table2. List of primers and DNA sequences used for real-time PCR.

Name of Primer	DNA sequence (5' to 3')
Act2-F	CTGGATCGGTGGTTCCATTC
Act2-R	CCTGGACCTGCCTCATCATAC
TGG1-F(ex7)	TAAGGATGACCAAAAAGGTATGA
TGG1-R(ex8)	CACTGAACTCTGGAAGCCGA
TGG2-F(ex7)	AAACAAGCAACTTGGAGGGCC
TGG2-R(ex8)	AGGGCGTGGGCGTATTGAGT