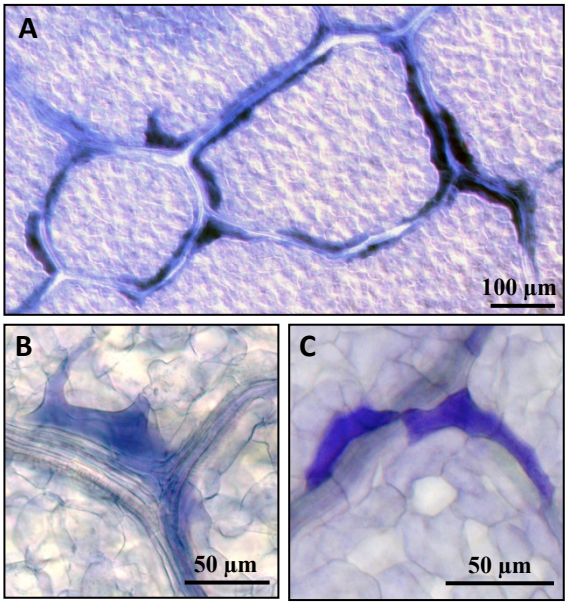
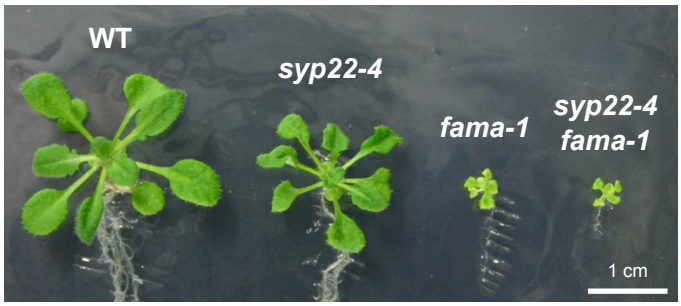
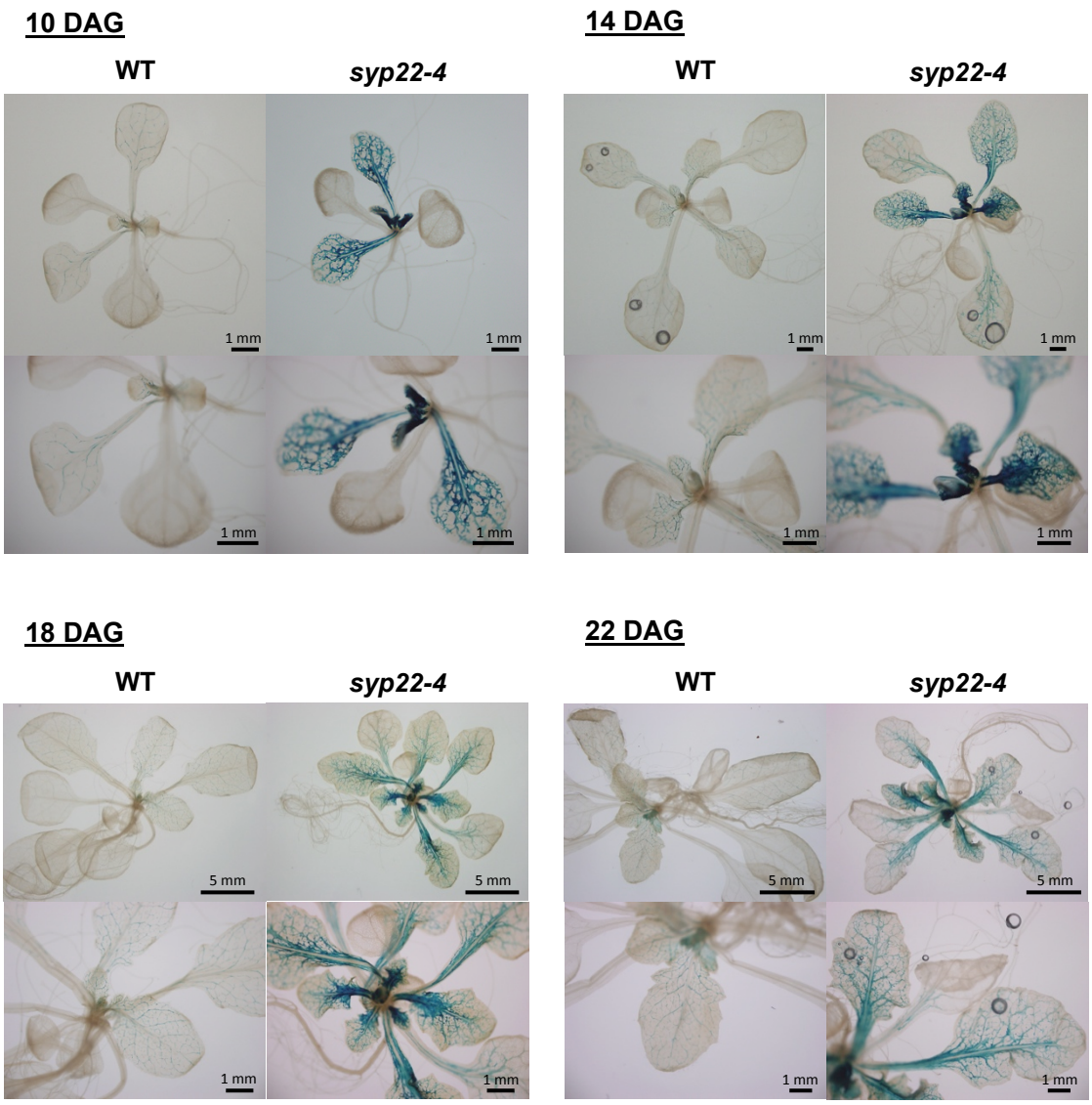


Supplemental Figure 1. Myrosin Cells Are Associated with Vascular Cells.

Coomassie brilliant blue-stained rosette leaves of *Cardamine schinziana* **(A)**, *Arabidopsis thaliana* **(B)**, and *Nasturtium officinale* **(C)**. Note that myrosin cells are distributed along the leaf veins.

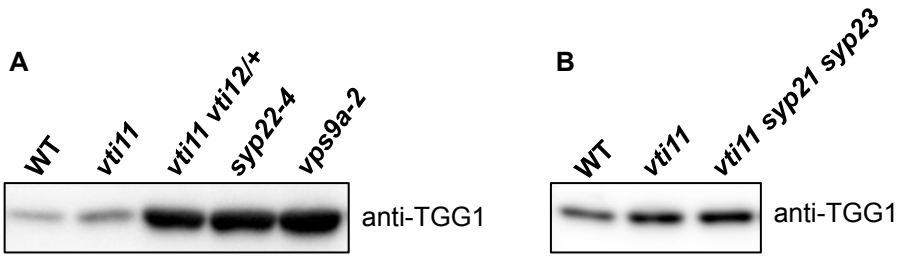


Supplemental Figure 2. Plant Morphology of *syp22-4 fama-1*. Images of wild type (WT), *syp22-4*, *fama-1*, and *syp22-4 fama-1*. The plants shown are 25 days after germination.



Supplemental Figure 3. Patterning of Myrosin Cells in Wild Type and *syp22-4*.

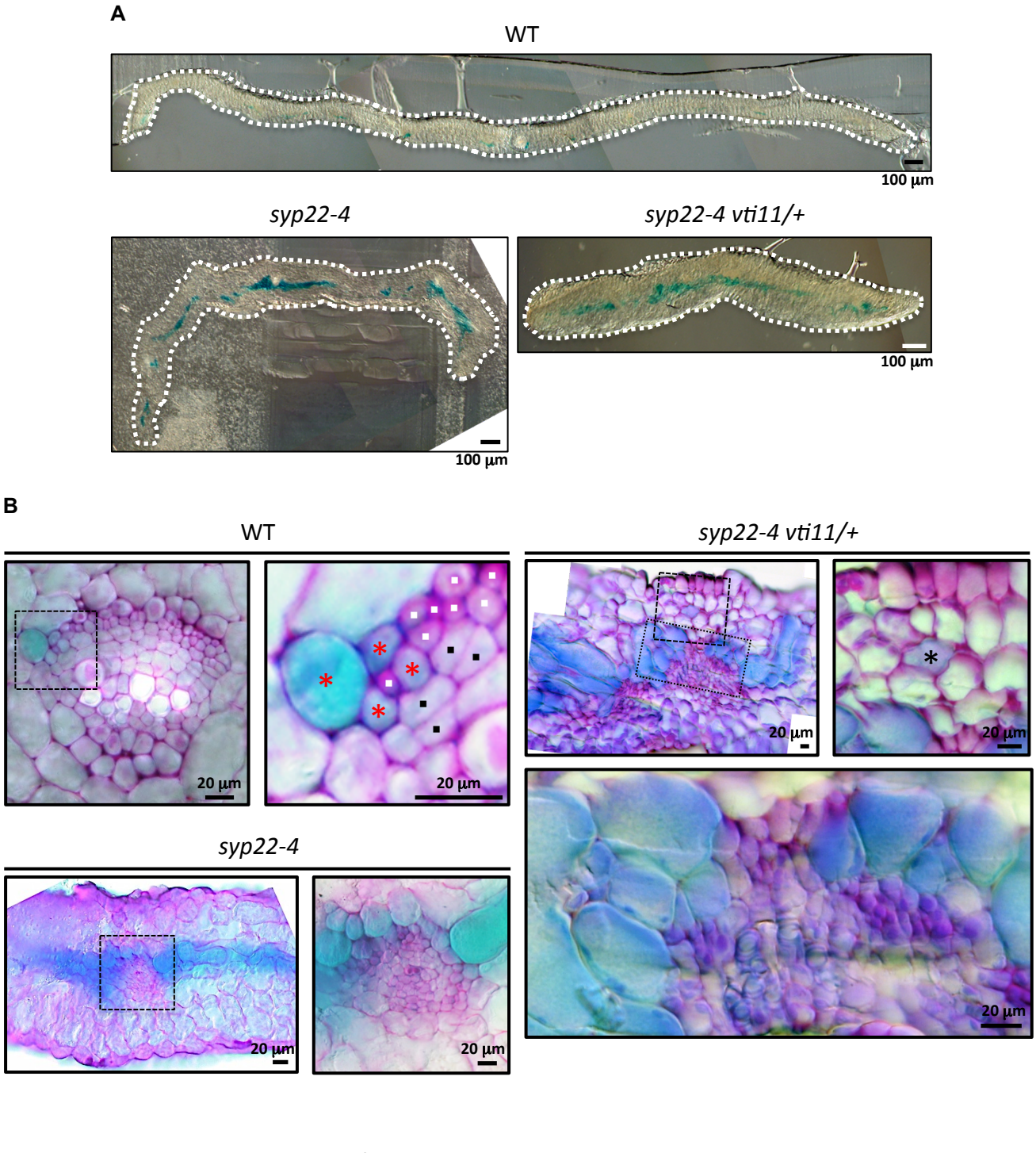
Developmental series of myrosin cells in wild type (WT) and *syp22-4* expressing *MYR001:GUS* at 10, 14, 18, and 22 DAG.



Supplemental Figure 4. Levels of TGG1 Accumulation in Multiple Mutants.

(A) Rosette leaves of wild type (WT), *vti11*, *vti11 vti12/+, syp22-4, vps9a-2* were subjected to immunoblotting with anti-TGG1 antibody.

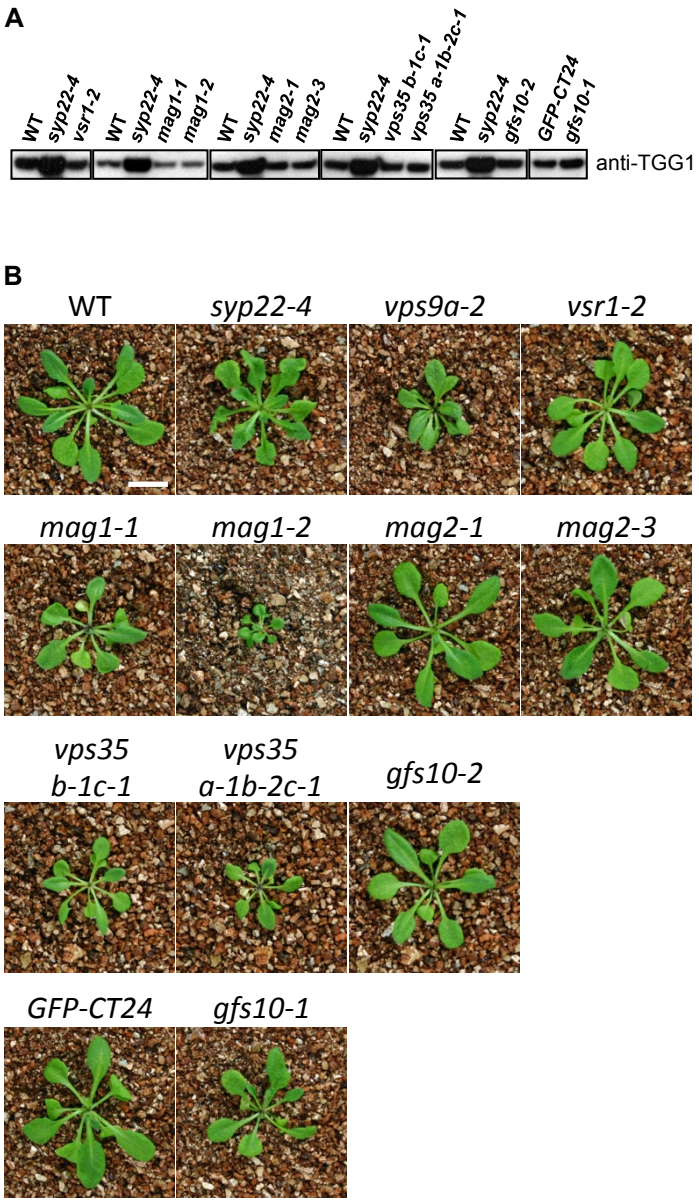
(B) Rosette leaves of wild type (WT), *vti11*, and *vti11 syp21 syp23* triple mutant were subjected to immunoblotting with anti-TGG1 antibody.



Supplemental Figure 5. Distribution of *MYR001:GUS*-Expressing Cells in Wild-Type, *syp22-4*, and *syp22-4 vti11/+* Plants.

(A) Cross sections of whole leaves harboring *MYR001:GUS* in a wild-type (WT), *syp22-4*, and *syp22-4 vti11/+* background. GUS-positive cells show extensive proliferation along the central region of *syp22-4* and *syp22-4 vti11/+* leaves.

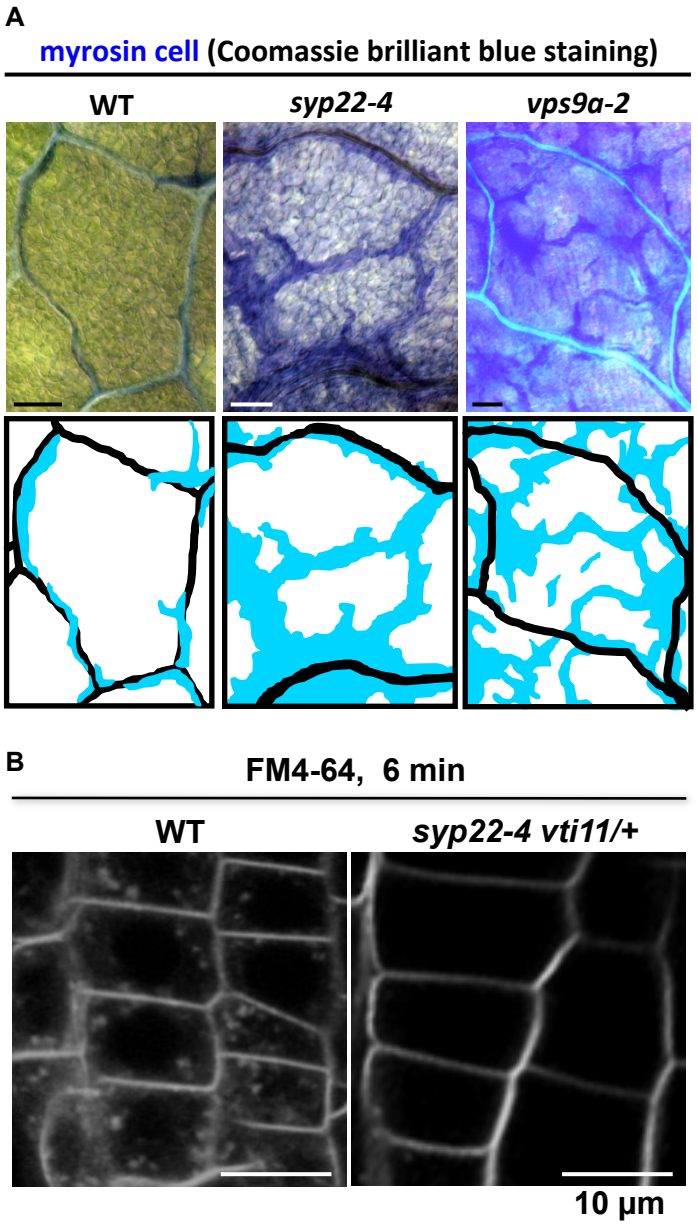
(B) Close-up images of cross sections. In wild-type (WT), GUS-positive myrosin cells (red asterisks) are adjacent to phloem cells (white squares) and procambium cells (black squares). Myrosin cells overproliferate along vascular bundles in *syp22-4* and *syp22-4 vti11/+* plants. Occasionally, a myrosin cell in *syp22-4 vti11/+* (black asterisk) is surrounded by mesophyll cells.



Supplemental Figure 6. Levels of TGG1 Accumulation and Morphology of Various Membrane-Trafficking Mutants.

(A) Rosette leaves of wild type (WT) and indicated membrane-trafficking mutants were subjected to immunoblotting with anti-TGG1 antibody. No significant increases in the levels of TGG1 accumulation were found in the membrane-trafficking mutants, except for *syp22-4*.

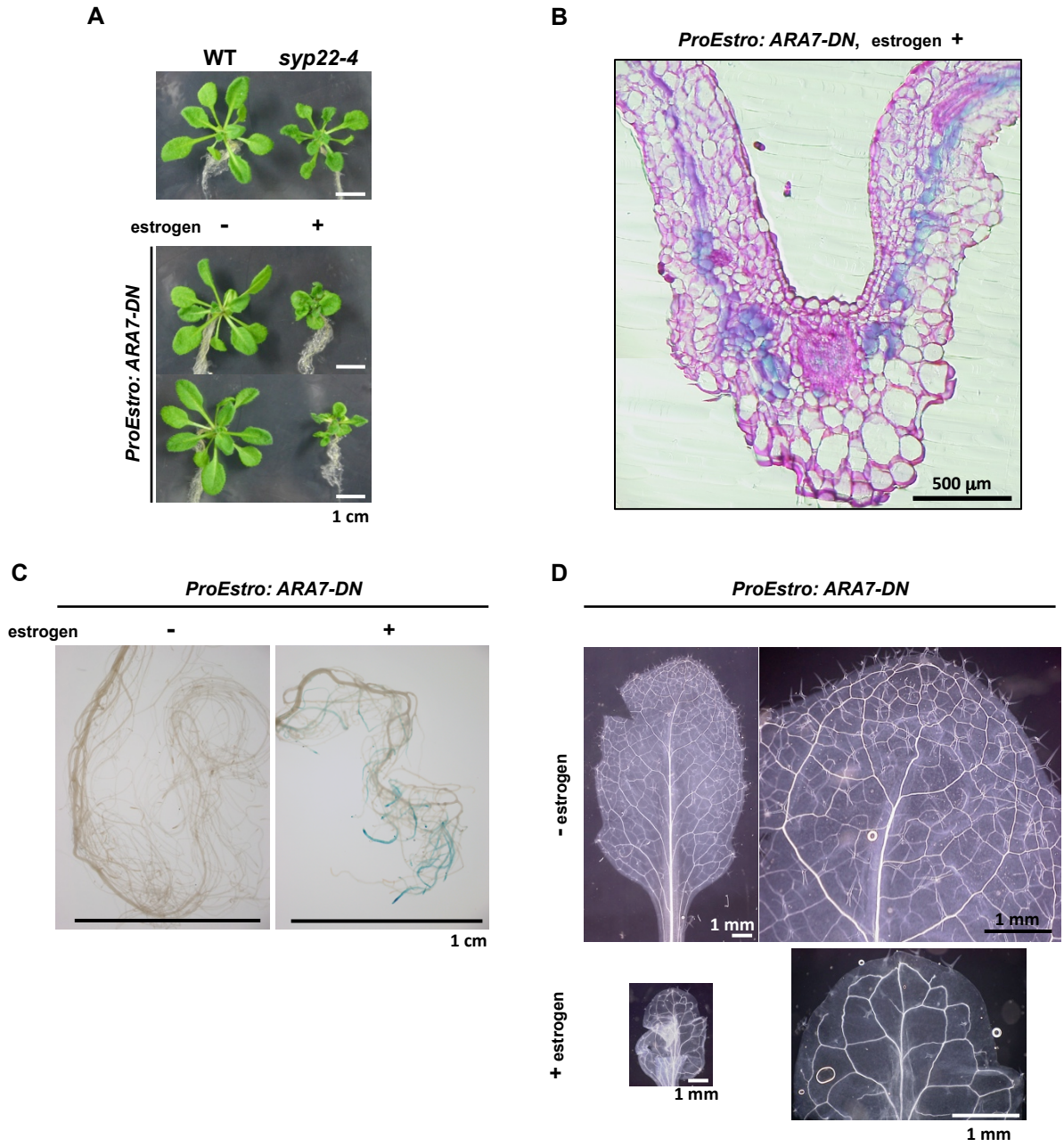
(B) Plant morphology of wild type (WT) and membrane-trafficking mutants at 31 DAG. Scale bar = 1 cm.



Supplemental Figure 7. Endocytic Pathway and Myrosin Cell Development.

(A) Distribution pattern of myrosin cells in *vps9a-2* Leaves. Distribution pattern of myrosin cells in the leaves of wild type (WT), *syp22-4*, and *vps9a-2* at 12 days after germination. Myrosin cells were visualized with Coomassie brilliant blue staining. Schematic illustrations of the patterns are shown in the respective lower panels. Myrosin cells and vascular veins are shown in blue and black, respectively. Scale bars = 100 µm.

(B) Endocytosis of FM4-64 is reduced in *syp22-4 vti11/+*. Internalization of the endocytic tracer FM4-64 in wild type (WT) and *syp22-4 vti11/+* roots after 6 min incubation. Images were captured with the same microscopic and processing settings.



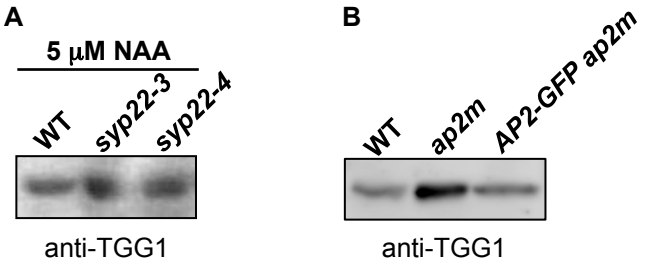
Supplemental Figure 8. Phenotypes of *ProEstro:ARA7-DN* Transgenic Plants.

(A) Comparison of wild type (WT), *syp22-4*, and transgenic plants expressing *ARA7-DN* under the control of the estrogen-inducible promoter (*ProEstro:ARA7-DN*). Two independent transgenic lines are presented.

(B) Cross section of a *MYR001:GUS* leaf expressing *ProEstro:ARA7-DN*. The 10-day-old plants were transplanted onto inductive medium containing 10 µM estrogen and incubated for two weeks. Myrosin cells are stained in blue.

(C) GUS staining of a *MYR001:GUS* root expressing *ProEstro:ARA7-DN*. The 10-day-old plants were transplanted onto inductive medium containing 10 µM estrogen and incubated for two weeks.

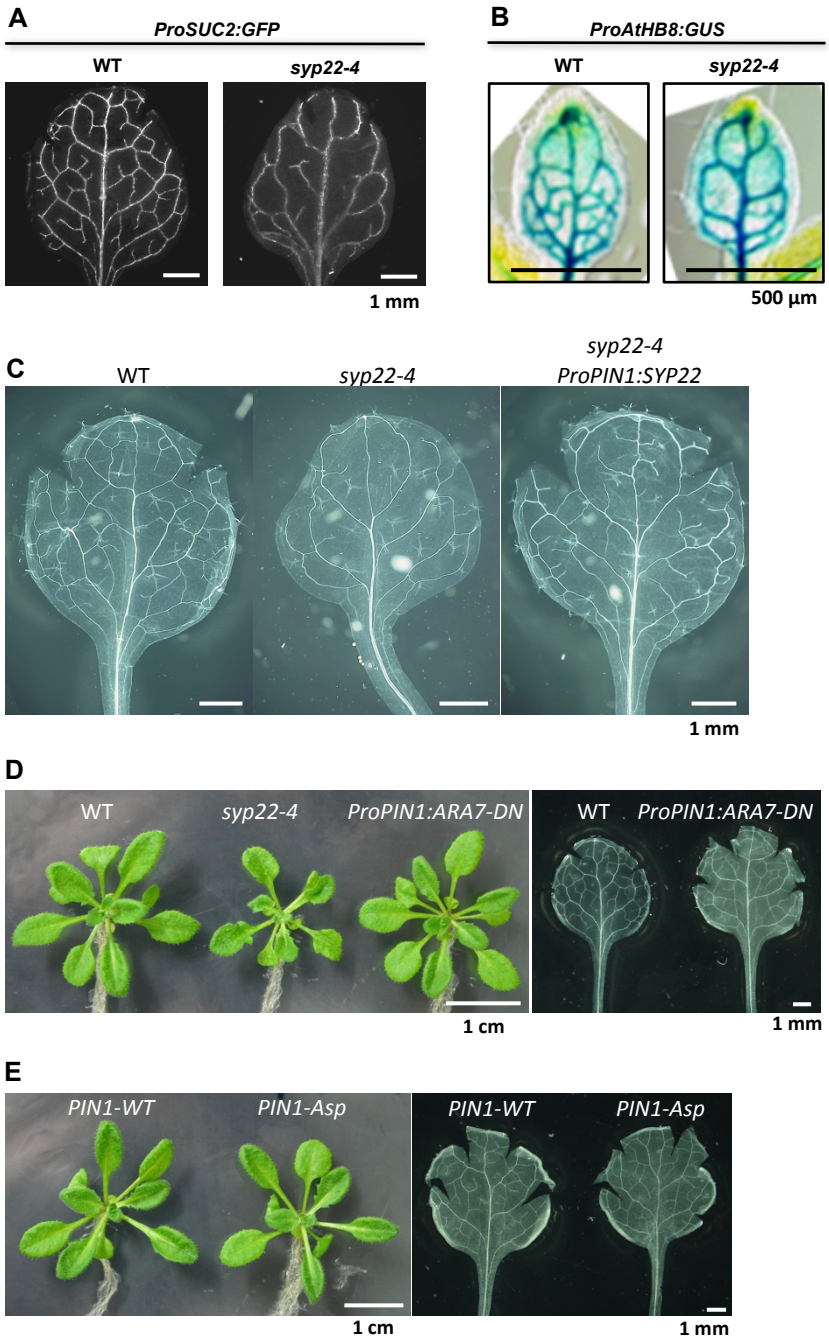
(D) Comparison of vascular patterns expressing *ProEstro:ARA7-DN*. The 10-day-old plants were transplanted onto inductive medium containing 10 µM estrogen and incubated for two weeks.



Supplemental Figure 9. Levels of TGG1 Accumulation in the *ap2m* Mutant and Auxin-Treated *syp22*.

(**A**) Rosette leaves of wild type (WT) and *syp22* mutants treated with synthetic auxin, NAA, were subjected to immunoblotting with anti-TGG1 antibody.

(**B**) Rosette leaves of wild type (WT), *ap2m*, and *AP2M-GFP ap2m* plants were subjected to immunoblotting with anti-TGG1 antibody. *AP2M-GFP ap2m* is a complemented line that was generated by introducing a genomic *AP2M-GFP* fusion construct into the *ap2m* mutant. The *ap2m* mutant exhibited increased TGG1 accumulation.



Supplemental Figure 10. Vascular Patterns and Plant Morphologies of Mutants and Transgenic Lines in This Study.

(**A**) Expression pattern of *ProSUC2:GFP* in wild type (WT) and *syp22-4*. Fluorescent images of rosette leaves at 17 days after germination of wild type and *syp22-4* expressing *ProSUC2:GFP*, a marker of phloem vascular cells (companion cells).

(**B**) Expression pattern of *ProAtHB8:GUS* in wild type (WT) and *syp22-4*. GUS staining of rosette leaves at 6 days after germination of the wild type and *syp22-4* expressing *ProAtHB8:GUS*, a marker of provascular cells.

(**C**) Vascular pattern of wild type (WT), *syp22-4*, and *syp22-4 ProPIN1:SYP22*.

(**D**) Plant morphology and vascular pattern of wild type (WT), *syp22-4*, and *ProPIN1:ARA7-DN*.

(**E**) Plant morphology and vascular pattern of *PIN1-WT* and *PIN1-Asp*.

Supplemental Table 1. Myrosin Cell Development, PIN1 Localization and Vascular Pattern in Mutants and Transgenic Plants.

ID	Line name	Development of myrosin cell	PIN1 localization	Vascular pattern	References
1	*syp22-4*	drastically increased	apolar localizatiion	simpler vascular pattern (reduced number of vein junctions)	This study; Shirakawa et al., 2009; Ueda et al., 2006
2	*syp22-4 vti11/+*	drastically increased	n.d.	simpler vascular pattern (reduced number of vein junctions)	This study; Shirakawa et al., 2009
3	*syp22-3 vti11/+*	drastically increased	n.d.	simpler vascular pattern (reduced number of vein junctions)	This study; Shirakawa et al., 2009
4	*syp22-3 vti11*	drastically increased	n.d.	simpler vascular pattern (reduced number of vein junctions)	This study; Shirakawa et al., 2009
5	*syp22-1 syp21^{ami}*	drastically increased	n.d.	simpler vascular pattern (reduced number of vein junctions)	Shirakawa et al., 2010
6	*syp22-3 syp21^{ami}*	drastically increased	n.d.	simpler vascular pattern (reduced number of vein junctions)	Shirakawa et al., 2010
7	*syp22-1 syp21^{ami} syp23*	drastically increased	n.d.	simpler vascular pattern (reduced number of vein junctions)	Shirakawa et al., 2010
8	*vps9a-2*	drastically increased	apolar localizatiion	simpler vascular pattern (reduced number of vein junctions)	This study; Shirakawa et al., 2009; Goh et al., 2007
9	*ProEstro:ARA7-DN*	drastically increased	n.d.	simpler vascular pattern (reduced number of vein junctions)	This study
10	*vti11 vti12/+*	drastically increased	n.d.	simpler vascular pattern (reduced number of vein junctions)	This study; Shirakawa et al., 2009
11	*syp22-1 rha1*	drastically increased	n.d.	n.d.	This study
12	*syp22-1 ara7*	drastically increased	n.d.	n.d.	This study
13	*syp22-1*	increased	n.d.	normal	Shirakawa et al., 2010
14	*syp22-3*	increased	n.d.	normal	Shirakawa et al., 2009; Ueda et al., 2006
15	*syp22-1 syp23*	increased	n.d.	normal	Shirakawa et al., 2010
16	*syp22-3 syp23*	increased	n.d.	normal	Shirakawa et al., 2010
17	*vti11*	increased	n.d.	normal	This study; Shirakawa et al., 2009
18	*vti11 syp21syp23*	increased	n.d.	n.d.	This study
19	*ProPIN1:ARA7-DN*	increased	n.d.	normal	This study
20	*PIN1-Asp*	increased	apolar localization	normal	This study; Zhang et al., 2010
21	*cov1-1*	increased	n.d.	V-shaped vein splice (not reduced number of vein junctions)	Shirakawa et al., 2014
22	*cov1-2*	drastically increased	n.d.	V-shaped vein splice (not reduced number of vein junctions)	Shirakawa et al., 2014
23	*ap2m*	increased	n.d.	n.d.	This study
24	*syp21*	normal	n.d.	normal	Shirakawa et al., 2010
25	*syp23*	normal	n.d.	normal	Shirakawa et al., 2010
26	*syp21 syp23*	normal	n.d.	normal	Shirakawa et al., 2010
27	*vsr1-2*	normal	n.d.	n.d.	This study
28	*mag1-1*	normal	n.d.	n.d.	This study
29	*mag1-2*	normal	n.d.	n.d.	This study
30	*mag2-1*	normal	n.d.*	n.d.	This study
31	*mag2-3*	normal	n.d.*	n.d.	This study
32	*vps35b-1c-1*	normal	n.d.**	n.d.	This study
33	*vps35a-1b-2c-1*	normal	n.d.**	n.d.	This study
34	*gfs10-1*	normal	n.d.	n.d.	This study
35	*gfs10-2*	normal	n.d.	n.d.	This study
36	*rha1*	normal	n.d.	n.d.	This study
37	*ara7*	normal	n.d.	n.d.	This study

* Polarity of PIN1 is normal but abnormal aggregation of PIN1 is formed and quantities of PIN1 is decreased in *vps29-3*, which is an another allele of *MAG1/VPS29*. (Jaillais et al., 2007; Kleine-Vehn et al., 2008)

** Polarity of PIN1 is normal but abnormal aggregation of PIN1 is formed in *pat3-1*, which is an another allele of *VPS35b*. (Nodzyński et al., 2013)

Supplemental Reference: **Nodzyński, T., Feraru, M.I., Hirsch, S., D Rycke, R., Niculaes, C., Boerjan, W., Van Leene, J., De Jaeger, G., Vanneste, S., and Friml, J.** (2013).

Retromer subunits VPS35A and VPS29 mediate prevacuolar compartment (PVC) function in *Arabidopsis*. Mol. Plant **6:** 1849-1862.

Supplemental Table 2. Primer Sets Used in This Study.

At4g19640 CDS:

RABF2B-F: 5’-CACCATGGCTGCAGCTGGAAACAAGAGCAT-3’

RABF2B-R: 5’-CTAAGCACAACAAGATGAGCTCACTGCCCT-3’

The site directed mutagenesis (S24N) of At4g19640 CDS:

S24N-S: 5’-GGAAAAAATAGTCTTGTGTTACGGTTTGTC-3’

S24N-AS: 5’-AAGACTATTTTTTCCAGCACCAACATCTCC-3’

At1g73590 promoter region:

At1g73590-F: 5’-ACTGGATCCGGTACATGATCCGATTGGATTCGGTCTGGAG-3’

At1g73590-R: 5’-GAGAAGAGACCACATTTTTATATTCTTTTT-3’

VAM3 CDS and NOS terminator:

InfAsc1VAM3-F:

5’-TCAAGGGTGGGCGCGATGAGTTTTCAAGATTTAGAATCAGGAAGAGGAAG-3’

InfAsc1NosT-R:

5’-AGCTGGGTCGGCGCGGATCTAGTAACATAGATGACACCGC-3’

Ara7-DN and 35S terminator:

InfAsc1Ara7-F:

5’-TCAAGGGTGGGCGCGATGGCTGCAGCTGGAAACAAGAGCATTAACGCCAA-3’

InfAsc135ST-R:

5’-AGCTGGGTCGGCGCGCGCATGCCTGCAGGTCACTGGATTTTGGTTTTAGG-3’

PIN1 genomic region (WT and Asp) fused to GFP:

InfuAsc1PIN1-F:

5’-TCAAGGGTGGGCGCGAACACTCACTTTACTCTTTTTTCCCTCTTCACCAC-3’

InfuAsc1PIN1-R:

5’-AGCTGGGTCGGCGCGGTGAACTTAACTTTTGAAATGGCTAAAACACTTCT-3’