

Table S1. List of primers used for genotyping assay.

Primer name	Forward primer (5' to 3')	Reverse primer (5' to 3')	Target sequence
P1 and P2	TCCAAAAATTGATAGGCATC	ATAAAGAAAAAGCTTACCCG	<i>ATML1</i>
P3 and P4	GATCAGTGCCTTGAAGGAAA	CTGTTGTCGACATTGTTGTC	<i>PDF2</i>
P5 and P6	TGGGATATAACAGGCAGAAGA	GTTTTGGAGCTACAGGGATCCAGA	<i>ATML1</i>
P7 and P8	GATCAGTGCCTTGAAGGAAA	ATCTTAGTTGTGGAATCATAC	<i>PDF2</i>
P9 and P10	ACATTCTCCTCCCATGGAAAC	TAGTACATGAACGAACAAGAC	<i>PAS2</i>
P11 and P12	AGGCGAGTGGTTCTTGTTT	TCACTCAACAACATCGAGCAC	<i>FLC</i>

Table S2. List of primers used for plasmid construction.

Name	Sequence (5' to 3')
ATML1m1-F	CACTGTTGATACATATGTATCCAAACATGTT
ATML1m1-R	AGCCACCAAAACGTTTGACCGAAAGCTAA
ATML1m2-F	AAACGTTGGTGGCTACACTTGACCGCCAA
ATML1m2-R	ATTCAAGATTGTCGAGGCTCCGTCGCAGGGCAGAGC
HSPp-F	CCAAGCTTGCATGCCAAGCTTTCTCTCATTTCTC
HSPp-R	TGTTCGTTGCTTTCTGGGAGA
NLS-mCherry-F	GAAAAGCAACGAACACATATGCGACCCCCAAAGAAGAAG
NLS-mCherry-R	ATTCAGAAATTGTCGATTACTTGACAGCTCGTCCAT
ATML1-F	GAAAAGCAACGAACACATATGTTATCATCCAAACATGTT
ATML1-R	ACTAGTGCTCCGTCGCAGGCCAGAGC
EGFP-F	GACGGAGCCACTAGTATGGTGAGCAAGGGCAGGGAG
EGFP-R	ATTCAAGATTGTCGATTACTTGACAGCTCGTCCAT
HSP_ATML1_F	CGGCCGCTGGATCCAAGCTTCTCTCATTTCTC
HSP_ATML1_R	TGATTACGAATTCCCCTTATCTTAATCATATTCCA
EGFP2-F	TTATGGATCCGAATTCTGTGAGCAAGGGCGAGGAGCTGTT
EGFP2-R	AAACATCGATTACTTGACAGCTCGTCCAT
ATML1ter-F	CAAGTAATCGATTTTGGGTAAGCTTTTT
ATML1ter-R	TACGAAGTTATGAATTGATGACTTGGTCTCCATAATTTC
gATML1-F	ACGAAGTTATGGATCCAAGCTTAGTTCTTATTGACATA
gATML1-R	CCTTGCTCACGGATCCGCTCCCTCCAAACATCACAAGAA
EGFP3-F	ACGAAGTTATGGATCCGTGAGCAAGGGCGAGGAGCTGTC
EGFP3-R	TGAAAAAAAACATTAACTTGACAGCTCGTCCATG
PDF2ter-F	GTAATAGTTTTTCAGGTATGATTC
PDF2ter-R	ACGAAGTTATGAATTCTTACCTTCTATGTTAGGCTGTTA
gPDF2-F	TACGAAGTTATGGATCCCATATAGTCCAACITTGCAAGACAT
gPDF2-R	CCCTTGCTCACGGATCCGCTCCCTCCAAACATCACAAGAA
loxEGFP-F	CCAAGCTTGCATGCCATACTCGTATAGCATACTTACATTACAGAAGTTATGGATCCGGTGGTGGTGGTGTGTCAC
loxEGFP-R	CCATGATTAACGAAATTAACTTGTATGCTATAACGAAGTTATGGATCCGGTGGTGGTGGTGTGTCAC
PAS2ter-F	GTACAAGTAAGAATTAAAGAAAAGATTAGAAAGAGACGAA
PAS2ter-R	ACGAAGTTATGGATCCGGAAATTCACCTGTAGCTT
gPAS2-F	ACGAAGTTATGGATCCGGAAATTCACCTGTAGCTT
gPAS2-R	CACCACCAACGGATCTCCCTCTGGATTGGAGAG
START-F	AGGGCCCGGGACATATGATACTTCTGAGGGTGATAAG
START-R	AGATTACCTATCTAGAACACAAGCCGAATTTGCTGGC

Table S3. List of primers and PCR conditions used for RT-PCR analysis.

Gene	Forward primer (5' to 3')	Reverse primer (5' to 3')	annealing temp.	cycles
<i>ATML1</i> ^{WT/W471L} -EGFP	TGGATGATCCGGGAAGACCT	CTCGCCCTTGCTCACCATAC	50	30
NLS-mCherry	GACCCCCAAAGAAGAACGTA	CTGCTTGATCTGCCCTCA	50	30
<i>TUB2</i>	CTCAAGAGGTTCTCAGCAGTA	TCACCTTCTTCATCCGCAGTT	50	30

Table S4. List of primers and probes used for RT-qPCR analysis.

Gene	Forward primer (5' to 3')	Reverse primer (5' to 3')	Probe
<i>ATML1</i>	GGAATAGTGTCTCCTTGCTTCG	TGATGCGTCCGTACAACTTT	122
<i>PDF2</i>	TCTCCTGCTCCGAGTCAATA	TGCATCTGTACAGCTCTCTGTAGA	106
<i>PP2A</i>	ATTCCGATAGTCGACCAAGC	AACATCAACATCTGGGTCTTCA	22

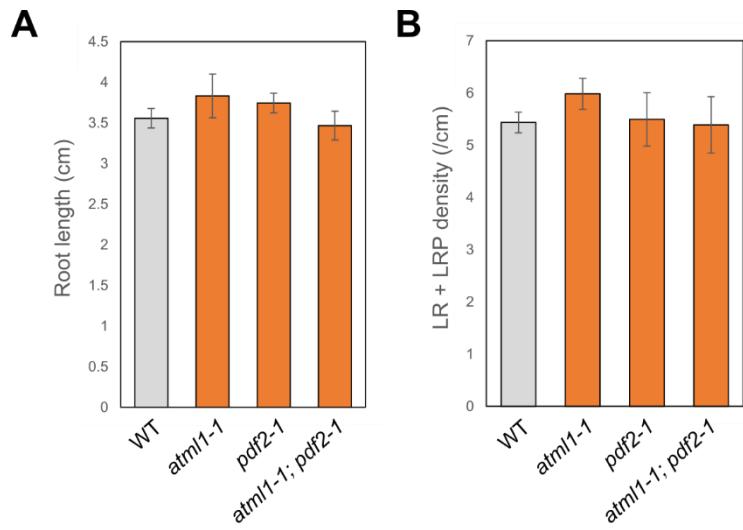


Fig. S1. Root phenotypes in the *atm1-1; pdf2-1* mutant.

(A) Primary root length of 10-day-old wild-type, *atm1-1*, *pdf2-1* and *atm1-1; pdf2-1* seedlings grown on MS medium. Data are mean \pm SE values (n = 10). **(B)** Number of LRs and LRPs per root length in 10-day-old wild-type, *atm1-1*, *pdf2-1* and *atm1-1; pdf2-1* seedlings grown on MS medium. Data are mean \pm SE values (n = 10).

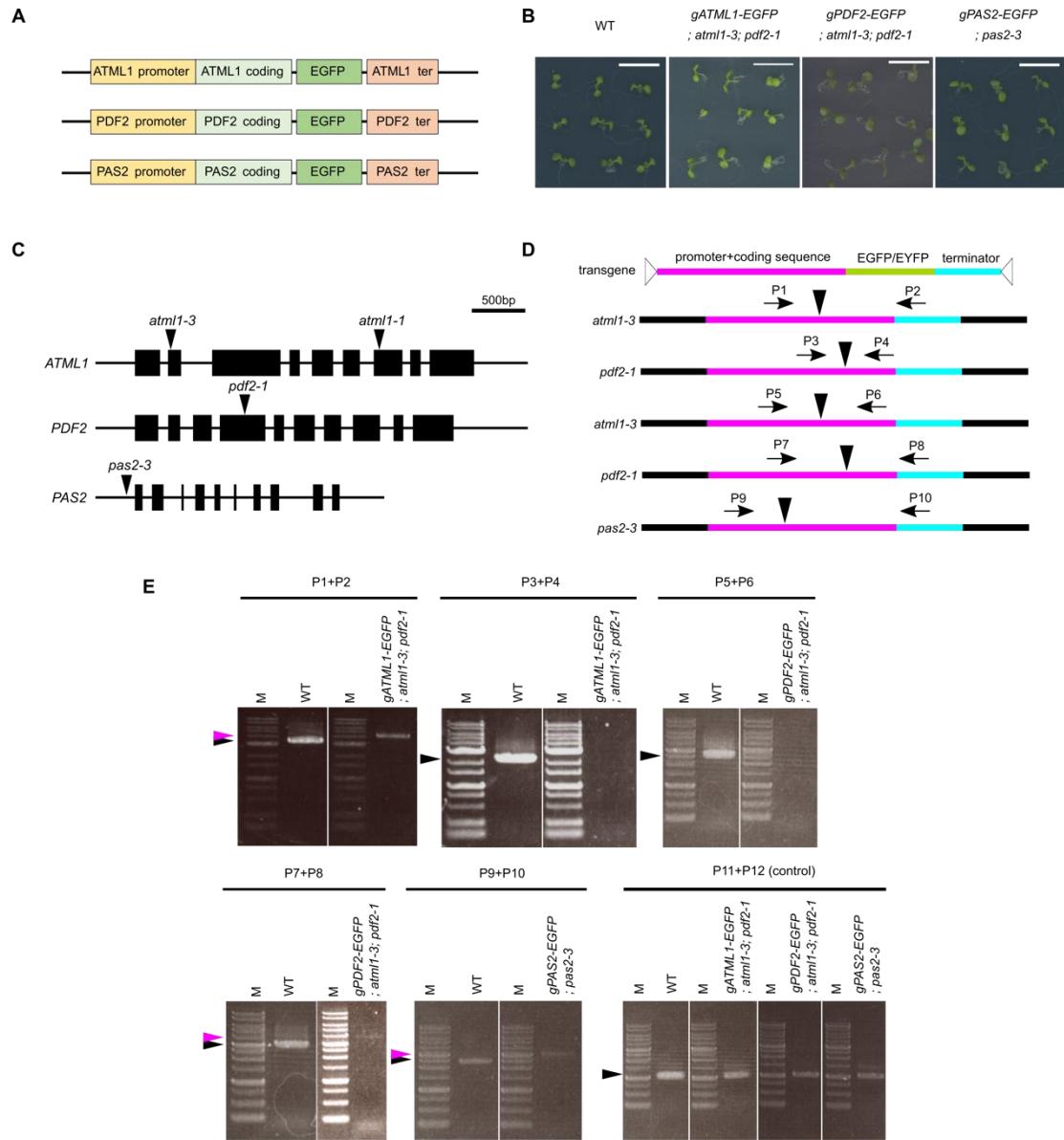


Fig. S2. Genetic complementation with reporter genes.

(A) Representation of plasmid constructions. (B) Phenotypes of 5-day-old seedlings. Wild type, *gATML1-EGFP; atm1-3; pdf2-1*, *gPDF2-EGFP; atm1-3; pdf2-1* and *gPAS2-EGFP; pas2-3* are shown (left to right). Scale bars: 1 cm. (C) Representation of T-DNA insertion sites in the mutants. (D) Primer design for confirmation of transgenes

in mutant background (primer information (P1-P10) are shown in Table S1). Arrows indicate primer locations and arrowheads indicate T-DNA insertion sites. **(E)** Genotyping assay. Black arrows indicate the expected amplicon size of the WT gene. As a control, *FLC* (AT5G10140) was amplified using primer set of P11 and P12 (Table S1). Magenta arrows indicate the expected amplicon size of the transgene. M: 1-kbp DNA ladder.

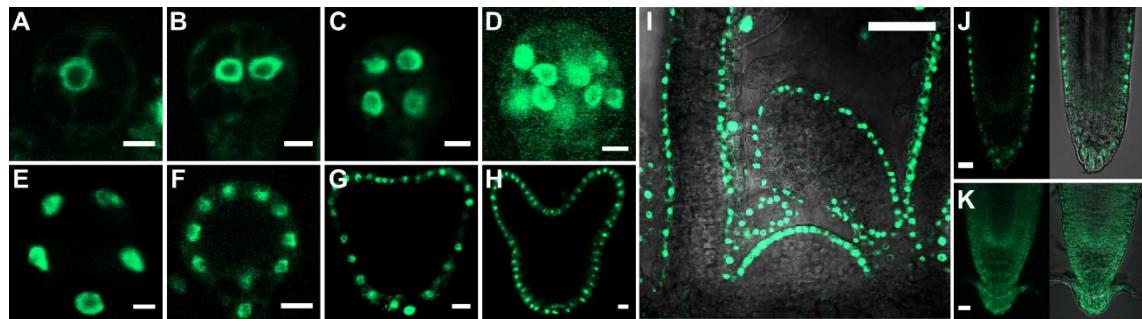
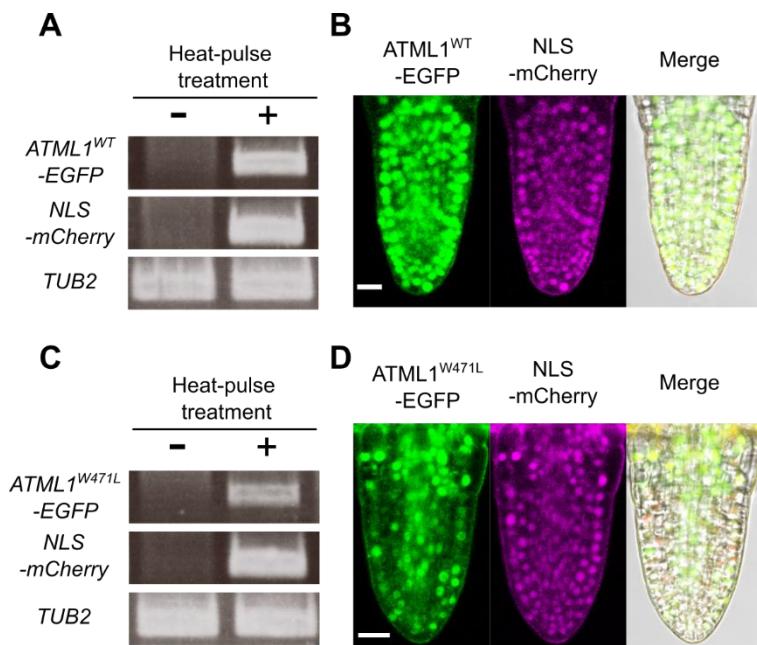


Fig. S3. Expression of *gATML1-EGFP* during embryogenesis.

(A) 1-cell stage embryo, (B) 2-cell stage embryo, (C) Octant (8-cell) stage embryo, (D) and (E) Two dermatogen (16-cell) stage embryos showing different fluorescence patterns, (F) Globular stage embryo, (G) Triangular stage embryo, (H) Heart stage embryo, (I) Shoot apical meristem and leaf primordia, (J) Radicle (Primary root before sloughing of the root cap cells) and (K) Primary root after sloughing of root cap cells. Scale bars: 5 μ m in (A-E); 10 μ m in (F-H); 20 μ m in (J, K); 50 μ m in (I).

**Fig. S4. Heat-pulse-dependent induction of transgene.**

(A) *ATML1^{WT}-EGFP* and *NLS-mCherry* mRNA in *HSP::NLS-mCherry; HSP::ATML1^{WT}-EGFP* seedlings. (B) Fluorescence images of EGFP (left) and mCherry (middle), and merged fluorescence and bright field image (right) in LRs of *HSP::NLS-mCherry; HSP::ATML1^{WT}-EGFP* plants before sloughing of root cap cells immediately after heat-pulse treatment (0-30 min). (C) *ATML1^{W471L}-EGFP* and *NLS-mCherry* mRNA in *HSP::NLS-mCherry; HSP::ATML1^{W471L}-EGFP* seedlings. (D) Fluorescence images of EGFP (left) and mCherry (middle), and merged fluorescence and bright field image (right) in LRs of *HSP::NLS-mCherry; HSP::ATML1^{W471L}-EGFP* plants before sloughing of root cap cells immediately after heat-pulse treatment (0-30 min). For RT-PCR analysis,

whole seedlings were harvested for RNA extraction before (–) and after (+) heat-pulse treatment. *TUB2* (AT5G62690) was amplified as a control.

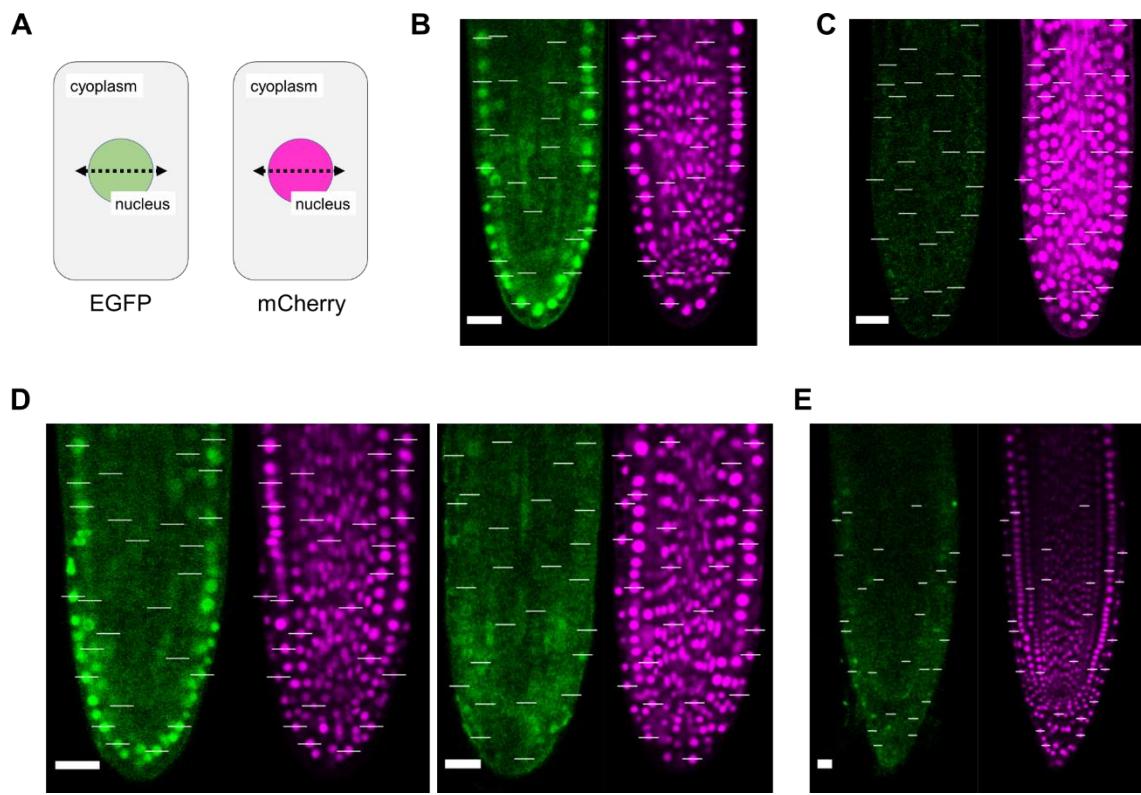


Fig. S5. Measuring the EGFP and mCherry intensity.

(A) Fluorescence intensities of EGFP and mCherry were measured by using the [Plot Profile] tools of ImageJ software. Intensity values of EGFP and mCherry were measured on the line crossing the nucleus of a single cell. (B-E) A series of intensity values was acquired from 13 inner cells and 13 outermost cells by using the fluorescence images in Fig. 3C, Fig. 3E, Fig 5A and Fig. S6A, corresponding to Fig. S5B, C, D and E, respectively. The lengths of the lines crossing the nucleus are the same in each pair of panels.

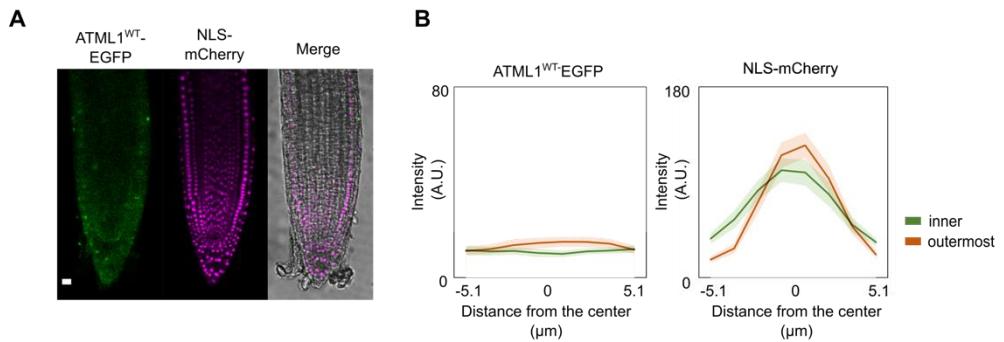
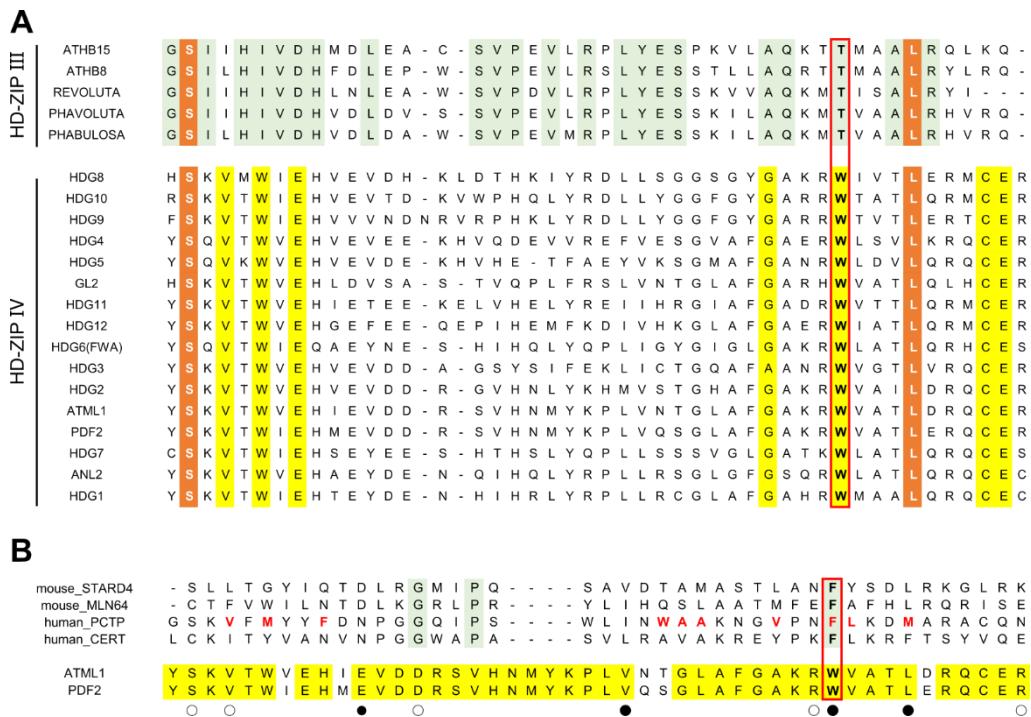


Fig. S6. Posttranslational regulation of ATML1 in LRs after sloughing of root cap

cells.

(A) Fluorescence images of EGFP (left) and mCherry (middle), and merged fluorescence and bright field image (right) in LRs of *HSP::NLS-mCherry; HSP::ATML1^{WT}-EGFP* plants after sloughing of root cap cells 2–3 hours after heat-pulse treatment. Scale bars: 20 μm . (B) Plot of EGFP (left) and mCherry (right) intensity in inner cells and outermost cells in (A) (see Fig. S5).

**Fig. S7. Amino acid sequences of the START domain.**

(A) Alignment of amino acid sequence of the C-terminal region of the START domain.

(B) Alignment of amino acid sequence of the C-terminal region of the START domain

from ATML1, PDF2, STARD4, MLN64, PCTP, and CERT. Red font indicates ligand

contact points, as derived from the PCTP-PtCho co-crystal. Red box indicates the amino

acid targeted for site-directed mutagenesis of ATML1. Yellow background indicates

amino acids conserved in HD-ZIP class IV proteins but not in HD-ZIP class III proteins

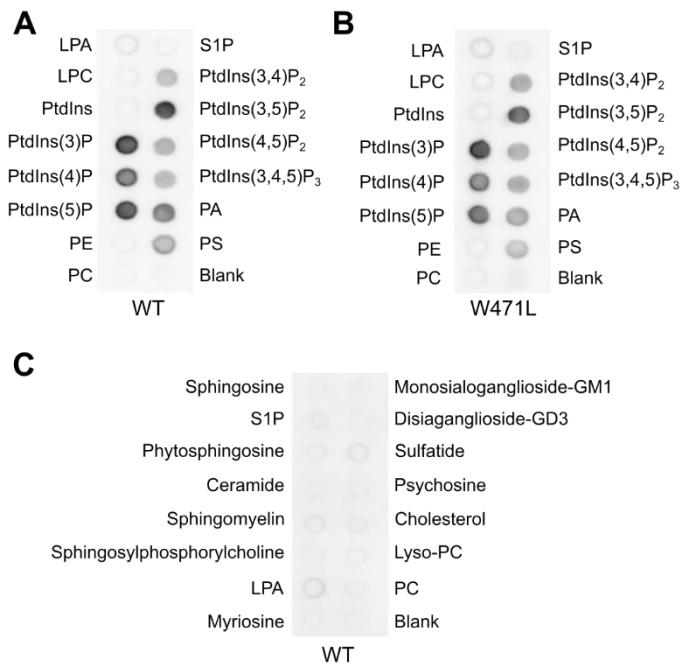
(A), or conserved in ATML1 and PDF2, but not in mammalian proteins (B). Green

background color indicates amino acids conserved in HD-ZIP class III proteins, but not

in HD-ZIP class IV proteins (A), or in mammalian proteins but not in ATML1 or PDF2

(B). Orange background color indicates amino acids conserved among HD-ZIP class III

proteins and HD-ZIP class IV proteins. Open circles indicate a site where the amino acids exhibit weak similarity. Closed circles indicate a site where the amino acids exhibit strong similarity.

**Fig. S8. Interaction of the GST tagged START domain of ATML1 (GST-START)****and ATML1^{W471L} (GST-START^{W471L}) with various lipids.**

(A and B) Interaction of GST-START (A) or GST-START^{W471L} (B) with all phosphoinositides and other biological important lipids. **(C)** Interaction of GST-START with various sphingolipids and other lipids. S1P, sphingosine 1-phosphate; LPA, lysophosphatidic acid; Lyso-PC, lysophosphatidylcholine; PC, phosphatidylcholine; LPA, lysophosphatidic acid; LPC, lysophosphocholine; PtdIns, phosphatidylinositol; PtdIns(3)P, phosphatidylinositol (3)-phosphate; PtdIns(4)P, phosphatidylinositol (4)-phosphate; PtdIns(5)P, phosphatidylinositol (5)-phosphate; PE, phosphatidylethanolamine; PtdIns(3,4)P₂, phosphatidylinositol (3,4)-bisphosphate; PtdIns(3,5)P₂, phosphatidylinositol (3,5)-bisphosphate; PtdIns(4,5)P₂, phosphatidylinositol (4,5)-bisphosphate.

phosphatidylinositol (4,5)-bisphosphate; PtdIns(3,4,5)P₃, phosphatidylinositol (3,4,5)-trisphosphate; PA, phosphatidic acid; PS, phosphatidylserine.

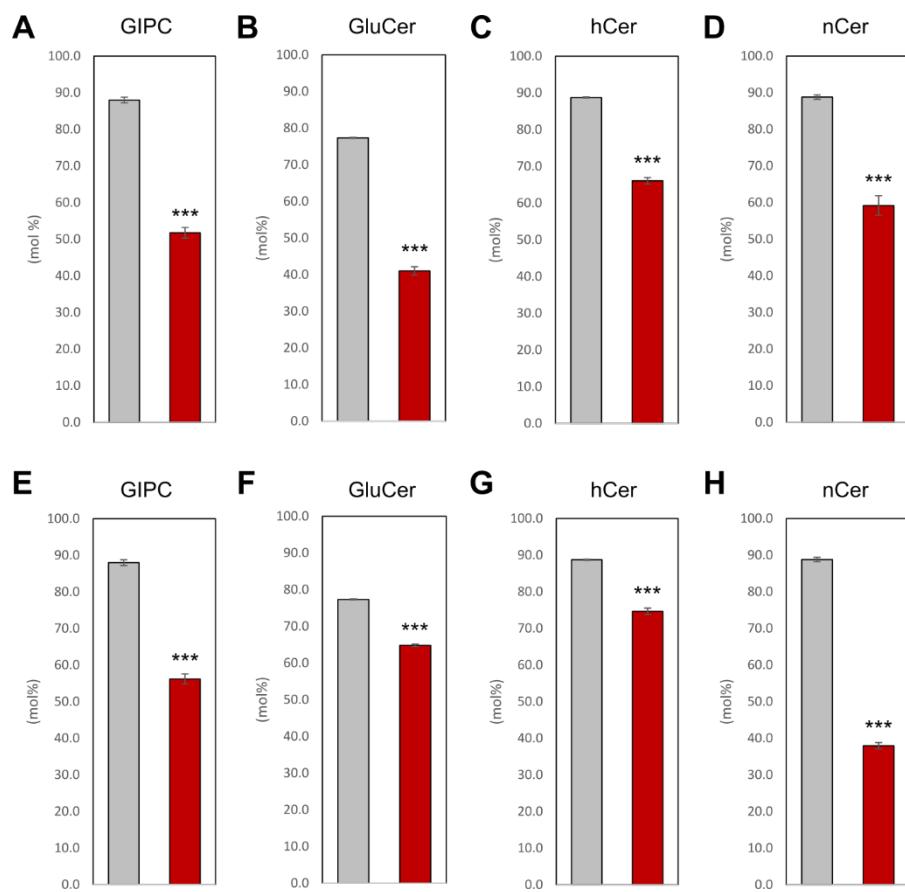


Fig. S9. Effect of cafenstrole and FB1 treatment on the sphingolipid metabolism.

The molar fraction of VLCFA-containing species in each sphingolipid class was measured in wild-type seedlings grown for 5 days in the presence of 0.3 μ M cafenstrole (**A-D**) or 0.5 μ M FB1 (**E-H**). (A, E), VLCFA-containing GIPC. (B, F), VLCFA-containing GluCer. (C, G) Hydroxy VLCFA containing ceramide. (D, H) Non-hydroxy VLCFA containing ceramide. Data are mean \pm SE values (***($P < 0.01$, Student's *t* test, $n = 3$).

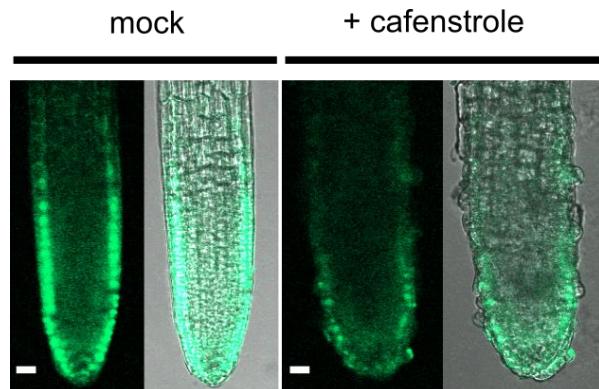


Fig. S10. Effect of cafenstrole treatment on the expression of *gATML1-EGFP*.

Shown are LRs before sloughing of root cap cells of *gATML1-EGFP; atml1-3; pdf2-1* plants grown for 8 days on MS medium without (mock) or with 0.3 µM cafenstrole. Scale bars: 20 µm.

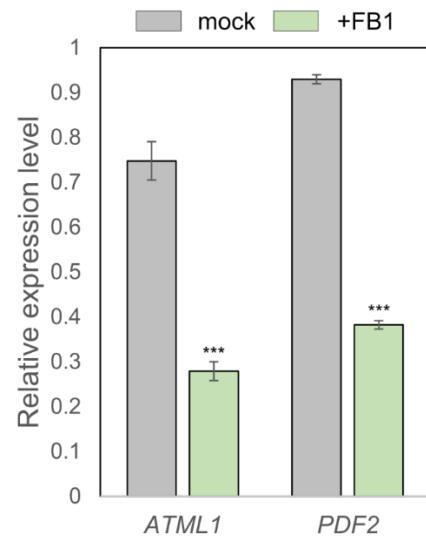


Fig. S11. Effect of the inhibition of ceramide synthase activity on the expression of *ATML1* and *PDF2*.

Relative expression levels of *ATML1* and *PDF2* in 5-day-old wild-type seedlings grown on MS medium without (mock) or with 0.5 µM FB1. Data are mean ± SE values (**P < 0.01, Student's *t* test, n = 3).

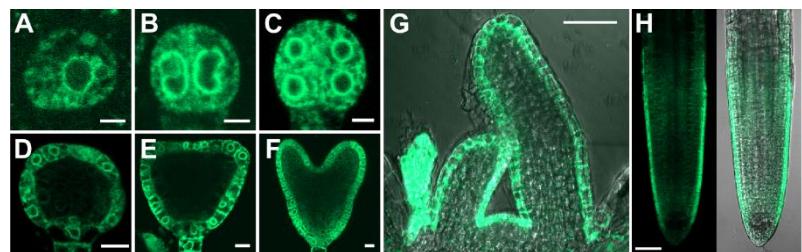


Fig. S12. Expression of *gPAS2-EGFP* during embryogenesis.

(A) 1-cell stage embryo, (B) 2-cell stage embryo, (C) Octant (8-cell) stage embryo, (D) Globular stage embryo, (E) Triangular stage embryo (F) Heart stage embryo, (G) Shoot apical meristem and leaf primordia and (H) Primary root. Scale bars: 5 μm in (A-C); 10 μm in (D-F); 20 μm in (H); 50 μm in (G).

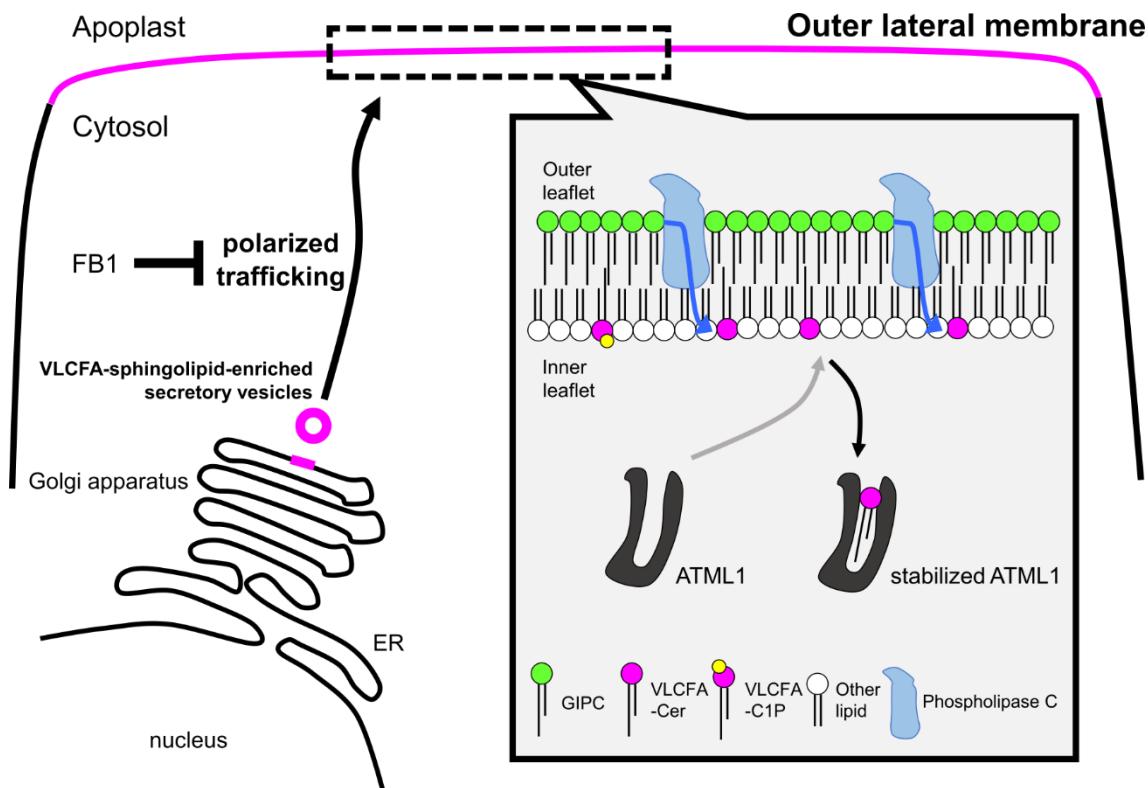


Fig. S13. Model of the polarized trafficking of VLCFA-sphingolipids to the outer lateral membrane and generation of VLCFA-Cers, which interact with intracellular ATML1.

VLCFA-sphingolipids are transported to the outer lateral membrane by a mechanism that is inhibited by FB1 treatment. Subsequently, VLCFA-sphingolipids (such as GIPCs) in the outer leaflet of the outer lateral membrane are hydrolyzed by specific enzymes (such as phospholipase C), generating VLCFA-Cers in the inner leaflet of the outer lateral membrane. Magenta line indicates apical membrane components. ER, endoplasmic reticulum.