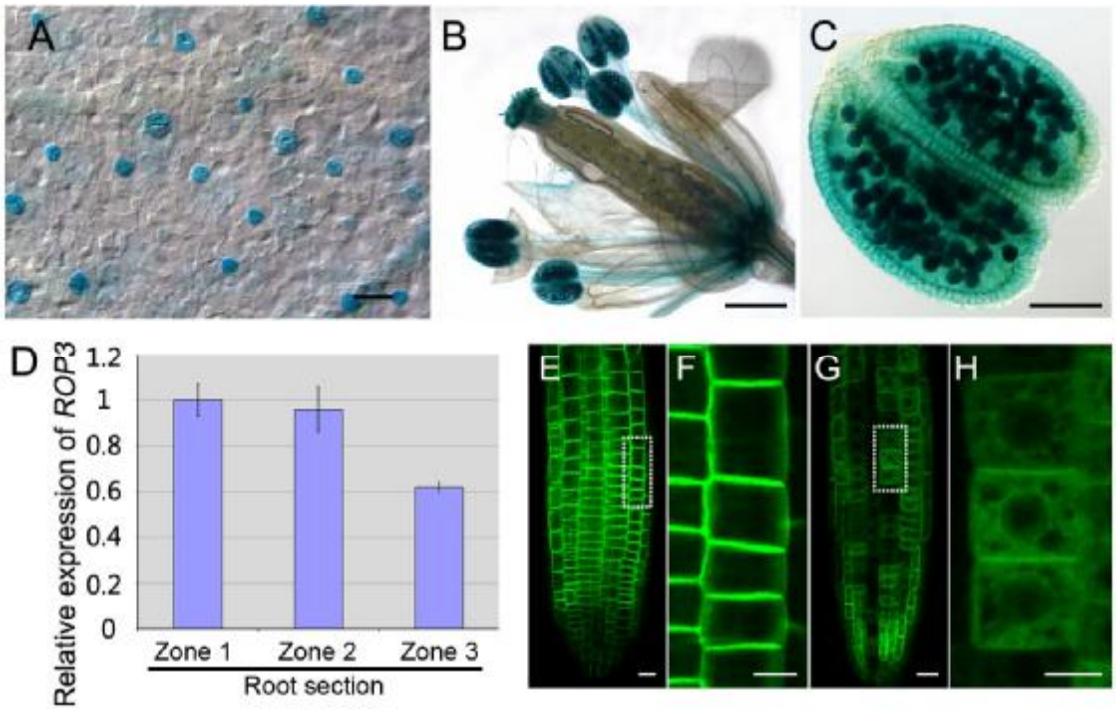
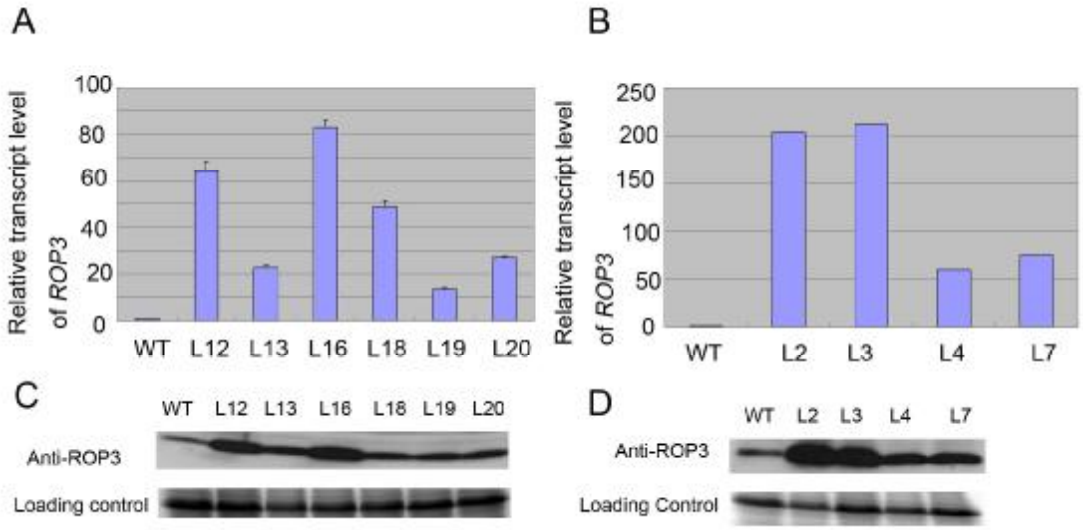


Supplemental Figure 1. Analysis of *ROP3* expression pattern and subcellular localization

(A) *ROP3*$_{pro}$*:GUS* is expressed at stomata of cotyledons of a 7-d-old seedling, (B) and (C) *ROP3*$_{pro}$*:GUS* is strongly expressed in anthers (B) and pollen grains (C). (D) RT-qPCR analysis on *ROP3* relative expression level in different root sections. Root sections: Zone1 contains root columella and meristem region; Zone 2 represents root elongation region; Zone 3 represents root maturation region. (E) YFP:ROP3 is predominantly localized in the PM of root cells of *35S*$_{pro}$*:YFP:ROP3* seedlings. (F) A magnified view of the dotted area in (E). (G) YFP:DN-rop3 is mainly localized in the cytosol and perinuclear region of root cells of *35S*$_{pro}$*:YFP:DN-rop3* seedlings. (H) A magnified view of the dotted area in (G).

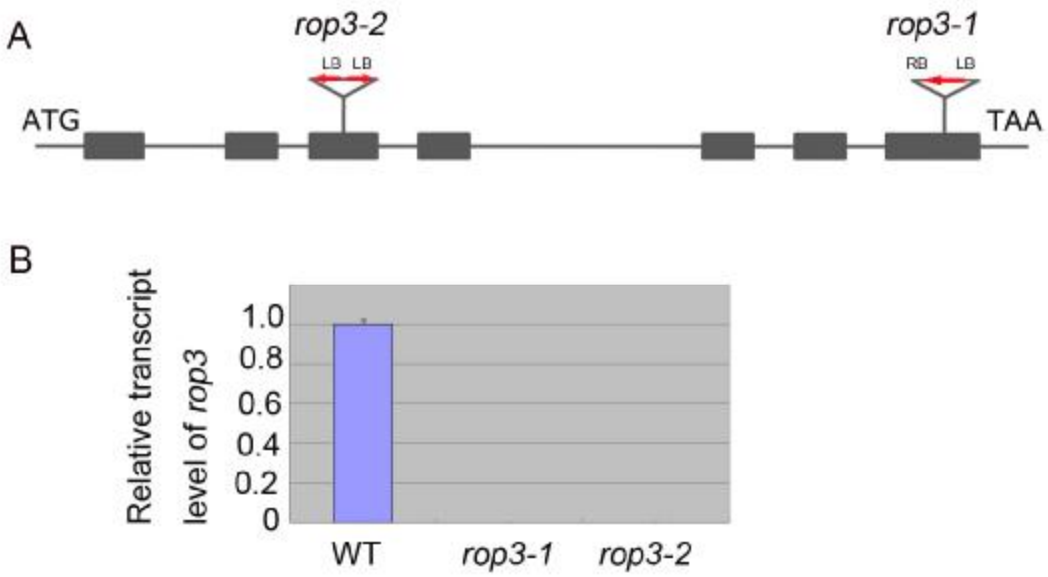
Bars =10 μm in (F) and (H); 20 μm in (A) to (C), (E) and (G).



Supplemental Figure 2. RT-qPCR analysis of *DN-rop3* transcripts and immunoblot analysis of DN-rop3 protein levels in the *DN-rop3* transgenic lines

(A) and (B) RT-qPCR analysis. Over-production of *DN-rop3* was confirmed in the embryos of *RPS5A*$_{pro}$***:DN-rop3*** lines (A) and the seedlings of *35S*$_{pro}$*:DN-rop3* lines (B). Data are means±SD (n=3).

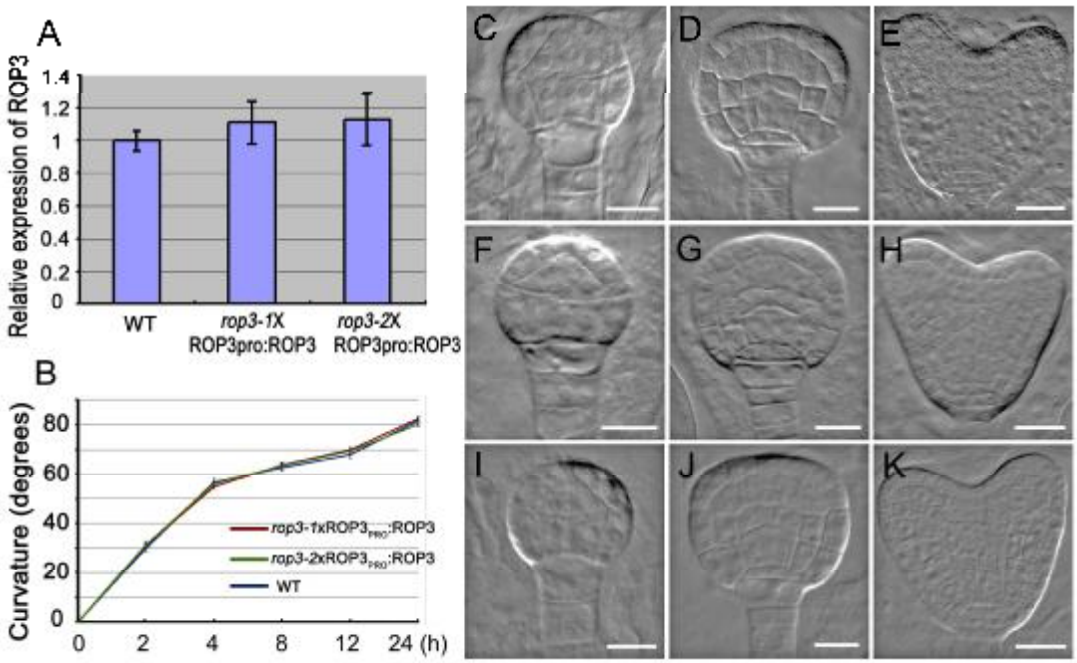
(C) and (D) Immunoblot analysis. Embryos of *RPS5A*$_{pro}$***:DN-rop3*** (C) and 7-d-old seedlings of *35S*$_{pro}$*:DN-rop3* (D) lines were harvested for protein extraction. Wild type embryos or seedlings were used as a control. Immunoblot was performed using anti-ROP3 antibody. Lower panels indicate coomassie-blue staining of the protein samples as loading controls



Supplemental Figure 3. Identification of the *rop3* T-DNA insertion mutants

(A) Scheme of the *ROP3* gene. Exons are indicated by boxes and introns by lines. For *rop3-1*and *rop3-2*, the orientations of T-DNA are indicated (RB, right border of T-DNA, LB, left border of T-DNA).

(B) RT-qPCR analysis for ROP3 transcript confirms *rop3-1* and *rop3-2* to be null-alleles of *ROP3*. Wild type (WT) is used as control.



Supplemental Figure 4. The *ROP3*$_{pro}$*: ROP3* transgene rescues the phenotypes in *rop3* mutants

(A) RT-qPCR analysis confirmed the complemented expression of *ROP3* in *rop3* mutants.

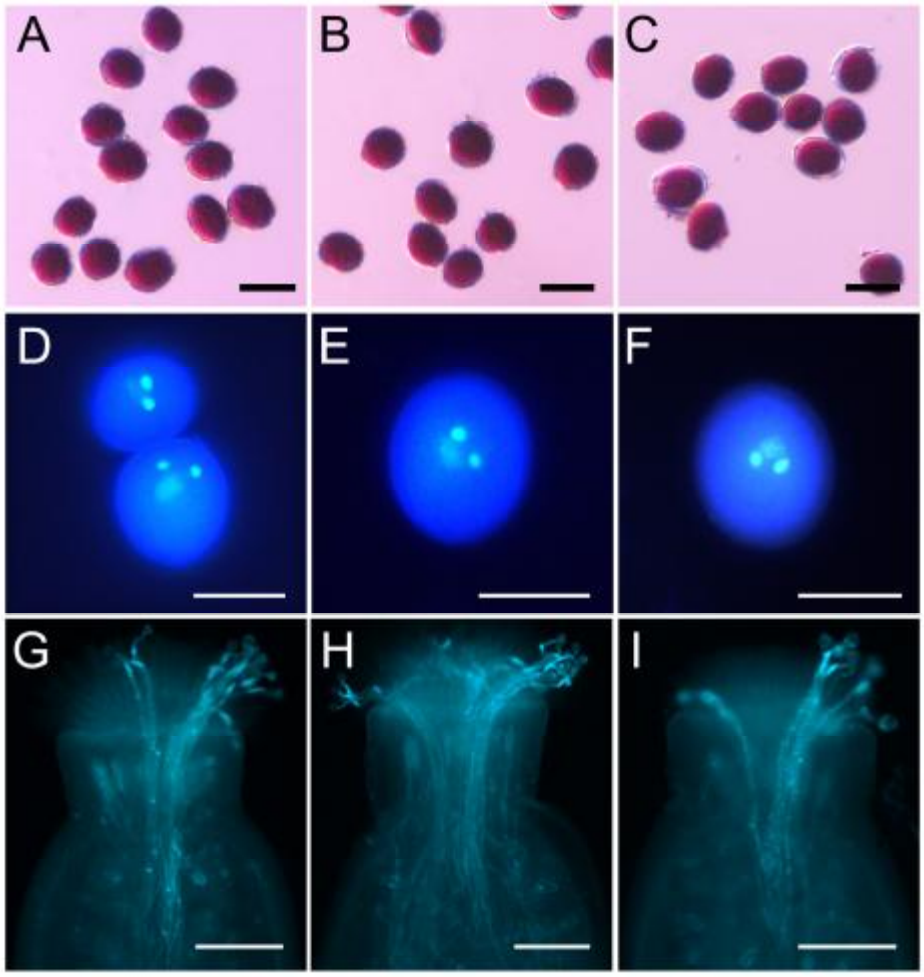
(B) Time course of curvature in root gravitropic response tests. Root gravitropic responses of *rop3* mutants (*rop3-1* and *rop3-2*) are complemented by *ROP3*$_{pro}$*:ROP3*, compared with the wild type controls. Curvatures were measured at different time as indicated after reorientation. Data are means±SD (n=30-50).

(C) to (E) Wild type embryos at 32 cell (B), globular (C) and heart (D) stages.

(F) to (H) Normal embryo development at 32 cell (E), globular (F) and heart (G) stages with rescued expression of *ROP3* in *rop3-1* mutant background.

(I) to (K) Normal embryo development at 32 cell (H), globular (I) and heart (J) stages with rescued expression of *ROP3* in *rop3-2* mutant background.

Bars = 10 μm in 4C, 4Fand 4I; 20 μm in 4D, 4E, 4G, 4H, 4J and 4K.

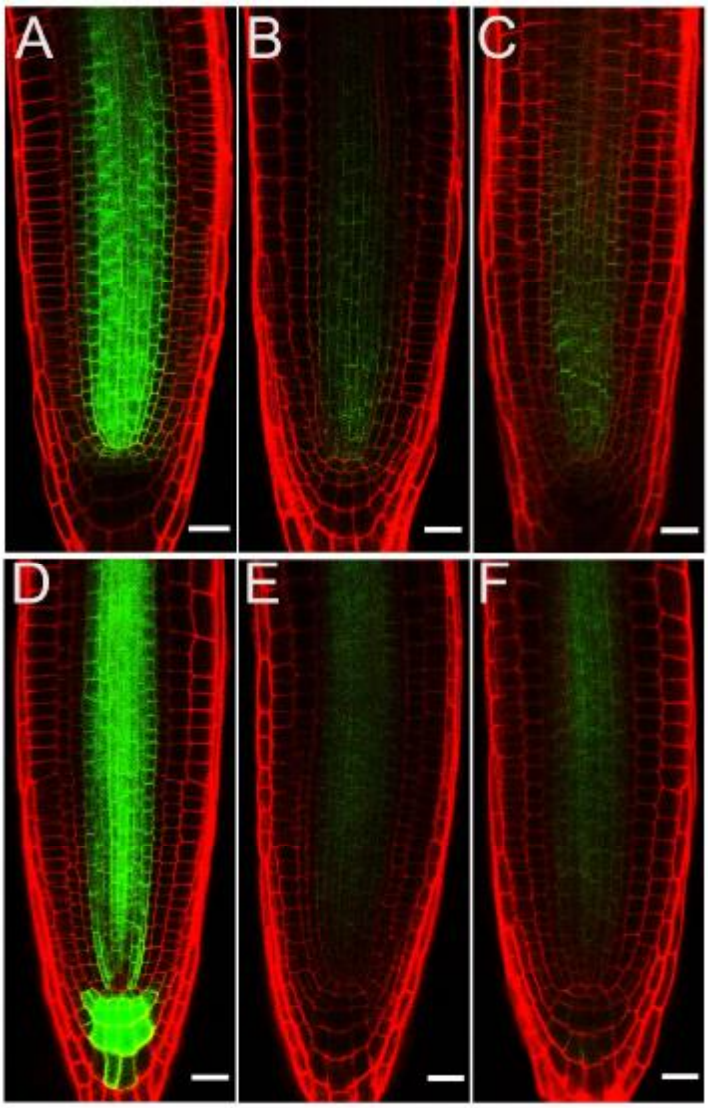


Supplemental Figure 5. Pollen development of *rop3* mutants is normal

(A) to (C) Pollen viability staining of wild type (A), *rop3-1* (B) and *rop3-2* (C) by Alexander solution. Pollen viability in *rop3* mutants were indistinguishable from those in wild type.

(D) to (F) DAPI staining of pollen grains from wild type (D), *rop3-1* (E) and *rop3-2* (F). Pollen morphology in *rop3* mutants were also similar to those in wild type.

(G) to (I) *In vivo* pollen germination and pollen tube growth under limited pollination. Pollen tubes were stained by aniline blue. Wild type pollen grains germinated on the wild type stigma and grown for 2 h (G). *rop3-1* pollen grains germinated on the *rop3-1* stigma and grown for 2 h (H). *rop3-2* pollen grains germinated on the *rop3-2* stigma and grown for 2 h (I). *rop3* pollen germinated normally on the stigma (*rop3-1*: 92.4%, n=486,; *rop3-2*: 92.6%, n=422; WT: 93.5%, n=470) and pollen tubes grew into style and transmitting tract at the same growth rate compared with wild type. Bars= 50 μm in (A) to (C); 30 μ m in (D) to (F); 200 μm in (G) to (I).

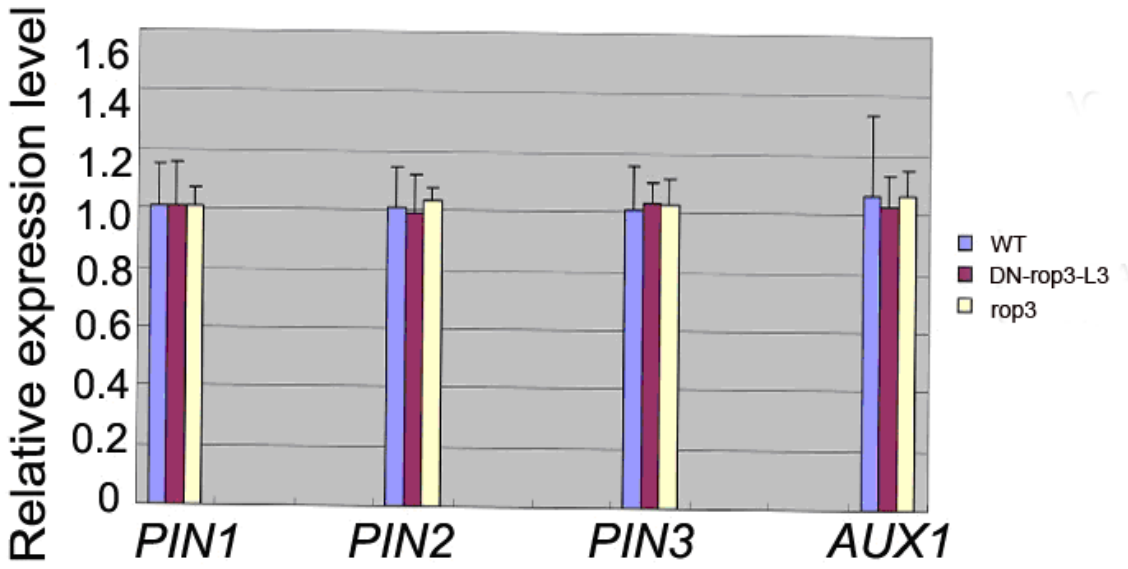


Supplemental Figure 6. ROP3 is required for the accumulation of PIN1$_{pro}$:PIN1:GFP and PIN3$_{pro}$:PIN3:GFP

(A) to (C) PIN1$_{pro}$:PIN1:GFP expression in roots of 4-d-old wild type (A), *DN-ROP3* (B) and *rop3* (C) seedlings.

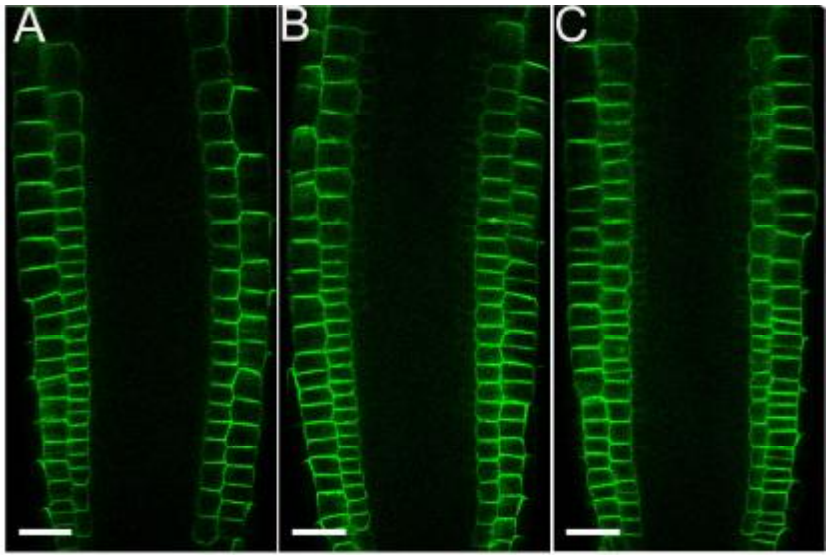
(D) to (F) PIN3$_{pro}$:PIN3:GFP expression in roots of 4-d-old wild type (D), *DN-rop3* (E) and *rop3* (F) seedlings.

Bars = 20 μm.



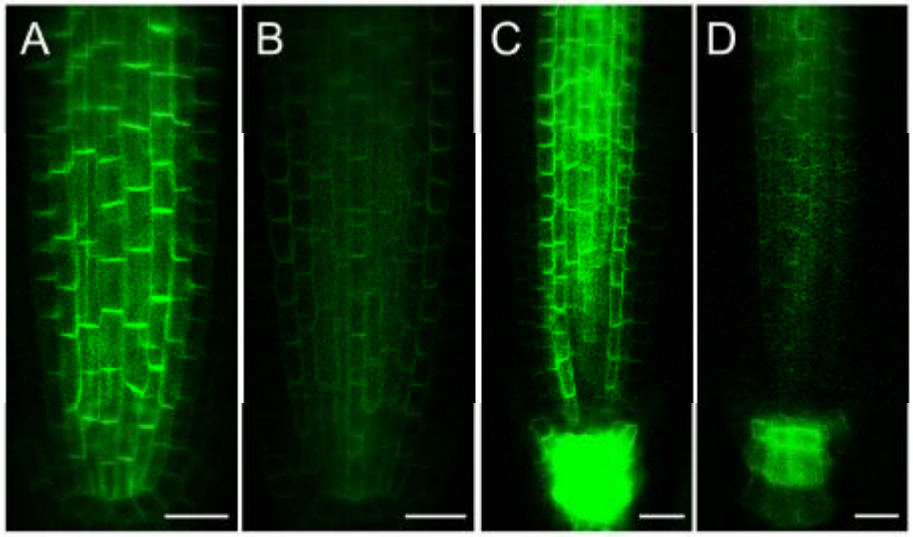
Supplemental Figure 7. ROP3 has no effect on the transcript levels of *PINs* and *AUX1* genes

PIN1, PIN2, PIN3 and *AUX1* transcripts in wild type (WT), *35S*$_{pro}$*:DN-rop3*-L3 line and *rop3-1* roots, as determined by RT-qPCR. Data represent means±SD (n=3).



Supplemental Figure 8. ROP3 does not affect the localization of PIN2:GFP

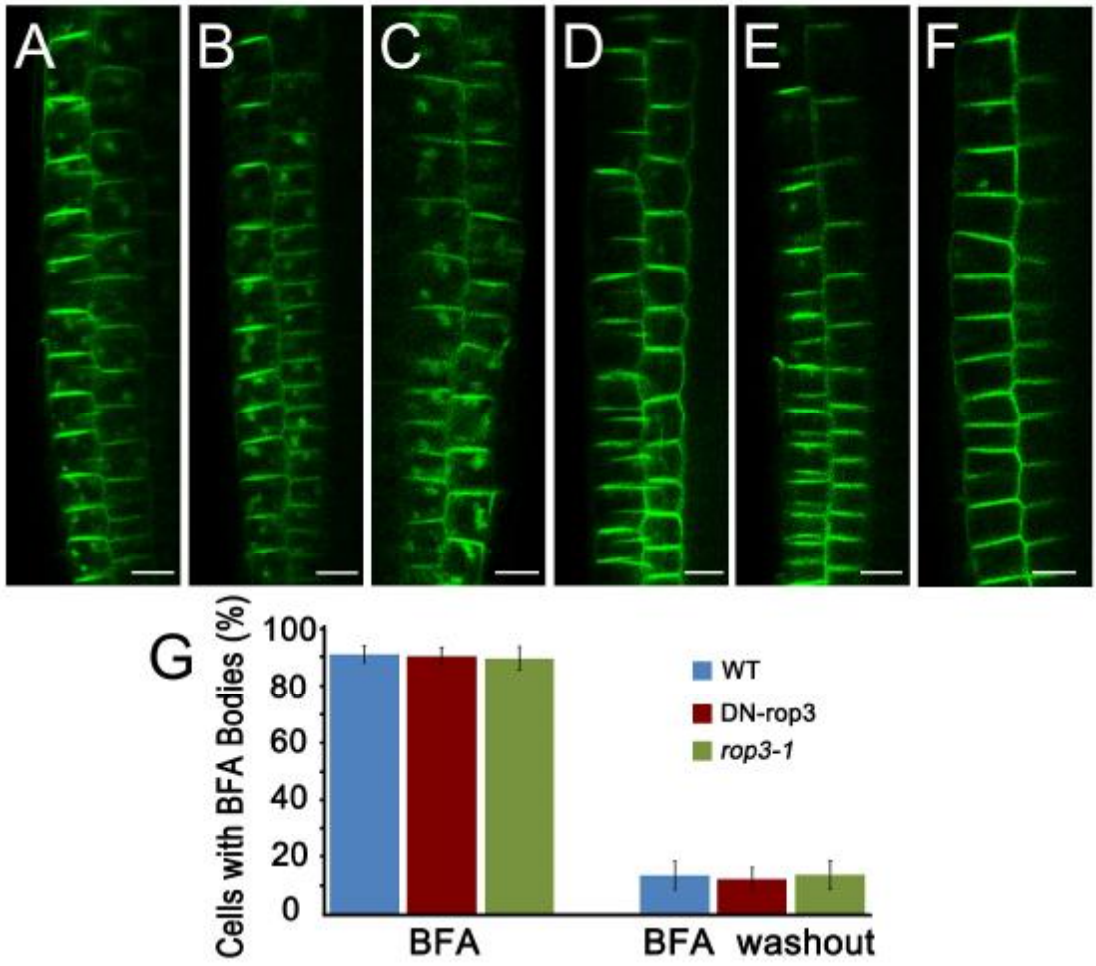
(A) to (C) $PIN2_{pro}$:PIN2:GFP localization in roots of 4-d-old wild type (A), *DN-rop3* (B) and *rop3* (C) seedlings. Bars = 45 μm.



Supplemental Figure 9. RopGEF7 does not affect the polarity of PIN1 and PIN3

(A) and **(B)** $PIN1_{pro}$:PIN1:GFP localization in roots of 4-d-old wild type (A) and *RopGEF7RNAi* (B) seedlings.

(C) and **(D)** $PIN3_{pro}$:PIN3:GFP localization in roots of 4-d-old wild type (C) and *RopGEF7RNAi* (D) seedlings. Bars=10 μm

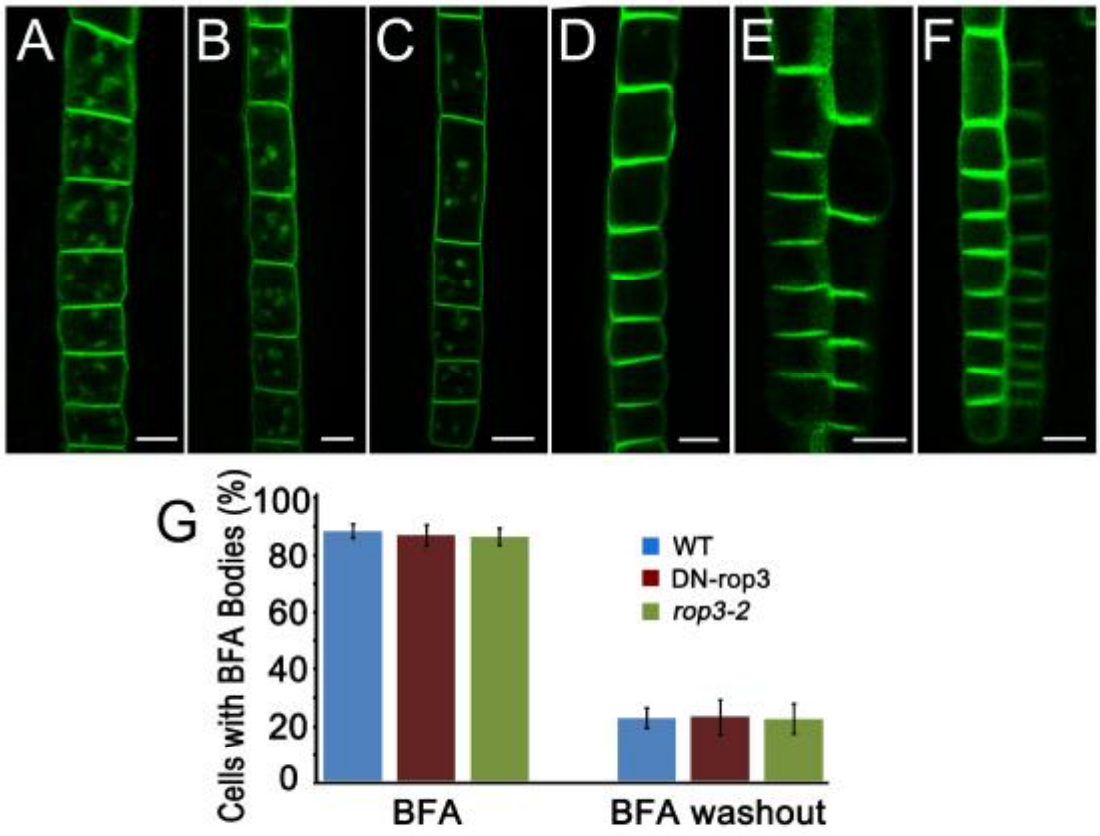


Supplemental Figure 10. ROP3 does not affect the trafficking of PIN2:GFP in roots

(A) to (C) BFA-treated seedlings expressing PIN2:GFP at 3 DAG of wild type (A), *35S$_{pro}$:DN-rop3* (B) and *rop3* (C).

(D) to (F) BFA washout in PIN2:GFP (D), *DN-rop3* (E) and *rop3* (F).

(G) Percentage of cells with PIN2:GFP-labeled BFA bodies before and after BFA washout in wild type, *35S$_{pro}$:DN-rop3* and *rop3*. Data are means ±SD (n=cell numbers from 15-30 roots). Bars=20 μm.

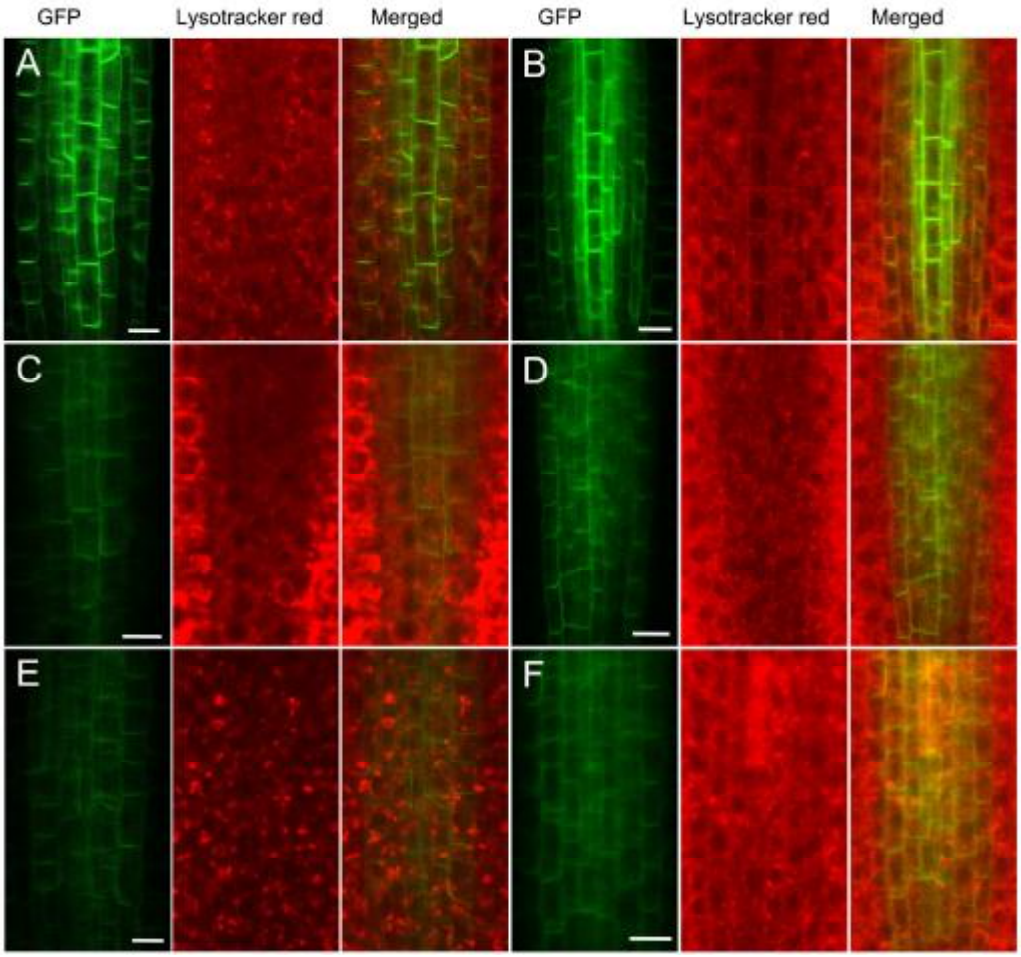


Supplemental Figure 11. ROP3 does not affect the trafficking of PIP2:GFP in roots

(A) to (C) BFA-treated seedlings expressing PIP2:GFP at 3 DAG of wild type (A), *35S$_{pro}$:DN-rop3* (B) and *rop3* (C).

(D) to (F) BFA washout in PIP2:GFP (D), *DN-rop3* (E) and *rop3* (F).

(G) Percentage of cells with PIP2:GFP-labeled BFA bodies before and after BFA washout in wild type, *35S$_{pro}$:DN-rop3* and *rop3*. Data are means ±SD (n=cell numbers from 15-30 roots). Bars=10 μm.



Supplemental Figure 12. PIN3:GFP does not accumulate in vacuole compartments in MG132-treated roots of *DN-rop3* and *rop3*

(A) and (B) PIN3:GFP localization in roots of 5-d-old seedlings untreated (A) or treated with MG132 (B).

(C) and (D) PIN3:GFP localization in roots of $35S_{pro}$: *DN-rop3* seedlings untreated (C) or treated with MG132 (D).

(E) and (F) PIN3:GFP localization in roots of *rop3* seedlings untreated (E) or treated with MG132 (F).

Lysotracker red was used for labeling of vacuolar compartments. Bars=10 μm.

Supplemental Data. Huang et al. (2014). Plant Cell 10.1105/tpc.114.127902

Supplemental Table 1. Primers used in this study

Vector construction	ROP3pro-F	5'-GTCGACTTCATGGAGTTCCAAAAGCAG -3'
	ROP3pro-R	5'-GGATCCTTCTCTTCTTCTTCCTTCTCCA -3'
	ROP3-F	5'-GTCGACATGAGCGCTTCGAGGTTCA-3'
	ROP3-R	5'-CCCGGGAAGCCTTCACTAGAAGAGTGGAAG-3'
	Rop3DN-F	5'-CTTGTTGGAACCAAACTAGCTCTTCGGGATGACAAACAG-3'
	Rop3DN-R	5'-CTGTTTGTCATCCCGAAGAGCTAGTTTGGTTCCAACAAG-3'
	RPS5A-F	5'- AGCAGCAGGAGATCTATCAGTGCA -3'
	RPS5A-R	5'- GGCTGTGGTGAGAGAAACAGAGC -3'
rop3 T-DNA mutant characterization	rop3-1-LP	5'-GGGTTTTTCTTGTTCCCTTTG-3'
	rop3-1-RP	5'-CAAAATCCAGGCCTAGTTTCC-3'
	rop3-2-LP	5'-CGAAATGAAGTGCATGTTGTG-3'
	rop3-2-RP	5'-CCTGCTGTCTGATCCGTCTAG-3'
RT-qPCR	ACTIN2-F	5'-ATGGCTGAGGCTGATGATATTCAAC-3'
	ACTIN2-R	5'-TACAAGGAGAGAACAGCTTGGATG-3'
	ROP3-F	5'-CCATTTCTGGTGGAGAAGGAAG-3'
	ROP3-R	5'-CCCATAGCCCAAGATTCACAGT-3'
	rop3 T-DNA-F	5'-TCCAGAGTTCGTTGTGATAAGCTCT-3'
	rop3 T-DNA-R	5'-GGTCTCAGCTAAAGCTGGAAACTAT-3'
	PIN1-F	5'-TGGAAGACAACCTTTGGAAACT-3'
	PIN1-R	5'-TGAAGCATTAGAACGACGAACA-3'
	PIN2-F	5'-CCTCGCCGCACTCTTTCTTT-3'
	PIN2-R	5'-CGTACATCGCCCTAAGCAAT-3'
	PIN3-F	5'-CAAGTGGAGATTTCGGAGGA-3'
	PIN3-R	5'-GCGTCTTTTGGTCTCTCTGC-3'
	AUX1-F	5'-CTTTCCTCCTCTGCACATTTCT-3'
	AUX1-R	5'-AAGAGTGGTTTTTGTCCGTTTG-3'

Supplemental Data. Huang et al. (2014). Plant Cell 10.1105/tpc.114.127902

Supplemental Table 2. Quantitative analysis of *DN- rop3* and *rop3* embryonic phenotypes

Genotype	1 C	2-8 C	16-32 C	Globular	Heart	Defect/Total	Percentage
WT	0/108 (0)	0/89 (0)	1/123 (0.8%)	1/157 (0.64%)	0/180 (0)	2/657	0.30%
DN-rop3-L12	7/60 (11.7%)	9/68 (13.2%)	18/133 (13.5%)	31/155 (20.0%)	21/121 (17.4%)	86/537	16.01%
DN-rop3-L16	5/40 (12.5%)	58/268 (21.6%)	49/191 (25.7%)	33/135 (24.4%)	29/114 (25.4%)	174/748	23.3%
Rop3-1	8/50 (16.0%)	10/189 (5.3%)	14/213 (6.57%)	20/240 (8.3%)	19/175 (10.9%)	71/867	8.19%
Rop3-2	6/46 (13.0%)	23/278 (8.27%)	18/258 (6.98%)	16/231 (6.98%)	20/237 (8.4%)	83/1043	7.96%

DN-rop3-L12 and *DN-rop3*-L16 denote two strong transgenic lines of *RPS5A*$_{pro}$*:DN-rop3*, L12 and L16, respectively. These two lines are homozygotes carrying single-locus *RPS5A*$_{pro}$*:DN-rop3* transgene. Five different developmental stages of the embryo were analyzed (from the one-cell [1C] stage to the heart stage).

Supplemental Data. Huang et al. (2014). Plant Cell 10.1105/tpc.114.127902

Supplemental Table 3. Quantitative analysis of *DN-rop3* and *rop3* seedling phenotypes

Genotype	Percentage of seedlings with no roots	Percentage of seedlings with short roots	Percentage of seedlings with fused, one or three cotyledons	Percentage of total defects	No. analyzed
WT	0%	1.8%	0%	1.8%	327
DN-L2	1.4%	29.3%	5.6%	36.3%	215
DN-L3	1.7%	36.21%	3.4%	41.31%	232
rop3-1	2.0%	12.8%	1.3%	15.8%	298
rop3-2	1.7%	8.1%	0.84%	10.64%	356

DN-L2 and DN-L3 denote two strong transgenic lines of *$35S_{pro}$:DN-rop3*, L2 and L3, respectively. These two lines are homozygotes carrying single-locus *$35S_{pro}$:DN-rop3* transgene. 7-d-old seedlings were examined under a stereo microscope and seedlings with defective root or cotyledon phenotypes were scored at T3 generation for transgenic lines.

Supplemental Table 4. Quantitative analysis of embryo phenotypes in the *$ROP3_{pro}$: ROP3* transgene rescued *rop3* background

Genotype	32 C	globular	heart	Defect/Total	Percentage (%)
WT	2/115 (1.74%)	2/145 (1.38%)	1/105 (0.95%)	5/365	1.37%
*rop3-1*x *$ROP3_{pro}$:ROP3*	4/204 (1.96%)	2/174 (1.49%)	1/185 (0.54%)	7/563	1.24%
*rop3-2*x *$ROP3_{pro}$:ROP3*	3/180 (1.67%)	1/155 (0.645%)	2/169 (1.18%)	6/504	1.19%