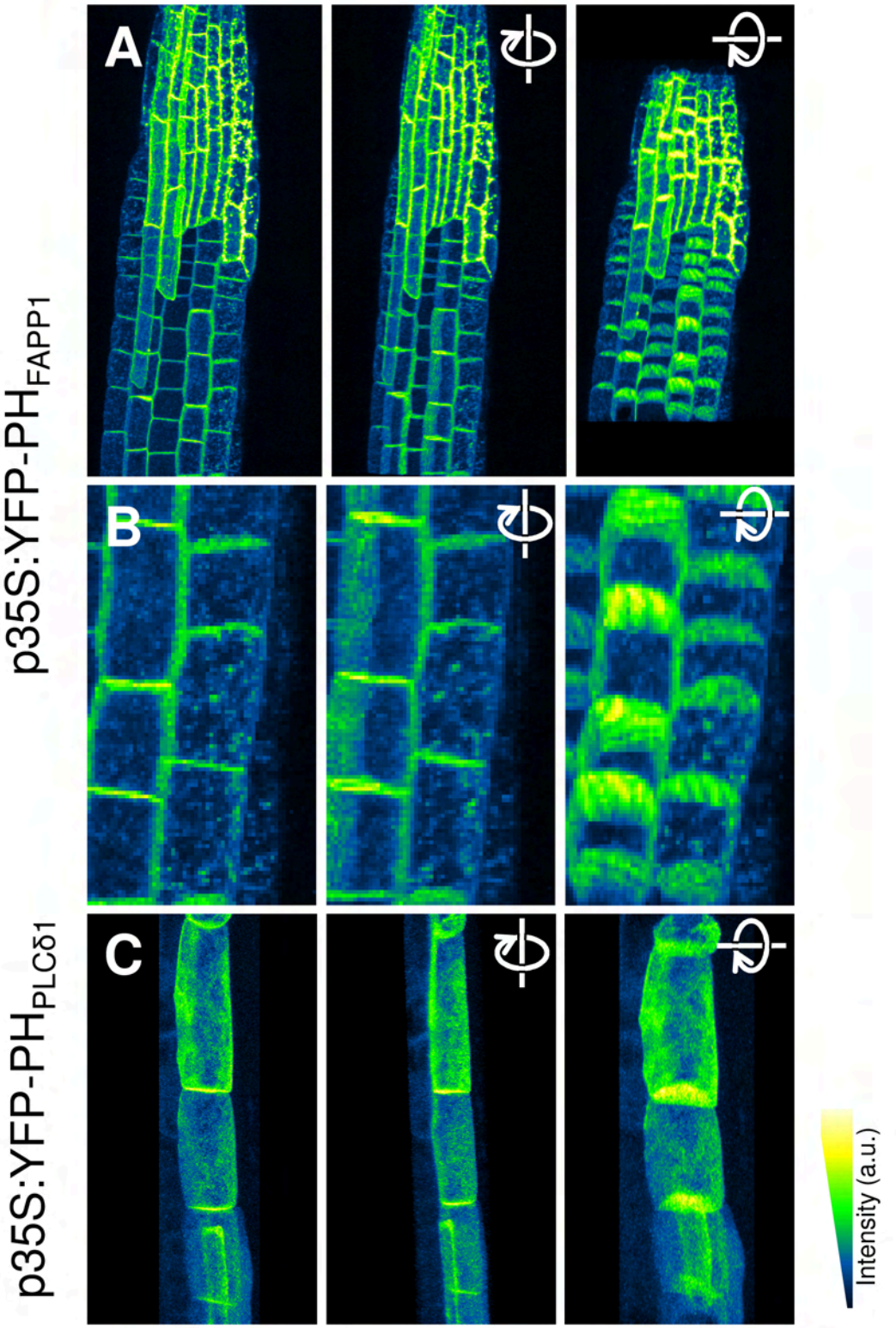
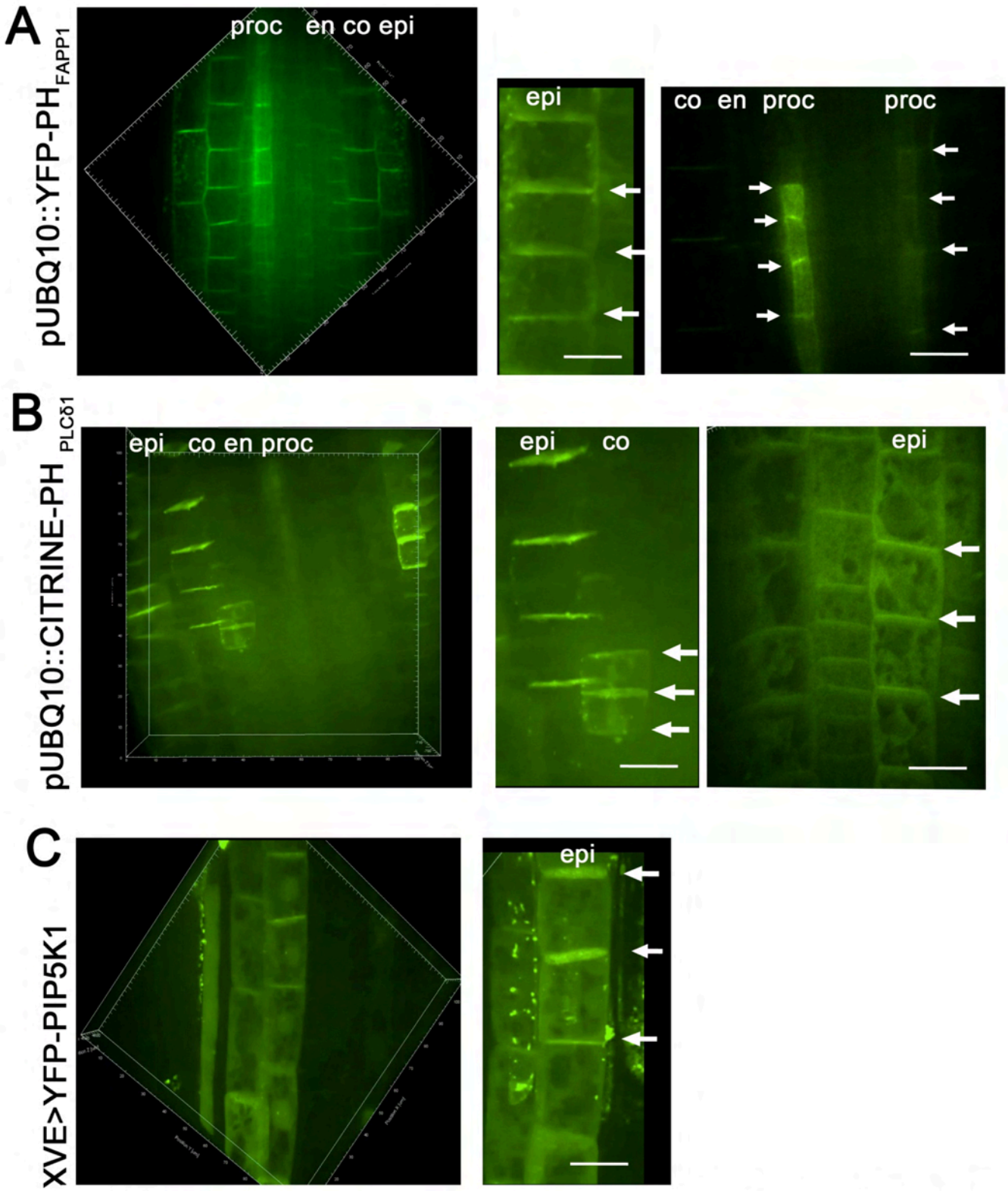
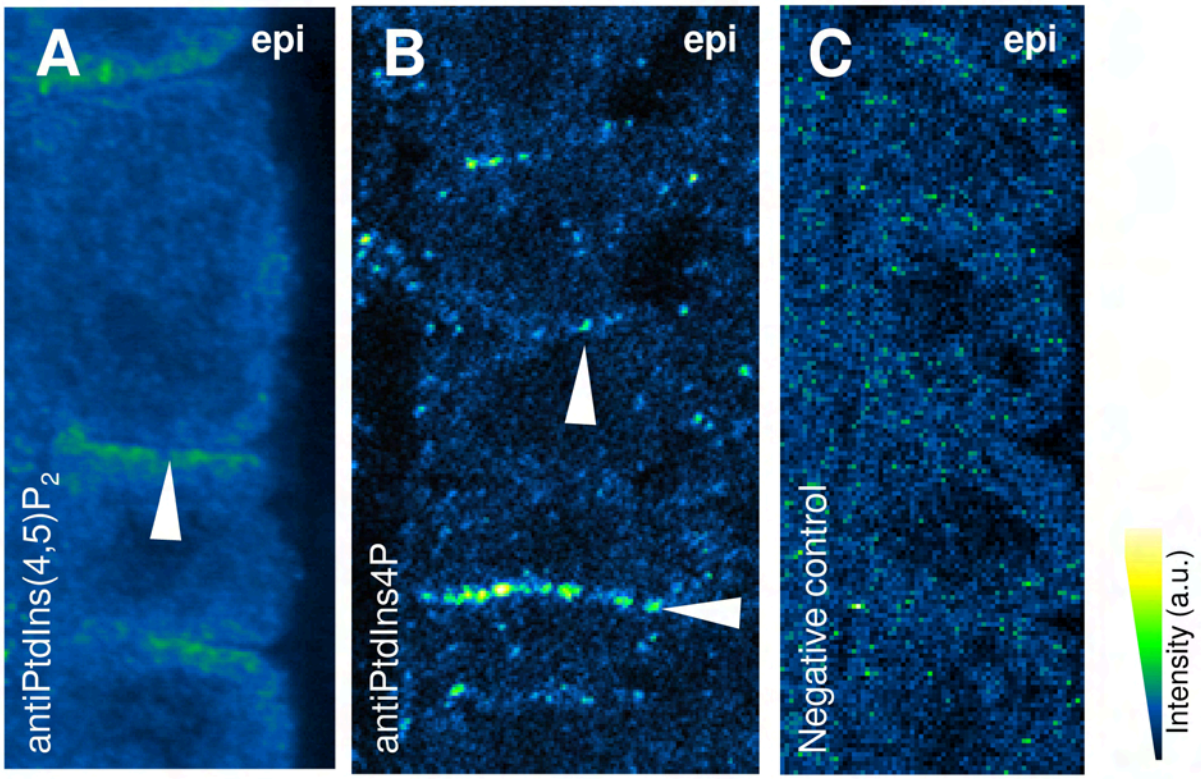


Supplemental Figure 1. 3D reconstructions of root epidermal cells imaged by confocal microscopy of seedlings expressing the phosphoinositide biosensors.

3D-projections for a series of confocal pictures taken on the Z axis and merged using the ZEN image viewer (Zeiss) in seedlings expressing the phosphoinositide biosensors PH_{FAPP1} (A, B) or $PH_{PLC\delta1}$ (C), driven by the 35S promoter. In each panel, middle pictures represent a 30°-clockwise rotation in the vertical axis and left pictures represent a 30°-rotation upwards in the horizontal axis, starting from the initial 3D-projection shown in the left picture.

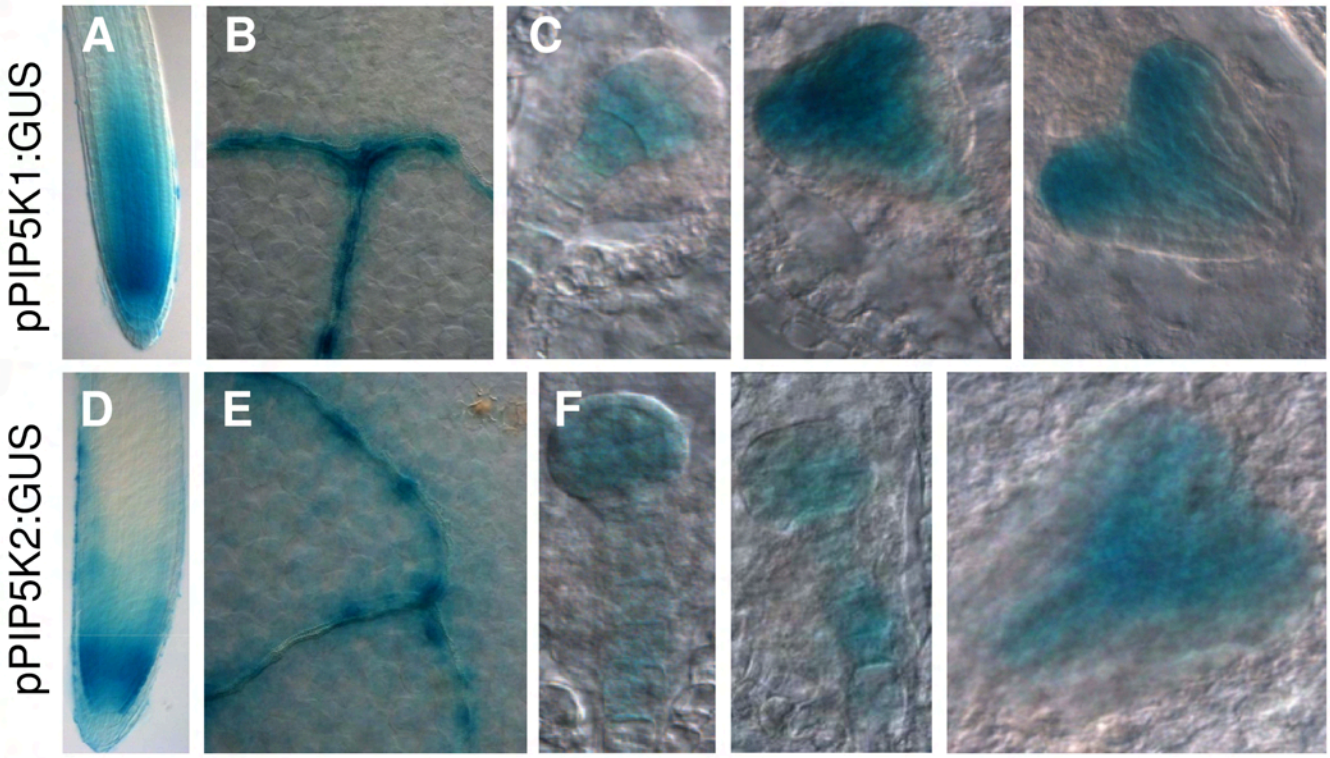


Supplemental Figure 2. 3D reconstructions of PIP5K marker lines and phosphoinositide biosensors imaged by spinning disc microscopy.
pUBQ10:YFP-PH_{FAPP1} (**A**) and pUBQ10:CITRINE-$PH_{PLC\delta1}$ (**B**), and the estradiol-inducible XVE>PIP5K1-YFP line (**C**). The localization pattern of these marker lines in other cell types show bipolar localization in epidermis (**A,** arrows in middle panel; **B** and **C**) and procambial cells (**A,** arrows in right panel) for pUBQ10:YFP-PH_{FAPP1}, and bipolar in cortex for pUBQ10:CITRINE-$PH_{PLC\delta1}$ (**B,** arrows in middle panel). Four-day-old seedlings were used. The arrows highlight the bipolar localizations. epi: epidermis, co: cortex, proc: procambium. Scale bar 10 µm.

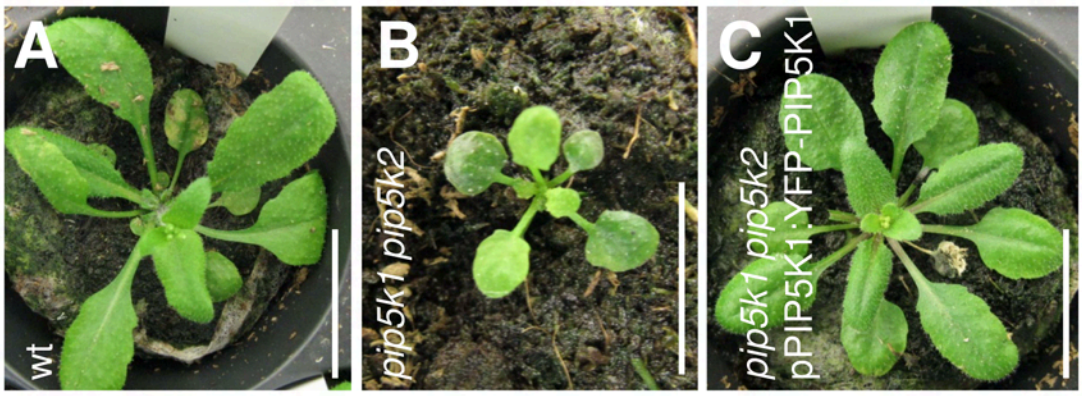


Supplemental Figure 3. Whole mount immunolocalization on epidermal cells on four-day old seedling root tips using antiPtdIns(4,5)P_2 and antiPtdIns4P antibodies

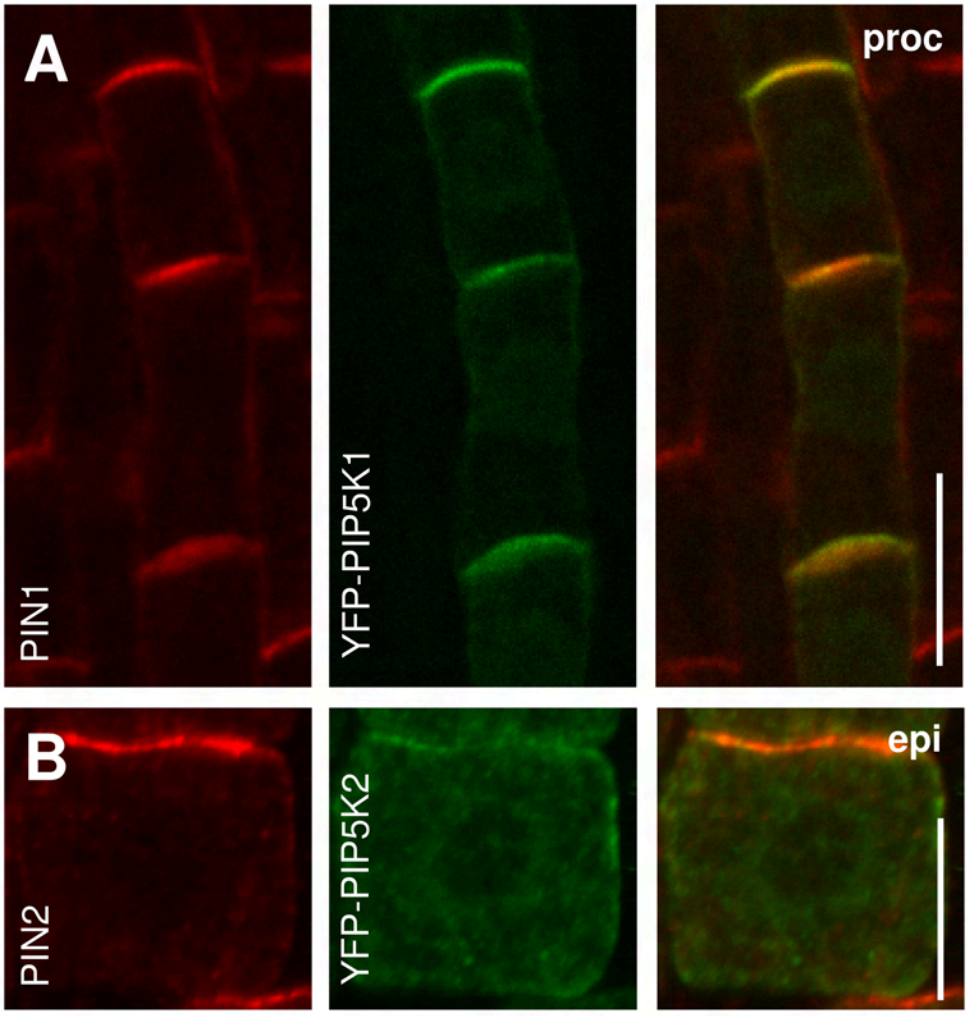
Whole mount immunolocalization on epidermal cells on four-day old seedling root tips using antiPtdIns(4,5)P_2 (**A**) and antiPtdIns4P (**B**) antibodies. Negative control without primary antibody (**C**). Arrowheads highlight the polar phosphoinositide signals. epi: epidermal cells.



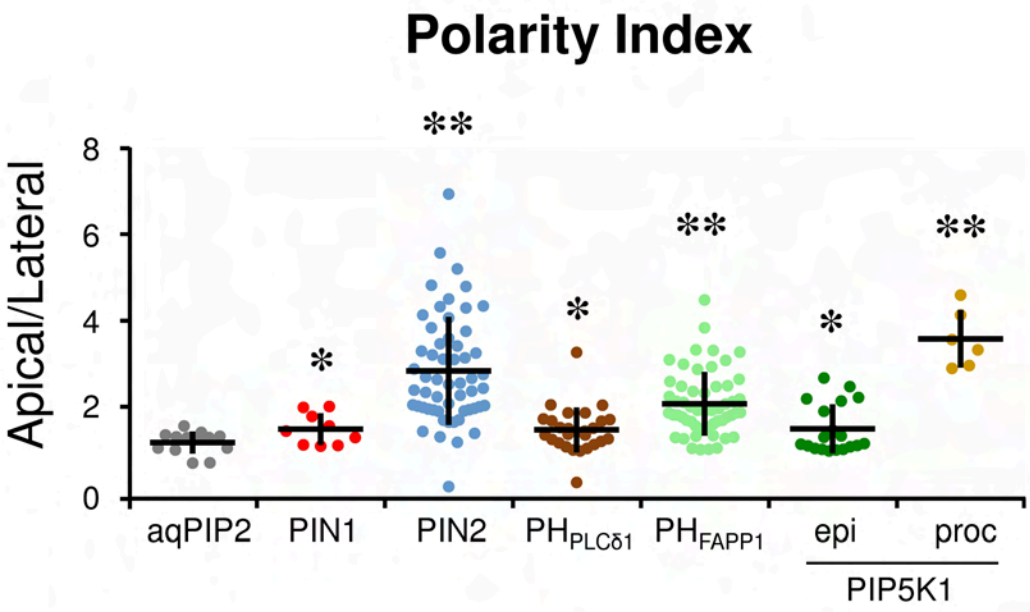
Supplemental Figure 4. Expression patterns of pPIP5K1:GUS and pPIP5K2:GUS lines.
Expression patterns of pPIP5K1:GUS (**A-C**) and pPIP5K2:GUS (**D-F**) in root tips (**A, D**), cotyledon vasculature (**B, E**), and during early embryo development (**C, F**).



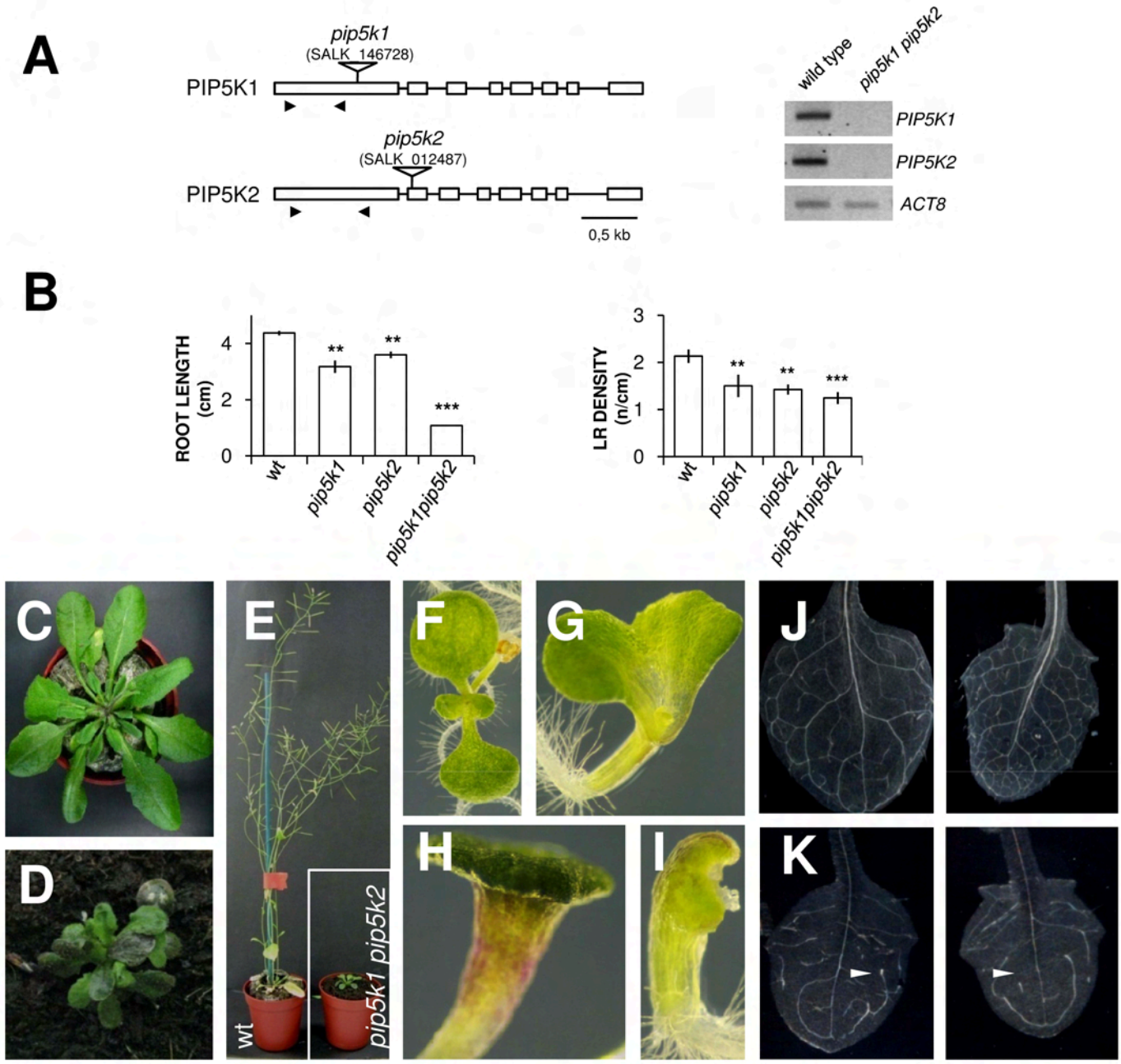
Supplemental Figure 5. Functional complementation of *pip5k1*$^{-/-}$*pip5k2*$^{-/-}$ double mutants with pPIP5K1:YFP-PIP5K1 construct.
From the F2 progeny of the cross *pip5k1*$^{+/-}$*pip5k2*$^{-/-}$ X pPIP5K1:YFP-PIP5K1, we preselected the YFP-positive plants using confocal microscopy and looked in those for the *pip5k1*$^{-/-}$*pip5k2*$^{-/-}$ double mutant genetic background. We show here a representative example of the complementation.
A. Wild type Col-0. **B.** *pip5k1*$^{-/-}$ *pip5k2*$^{-/-}$ double mutant. **C.** *pip5k1*$^{-/-}$ *pip5k2*$^{-/-}$ double mutant complemented by pPIP5K1:YFP-PIP5K1.



Supplemental Figure 6. Root whole mount immunolocalization on four-days old seedlings of pPIP5K1:YFP-PIP5K1 (A) and pPIP5K2:YFP-PIP5K2 (B) using anti-PIN1 (A, left panel), anti-PIN2- (B, left panel) and anti-GFP (A, B, middle panels) antibodies. PIP5K1 colocalizes with PIN1 in procambial root cells (A, right panel) and PIP5K2 colocalizes with PIN2 in epidermis root cells (B, right panel). proc: procambial cells; epi: epidermal cells. Size bar = 10 µm.



Supplemental Figure 7. Apical to lateral fluorescent ratio (Polarity index) of phosphoinositide biosensors expressed under the 35S promoter and PIP5K1 proteins in root epidermis (epi) and procambial (proc) cells expressed under the *DNAjc17* promoter. As an apolar marker we measured the plasma membrane aquaporin aqPIP2-GFP in root epidermis cells, and we used PIN1-GFP and PIN2-GFP as polar markers in stele and epidermal root cells, respectively. The data correspond to those plotted in Figure 3, but here single dots correspond to individual cell measurements, and the arithmetic mean (horizontal line) and the standard deviations (vertical lines) are indicated. Root cells of seedlings 4 days after germination were quantified using ImageJ. Two-tailed unpaired Student *t*-test compared to the aquaporin aqPIP2-GFP. * $P < 0.05$. $n > 20$ cells corresponding to 10 different roots imaged under comparable conditions.



Supplemental Figure 8. Characterization of *pip5k* mutants.

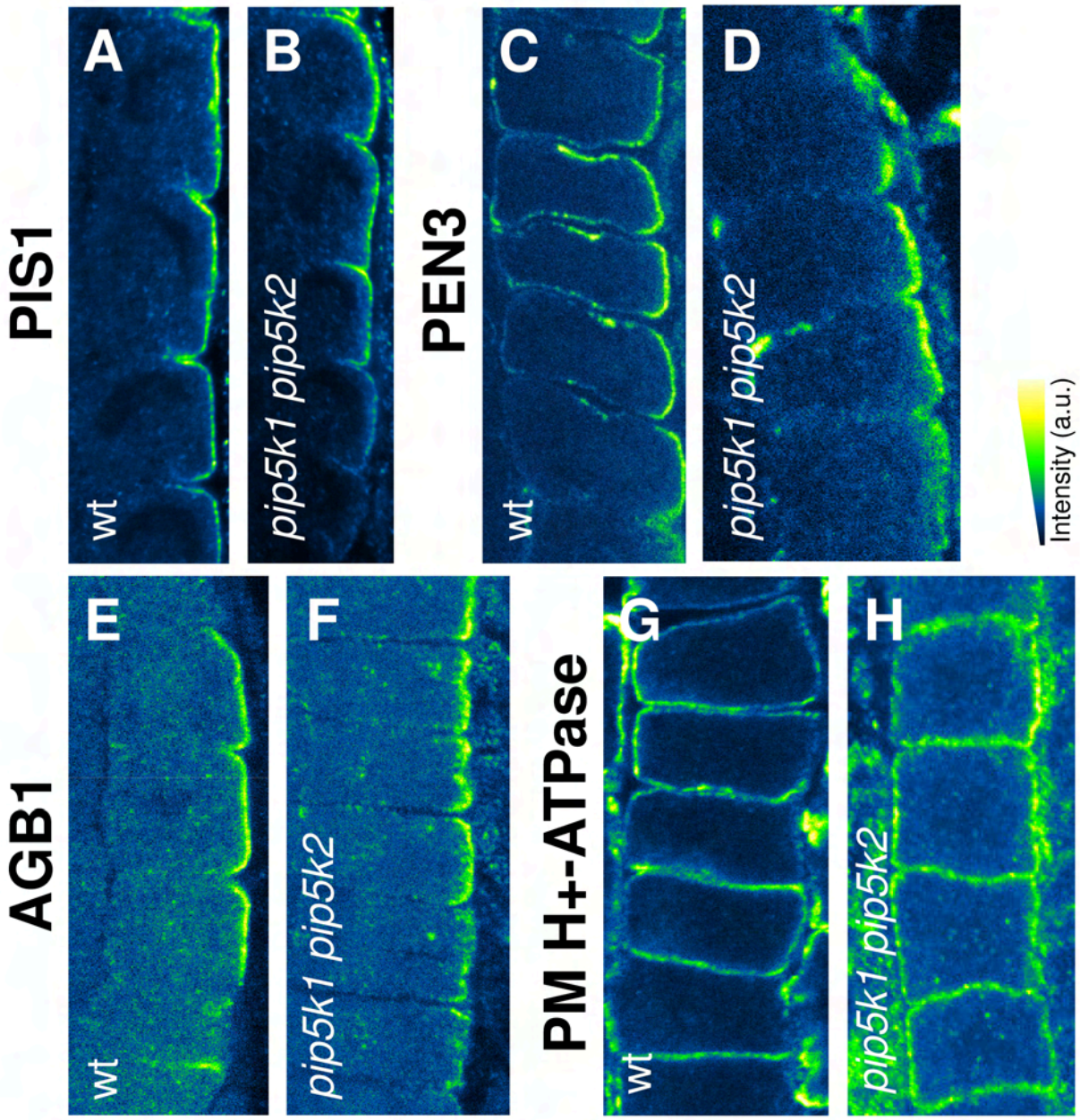
(A) Intron-exon structure of *PIP5K1* and *PIP5K2* genes with indication of the position of the T-DNA insertion sites (left panel). Exons indicated as boxes, introns as lines. The arrowheads indicate the positions of the primers used for the RT-PCR analysis (right panel). No cDNA could be amplified from *pip5k1*$^{-/-}$ *pip5k2*$^{-/-}$ double mutants, indicating these mutant alleles represent full knock-outs. *ACTIN8* (*ACT8*) was used as a constitutive expressed gene control.

(B) Root length and lateral root density in single and double mutant 12-day old seedlings. Graphs show Mean ± S.E. Asterisks indicate significant differences compared to wild type (wt). Two tailed Student's *t*-test; $^{**}P < 0{,}05$, $^{***}P < 0{,}001$. n > 20.

(C-E) Adult plants of *pip5k1*$^{-/-}$ *pip5k2*$^{-/-}$ mutant (**D, E**) compared to wt (C). Notice the reduced size and the accumulation of anthocyanins (dark leaves in D), and the infertile double mutants (E).

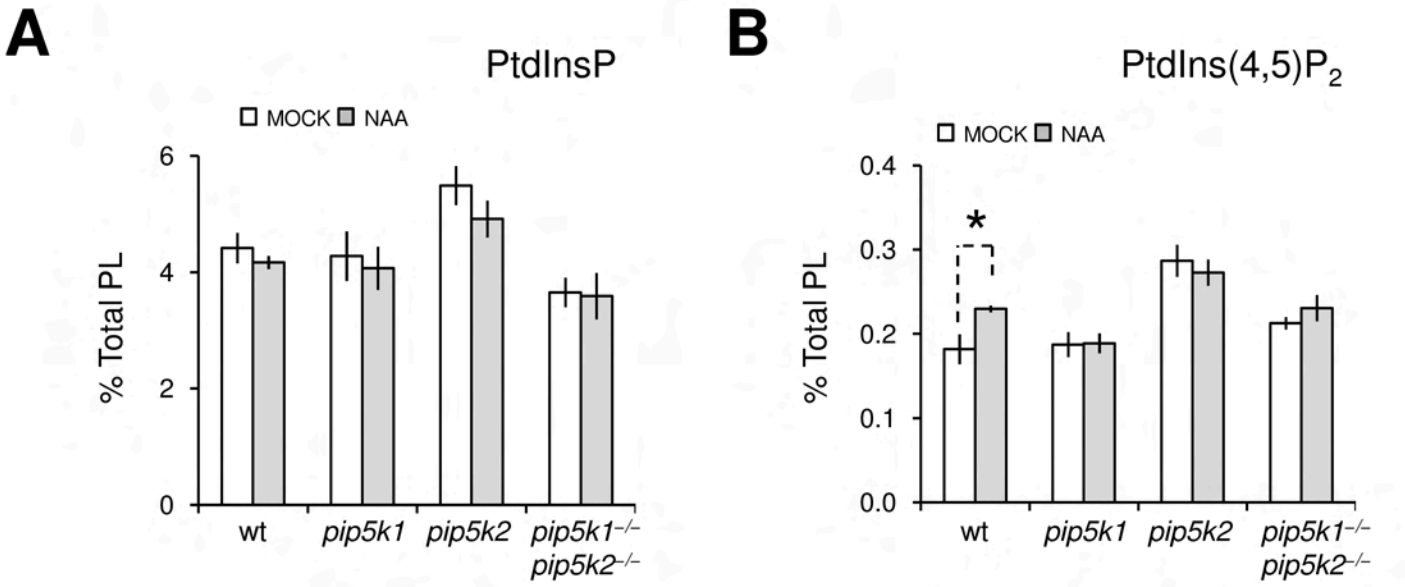
(F-I) Seven-day-old wt seedlings (F) and seedlings of a self-pollinated *pip5k1*$^{-/-}$ *pip5k2*$^{+/-}$ plant progeny (G-I).

(J, K) Vascular tissues of wt (J) and *pip5k1*$^{-/-}$ *pip5k2*$^{-/-}$ (K) adult plants. Arrowhead indicates disconnected vascular strands.



Supplemental Figure 9. Apolar and outer lateral localized proteins are not affected in *pip5k1*$^{-/-}$ *pip5k2*$^{-/-}$ double mutant.

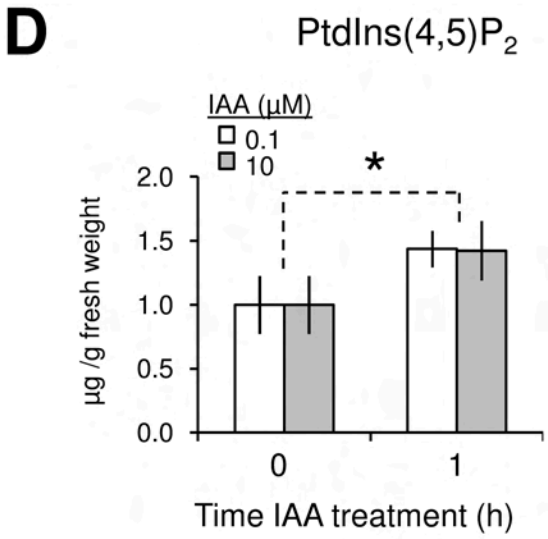
Root whole-mount immunolocalization using anti-PIS1 (A, B), anti-PEN3 (C, D), anti-AGB1 (E,F) and anti-PM H$^+$-ATPase antibodies (G, H) in wild-type (A, C, E, G) and *pip5k1*$^{-/-}$ *pip5k2*$^{-/-}$ (B, D, F, H) seven-day-old seedlings. The images for the lateral localized proteins (A-F) were obtained taking the middle section of one epidermal cell file so the outer/surface-facing side of the cell is placed to the left side in each panel. The apolar protein PM H$^+$-ATPase was imaged by scanning the middle section of the epidermal cell layer of the surface of the root so at least three cell files appear in each picture and in this case the outer/surface-facing side of the cell is pointing towards the observer.



C

Student's *t* Test *P* values

			vs wt	
		mock vs NAA	mock	NAA
wt	$PtdIns(4,5)P_2$	0.0243		
	PtdinsP	0.4022		
pip5k1	$PtdIns(4,5)P_2$	0.9362	0.8176	0.0084
	PtdinsP	0.7192	0.791	0.8096
pip5k2	$PtdIns(4,5)P_2$	0.5802	0.0023	0.0222
	PtdinsP	0.2411	0.0302	0.049
pip5k1 pip5k2	$PtdIns(4,5)P_2$	0.3236	0.1379	0.9528
	PtdinsP	0.8961	0.0638	0.1928



Supplemental Figure 10. Phosphoinositide measurements in response to auxin in wild type and *pip5k* mutant seedlings.

A, B. For the quantification of PtdInsP and $PtdIns(4,5)P_2$, seedlings were pre-labelled overnight with ^{32}P-orthophosphate and treated next day with auxin (NAA 10 µM) for 30 min, after which lipids were extracted, separated by thin layer chromatography and quantified using a phosphorimager.

C. Student's *t*-test *P* values for the conditions plotted in A and B. In the table, the "NAA *vs* mock" row is the comparison of the values between the mock- and the NAA-treated condition within each genotype. The following two columns represent the comparison to wt. The values $P < 0.01$ are highlighted in orange and the values $P < 0.05$ in blue.

D. Total $PtdIns(4,5)P_2$ measurements in seedlings after auxin treatment. Four-days after germination, 50 to 60 seedlings were treated with IAA for 1 h, and then harvested and $PtdIns(4,5)P_2$ was extracted and analyzed as previously described (König et al., 2008).

Supplemental Table 1. Mutant phenotype segregation in seedlings and embryo in wild type and *pip5k1−/− pip5k2+/−* double mutant.

GENOTYPE	PHENOTYPES							
	SEEDLING				EMBRYO			
	observed (n)		expected		observed (n)		expected	
	wild type	mutant	wild type	mutant	wild type	mutant	wild type	mutant
wild type	200	0	200 (100%)	0	150	0	150 (100%)	0%
pip5k1−/− pip5k2+/−	162 (82.2%)	35 (17.8%)	184.69 (75%)	12.31 (25%)	106 (88%)	15 (12%)	90.75 (75%)	30.25 (25%)

Supplemental Table 2. Quantification of vascular tissues defects in double pip5k1−/− pip5k2−/− mutants.

The *pip5k1 pip5k2* mutant frequently shows disconnected vascular fragments (vascular islands, VI), as well as reduced complexity in the vascular tissues, i.e. less branching points, secondary veins and closed areoles.

GENOTYPE	VI (%)	Free Ends* (n)	Closed areoles* (n)	Branching points* (n)	Secondary Veins* (n)
wild type	7.4	4.41 ± 1.2	5.55 ± 1.8	16.41 ± 2.7	6.74 ± 1.1
pip5k1−/− pip5k2−/−	72	5.64 ± 0.9	1.12 ± 0.5	3.68 ± 2.1	3.12 ± 0.9

Quantification of vascular development parameters in wild type and *pip5k1 pip5k2* double mutant were obtained from PIN1 immunolocalization in whole mount 7-9 DAG primary leaves. VI: Vascular Island. * Mean ± s.d. $n \geq 25$

Supplemental Table 3. Primers used for expression analysis and cloning procedures.

Gene	Sequence[#]	Orientation	Application
EIF4A	ACGGAGACATGGACCAGAAC	Fw	qPCR
	GCTGAGTTGGGAGATCGAAG	Rv	
CBP20	GATTACGGTACTGGCTCATTGG	Fw	
	GATTACGGTACTGGCTCATTGG	Rv	
PIP5K1	GGAACATTGTGAATCGAGGACTG	Fw	
	CCGTCTCGTCTCTCTACTTCTT	Rv	
IAA3	CAACCCAAGCACAGACAGAG	Fw	
	TGATTGGATGCTCATTGGTG	Rv	
BDL	TTGGGTCTAAACGCTCTGCT	Fw	
	AAGCCCTGAACTTTCGGATT	Rv	
ACTIN8	ACCTTGCTGGTCGTGACCTTACTG	Fw	RT-PCR
	GATCCCGTCATGGAAACGATGTCTC	Rv	
PIP5K1	ATGAGTGATTCAGAAGAAG	Fw	
	ATCACACCACTACGCCACTCT	Rv	
PIP5K2	ATGATGCGTGAACCGCTTG	Fw	
	TTCCATGCTGCAGGTTGAGCA	Rv	
LBa1	TGGTTCACGTAGTGGGCCATCG		Genotyping
PIP5K1	AAGATGGGTGCATGTACGAAG	Right	
	TTCCACCTGAAATCCACTGAC	Left	
PIP5K2	GGAAGTTTGACTGGGGAGAAG	Right	
	TCATACTGGCAGACGTGTTT	Left	
DNAJc17	ATTTGGAGGTATAATTGGT	Fw[1]	promoter cloning
	CTTCTTTCTTCTTCTAAACC	Rv[2]	
PIP5K1	CAAAATGTGTATTCTATGAC	Fw[1]	
	CAGAGAATCTTCACTCCAG	Rv[2]	
PIP5K2	AGGCAAAATAATGTATACTC	Fw[1]	
	CTACTCATCAGAGAAACCC	Rv[2]	
PIP5K1	GTAATGGTGAGCAAGGGCGAGGAG	Fw[3]	YFP-PIP5K1 cloning
	TTCTTAGCCCTCTTCAATGAAGATC	Rv[4]	
PIP5K2	GTAATGGTGAGCAAGGGCGAGGAG	Fw[3]	YFP-PIP5K2 cloning
	TTAGCCGTCTTCGATGAAG	Rv[4]	
PH$_{FAPP1}$	ATGGAGGGGGTGTTGTAC	Fw	PH domain cloning
	ATTCTTATGTATCAGTCAAACATG	Rv	
PH$_{PLC\delta1}$	ATGCAGTGCCTGGGGATCC	Fw	
	TTATTTGTCAGCTTTTCGCA	Rv	

[#] All primers are indicated in the 5'>3' orientation; [1] Plus attb4 site at the 5' of the primer sequence; [2] Plus attb1r site at the 5' of the primer sequence; [3] Plus attb1 site at the 5' of the primer sequence; [4] Plus attb2 site at the 5' of the primer sequence. Fw: forward orientation; Rv: reverse orientation.