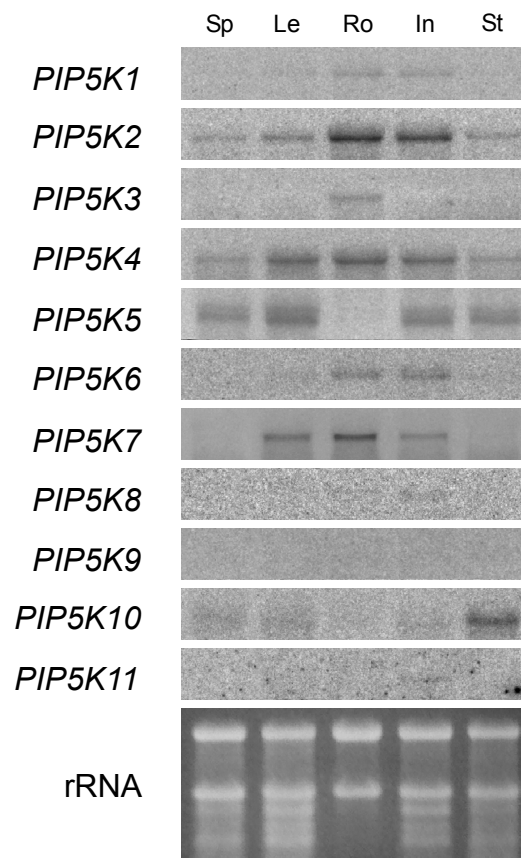


Supplemental Data. Kusano et al. (2008). The *Arabidopsis* phosphatidylinositol phosphate 5-kinase PIP5K3 is a key regulator of root hair tip growth.



Supplemental Figure 1. Northern hybridization analysis of *Arabidopsis* PIP5K genes.

Northern hybridization analysis was performed using probes specific to each *PIP5K* gene and total RNA prepared from seedpods and pollinated flowers (Sp), rosette leaves (Le), roots (Ro), inflorescences without pollinated flowers (In), and stems (St). The same RNA preparations, electrophoresed on an agarose gel and stained with ethidium bromide are shown below the autoradiogram.

A

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-----TATAATTTCTGTTGAATTACGTTAAGCATGTAATAATTAACATGT
AATGCATGACGTTATTTATGAGATGGGTTTTTATGATTAGAGTCCCGCAATTATACATTTAAT
ACGCGATAGAAAACAAAATATAGCGCGCAAAC TAGGATAAATTATCGCGCGCGGTGTCATCTA
TGTTACTAGATCGGGAATTCAC TGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGC
GTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAG
GCCCCACCCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGCCCGCTCCTTTTCGCTTT
CTTCCCTTCTTTCTCGCCACGTTTCGCCGGCTTTCCCGTCAAGCTCTAAATCGGGGGCTCCC
TTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAAACTTGATTTGGGTGATGG
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TGATTTATAAGGGATTTTGCCGATTTTCGGAACCACCATCAAACAGGATTTTCGCCCTGCTGGGG
CAAACCAGCGTGGACCGCTTGCTGCAACTCTCTCAGGGCCAGGCGGTGAAGGGCAATCAGCTG
TTGCCCGTCTCACTGGTGAAAAGAAAACCACCCAGTACATTA AAAACGTC CGCAATGTGTT
ATTAAGTTGTCTAAGCGTCAATTTGTTTACACCACAATATATTTGAATAAGACATATATAATA
ATGCAAGAGACAGTGTTCTCTTACGGAAGAGAATCTGAATAAAGAGCAATCGTTGGGGGTC
AAGTACAAACAAAGCTCCCGGCGGGTGGTTCCCATGACAAGTTGCGAGGTGAGTGATACGGCT
GCCGAGATTAGAATAGTAGAGAAGGTGCTAAAGAACGGCGACCTTTAT-----
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B

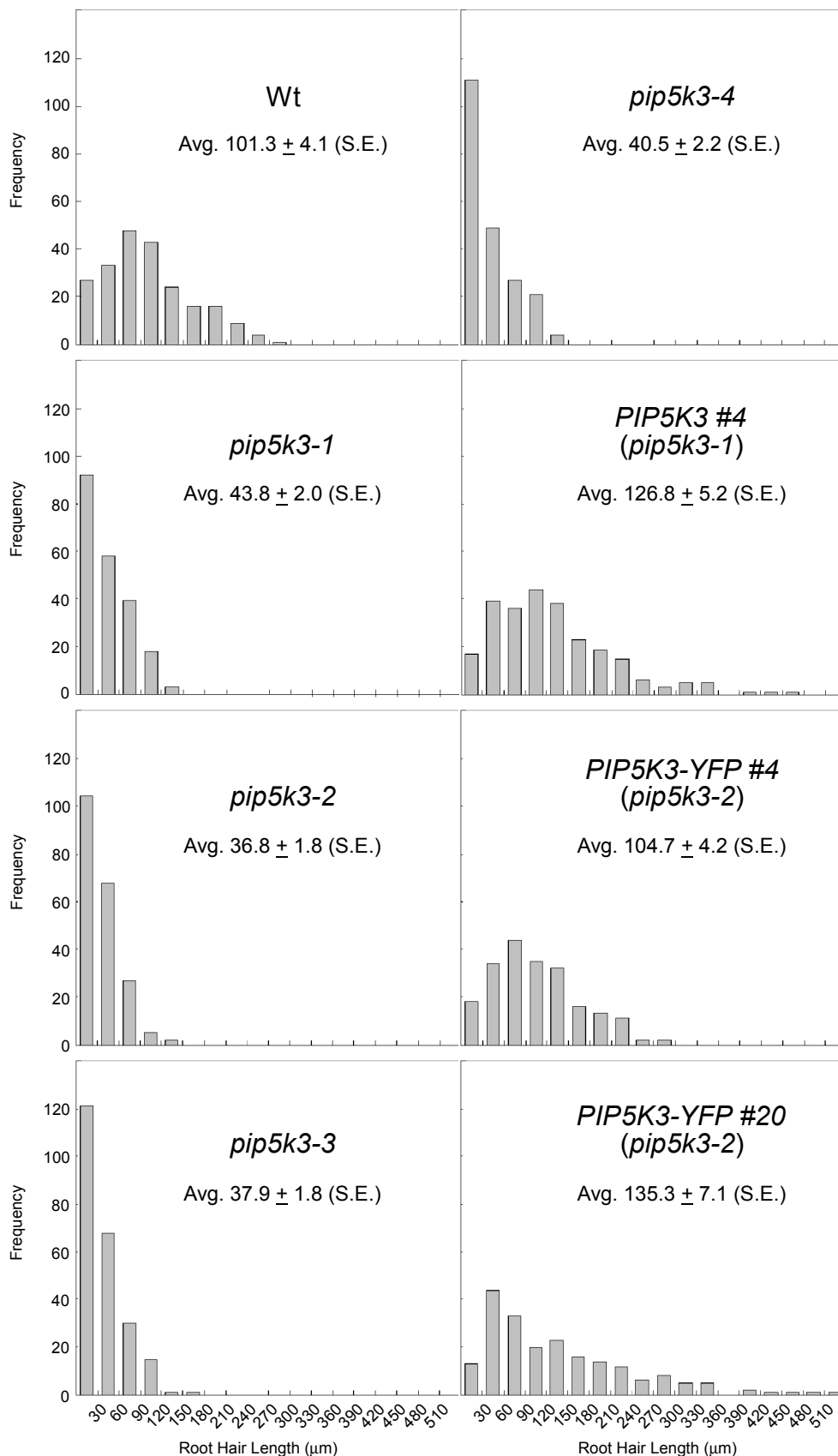
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-----GAGCAGGTGAGCAGATTTGAGGAAGAGACGTGGGAAGAAGATGCAATTGACAACTCA
- - E Q V S R F E E E T W E E D A I D N S
AATCCTAAGGGTACAAGGAAAGAAGCCGTTGAGGTGATCCTTTATTTTCGGTGTATTGACATT
N P K G T R K E A V E V I L Y F G V I D I
CTTCAAGTCTACCGCGGACAAC TTGACAAATTGACGAGGATATATTGTGGTGTAACAAATTG
L Q V Y R G Q L D K L T R I Y C G V N K L
ACGCTTAGACAAC TTAATAACACATTGCGGACGTTTTTAATGTACTGGGGTGGTTTTTCTTTT
T L R Q L N N T L R T F L M Y W G G F S F
CACCAGTGAGACGGGCAACAGCTGATTGCCCTTC-----
H Q *
```

```
-----GAGCAGGTGAGCAGATTTGAGGAAGAGACGTGGGAAGAAGATGCAATTGACAACTCA
- - E Q V S R F E E E T W E E D A I D N S
AATCCTAAGGGTACAAGGAAAGAAGCCGTTGAGTGAAGACGGGCAACAGCTGATTGCCCTTC--
N P K G T R K E A V E *
```

Supplemental Figure 2. Sequence information of mutant transcripts.

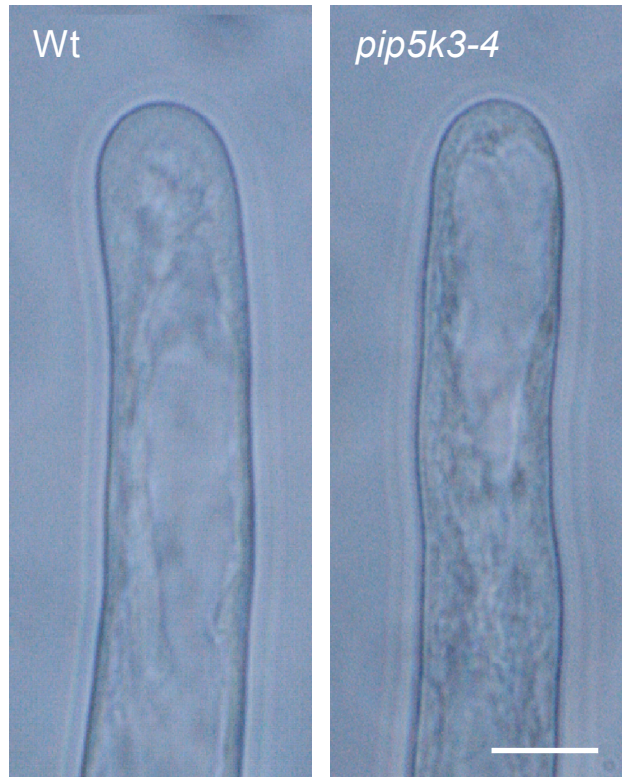
(A) Sequence of cDNA derived from *pip5k3-2* mutant transcripts. Transcripts of the *pip5k3-2* mutant gene contain 5'-extension exceeding 1,000 nucleotides. The cDNA sequence of a part of the 5'-extension is shown. The sequence originating from the T-DNA insertion is highlighted by blue. ATG sequences appearing in the 5'-extension and the authentic initiation codon are highlighted by yellow and red, respectively.

(B) Sequence of cDNA derived from *pip5k3-4* mutant transcripts. Transcripts of the *pip5k3-4* mutant gene include two splicing variants. The cDNA sequences corresponding to the ends of their coding regions are shown with their predicted amino-acid sequences. The sequence originating from the T-DNA insertion and termination codons are highlighted by blue and yellow, respectively.



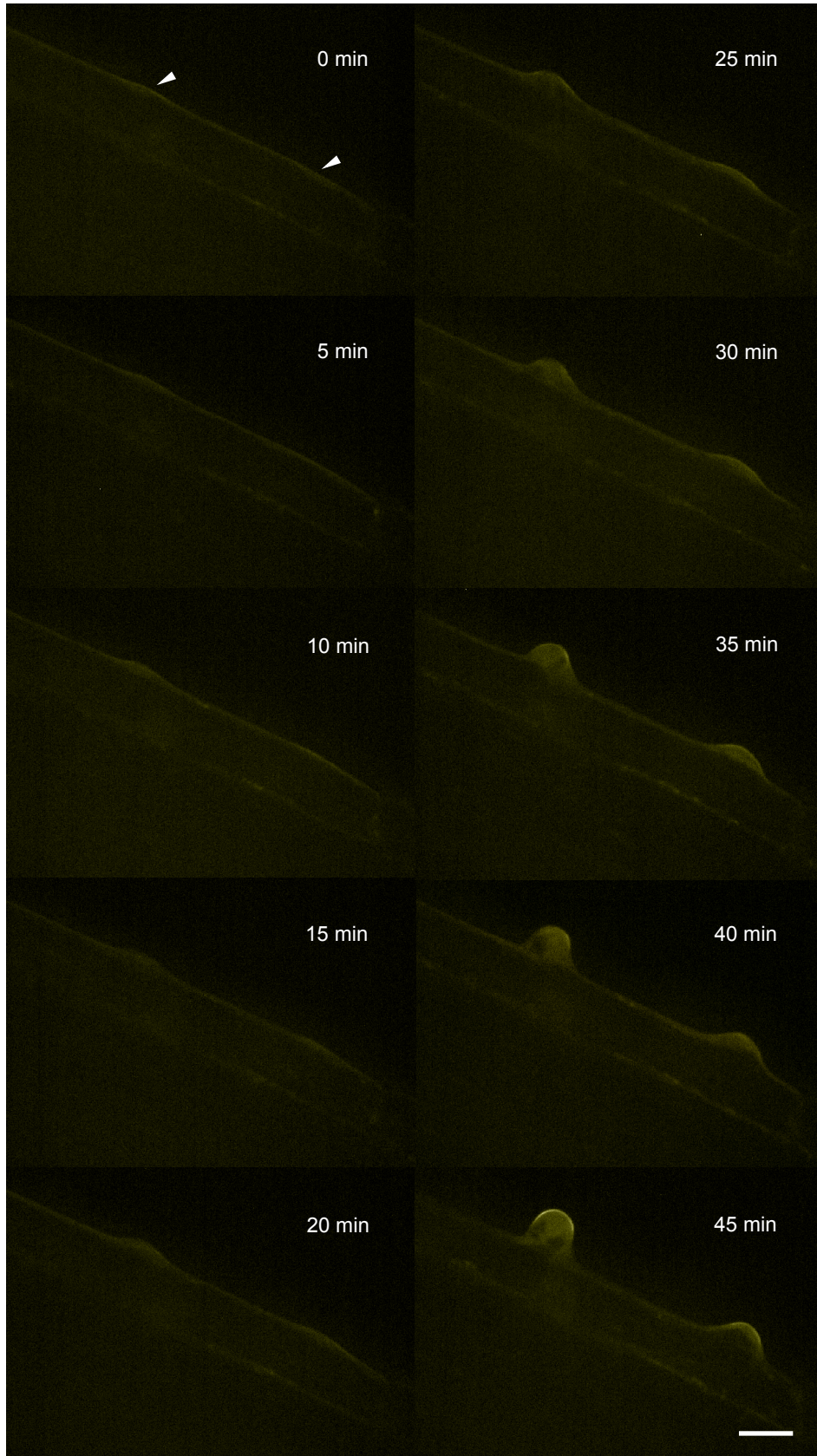
Supplemental Figure 3. Distributions of root hair lengths of wild-type, mutant, and complementation lines.

Histograms of the distributions of root hair lengths on the primary root surface of the wild type (Wt), mutant, and complementation lines grown on vertical standing 2% agar medium for 7 DAG are shown in histograms.



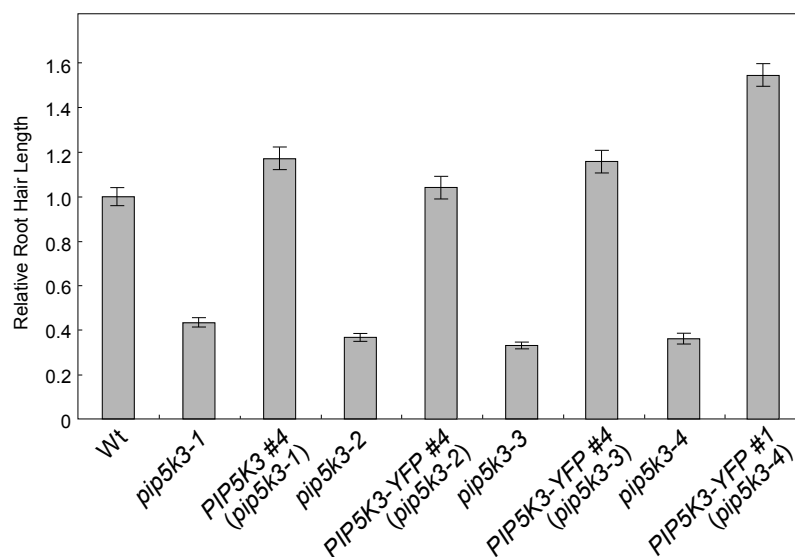
Supplemental Figure 4. Apices of wild-type and mutant root hairs.

Apices of mature root hairs of wild-type (Wt) and *pip5k3-4* primary roots are shown. Plants grown on vertical standing 2% agar medium for 10 DAG were transferred to liquid medium a day before the observation. Bar = 10 μ m.



Supplemental Figure 5. Continuous observation of PIP5K3-YFP in the sites of bulge formation.

The fluorescence of PIP5K3-YFP in consecutive sites expected to form bulges (indicated by arrowheads in the panel at 0 min) was observed every 5 min by confocal laser scanning microscopy. Bar = 20 μ m.



Supplemental Figure 6. Complementation of the mutant phenotype by the PIP5K3 or PIP5K3-YFP transgenes.

Lengths of root hairs on the primary root surface of wild type (Wt), *pip5k3* mutants, and the complementation lines with the PIP5K3 or PIP5K3-YFP transgenes under *pip5k3* mutant backgrounds were measured, and the means and SE of relative root hair length are indicated by the box and error bars (n>200), respectively.



Supplemental Figure 7. Phenotype caused by prolonged overexpression of *PIP5K3*.

ER8-PIP5K3, *ER8-GFP*, and wild-type (Wt) seedlings grown under the induction condition for 14 days are shown. Bar = 1 cm.