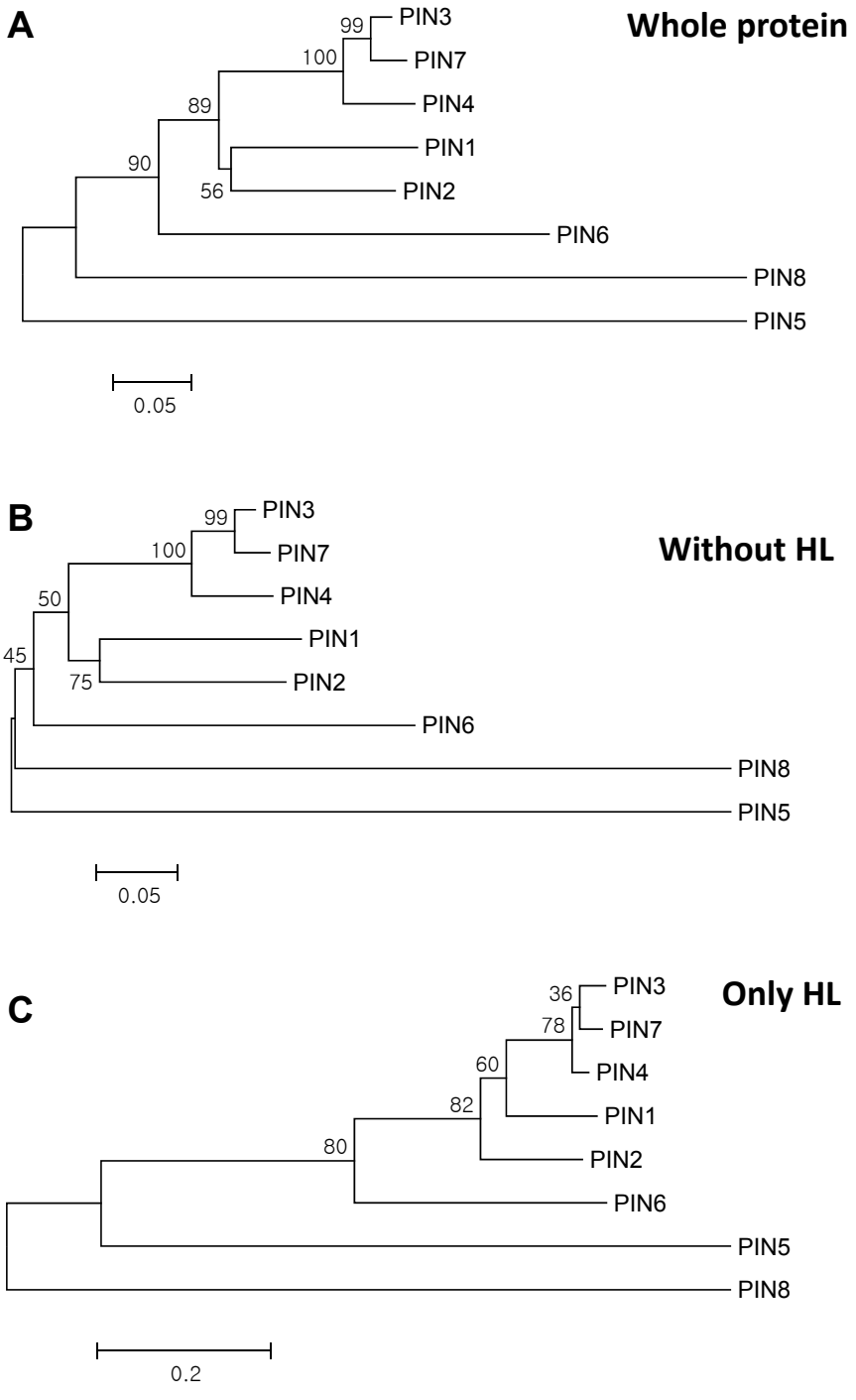
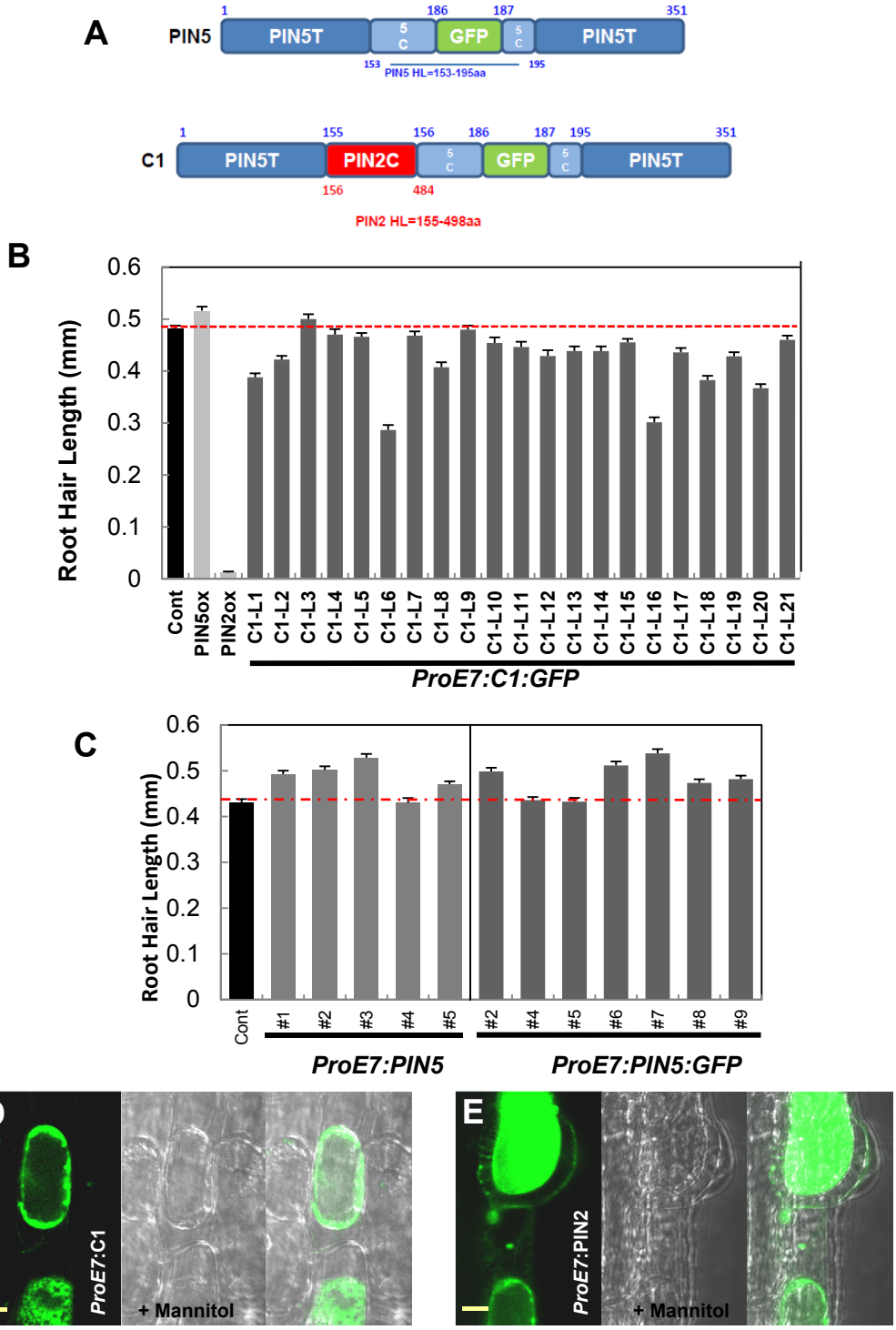
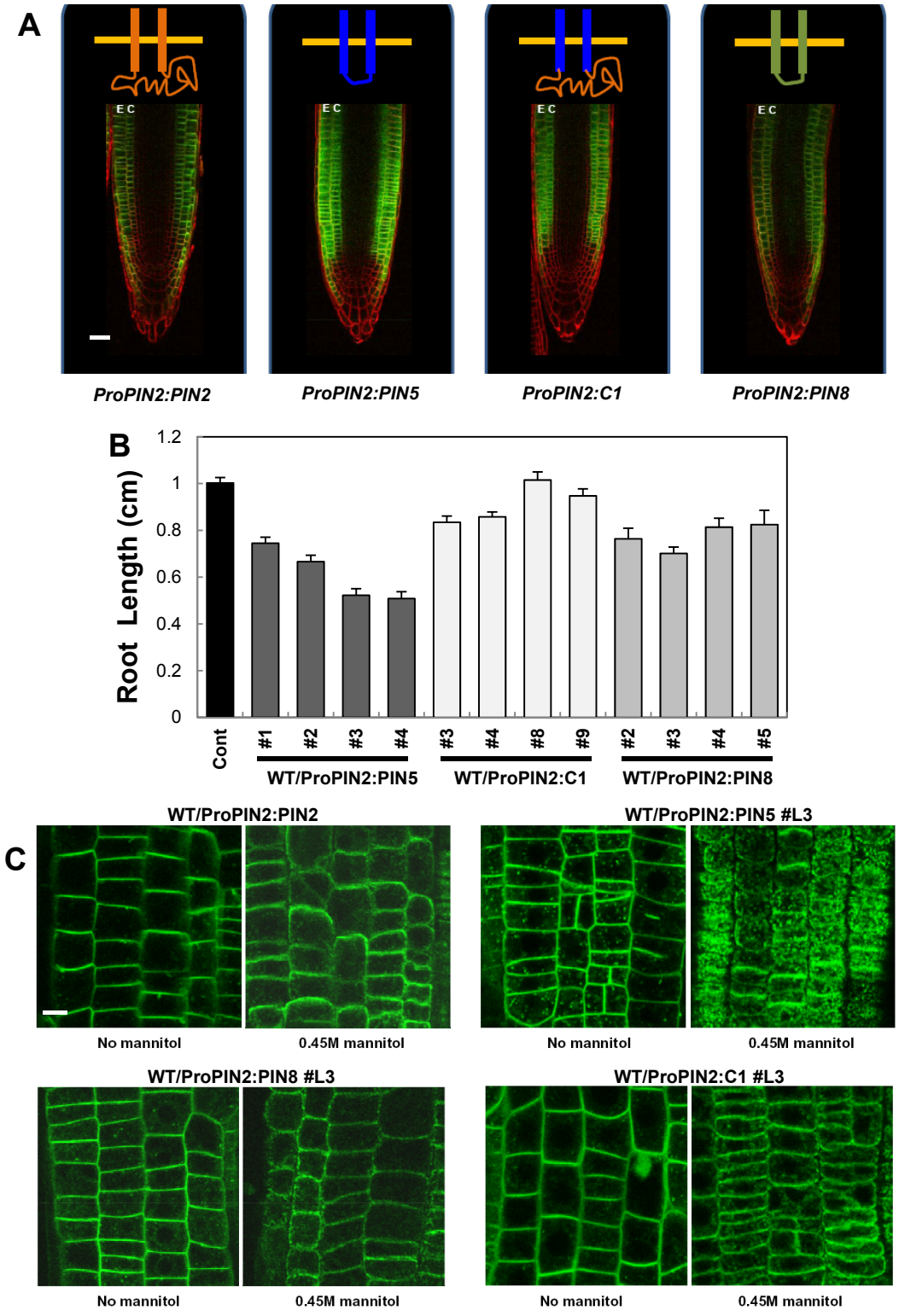


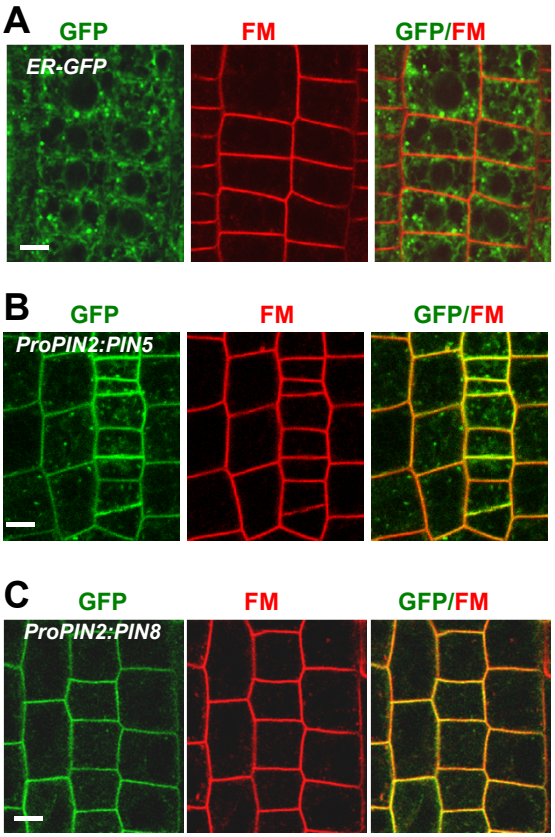
Supplemental Figure 1. Phylogenetic relationship of *Arabidopsis* PINs.
The phylogenetic trees were constructed by neighbor-joining (MEGA4; Tamura et al., 2007) with the whole protein (**A**), only transmembrane domain (**B**, Without HL), or only HL (**C**) of *Arabidopsis* PINs.



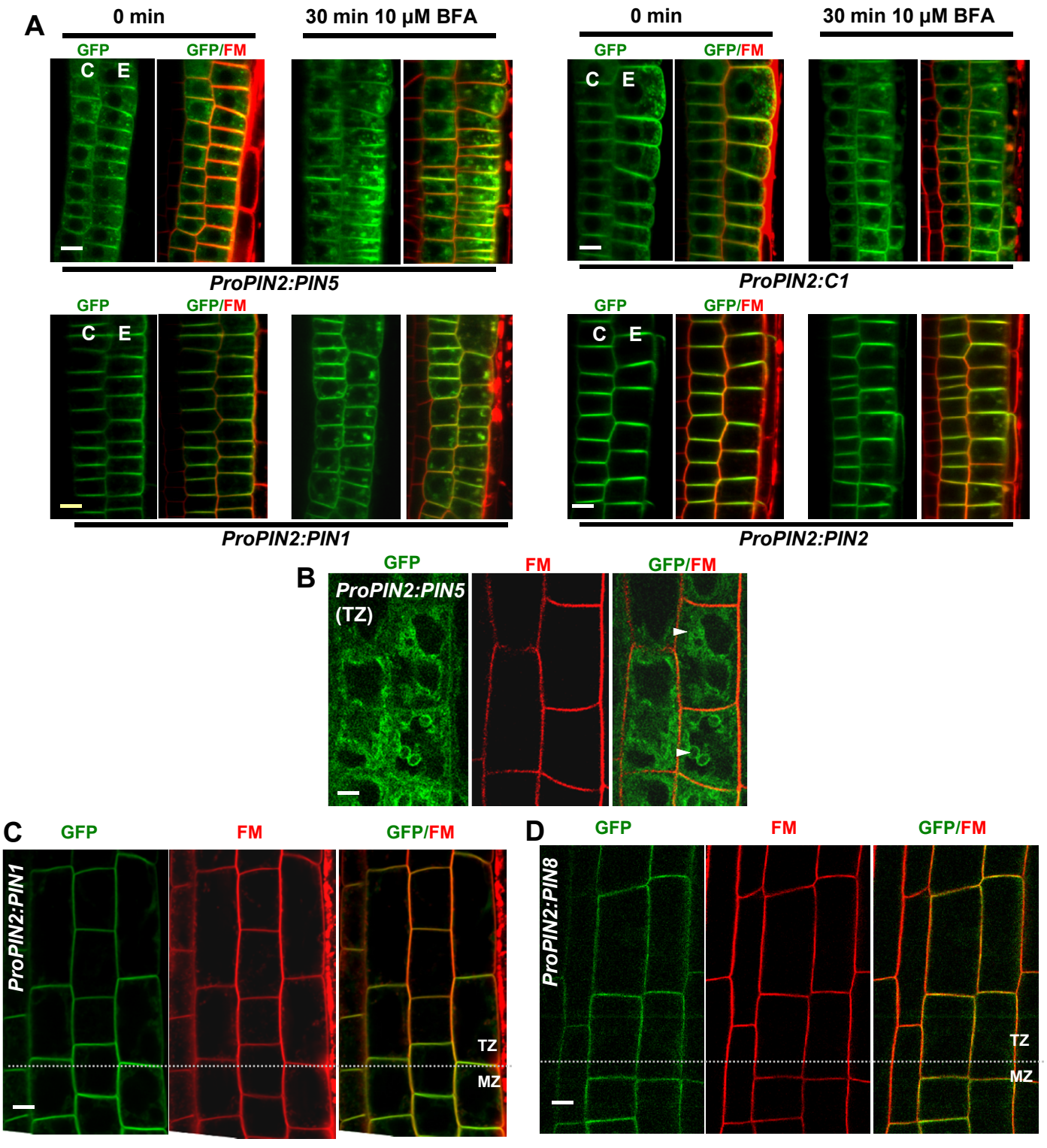
Supplemental Figure 2. Root hair lengths of different PINs expressed under *ProE7*.
(A) Schematic representation of the PIN5 and C1 (PIN5:PIN2-HL) construct with GFP insertion sites. The numbers in blue and red indicate the corresponding amino acid number with respect to PIN5 and PIN2 protein sequence.
(B) and **(C)** Root hair lengths of individual transgenic lines of *ProE7:C1, ProE7:PIN5* and *ProE7:PIN5:GFP* plants, respectively. The dashed red line indicates root hair length of the control seedlings. Data represent means±SE (n =115-178 hairs from 10 roots for each line).
(D) and **(E)** PIN2, unlike C1, shows distinct localization in the cell wall region after 10 min of 0.45 M mannitol treatment in the root hair cell (n=7). Bar=10 µm for **D** and **E**.



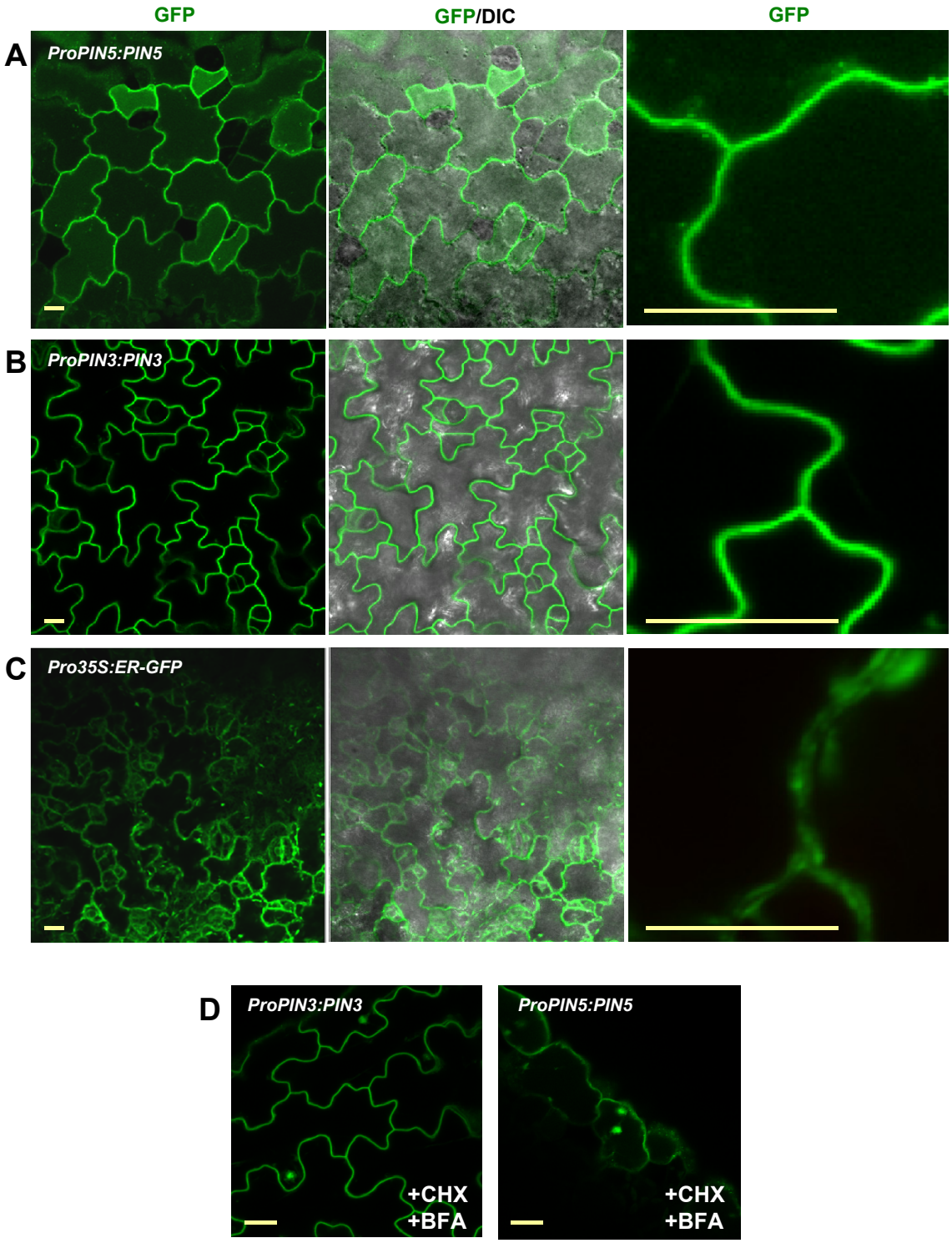
Supplemental Figure 3. PINs expression under the *PIN2* promoter.
(A) Expression patterns of PINs in the PIN2 domain under *ProPIN2*. C=cortex, E=epidermis.
(B) Root lengths of *ProPIN2:PINs* transformants in the wild type (WT) background. Data represent means±SE (n=3 1-36 roots per transgenic line, 4 independent transgenic line).
(C) Mannitol treatment (0.45 M, <5 min) leads to rapid internalization of PIN5 from the PM as compared to C1, PIN 8 and the PIN2 protein (n=8). Bar=50 µm for **A**, 5 µm for **C**.



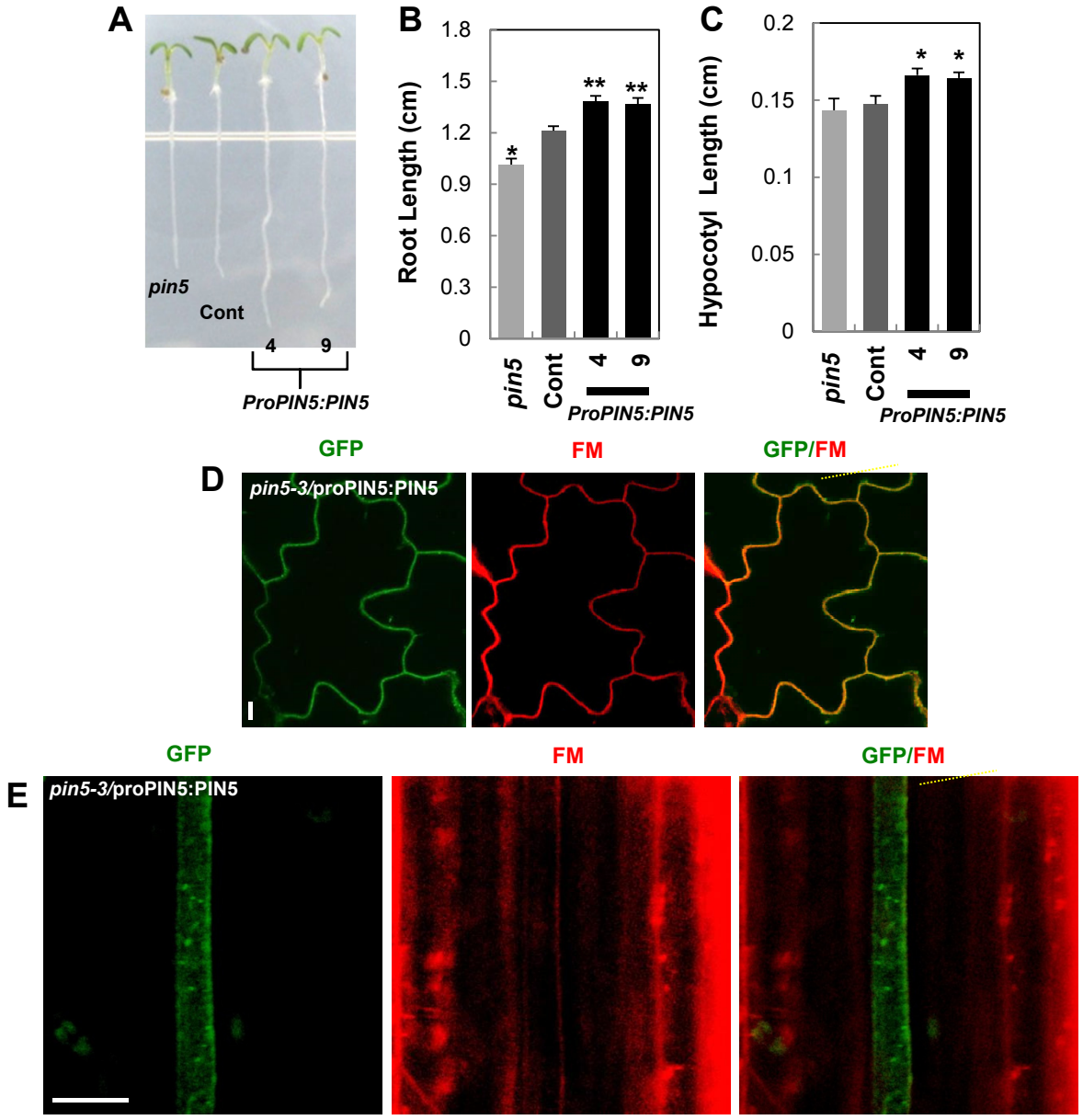
Supplemental Figure 4. PIN5 and PIN8 localize to the PM in the root meristem epidermis.
(A) The localization pattern of ER-GFP in the root meristematic epidermal cells (n=6 roots).
(B) and **(C)** PIN5 and PIN8 colocalize with FM4-64 in the root meristematic epidermal cells (n=16 roots).
Bar=5 µm for all.



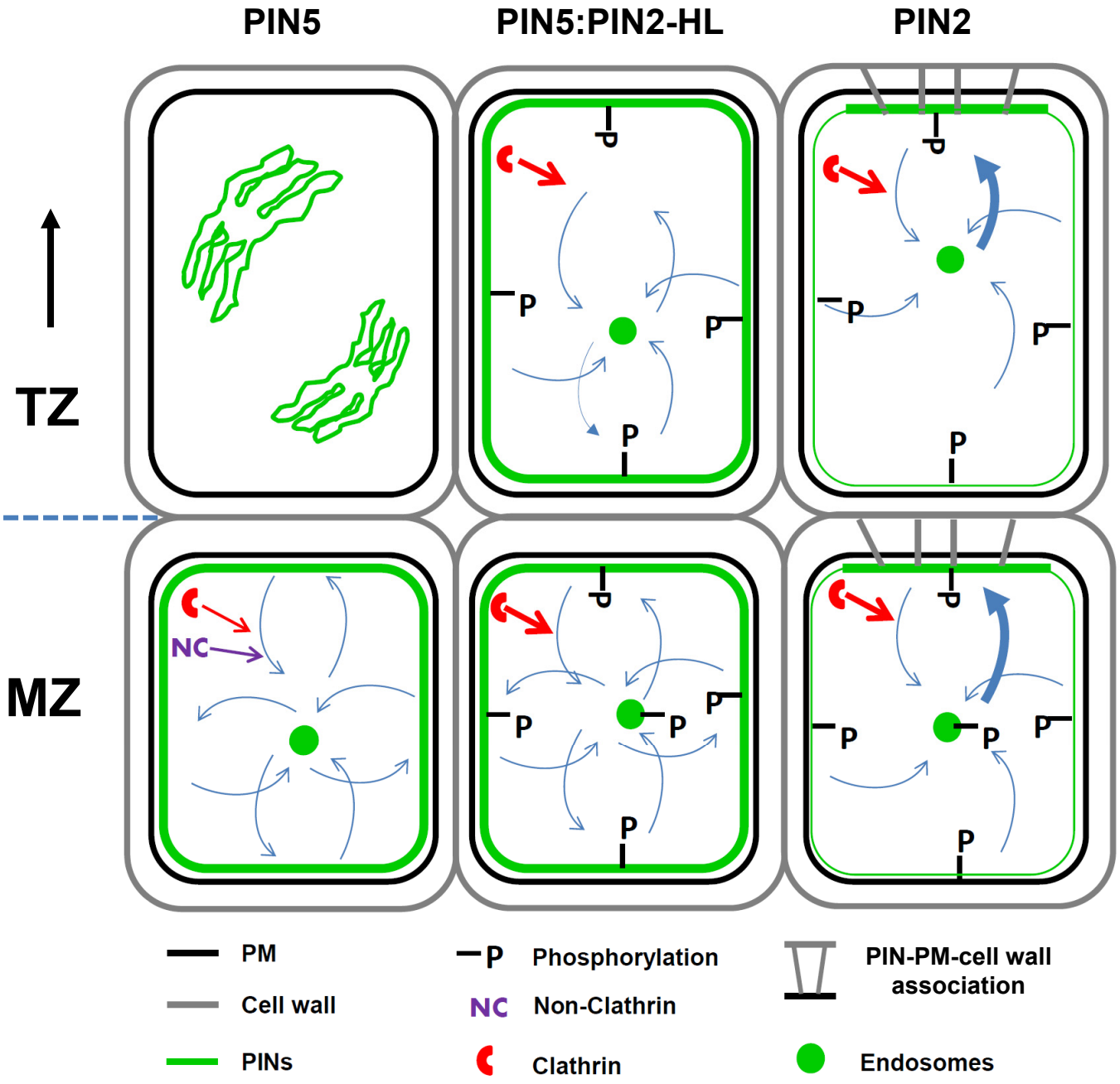
Supplemental Figure 5. Different PINs show differential response to BFA treatment.
(A) Both PIN5 and PIN1 are more sensitive to BFA treatment as compared to C1 and PIN2 (n=7). C=cortex, E=epidermis.
(B) PIN5 predominantly localizes to internal compartments in the epidermal cells of root transition zone (TZ) (n=12).
(C) and **(D)** Representative images showing PIN1 and PIN8 localization to the PM in the root meristem (MZ) and tra nsition/elongation zone (TZ). (n=9-10 roots per construct). Bar=5 µm for all.



Supplemental Figure 6. PIN5 shows PM localization in the pavement cells of the cotyledon.
(A) to **(C)** The localization pattern of PIN5, PIN3, and ER-GFP in the pavement cell when expressed under the PIN5, PIN3, and 35S promoter, respectively.
(D) Both PIN3 and PIN5 show BFA compartment formation in the pavement cell. CHX pretreatment (50 µM, 30 min) was followed by BFA treatment (25 µM, 30 min) before imaging. Bar=10 µm for all.



Supplemental Figure 7. PIN5:GFP is functional in seedling growth.
The loss of PIN5 (*pin5*) mutant grew a shorter primary root but the *ProPIN5:PIN5:GFP* transformants grew sligh tly longer roots than that of wild type (WT, Cont).
(A) Seedling phenotypes of 4-day-old *pin5-3*, WT, and two independent *ProPIN5:PIN5:GFP* transformants (in WT background) seedlings. The two *ProPIN5:PIN5:GFP* lines (#4 and 9) were randomly chosen.
(B) Root growth of 4-day-old *pin5-3*, WT, and two *ProPIN5:PIN5:GFP* lines.
(C) Hypocotyl growth of 4-day-old *pin5-3*, WT, and two *ProPIN5:PIN5:GFP* lines. For **B** and **C**, experiments were repeated twice with 12 seedlings each for each line. Data represent means±SE (n=24; *P<0.01, **P<0.001).
(D) PIN5:GFP protein localize to the PM in the cotyledon pavement cells in the *pin5-3* background (n=6 cotyledons). Seedlings were treated with 5 μM FM4-64 for 3-5 min before imaging.
(E) PIN5:GFP protein localizes to internal compartments in the root vascular cells in the *pin5-3* background (n=4 roots). Seedlings were treated with 10 μM FM4-64 for 20-30 min before imaging.
Bar=5 μm for **D** and **E.**



Supplemental Figure 8. A model illustrating the role of PIN-HL in PIN trafficking during root development.

The long PIN-HL have multiple functions in PIN protein trafficking. In the epidermal cells of the root meristem (MZ) PIN5, PIN2 and PIN5:PIN2-HL localize to the PM. While PIN2 shows apical/shootward polarity, PIN5 and PIN5:PIN2-HL shows largely non-polar PM localization in the root epidermis. The long PIN2-HL undergoes phosphorylation, clathrin-mediated endocytosis and is recycled back to the apical PM thereby making polar PIN2 localization. PIN5, on the other hand, also undergoes clathrin-mediated, along with clathrin-independent, endocytosis and recycling but lacks the phosphorylation capability. Although PIN2-HL provides the PIN5:PIN2-HL fusion protein with phosphorylation capability and increased clathrin dependency but fails to confer the PIN2 polarity possibly due to the limitation of the HL to form strong cell wall-PM-PIN association. However, PIN5 localization switches from being predominantly PM in MZ to being predominantly ER/endomembrane in TZ, whereas PIN2 exhibits a consistent trafficking and localization pattern throughout the developmental stages.

Supplemental Table 1. The list of primers

Subject		Name	Sequence (5' to 3')2
PIN2 promoter		pPN2-PmeI-F	CGA CGT TTA AAC TGC AAG GAT ATC ATT ACC AGT ACC G
		pPN2-SalI-R	CTG GTC GAC TTT GAT TTA CTT TTT CCG GCG AGA G
PIN5-GFP	1st fragment	PN5-CD-Xh-F	ATT TTC TCG AGA TGA TAA ATT GTG GAG ATG TTT ACA AGG
		PN5Xm995R	AGG AAC CCG GGC TCT CCC ACA ACC AC
	2nd fragment	PN5Av996F	TTG TGC CTA GGA AGT CGT TCC TTG AGG TC
		PN5-pt-New-R	TATACTGCAGTCAATGAATAAACTCCAGAGCTGC
GFP		EGFP-Xm-F	TAT ACC CGG GGA TGG TGA GCA AGG GCG
		EGFP-Av-R	CGG CCC CTA GGC TTG TAC AGC TCG TCC
PIN5:PIN2-HL (C1)	megaprimer	P5-P2C156-F-new	CTTGTTGTTTGTCTTAGAGTTTAGAGGGGCTAAGCTTCTCATCTCCGAGC
	megaprimer	P5-P2C484-R-new	CTGATATTATTACTACTAAATCCGGCTTTACTCGCCGGCGGCATCTGC
		PN5-CD-Xh-F	ATT TTC TCG AGA TGA TAA ATT GTG GAG ATG TTT ACA AGG
		PN5Xm995R	AGG AAC CCG GGC TCT CCC ACA ACC AC
PIN5 promoter	digested with Xho1 and Sal1	PIN5Pr-Xh-F	TATACTCGAGGTAGGTGCTGTAGCATATATTGAGTGACG
		PN5Xm995R	AGG AAC CCG GGC TCT CCC ACA ACC AC
PIN5:GFP and PIN5:PIN2-HL for ProTA vector		PN5-CD-Xh-F	ATT TTC TCG AGA TGA TAA ATT GTG GAG ATG TTT ACA AGG
		PN5-Sp-New-R	TATAACTAGTTCAATGAATAAACTCCAGAGCTGC

[1]Abbreviations of restriction enzyme sites in primers: Av, AvrII; Xm, XmaI; Sl, Sal1; Pm, Pme1; Xh, Xho1; Pt, Pst1; Sp, Spe1

Supplemental Reference :

Tamura, K., Dudley, J., Nei, M., and Kumar, S. (2007), MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol. Biol. Evol. **24**: 1596-1599.