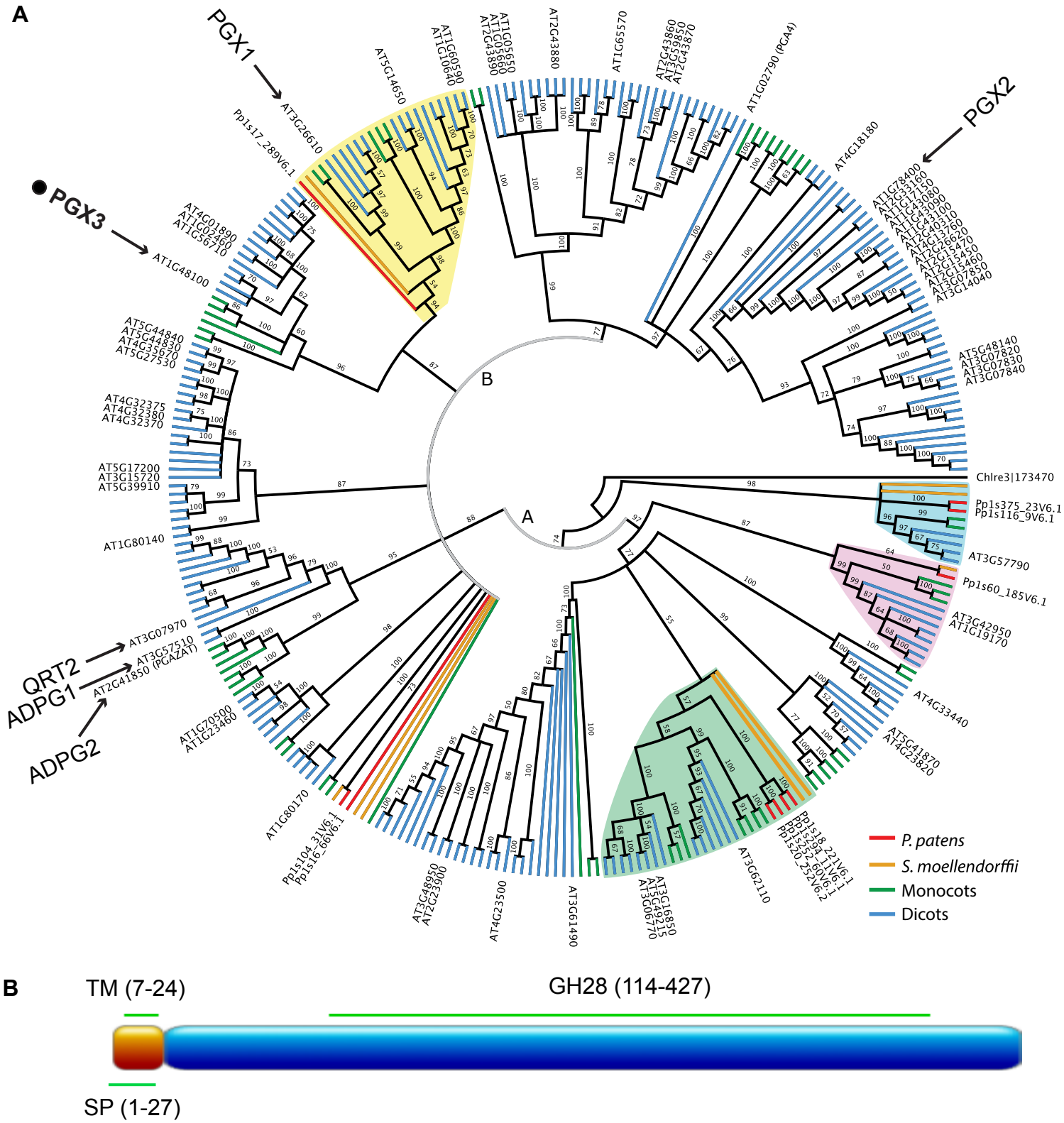
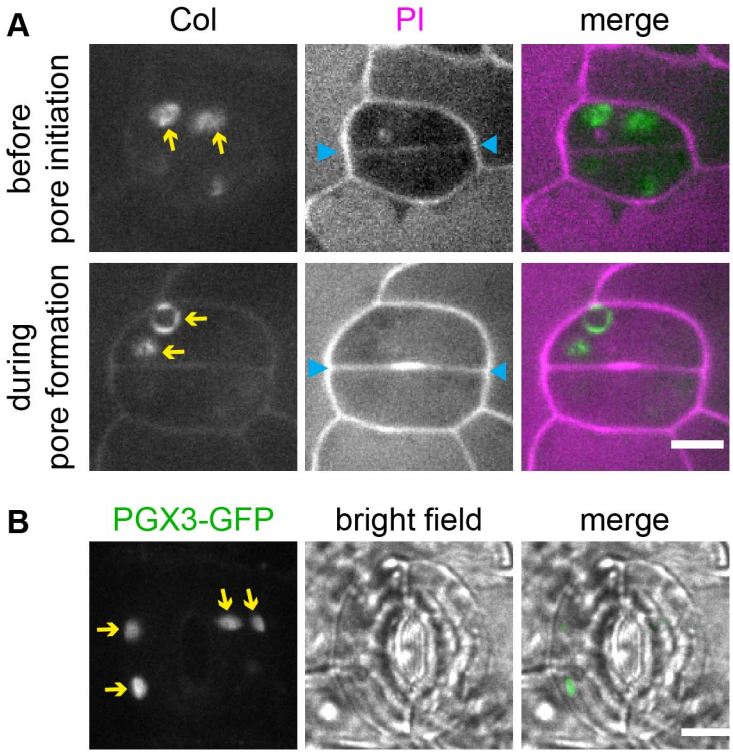


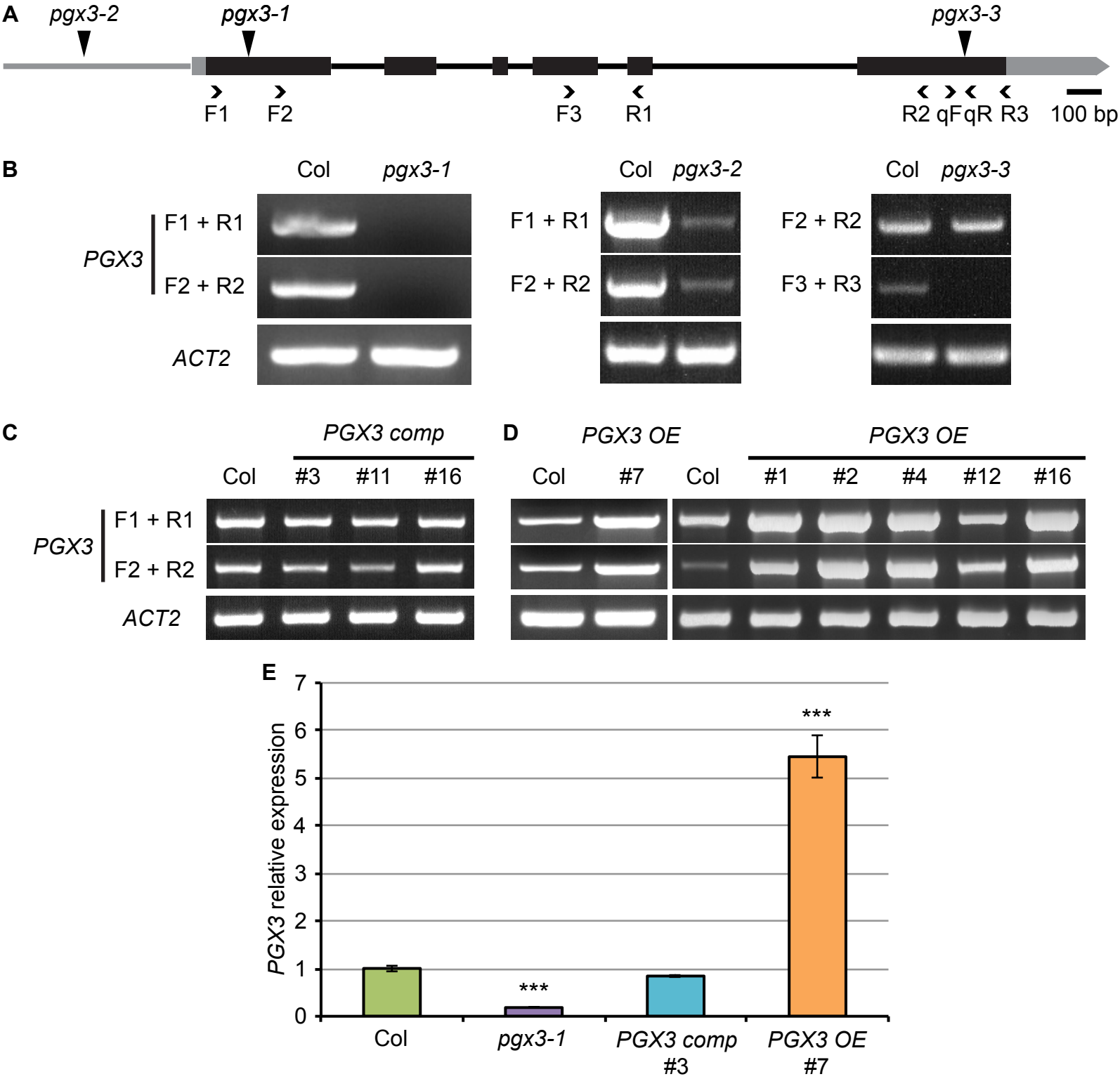
Supplemental Figure 1. Phylogenetic Analysis of the Polygalacturonase Family and the Schematic Protein Domains of PGX3. (Supports Figure 1 and Figure 2.)

(A) A phylogenetic tree of the polygalacturonase family, adapted from McCarthy et al., BMC Plant Biology, 2014. PGX1 functions in cell expansion and floral patterning (Xiao et al., Plant Cell, 2014). PGX2 promotes cell expansion and stem lignification (Xiao et al., Plant Journal, 2017). QRT2 is required for microspore separation (Rhee and Somerville, Plant Journal, 1998). ADPG1 and ADPG2 are essential for silique dehiscence and contribute to floral organ abscission (Ogawa et al., Plant Cell, 2009).

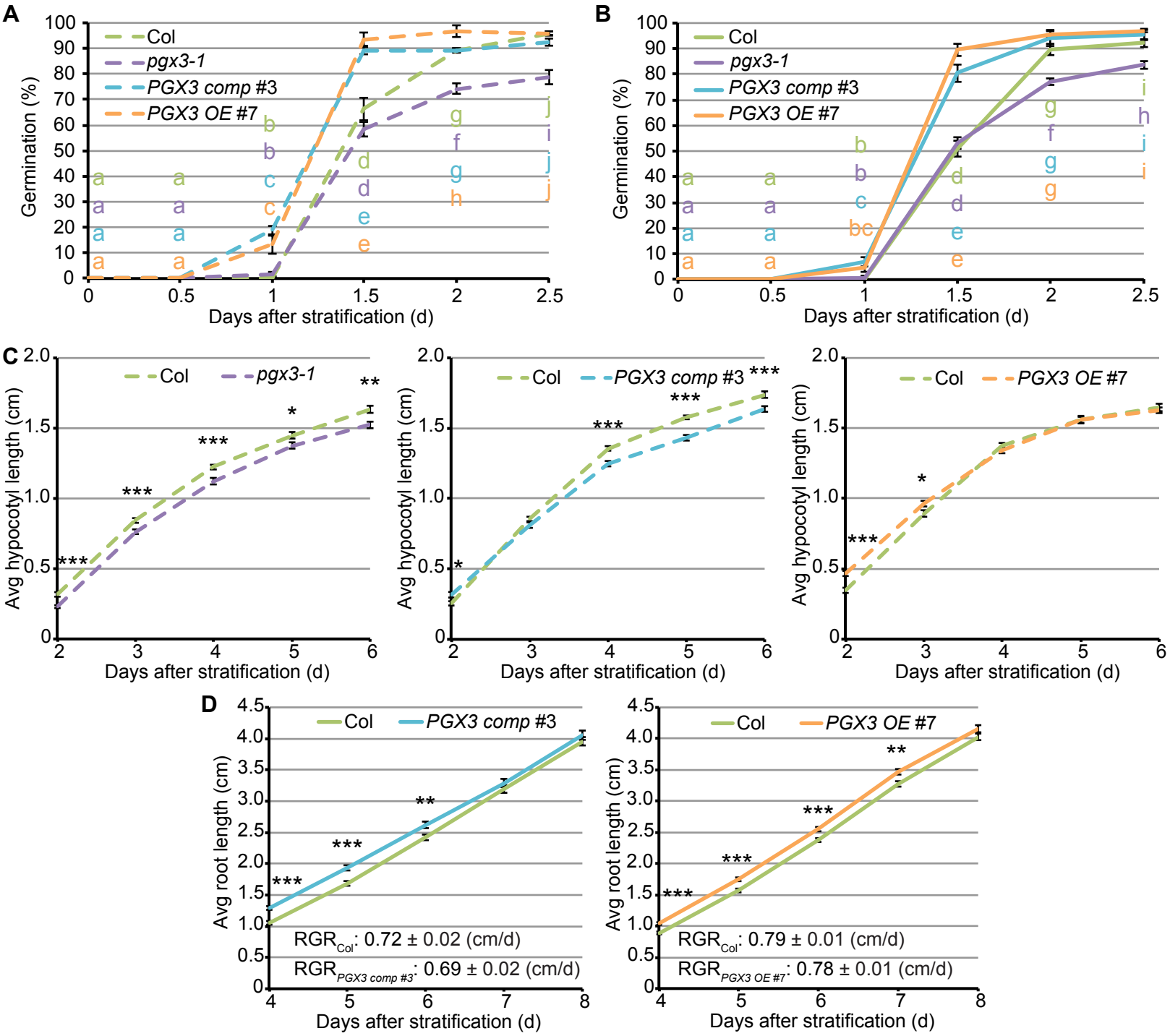
(B) Schematic protein domains of PGX3, including a signal peptide (SP), a transmembrane domain (TM), and a GH28 domain. The predicted cleavage site of the signal peptide is between amino acids 27 and 28. The signal peptide and transmembrane domain were predicted by SignalP 4.1 (http://www.cbs.dtu.dk/services/SignalP/) and TMHMM 2.0 (http://www.cbs.dtu.dk/services/TMHMM/).



Supplemental Figure 2. Negative Controls for PI-stained Stomata in 4-d-old Col Seedlings and No Detectable PGX3-GFP Signals in 3-week-old Rosette Leaves.
(Supports Figure 2.)
(A) PI staining in developing guard cells of 4-d-old Col seedlings. Blue arrowheads indicate cell plates between sister guard cells. Yellow arrows indicate autofluorescence from chloroplasts.
(B) Fluorescence and bright-field imaging in guard cells from epidermal peels made from 3-week-old transgenic plants expressing *ProPGX3:PGX3-GFP*. Yellow arrows indicate autofluorescence from chloroplasts.
Scale bars: 5 μm in **(A)** and **(B)**.



Supplemental Figure 3. *PGX3* Gene Structure and Transcript Detection.
(Supports Figure 3 and Figure 4.)
(A) Schematic gene model of *PGX3*, with the promoter as a gray line, UTRs as grey boxes, exons as solid black boxes, introns as black lines, T-DNA insertion sites as triangles, and primers used for RT-PCR **(B)** to **(D)** and qPCR **(E)** as arrowheads.
(B) to **(D)** RT-PCR results in *PGX3* T-DNA insertional mutants **(B)**, *PGX3* complementation (*PGX3 comp*) lines **(C)**, and *PGX3* overexpression (*PGX3 OE*) lines **(D)**, with *ACT2* used as an internal control.
(E) qPCR quantification of *PGX3* expression level in Col, *pgx3-1*, *PGX3 comp* #3, and *PGX3 OE* #7 plants. *ACT2* was used as an internal control, and *PGX3* expression in Col was normalized to 1. Error bars are SE and asterisks indicate significant differences compared to Col controls (n = 3 technical replicates from pooled rosette leaves for each genotype; *** $P < 0.001$, Student's t-test).



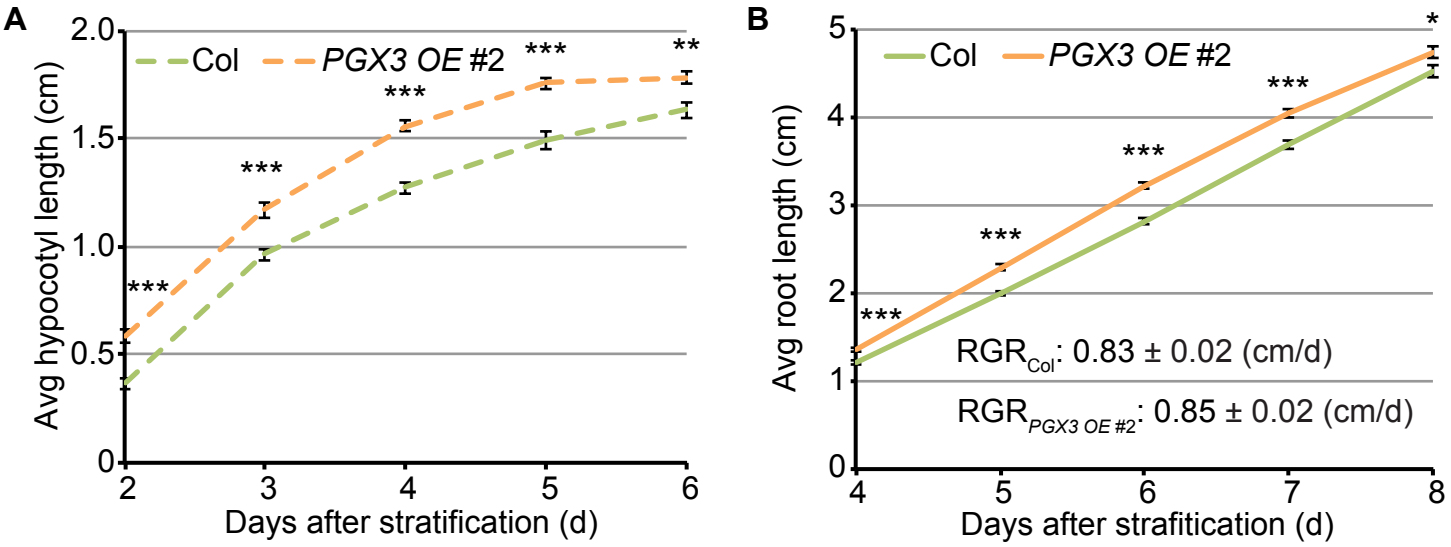
Supplemental Figure 4. *PGX3* Functions in Seed Germination, Etiolated Hypocotyl Elongation, and Root Elongation. (Supports Figure 3.)

(A) and **(B)** Analysis of seed germination in Col, *pgx3-1*, *PGX3 comp* #3, and *PGX3 OE* #7 under the dark-grown condition **(A)** or the light-grown condition **(B)** from 0 to 2.5 days after stratification. Error bars are SE. Lowercase letters represent significantly different groups and colors of the letters correspond to the colors of genotypes ($n \geq 120$ seeds per genotype per time point from three independent experiments; $P < 0.05$, one-way ANOVA and Tukey test).

(C) Etiolated hypocotyl length from 2- to 6-d-old dark-grown seedlings in Col, *pgx3-1*, *PGX3 comp* #3, and *PGX3 OE* #7. Error bars are SE ($n \geq 62$ seedlings per genotype per day from three independent experiments; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, Student's *t*-test).

(D) Primary root length from 4- to 8-d-old light-grown seedlings in Col, *PGX3 comp* #3, and *PGX3 OE* #7. Error bars are SE ($n \geq 100$ seedlings per genotype per day from three independent experiments; ** $P < 0.01$, *** $P < 0.001$, Student's *t*-test). Relative Growth Rates (RGRs) of roots in each genotype are indicated on each graph.

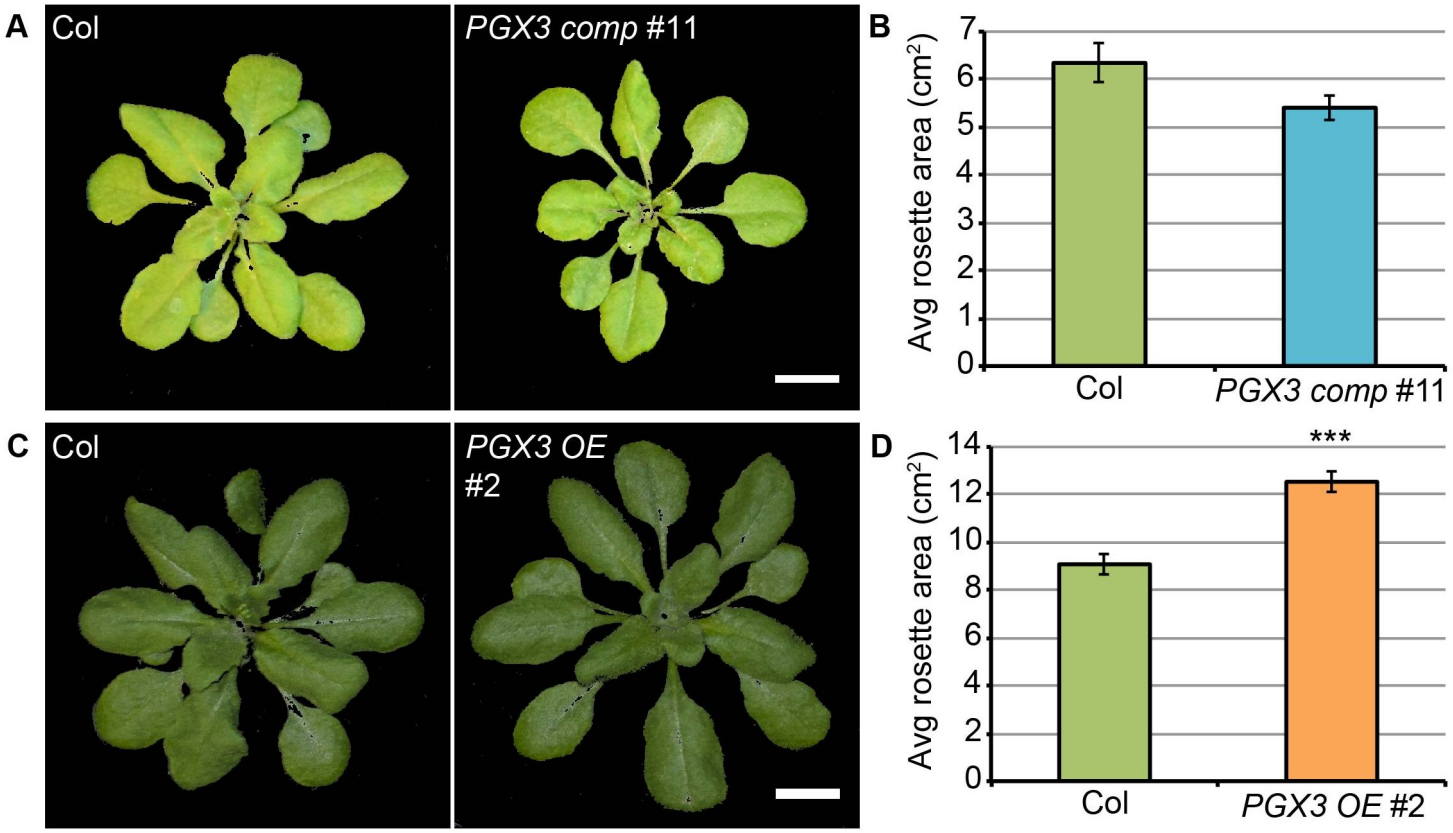
Note that side-by-side controls were always used for each genotype in every independent experiment in **(A)** to **(D)**.



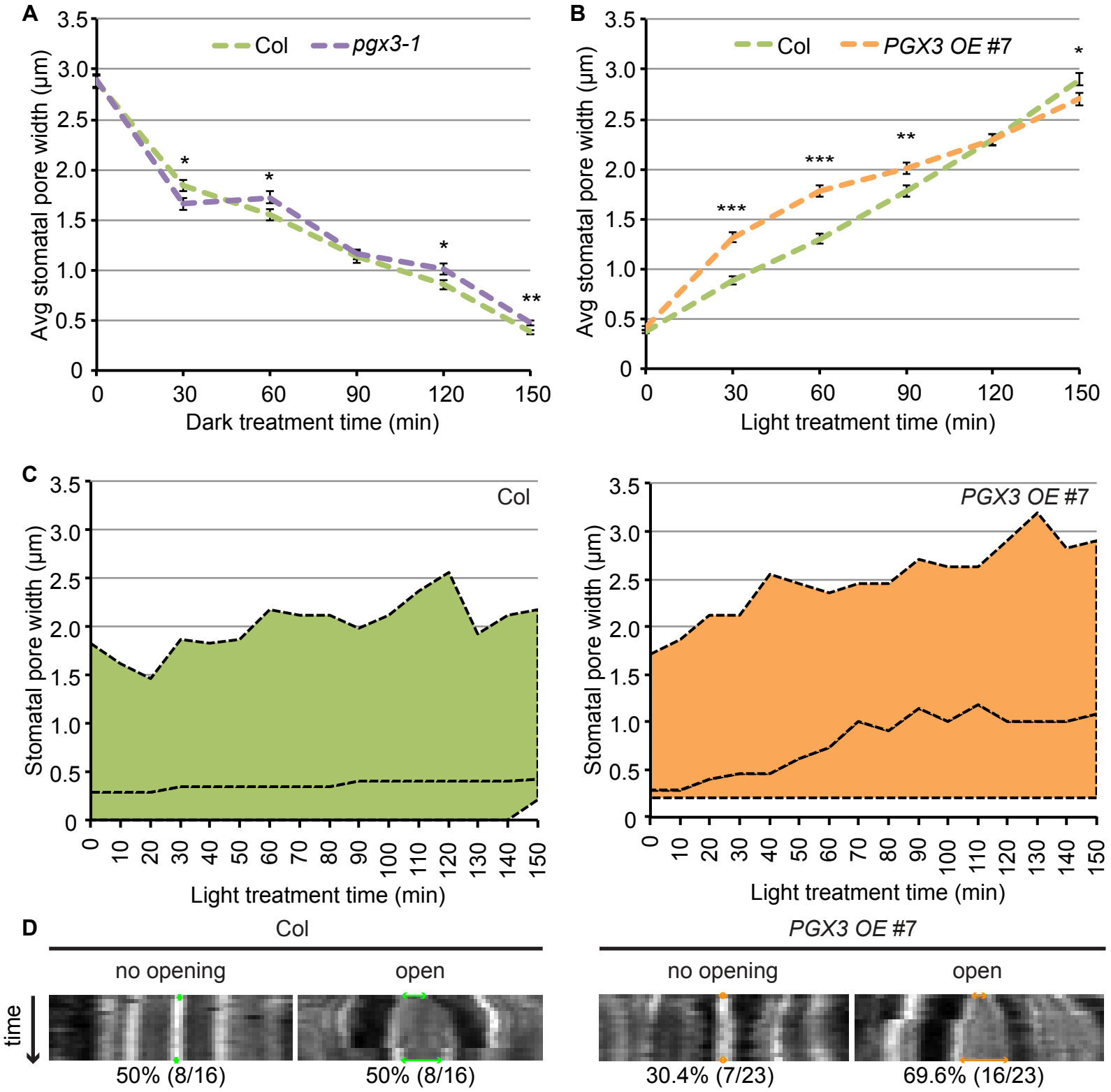
Supplemental Figure 5. Hypocotyl Growth and Root Length Are Enhanced in an Additional *PGX3* Overexpression Line. (Supports Figure 3.)

(A) Etiolated hypocotyl length from 2- to 6-d-old dark-grown seedlings in Col and *PGX3 OE* #2. Error bars are SE ($n \geq 74$ seedlings per genotype per day from three independent experiments; ** $P < 0.01$, *** $P < 0.001$, Student's *t*-test).

(B) Primary root length from 4- to 8-d-old light-grown seedlings in Col and *PGX3 OE* #2. Error bars are SE ($n \geq 97$ seedlings per genotype per day from three independent experiments; * $P < 0.05$, *** $P < 0.001$, Student's *t*-test). Relative Growth Rates (RGRs) of roots in each genotype are indicated on the graph.



Supplemental Figure 6. Rosette Phenotypes in Other *PGX3* Complementation and Overexpression Lines.
(Supports Figure 4.)
(A) and **(B)** Representative segmented images of rosettes **(A)** and measurements of rosette area **(B)** in 3-week-old Col and *PGX3 comp* #11 plants. Error bars are SE ($n \geq 36$ plants per genotype from two independent experiments; $P = 0.06$, Student's *t*-test).
(C) and **(D)** Representative segmented images of rosettes **(C)** and measurements of rosette area **(D)** in 3-week-old Col and *PGX3 OE* #2 plants. Error bars are SE ($n = 48$ plants per genotype from three independent experiments; *** $P < 0.001$, Student's *t*-test).
Scale bars: 1 cm in **(A)** and **(C)**. Note that side-by-side controls were always used for each genotype in each independent experiment in **(A)** to **(D)**.

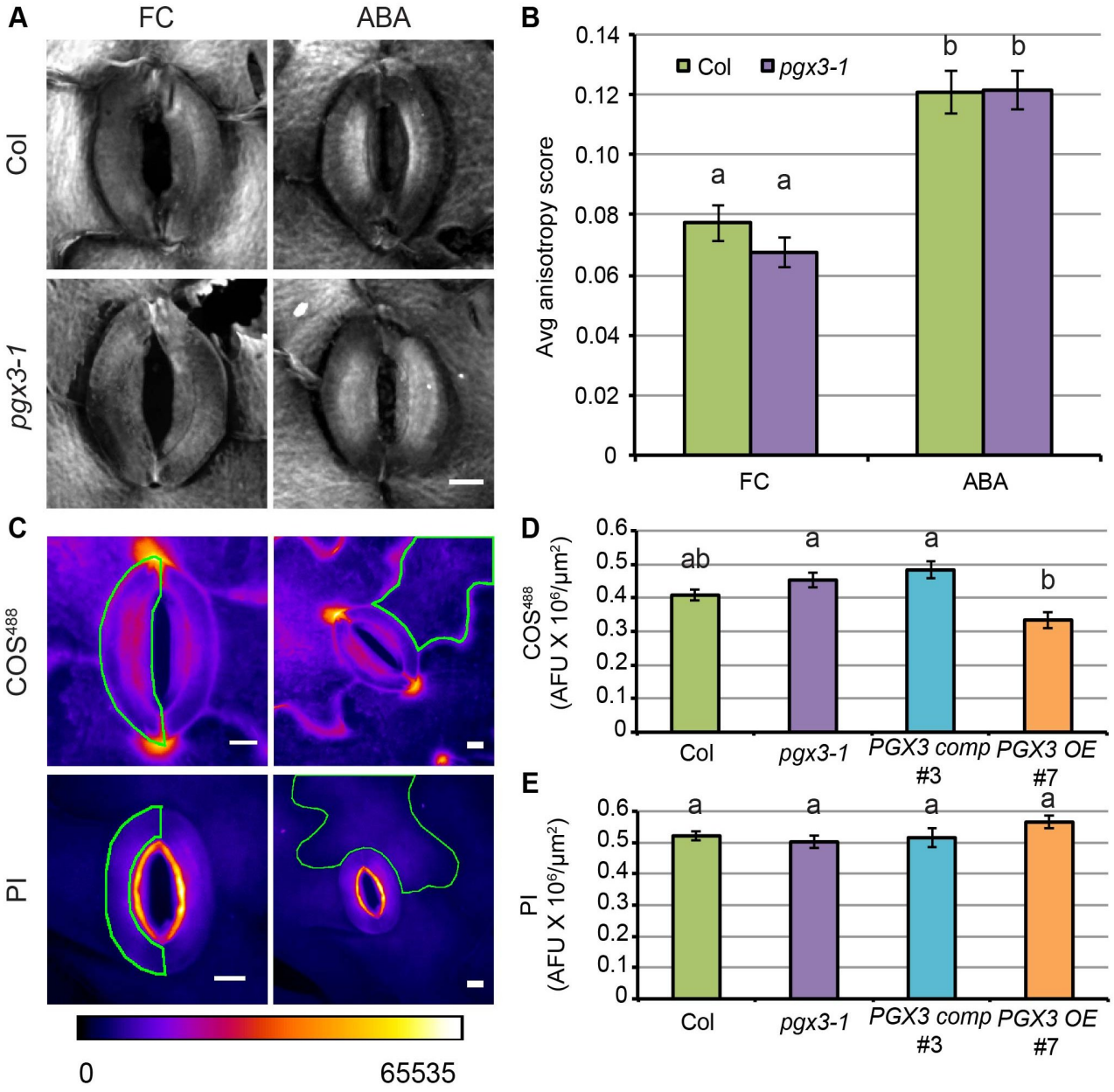


Supplemental Figure 7. *PGX3* Regulates Dark- or Light-induced Stomatal Dynamics in True Leaves.
(Supports Figure 5.)

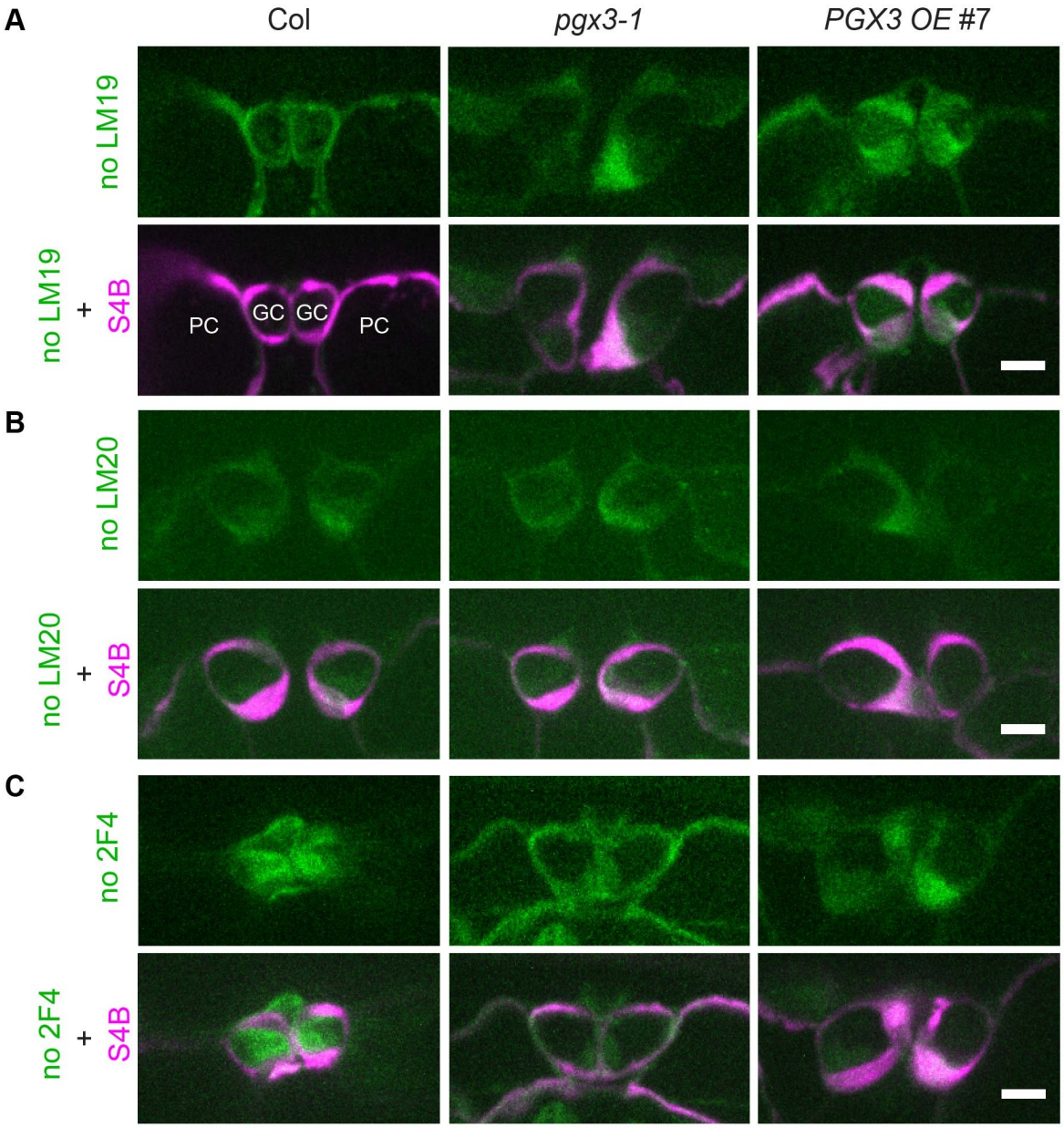
(A) Average stomatal response to dark-induced closure on the population level in 3- to 4-week-old Col and *pgx3-1* plants. Error bars are SE ($n \geq 87$ stomata per genotype per time point from three independent experiments; * $P < 0.05$, ** $P < 0.01$, Student's *t*-test).

(B) Average stomatal response to light-induced opening on the population level in 3- to 4-week-old Col and *PGX3 OE* #7 plants. Error bars are SE ($n \geq 88$ stomata per genotype per time point from three independent experiments; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, Student's *t*-test).

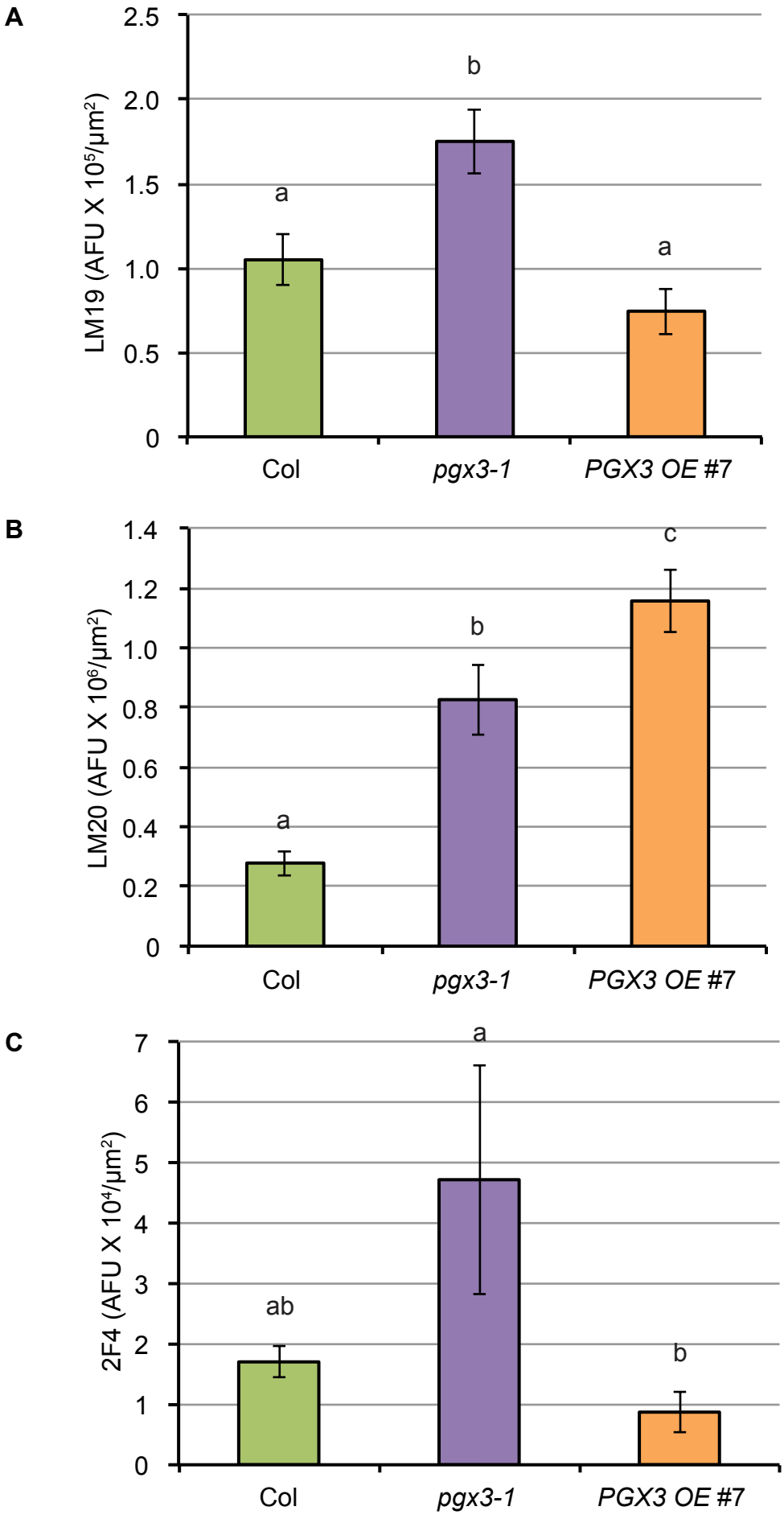
(C) and **(D)** Individual stomatal dynamics in 3- to 4-week-old Col controls and *PGX3 OE* #7 plants during light-induced opening ($n \geq 16$ stomata per genotype from four independent experiments). For each graph in **(C)**, top, middle, and bottom lines correspond to the maximum, median, and minimum stomatal pore width values at each time point, respectively. Kymographs with quantifications in **(D)** were generated from the same set of images in Supplemental Movie 2. Double-headed arrows in each kymograph indicate stomatal pore width at the beginning or the end of light-induced opening.



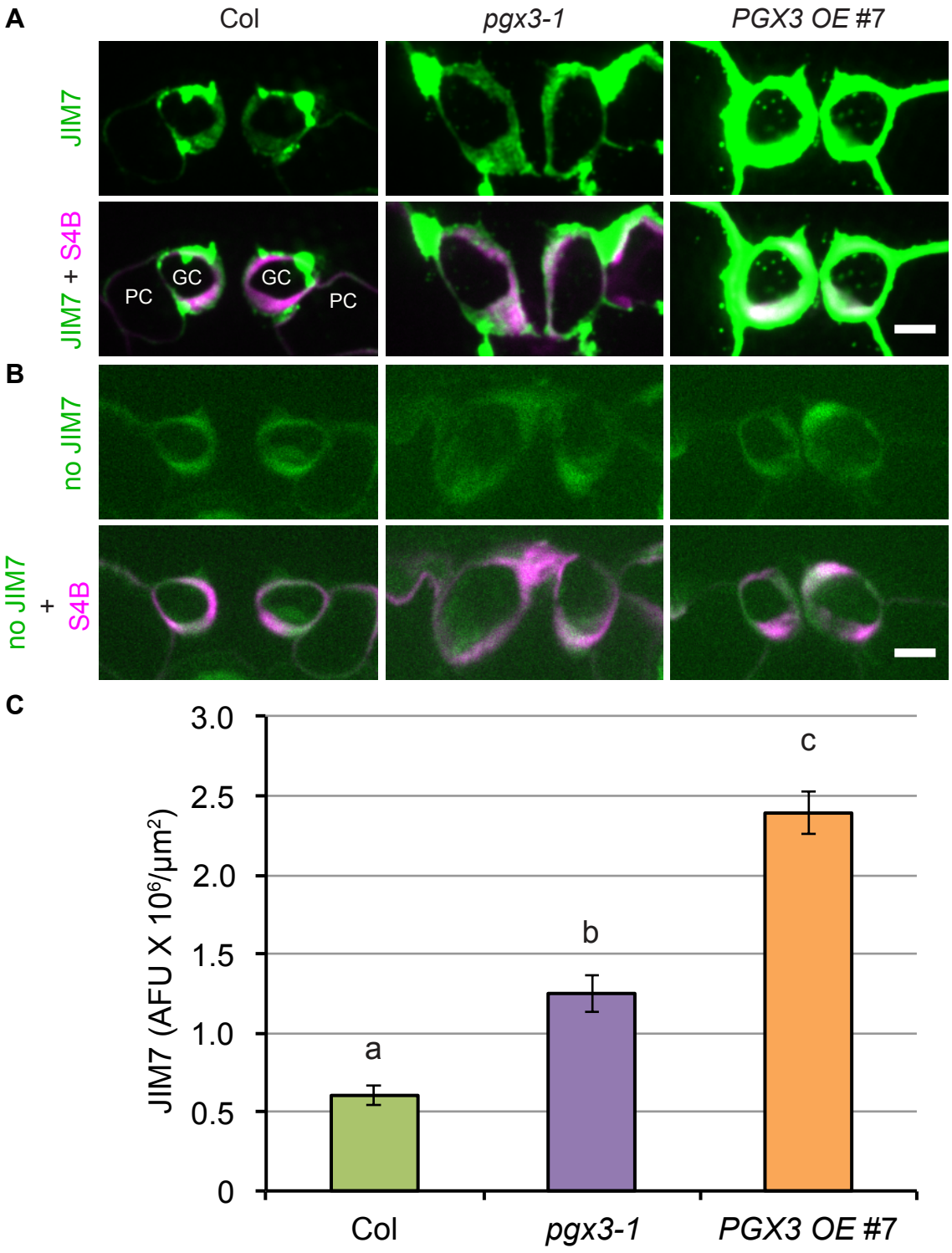
Supplemental Figure 8. Cellulose Organization in *pgx3-1* Mutant Guard Cells, and Pectin Labeling by COS^{488} or PI in Guard Cells and Neighboring Pavement Cells.
(Supports Figure 6.)
(A) and **(B)** S4B labeling of cellulose **(A)** and anisotropy quantifications **(B)** in Col and *pgx3-1* guard cells with open or closed stomata. Stomatal opening was induced by 1 μM FC for 2.5 h, whereas stomata closure was induced by 50 μM ABA for 2.5 h. Scale bar in **(A)** is 5 μm. Error bars in **(B)** are SE, and lowercase letters represent significantly different groups ($n \geq 13$ guard cell pairs per genotype per treatment from two independent experiments; $P < 0.05$, one-way ANOVA and Tukey test).
(C) Representative maximum projection images of COS^{488} and PI labeling in guard cells and neighboring pavement cells with ROIs outlined in green. Note that for PI staining in guard cells, the phenolic ester ring is excluded from ROIs. Images were applied with a fire look-up table. Scale bars: 5 μm.
(D) and **(E)** Quantifications of COS^{488} **(D)** and PI **(E)** fluorescence intensity in neighboring pavement cells of Col, *pgx3-1*, *PGX3 comp* #3, and *PGX3 OE* #7. Error bars are SE and lowercase letters represent significantly different groups ($n \geq 23$ pavement cells from 6 individual plants per genotype, two independent experiments; $P < 0.05$, one-way ANOVA and Tukey test).



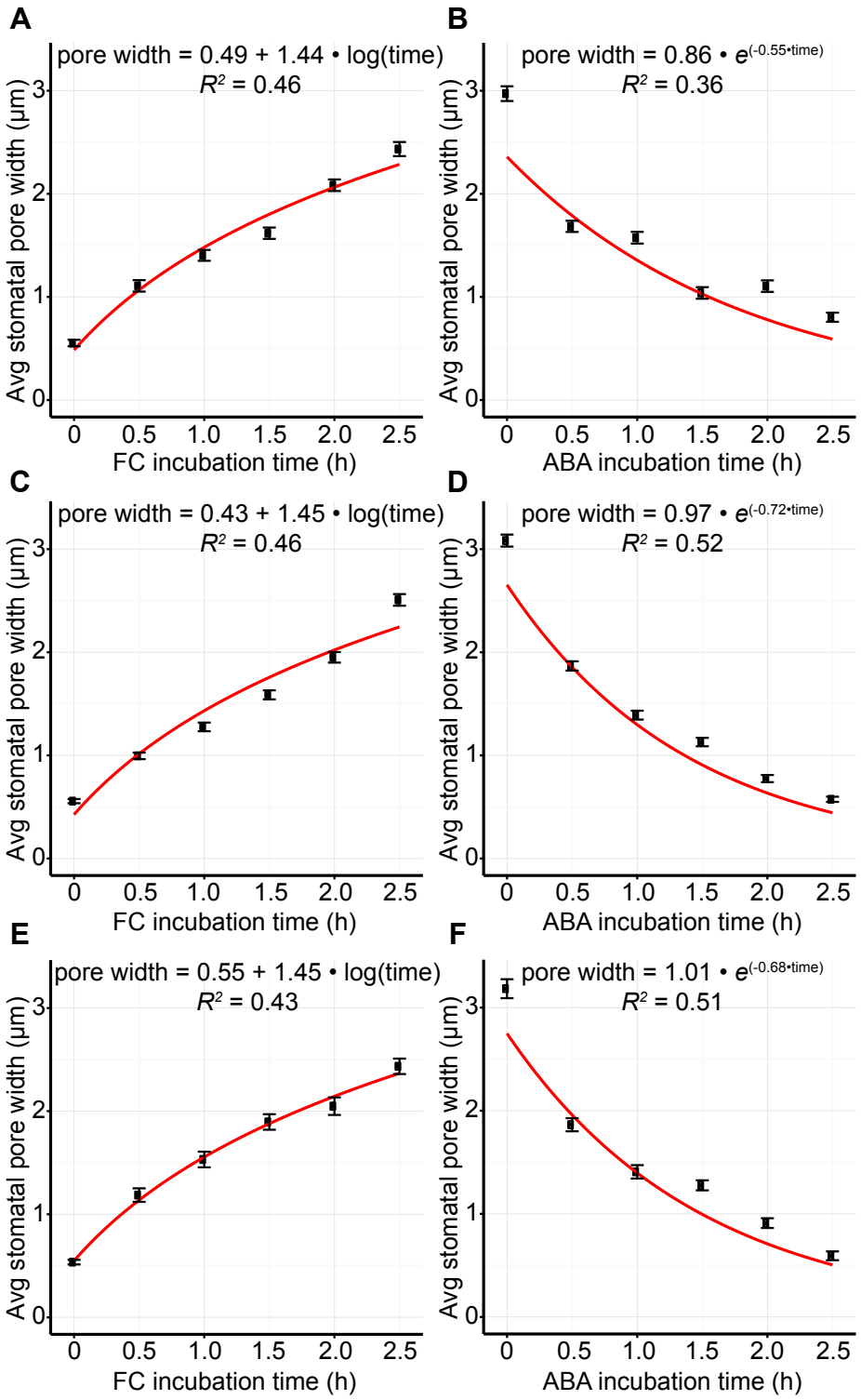
Supplemental Figure 9. Controls for Immunolabeling in Guard Cell Walls.
(Suppors Figure 7.)
(A) Controls without LM19 labeling but stained with S4B in cross-sections of guard cell pairs in 3- to 4-week-old Col, *pgx3-1*, and *PGX3 OE* #7. Top panel, green channel without LM19 labeling; bottom panel, green channel merged with S4B signals (magenta) in the same guard cell pair. GC, guard cells; PC, pavement cells.
(B) Controls without LM20 labeling but stained with S4B in cross-sections of guard cell pairs in 3- to 4-week-old Col, *pgx3-1*, and *PGX3 OE* #7. Top panel, green channel without LM20 labeling; bottom panel, green channel merged with S4B signals (magenta) in the same guard cell pair.
(C) Controls without 2F4 labeling but stained with S4B in cross-sections of guard cell pairs in 3- to 4-week-old Col, *pgx3-1*, and *PGX3 OE* #7. Top panel, green channel without 2F4 labeling; bottom panel, green channel merged with S4B signals (magenta) in the same guard cell pair.
Scale bars: 5 μm in **(A)** to **(C)**.


A
LM19 (AFU X 10⁵/μm²)
2.5
2.0
1.5
1.0
0.5
0
a
b
a
Col
pgx3-1
PGX3 OE #7
B
LM20 (AFU X 10⁶/μm²)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
a
b
c
Col
pgx3-1
PGX3 OE #7
C
2F4 (AFU X 10⁴/μm²)
7
6
5
4
3
2
1
0
ab
a
b
Col
pgx3-1
PGX3 OE #7

Supplemental Figure 10. Quantifications of LM19, LM20, and 2F4 Immunolabeling Intensity in Guard Cell Walls.
(Supports Figure 7.)
(A) Measurements of LM19 immunolabeling fluorescence intensity in guard cell walls of 3- to 4-week-old Col, *pgx3-1*, and *PGX3 OE* #7 plants. Error bars are SE and lowercase letters represent significantly different groups ($n \geq 32$ guard cells from three to four individual plants per genotype, three independent experiments; $P < 0.05$, one-way ANOVA and Tukey test).
(B) Measurements of LM20 immunolabeling fluorescence intensity in guard cell walls of 3- to 4-week-old Col, *pgx3-1*, and *PGX3 OE* #7 plants. Error bars are SE and lowercase letters represent significantly different groups ($n \geq 41$ guard cells from three to four individual plants per genotype, three independent experiments; $P < 0.05$, one-way ANOVA and Tukey test).
(C) Measurements of 2F4 immunolabeling fluorescence intensity in guard cell walls of 3- to 4-week-old Col, *pgx3-1*, and *PGX3 OE* #7 plants. Error bars are SE and lowercase letters represent significantly different groups ($n \geq 35$ guard cells from three to four individual plants per genotype, three independent experiments; $P < 0.05$, one-way ANOVA and Tukey test).



Supplemental Figure 11. JIM7 Immunolabeling in Guard Cell Walls.
(Supports Figure 7.)
(A) Co-labeling of JIM7, an antibody that recognizes highly methyl-esterified HG, and S4B in cross-sections of guard cell pairs in 3- to 4-week-old Col, *pgx3-1*, and *PGX3 OE* #7 plants. Top panel, JIM7 labeling; bottom panel, JIM7 labeling (green) merged with S4B signals (magenta) in the same guard cell pair. GC, guard cells; PC, pavement cells. Scale bar is 5 µm.
(B) Controls without JIM7 labeling but stained with S4B in cross-sections of guard cell pairs in 3- to 4-week-old Col, *pgx3-1*, and *PGX3 OE* #7 plants. Top panel, green channel without JIM7 labeling; bottom panel, green channel merged with S4B signals (magenta) in the same guard cell pair. Scale bar is 5 µm.
(C) Measurements of JIM7 immunolabeling fluorescence intensity in guard cell walls of 3- to 4-week-old Col, *pgx3-1*, and *PGX3 OE* #7 plants. Error bars are SE and lowercase letters represent significantly different groups ($n \geq 32$ guard cells from three to four individual plants per genotype, three independent experiments; $P < 0.05$, one-way ANOVA and Tukey test).



Supplemental Figure 12. Mathematical Fits of Stomatal Opening and Closure in Col, *pgx3-1*, and *PGX3 OE* #7 Plants.
(Supports Figure 9.)
(A), **(C)**, and **(E)** Mathematical fits of FC-induced stomatal opening in *pgx3-1* mutant **(A)**, Col **(C)**, and *PGX3 OE* #7 **(E)**, which show monotonic pore width increase. Data points are the same as in Figure 5A, with error bars representing SE.
(B), **(D)**, and **(F)** Mathematical fits of ABA-induced stomatal closure in *pgx3-1* mutant **(B)**, Col **(D)**, and *PGX3 OE* #7 **(F)**, which show larger pore width decrease in the first 30 minutes followed by attenuated pore width decrease. Data points are the same as in Figure 5B, with error bars representing SE.

Supplemental Table 1. Measurement of Stomatal Pore Dimensions, Guard Cell Pair Dimensions, and Guard Cell Dimensions in Wild Type and *PGX3 OE* #7 Plants during FC Treatment.

(Supports Figure 5.)

Treatment	Time after treat-ment (h)	Genotype	Avg stomatal pore width (µm)	Avg stomatal pore length (µm)	Avg ratio of pore width/pore length	Avg guard cell pair height (µm)	Avg guard cell pair width (µm)	Avg aspect ratio of guard cell pairs	Avg ratio of pore width /guard cell pair width	Avg guard cell diameter (µm)	Avg guard cell length (µm)	Δ avg guard cell length (µm)
FC	0	Col	0.5 ± 0.0^{a}	7.3 ± 0.1^{a}	0.05 ± 0.00^{a}	23.5 ± 0.2^{a}	17.7 ± 0.1^{a}	1.33 ± 0.01^{a}	0.02 ± 0.00^{a}	7.8 ± 0.1^{a}	22.2 ± 0.2^{a}	NA
		PGX3 OE #7	0.5 ± 0.0^{a}	7.0 ± 0.1^{a}	0.05 ± 0.00^{a}	22.9 ± 0.2^{ac}	17.1 ± 0.1^{b}	1.34 ± 0.01^{a}	0.02 ± 0.00^{a}	7.6 ± 0.0^{a}	21.6 ± 0.2^{a}	NA
	2.5	Col	2.5 ± 0.1^{b}	7.9 ± 0.2^{b}	0.31 ± 0.01^{b}	22.5 ± 0.2^{bc}	19.7 ± 0.2^{c}	1.15 ± 0.01^{b}	0.12 ± 0.00^{b}	7.8 ± 0.0^{a}	23.2 ± 0.2^{b}	1.0
		PGX3 OE #7	2.4 ± 0.1^{b}	7.4 ± 0.2^{ab}	0.32 ± 0.01^{b}	22.1 ± 0.2^{b}	19.4 ± 0.1^{c}	1.14 ± 0.01^{b}	0.12 ± 0.00^{b}	7.7 ± 0.0^{a}	22.6 ± 0.2^{a}	1.0

Stomatal pore width, stomatal pore length, ratio of pore width to pore length, guard cell pair height, guard cell pair width, aspect ratio of guard cell pairs (height/width), ratio of pore width to guard cell pair width, guard cell diameter, and guard cell length were measured on a single stoma basis using epidermal peels from 3-week-old plants. Values are presented as mean ± SE ($n \geq 100$ stomata for each genotype at each time point from three experiments). Lowercase letters indicate significantly different groups ($P < 0.05$, one-way ANOVA and Tukey test).

Supplemental Table 2. Measurement of Stomatal Pore Dimensions, Guard Cell Pair Dimensions, and Guard Cell Dimensions in Wild Type and *pgx3-1* Mutants during ABA Treatment.

(Supports Figure 5.)

Treatment	Time after treat-ment (h)	Genotype	Avg stomatal pore width (µm)	Avg stomatal pore length (µm)	Avg ratio of pore width /pore length	Avg guard cell pair height (µm)	Avg guard cell pair width (µm)	Avg aspect ratio of guard cell pairs	Avg ratio of pore width /guard cell pair width	Avg guard cell diameter (µm)	Avg guard cell length (µm)	Δ avg guard cell length (µm)
ABA	0	Col	3.0 ± 0.1^{a}	6.8 ± 0.1^{a}	0.41 ± 0.01^{a}	21.2 ± 0.2^{a}	18.9 ± 0.1^{a}	1.12 ± 0.01^{a}	0.14 ± 0.00^{a}	7.1 ± 0.0^{a}	22.5 ± 0.2^{a}	NA
		pgx3-1	3.0 ± 0.1^{a}	7.9 ± 0.1^{b}	0.37 ± 0.03^{b}	22.2 ± 0.2^{b}	19.2 ± 0.1^{a}	1.16 ± 0.01^{a}	0.15 ± 0.00^{a}	7.3 ± 0.0^{ab}	23.4 ± 0.2^{b}	NA
	2.5	Col	0.6 ± 0.0^{b}	6.9 ± 0.1^{a}	0.07 ± 0.00^{c}	22.8 ± 0.2^{bc}	17.3 ± 0.1^{b}	1.33 ± 0.01^{b}	0.02 ± 0.00^{b}	7.3 ± 0.1^{b}	22.1 ± 0.2^{a}	-0.4
		pgx3-1	0.8 ± 0.0^{b}	7.5 ± 0.2^{b}	0.07 ± 0.01^{c}	23.5 ± 0.2^{c}	17.3 ± 0.1^{b}	1.36 ± 0.02^{b}	0.03 ± 0.00^{b}	7.3 ± 0.0^{ab}	22.6 ± 0.2^{a}	-0.8

Stomatal pore width, stomatal pore length, ratio of pore width to pore length, guard cell pair height, guard cell pair width, aspect ratio of guard cell pairs (height/width), ratio of pore width to guard cell pair width, guard cell diameter, and guard cell length were measured on a single stoma basis using epidermal peels from 3-week-old plants. Values are presented as mean ± SE ($n \geq 134$ stomata for each genotype at each time point from three experiments). Lowercase letters indicate significantly different groups ($P < 0.05$, one-way ANOVA and Tukey test).

Supplemental Table 3. Primers Used in This Study.

Gene	Primer name	Primer sequence (5'-3')	Purpose
PGX3	SALK LBb1.3	ATTTTGCCGATTTCGGAAC	Genotyping
	SALK_010192C LP	ATAGTTACCCTGTGGGGCTTG	Genotyping
	SALK_010192C RP	CTCTTAAAAAGCCTCATGCCC	Genotyping
	SALK_019868 LP	CGCATCCTGAGTCAGAATCTC	Genotyping
	SALK_019868 RP	CGAGAGGCGAGATAATAGTGC	Genotyping
	SALK_022923C LP	TCCTGCTTCATCATACAAGCC	Genotyping
	SALK_022923C RP	TTCAAAACGCGATTTGAATTC	Genotyping
	PGX3 RT F1	CAGACTCAGTGGCATTCTCG	RT-PCR
	PGX3 RT R1	GCCACAAGTGACACCTTGAA	RT-PCR
	PGX3 RT F2	TTACGTCGTTTGGAGCAGTG	RT-PCR
	PGX3 RT R2	CGTTACGGCAATCTTTGGAT	RT-PCR
	PGX3 RT F3	AAATTCGACGGATGTCAAGG	RT-PCR
	PGX3 RT R3	AGCCCATAGAGACAGGCATC	RT-PCR
	PGX3 qF	AAGTCCACCGATTCATTTCG	qPCR
	PGX3 qR	TCCGGCAATAACTCAACCTC	qPCR
	PGX3 promoter F	AATCGATCAATCGCTGTCGT	Cloning
	PGX3 promoter R	TGTTTAGCTTTGAGAAGAGAGAAATGGAGAAA	Cloning
	PGX3 CDS F	ATGCGCAGACTCAGTGGCAT	Cloning
	PGX3 CDS Overlap F	TCTCAAAGCTAAACAATGCGCAGACTCAGTGGCAT	Cloning
	PGX3 CDS R	ACAACCATAATTCGAATCATA	Cloning
ACT2	ACT2 RT F	CACTGTGCCAATCTACGAGGGT	RT-PCR
	ACT2 RT R	CACAAACGAGGGCTGGAACAAG	RT-PCR
	ACT2 qF	CTTGCACCAAGCAGCATGAA	qPCR
	ACT2 qR	CCGATCCAGACACTGTACTTCCTT	qPCR