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Supplementary Materials for

Touch-induced seedling morphological changes are determined by ethylene-regulated pectin degradation

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This PDF file includes:

SUPPLEMENTARY MATERIALS

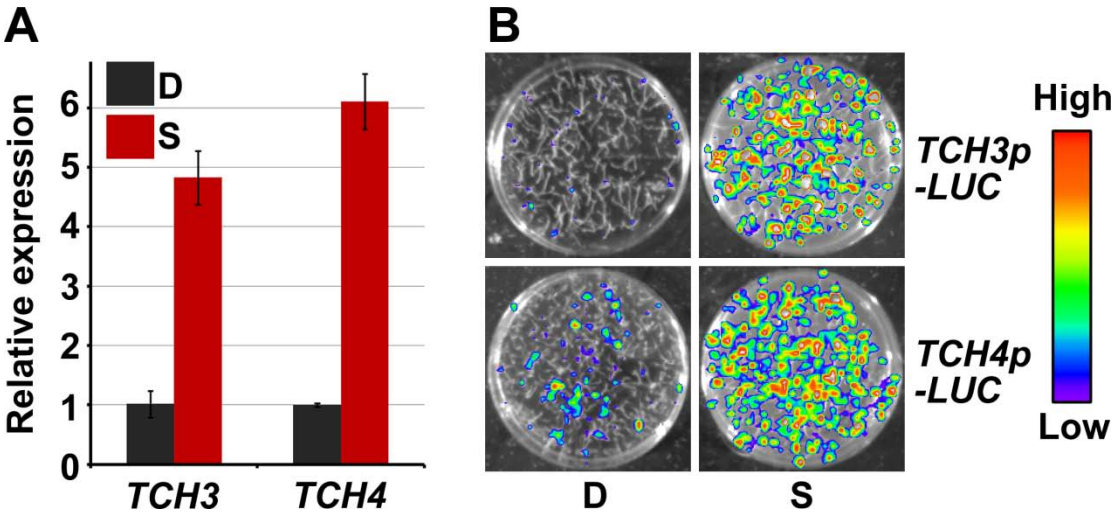

Fig. S1. Analysis of *TCH3* and *TCH4* gene expression following touch treatment in the S condition.

(**A**) RT-qPCR analysis of *TCH3* and *TCH4* gene expression in 3-day-old etiolated seedlings in the D or S condition. Mean ± SD; n = 3.

(**B**) Bioluminescent images of luciferase activity showing changes in the gene expression levels of *TCH3* and *TCH4* in response to touch. The *TCH3p-LUC* and *TCH4p-LUC* transgenic seedlings were grown in the D or S condition for 3 days.

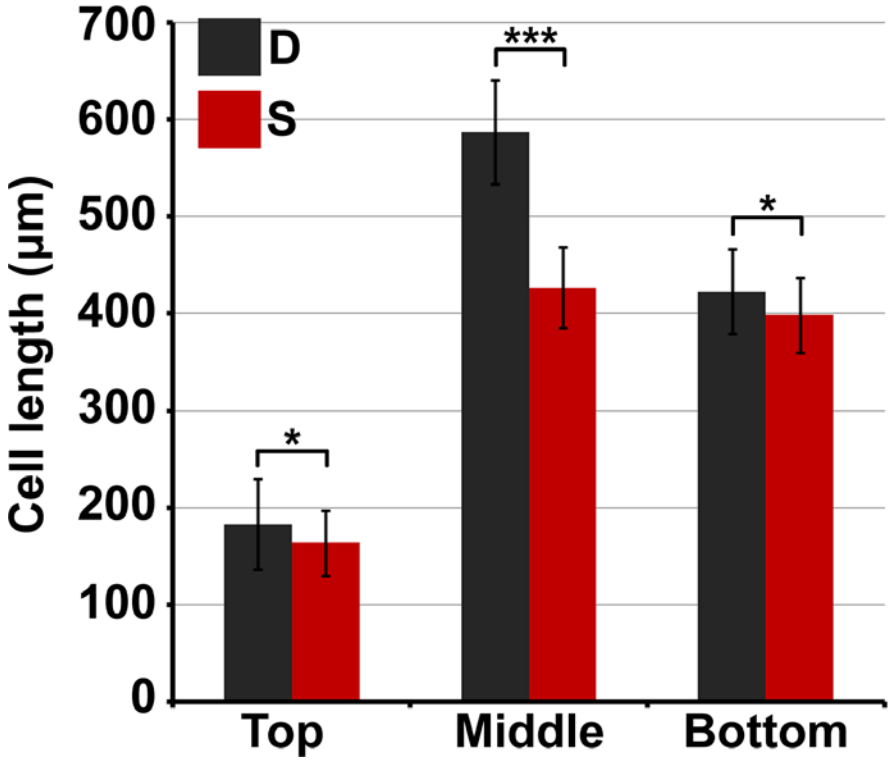

Fig. S2. Length of cells at the top, middle and bottom regions of the hypocotyl following mechanical stimulation.

Etiolated seedlings were grown in the D or S condition for three days. Mean ± SD; n ≥ 15. Significant differences (* $P < 0.05$ and *** $P < 0.001$, Student's t-test) are indicated by the asterisks.

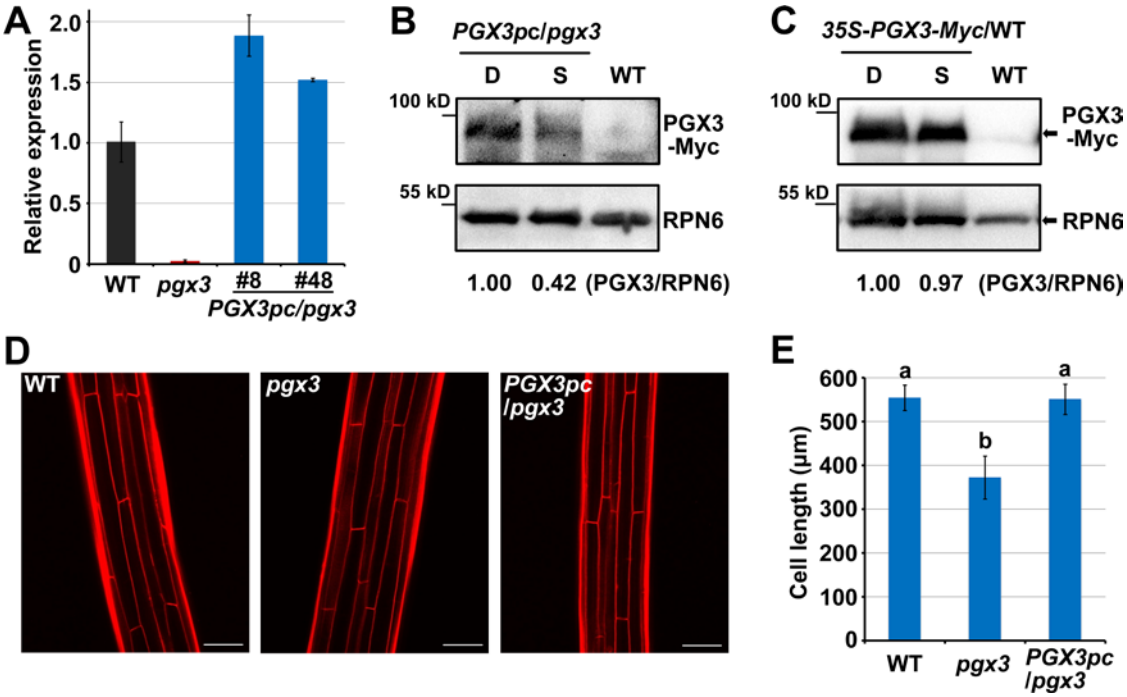

Fig. S3. Characterization of PGX3 protein accumulation and morphology of *pgx3* and *PGX3pc*/*pgx3* seedlings.

(**A**) RT-qPCR results showing *PGX3* gene expression in *pgx3* mutant and complementary *PGX3pc*/*pgx3* transgenic plants. Seedlings were grown in the dark for 3 days. #8 and #48 are two independent *PGX3pc*/*pgx3* transgenic lines. #8 was used in the studies unless specified otherwise. Mean ± SD; n = 3.

(**B** and **C**) Immunoblot analysis of PGX3 protein abundance in response to mechanical resistance. *PGX3p-PGX3-Myc*/*pgx3* (*PGX3pc*/*pgx3*) (**B**) or *35S-PGX3-Myc*/WT (**C**) seedlings were grown in the D or S condition for 3 days. Anti-Myc and anti-RPN6 antibodies were used for immunoblots. WT seedlings were used as the negative control group. RPN6 was used as the loading control. PGX3/RPN6 indicates the relative band intensities of PGX3-Myc normalized to RPN6 and is presented relative to that in the D condition set at unity.

(**D** and **E**) PI staining images (**D**) and cell length (**E**) of cells at the middle region of hypocotyls. WT, *pgx3* and *PGX3pc*/*pgx3* seedlings were grown in the D condition for 3 days. Scale bar = 100 μm. Mean ± SD; $\geq$n15. Lowercase letters above the bars represent significantly different groups, $P < 0.05$, one-way ANOVA and Tukey test.

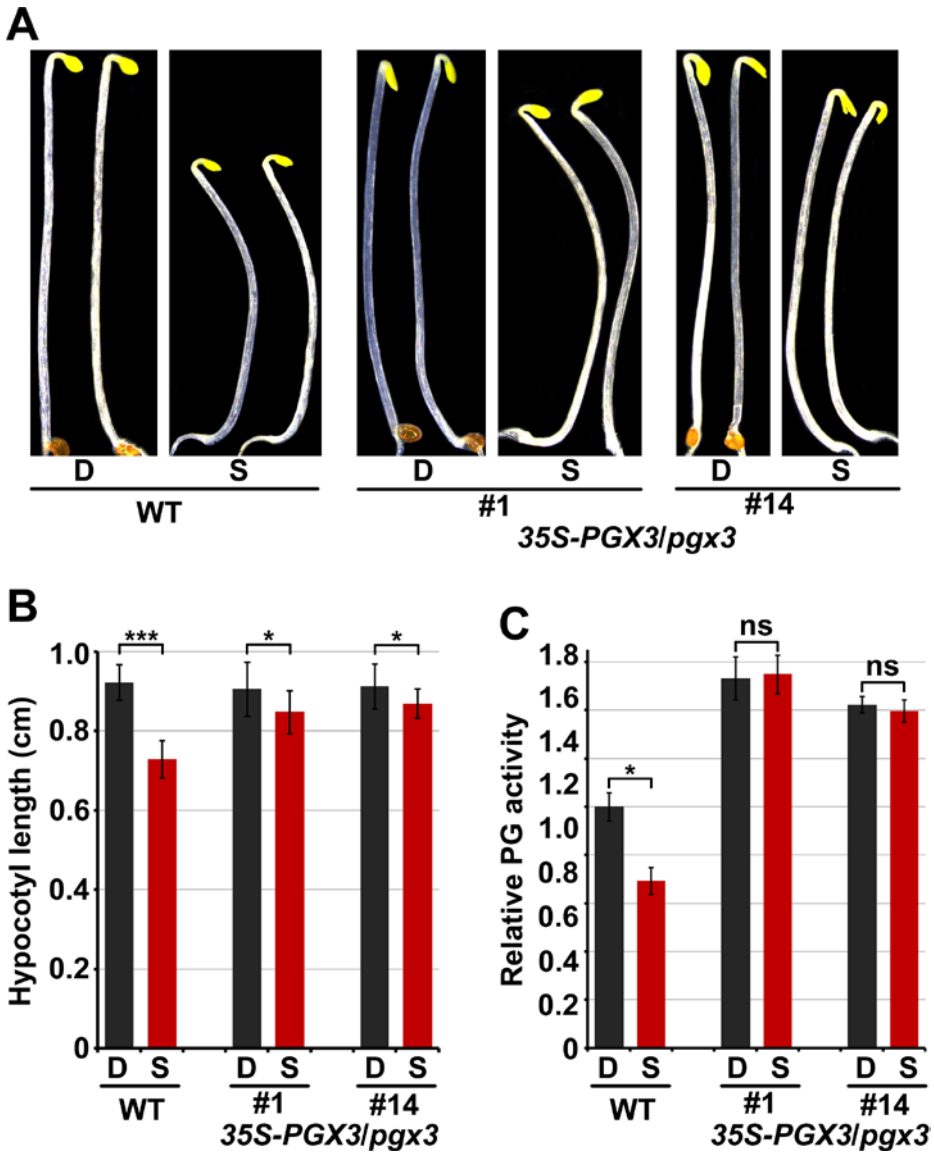

Fig. S4. Hypocotyl length and PG activity of *35S-PGX3*/*pgx3* in response to mechanical stimulation.

(**A** to **C**) Images (**A**), hypocotyl length (**B**), and PG activity (**C**) of etiolated seedlings in response to mechanical resistance. WT and *35S-PGX3*/*pgx3* were grown in the D or S condition for 3 days. #1 and #14 are two independent *35S-PGX3*/*pgx3* transgenic lines. Mean ± SD; n ≥ 20. Significant differences (* $P < 0.05$ and *** $P < 0.001$, Student's t-test) are indicated by the asterisks. The relative PG activities of the samples were normalized to the absorbance of WT seedlings grown in the D condition. Mean ± SD; n = 3. Significant differences (* $P < 0.05$, Student's t-test) are indicated by the asterisks.

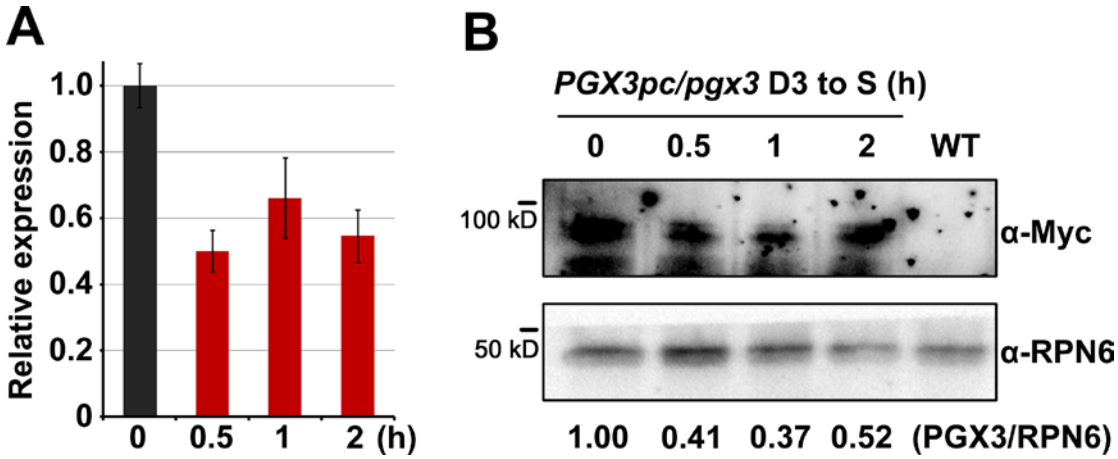

Fig. S5. Time-course analysis of *PGX3* expression and PGX3 protein accumulation in response to mechanical stimulation.

(**A**) RT-qPCR analysis of *PGX3* gene expression in response to a time course of touch treatment. WT seedlings were grown in the D condition for 3 days and then were touched by lid for the indicated periods. Mean ± SD; n = 3.

(**B**) Immunoblot analysis of PGX3 protein levels in response to a time course of touch treatment. *PGX3pc*/*pgx3* seedlings were grown in the D condition for 3 days and then were touched by lid for the indicated periods. WT seedlings were used as the negative control group. RPN6 was used as the loading control. PGX3/RPN6 indicates the relative band intensities of PGX3-Myc normalized to RPN6 and is presented relative to that in the D condition set at unity.

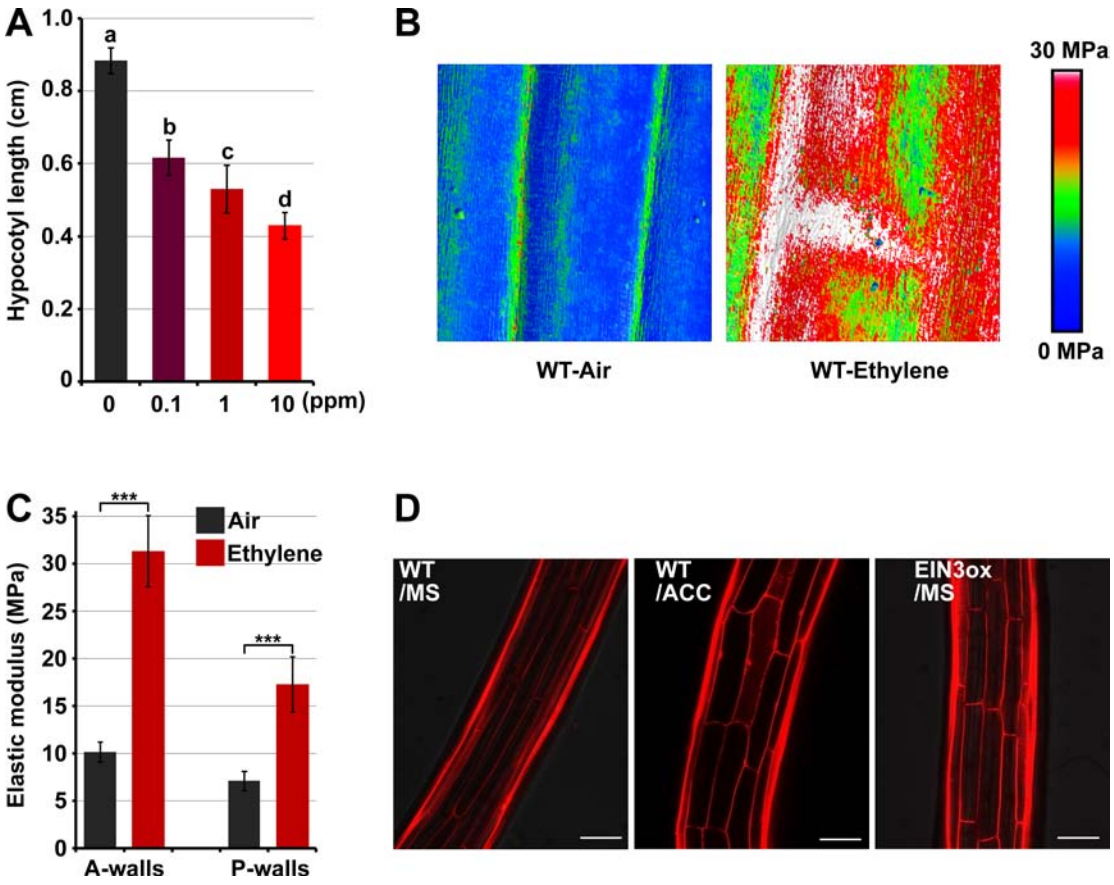

Fig. S6. Characterization of seedling morphology following ethylene treatment or EIN3 overexpression.

(**A**) Hypocotyl length of etiolated seedlings in response to ethylene treatment. Two-day-old WT etiolated seedlings in the D condition were treated with the indicated concentrations (air, 0.1, 1, 10 ppm) of ethylene for an additional day. Mean ± SD; n ≥ 20. Lowercase letters above the bars represent significantly different groups, $P < 0.05$, one-way ANOVA and Tukey test.

(**B** and **C**) Elastic modulus map (**B**) and quantification (**C**) of epidermal cell walls at the middle region of hypocotyls. WT seedlings were grown in the D condition for 2 days and then supplemented without (Air) or with (Ethylene) 10 ppm ethylene. The elastic modulus (stiffness) map is scaled by color. The elastic modulus on the anticlinal (A-walls) and outer periclinal (P-walls) cell walls was obtained by AFM in QNM mode. Mean ± SD; n≥ 10. Significant differences (*** $P < 0.001$, Student's t-test) are indicated by the asterisks.

(**D**) PI staining images of cells at the middle region of hypocotyls. Seedlings were grown in the D condition for 3 days on 1/2 MS medium (MS) or supplemented with ACC (ACC). Scale bar = 100 μm.

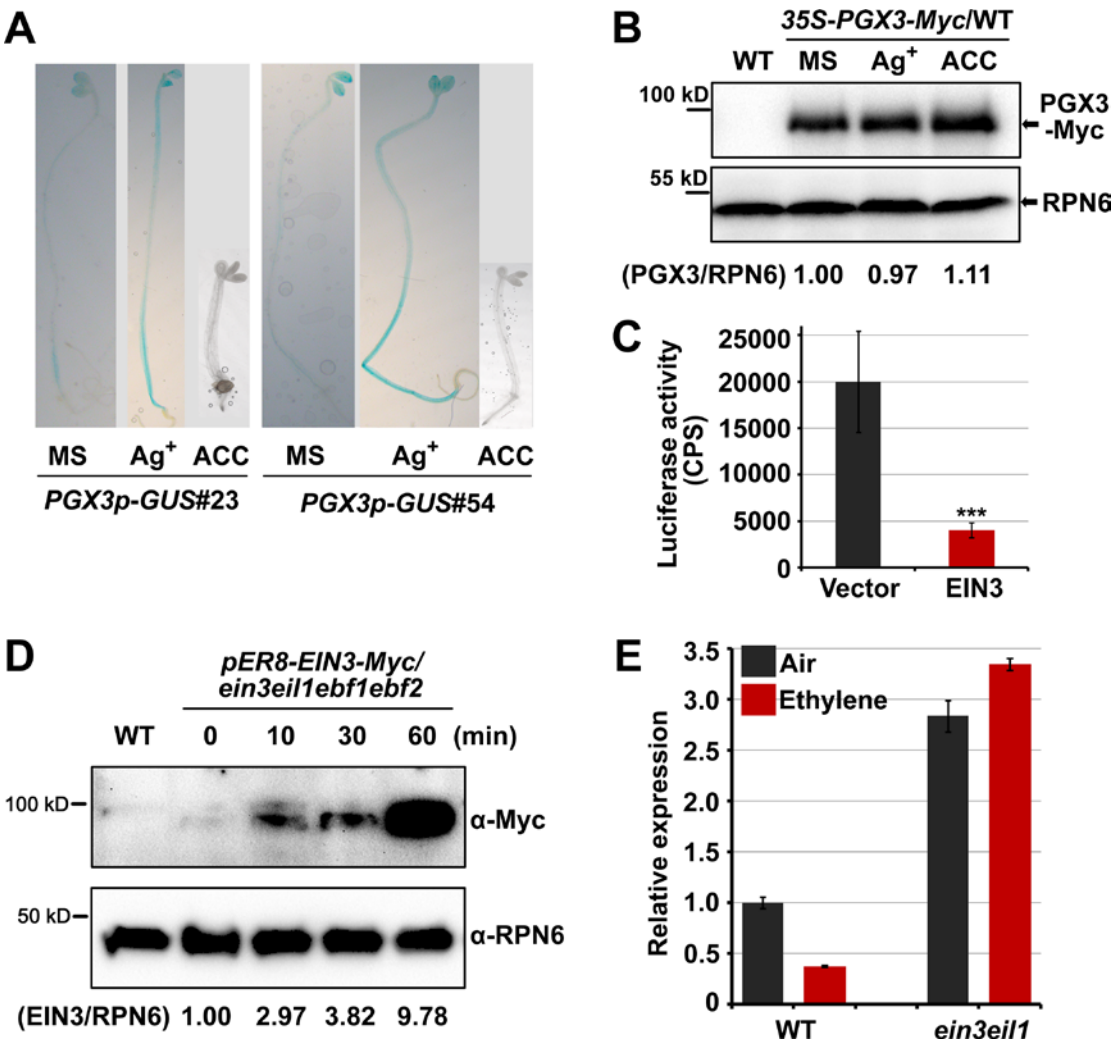

Fig. S7. Transcriptional regulation of *PGX3* by the ethylene-EIN3 pathway.

(**A**) GUS staining images of *PGX3p*-GUS etiolated seedlings. Seedlings were grown in the D condition for 3 days on 1/2 MS medium (MS), supplemented with ACC (ACC), or $AgNO_3$ (Ag^+). #23 and #54 are two independent *PGX3p-GUS* transgenic lines in the WT background.

(**B**) Immunoblot analysis of constitutively expressed PGX3 protein levels in *35S-PGX3-Myc*/WT seedlings. Seedlings were grown in the D condition for 3 days on 1/2 MS medium (MS), supplemented with ACC (ACC), or $AgNO_3$ (Ag^+). Anti-Myc and anti-RPN6 antibodies were used for immunoblotting. WT seedlings were used as the negative control group. RPN6 was used as the loading control. PGX3/RPN6 indicates the relative band intensities of PGX3-Myc normalized to RPN6 and is presented relative to that grown on 1/2 MS medium set at unity.

(**C**) EIN3 represses *PGX3* transcription in a transient expression system in tobacco leaves. *PGX3p-LUC* was co-expressed with the *35S* vector (Vector) or *35S*-EIN3 (EIN3) in tobacco leaves. Mean ± SD; $n \geq 5$. Significant differences (*** $P < 0.001$, Student's t-test) are indicated by the asterisks.

(**D**) Immunoblot analysis of induced-EIN3 protein levels in 3-day-old etiolated *pER8-EIN3-Myc*/*ein3eil1ebf1ebf2* seedlings treated with β-estradiol for the indicated periods. Anti-Myc and anti-RPN6 antibodies were used for immunoblotting. WT seedlings were used as the negative control group. RPN6 was used as the loading control. EIN3/RPN6 indicates the relative band intensities of EIN3-Myc normalized to RPN6 and is presented relative to that without β-estradiol treatment set at unity.

(**E**) RT-qPCR analysis of changes in *PGX3* gene expression in response to ethylene treatment. Three-day-old etiolated seedlings were treated without (Air) or with (Ethylene) 10 ppm ethylene for 0.5 h. Mean ± SD; n=3.

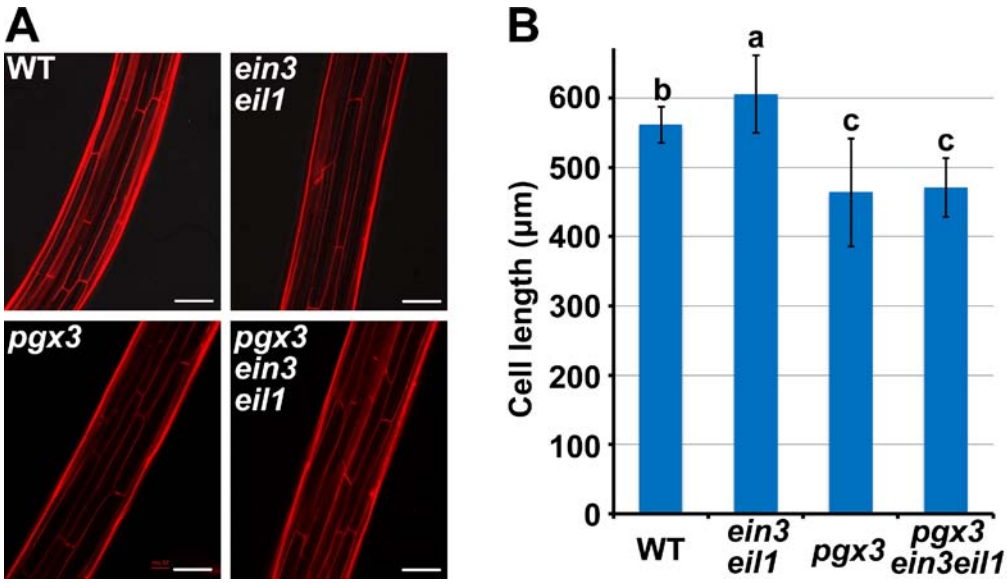

Fig. S8. Genetic relationship between PGX3 and EIN3 in regulating hypocotyl cell elongation.

PI staining images (**A**) and cell length (**B**) of the cells at the middle region of hypocotyls. Seedlings were grown in the D condition for 3 days. Scale bar = 100 μm. Mean ± SD; n≥ 15. Lowercase letters above the bars represent significantly different groups, $P < 0.05$, one-way ANOVA and Tukey test.

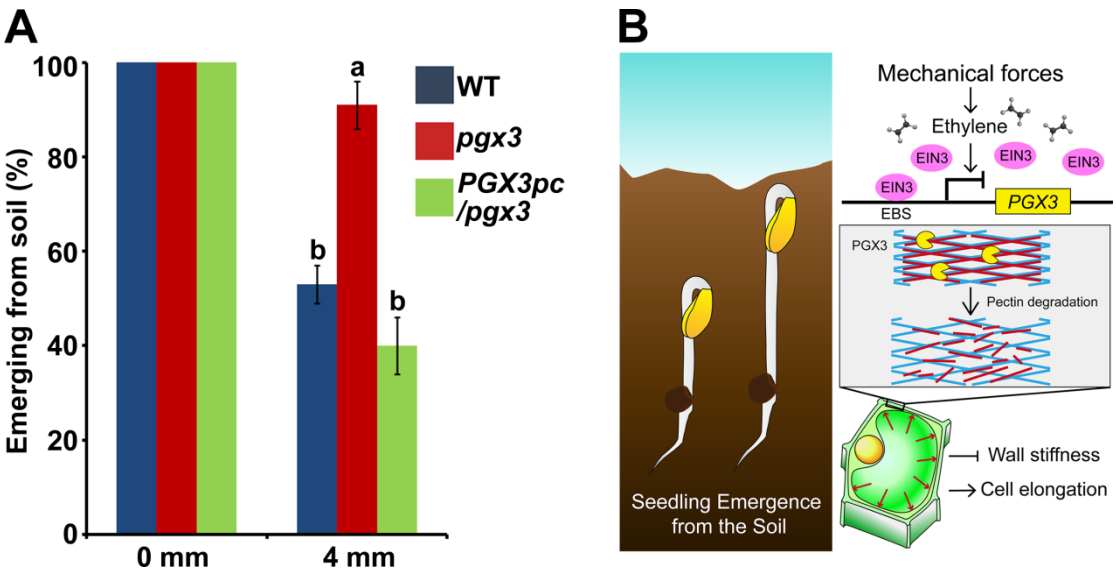

Fig. S9. EIN3-PGX3 mediates touch-induced morphological changes to facilitate soil emergence.

(**A**) Soil emergence rate of seedlings with soil cover. Seedlings were grown in the dark for 7 days on 1/2 MS medium without (0 mm) or with 4 mm sand cover, and the emergence rate was counted. Mean ± SE, n = 3. Lowercase letters above the bars represent significantly different groups, $P < 0.05$, one-way ANOVA and Tukey test.

(**B**) A proposed model showing that the EIN3-PGX3 module mediates touch-induced morphological changes of etiolated seedlings. Mechanical stimulation from the physical barrier of soil cover induces ethylene production and stabilizes EIN3 protein. EIN3 directly binds to the *PGX3* promoter and represses transcription. This EIN3-PGX3 module responds to touch by increasing hypocotyl cell wall stiffness and inhibiting cell elongation, improving resistance to force stresses and facilitating seedling soil emergence.

Table S1. Primers used in this study.

Primer's Name	Primer Sequence (5'→3')	Assay
SALK _LBb1.3	ATTTTGCCGATTTCGGAAC	Genotyping primers
SALK_010192 LP	CTTTCTCTGGCCATTCCTCT	
SALK_010192 RP	GTCAAATTTTCGACATAGATGAAAAAC	
RTM-PGX1-F	CGGAGAATAGCCCTAACACC	RT-qPCR primers
RTM-PGX1-R	TGATTCGAACACCAGCAAGAG	
RTM-PGX2-F	CGAAGCTATGGTGCTCGTGG	
RTM-PGX2-R	CCGTGAGATAGTCAACGTACC	
RTM-PGX3-F	AAGTCCACCGATTCATTTCG	
RTM-PGX3-R	TCCGGCAATAACTCAACCTC	
RTM-TCH3-F	TGGCACCAATTGGGAGAGGGACATA	
RTM-TCH3-R	CATGAAGTCGGAAATTGGCGAAGCG	
RTM-TCH4-F	CGCAGGAACAGTCACAACACTTTACTTGA	
RTM-TCH4-R	GTGTGTAAGGTTCTCCACTTGAATTCCCT	
RTM-PP2A-F	GTGACTTGGTTGAGCATTTCACTCC	
RTM-PP2A-R	GAGCTGATTCAATTGTAGCAGCAAAC	
RTM-SAND-F	CCAGAGACGAATTCGGAGCGT	
RTM-SAND-R	TCTCTCTCCATCGTAACCGCTACT	
ChIP-PGX3pro-A-F	GAAAAAGACAAGTCGTAAAAACATTAGAA TATTTG	Chip-qPCR primers
ChIP-PGX3pro-A-R	CTTTGAGAAGAGAGAAATGGAGAAAGAGA	
ChIP-PGX3pro-B-F	AAGAATTTTCTCGATACACAAGAAGG	
ChIP-PGX3pro-B-R	ATGTTTTTACGACTTGTCTTTTTCTCC	
ChIP-PGX3pro-C-F	CACTCTTATTGTATGTTGAAAGTTAACATAA GAT	
ChIP-PGX3pro-C-R	AATAAAACAAATCTTTTTAAGGTTTTGCAG ATTT	
PlacZi-PGX3pro-A-F	GAAAAAGACAAGTCGTAAAAAC	Cloning primers for yeast one-hybrid
PlacZi-PGX3pro-A-R	CTTTGAGAAGAGAGAAATGGA	
PlacZi-PGX3pro-B-F	AAGAATTTTCTCGATACACAAG	
PlacZi-PGX3pro-B-R	CAAATATTCTAATGTTTTTACGACT	
PlacZi-PGX3pro-C-F	CACTCTTATTGTATGTTGAAAGT	
PlacZi-PGX3pro-C-R	AATAAAACAAATCTTTTTAAGGTTTTG	
PGX3pro-A-Mutant-F	GTATTTGAATTATGTTTAAAAATTAAATATGA TTA	
PGX3pro-A-Control-F	GTATTTGAATTATGTTTCAAAATTAAATATGA TTA	
PGX3pro-C-Mutant-F	AAAAATTTTAGGTATTTAAATTATGTTTAAA AATTAAATA	
PGX3pro-C-Control-F	AAAAATTTTAGGTATTTGAATTATGTTTGAA AATTAAATA	
pB42AD-EIN3-F	ACTGCTAAAGAGAGTGCTACCTGG	

pB42AD-EIN3-R	CTTTTTCTGGCTTGAGCTCTTCCAC	
PGX3pro-A-F	TCGTCTCCACAAACTTCAAACTCTATTGTC TCTCTGTTCT	EMSA
PGX3pro-A-R	AGAACAGAGAGACAATAGAGTTTGAAGTT TGTGGAGACGA	
PGX3pro-C-F	AAAATTTTAGGTATTTGAATTATGTTTGAAA ATTAAATATGA	
PGX3pro-C-R	TCATATTTAATTTTCAAACATAATTCAAATAC CTAAAATTTT	