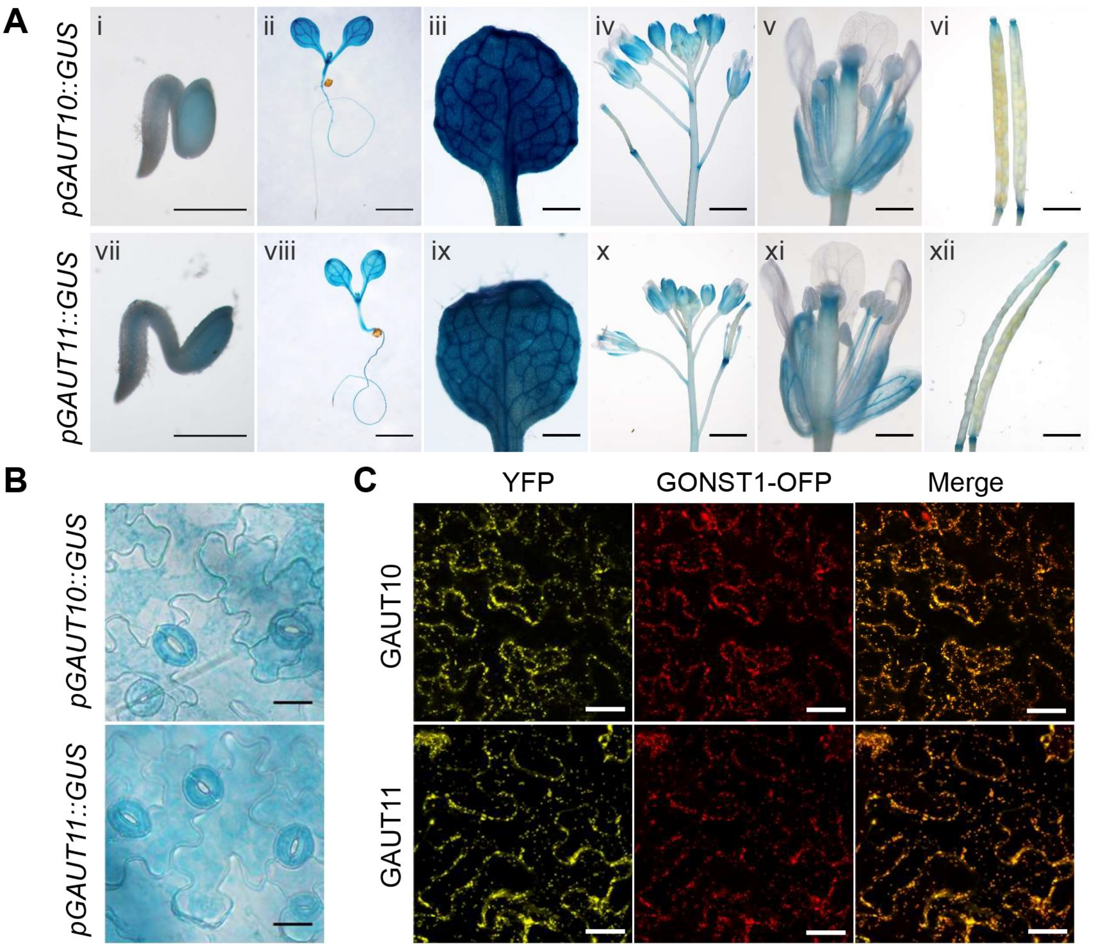
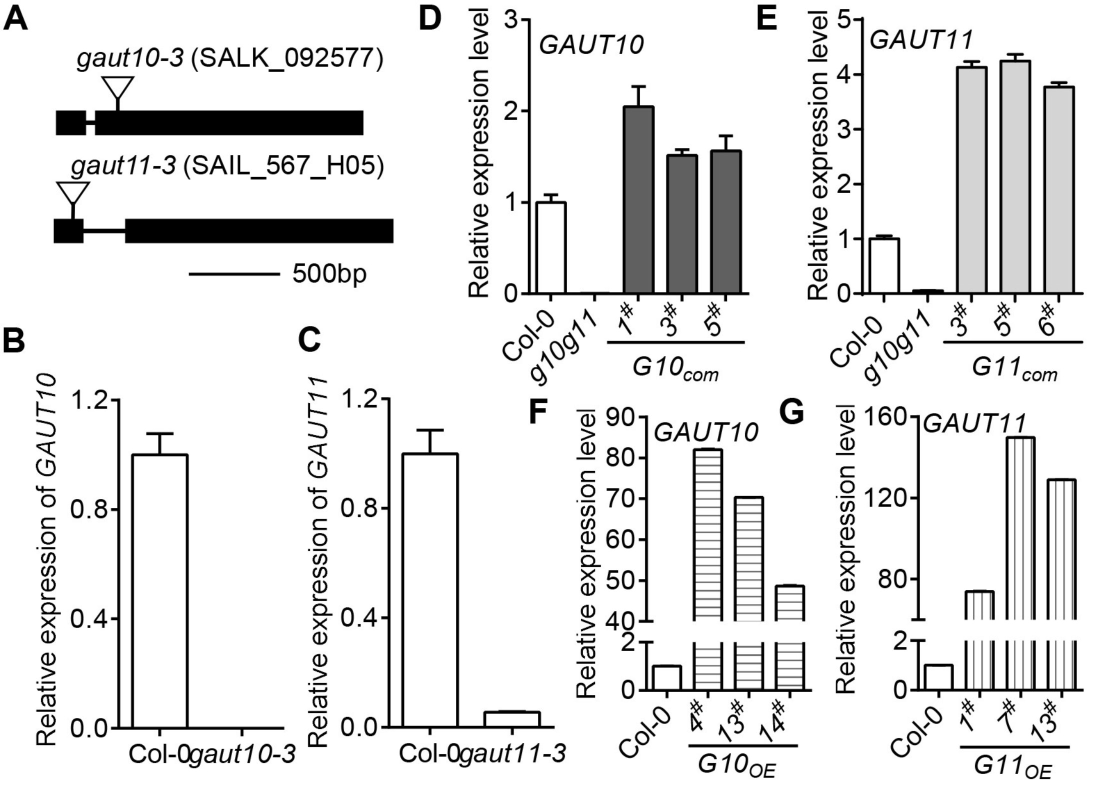


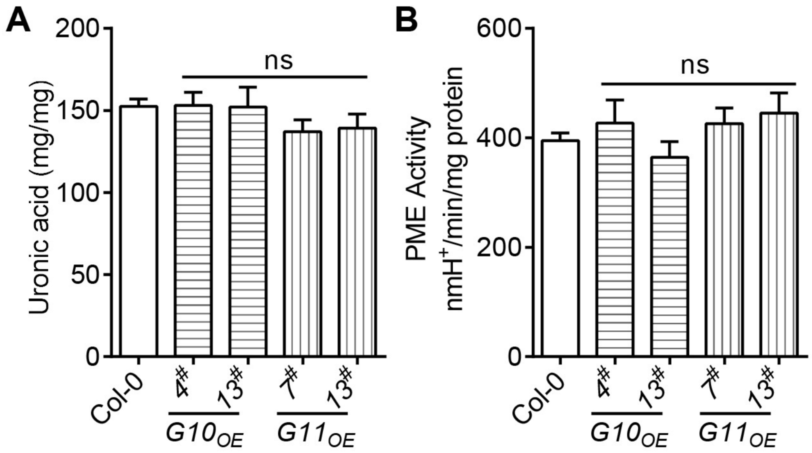
Supplemental Figure S1. Expression pattern and subcellular localization of GAUT10 and GAUT11.

A, GUS staining of tissues from *pGAUT10::GUS* and *pGAUT11::GUS* transgenic plants. (i) and (vii), 1-d-old embryo; (ii) and (viii), 6-d-old light-grown seedling; (iii) and (ix), true leaf from 9-d-old seedlings; (iv) and (x), inflorescence from 4-week-old plants; (v) and (xi), a flower; (vi) and (xii), siliques. Bars = 0.5 mm in (i), (iii), (v), (vii), (ix) and (xi), and 2 mm in (ii), (iv), (vi), (viii), (x) and (xii). B, GUS staining of rosette leaves from 3-week-old *pGAUT10::GUS* and *pGAUT11::GUS* transgenic lines. The GUS signals were abundant in guard cells. Bars = 20 μm. C, YFP-tagged GAUT10 and GAUT11 fusion proteins are co-localized with GONST1-OFP (Golgi apparatus marker) in Golgi in young *Nicotiana. benthamiana* leaf epidermis. The signals were visualized with a laser confocal microscope. Bars = 25 μm.



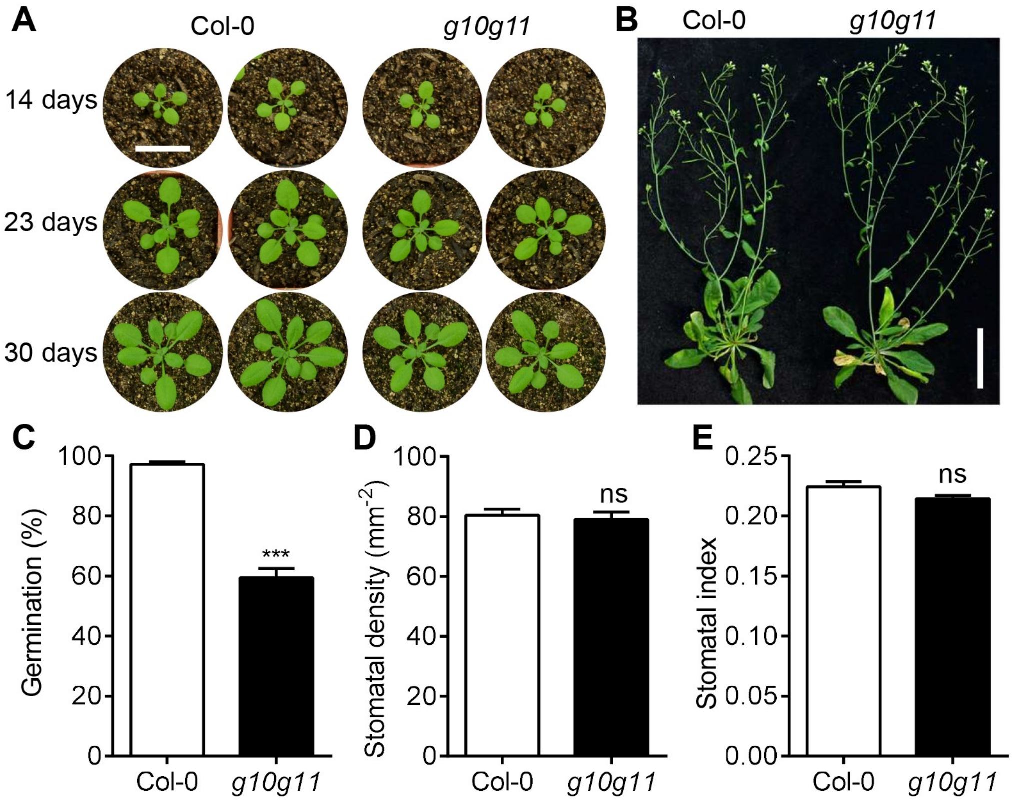
Supplemental Figure S2. Gene structure and transcript analyses of *GAUT10* and *GAUT11*.

A, Genomic structure of *GAUT10* and *GAUT11* genes and T-DNA insertions in the *gaut10-3* and *gaut11-3* mutant lines. Exons and introns are indicated as black box and black line, respectively. Inverted triangles represent the sites of T-DNA insertion. B and C, RT-qPCR analysis of *GAUT10* and *GAUT11* transcript abundance using gene-specific primers in *gaut10-3* (B) and *gaut11-3* (C) plants, respectively. Values are means ± SE. *n* ≥ 3. D and E, RT-qPCR quantification of *GAUT10* and *GAUT11* expression levels in Col-0, *g10g11*, *GAUT10* complementation (*$G10_{com}$ 1#, 3#, 5#*) lines (D) and *GAUT11* complementation (*$G11_{com}$ 3#, 5#, 6#*) lines (E), respectively. Values are means ± SE. *n* ≥ 3. F and G, RT-qPCR quantification of *GAUT10* and *GAUT11* expression levels in Col, *g10g11*, *GAUT10* overexpression (*$G10_{OE}$ 4#, 13#, 14#*) lines (F) and *GAUT11* overexpression (*$G11_{OE}$ 1#, 7#, 13#*) lines (G), respectively. Values are means ± SE. *n* ≥ 3.



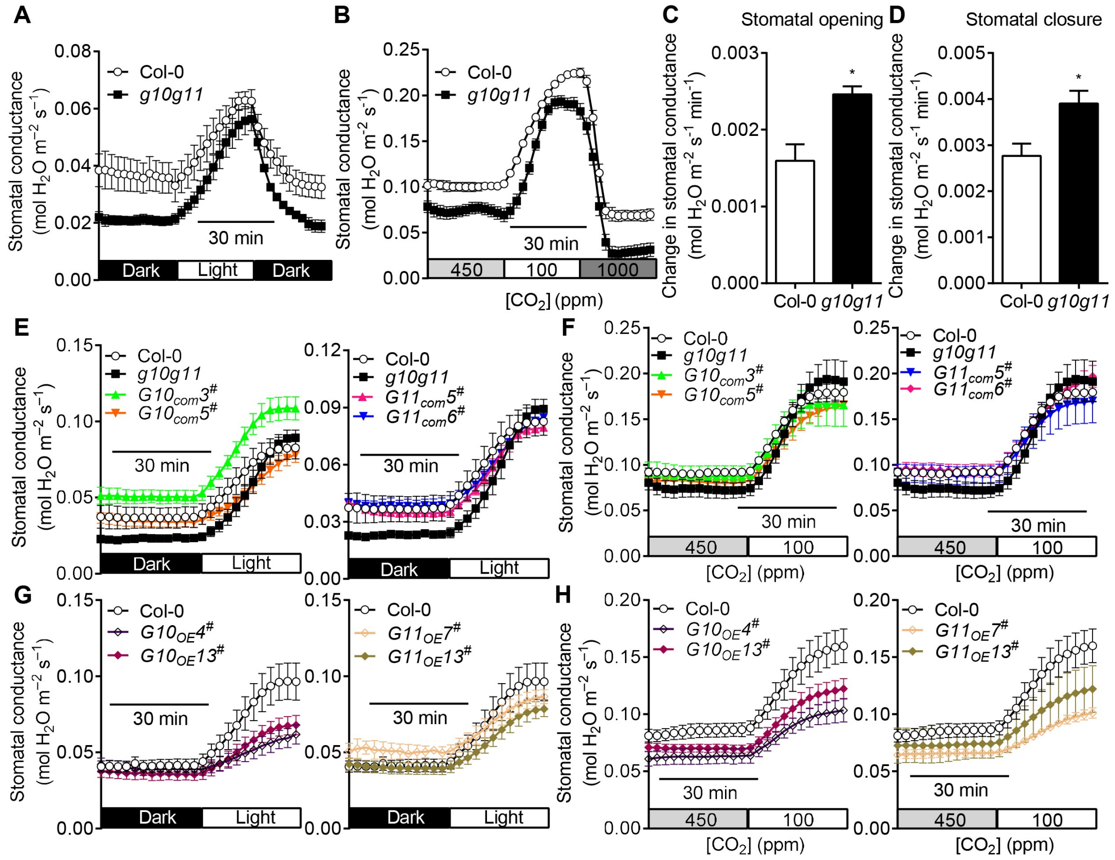
Supplemental Figure S3. Total uronic acid content and PME activity are not changed in *GAUT10* or *GAUT11* overexpression lines.

A, Uronic acid measurements in the leaves of 4-week-old Col-0, *GAUT10*-overexpression (*$G10_{OE}$ 4#*, *13#*) and *GAUT11*-overexpression (*$G11_{OE}$ 7#*, *13#*) plants. Values are means ± SE, three biological replicates. No significantly difference (ns), $P \geq 0.05$; Student's *t*-test. B, PME activity in the leaves of 4-week-old Col-0, *GAUT10*-overexpression (*$G10_{OE}$ 4#*, *13#*) and *GAUT11*-overexpression (*$G11_{OE}$ 7#*, *13#*) plants. Values are means ± SE, three biological replicates. No significantly difference (ns), $P \geq 0.05$; Student's *t*-test.



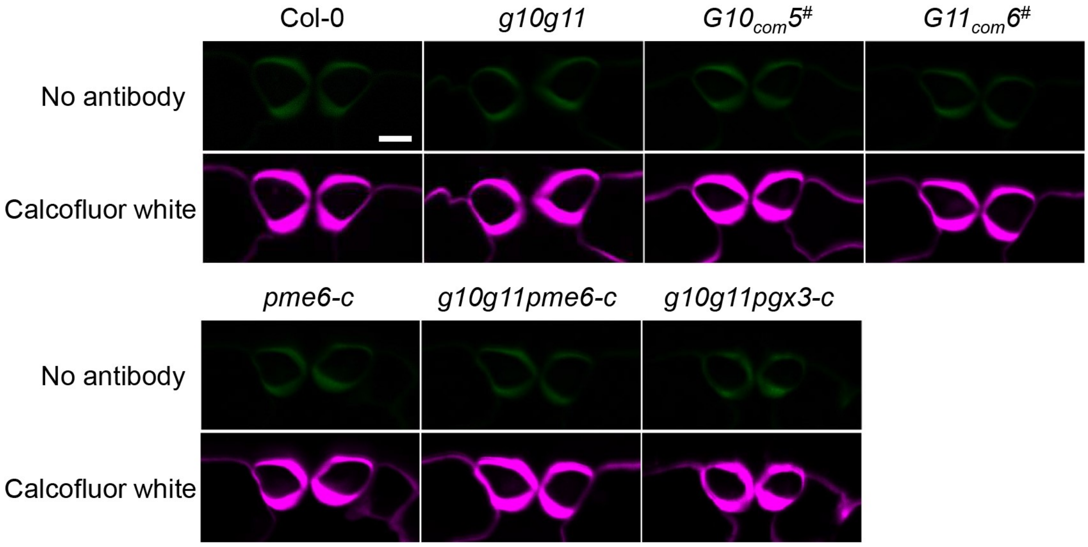
Supplemental Figure S4. Phenotypic characterization of *g10g11* mutant.

A, Images of Col-0 and *g10g11* plants. Bars = 3 cm. B, Col-0 and *g10g11* plants had similar plant height. Bars = 5 cm. C, Seed germination of Col-0 and *g10g11* under light conditions. Values are means ± SE. $n \geq 200$ seeds per genotype, ***$P < 0.001$; Student's *t*-test. D, Stomata density in the abaxial epidermis from Col-0 and *g10g11* plants grown under normal growth conditions. Values are means ± SE. $n \geq 10$ leaves per genotype, No significantly difference (ns), $P \geq 0.05$; Student's *t*-test. E, Stomata index (the ratio of the number of stomata per unit area to the number of epidermal cells) in the abaxial epidermis from Col-0 and *g10g11* plants grown under normal condition. Values are means ± SE. $n \geq 10$ leaves per genotype, No significantly difference (ns), $P \geq 0.05$; Student's *t*-test.



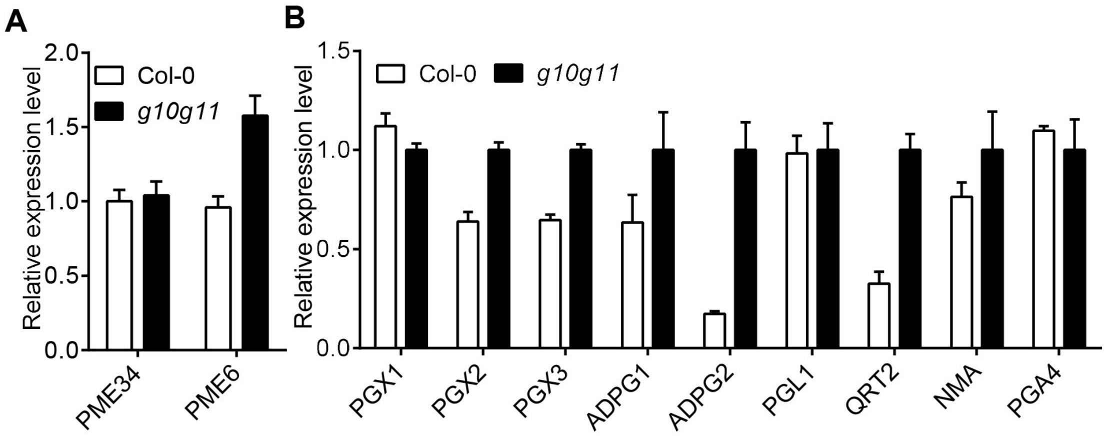
Supplemental Figure S5. *GAUT10* and *GAUT11* modulate stomatal dynamics in response to light and CO_2 changes.

A and B, Stomatal conductance of Col-0 and *g10g11* plants in response to changes in light intensity or CO_2 concentrations. Original data of Fig 5A-B. Experiments are repeated three times. Values are means ± SE, $n \geq 3$ leaves per genotype per experiment. C and D, Changes in stomatal conductance of Col-0 and *g10g11* plants per minute during stomatal opening (C) and stomatal closure (D) in response to CO_2 concentration changes in (B). Values are means ± SE, $^*P < 0.05$; Student's *t*-test. E and F, Stomatal conductance of Col-0, *g10g11*, *GAUT10* and *GAUT11* complementation lines in response to light intensity or CO_2 concentration changes. Experiments are repeated three times. Values are means ± SE, $n \geq 3$ leaves per genotype per experiment. Original data of Fig 5C-D. G and H, Stomatal conductance in Col-0, *g10g11*, *GAUT10* and *GAUT11* overexpression lines in response to light intensity or CO_2 concentrations changes. Original data of Fig 5E-F. Experiments are repeated three times. Values are means ± SE, $n \geq 3$ leaves per genotype per experiment.



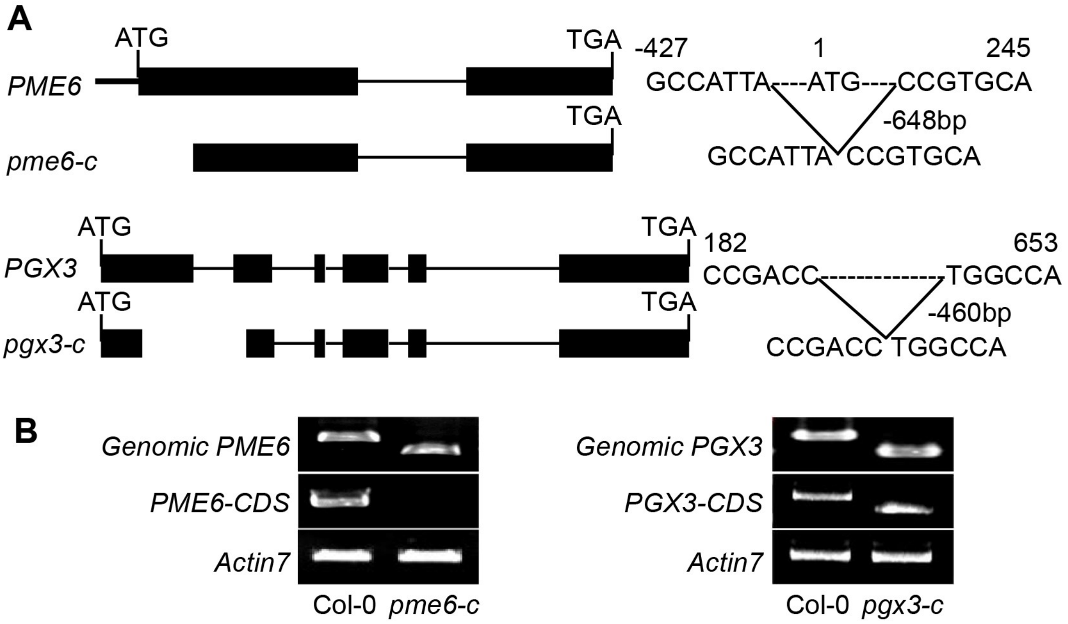
Supplemental Figure S6. Control images for immunolabeling in guard cell walls.

Sample controls were incubated with anti-rat-IgG coupled to fluorescein isothiocyanate (FITC) without primary antibody (LM19 or LM20). Samples show a very low level of fluorescence in the green channel, the magenta signal indicates Calcofluor White fluorescence in the same guard cell pairs. Bars = 5 μm.



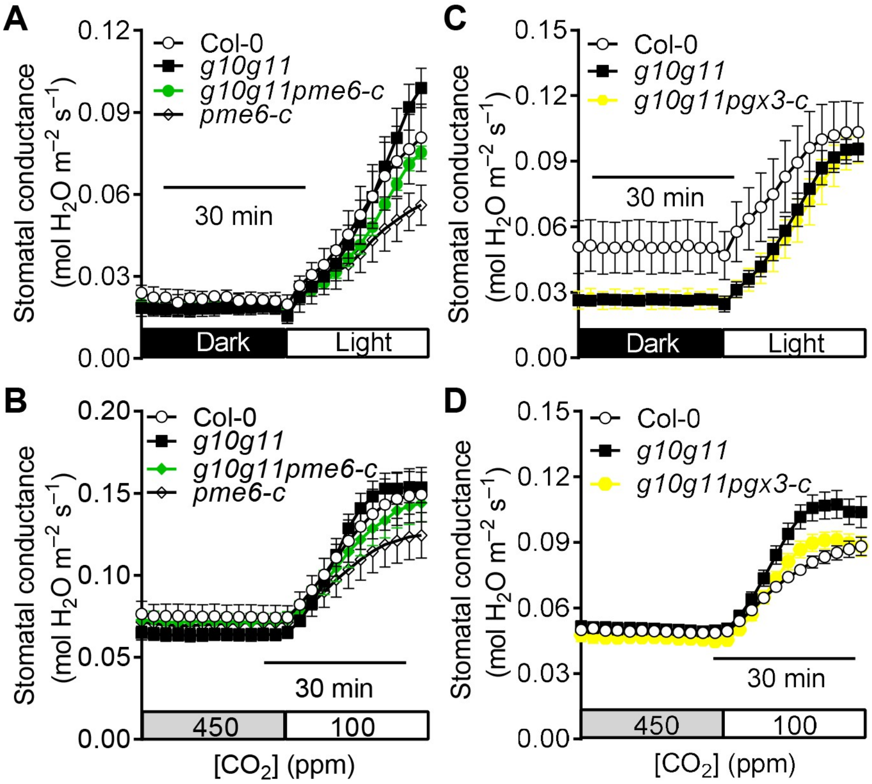
Supplemental Figure S7. Expression levels of *PME* and *PG* genes in the *g10g11* mutant.

A, Expression levels of *PME34* and *PME6* in rosette leaves from 3- to 4-week-old Col-0 controls and *g10g11* mutants. Values are means ± SE. *n* ≥ 3. B, Expression levels of *PGX1*, *PGX2*, *PGX3*, *ADPG1*, *ADPG2*, *PGL1*, *QRT2*, *NMA*, and *PGA4* in rosette leaves from 3- to 4-week-old Col-0 and *g10g11* plants. Values are means ± SE. *n* ≥ 3.



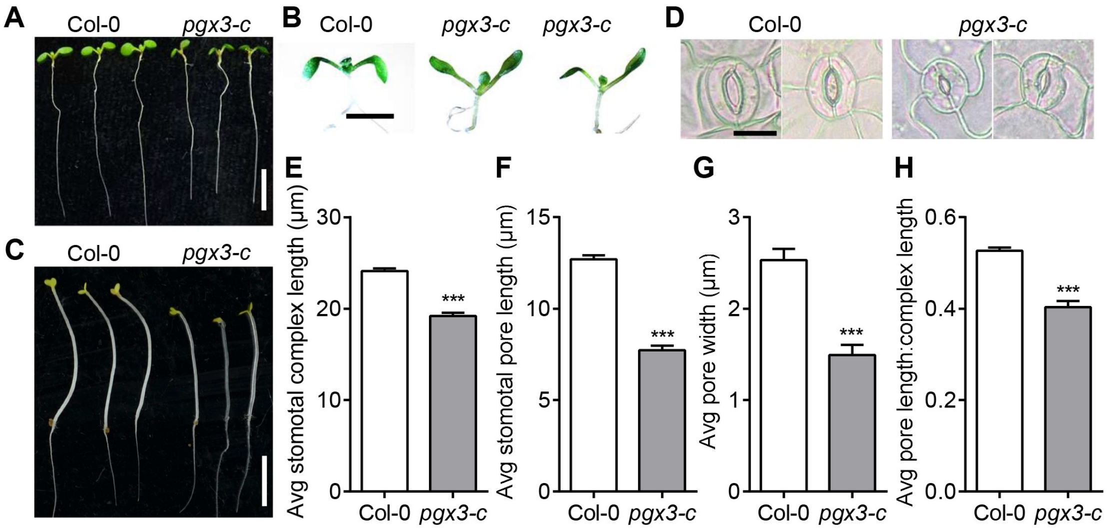
Supplemental Figure S8. Schematic of gene structure and transcript analyses of *PME6* and *PGX3*.

A, A brief structure diagrams show the editing sites of *PME6* and *PGX3* genes in *pme6-c* (*pme6-crispr*) and *pgx3-c* (*pgx3-crispr*), respectively. Exons are represented by boxes and introns by light lines, heavy black line indicates promoter region. Blank areas represent deletion regions. B, RT-PCR analyses of *PME6* and *PGX3* transcript levels in *pme6-c* and *pgx3-c* mutants, respectively. *Actin7* was served as a control.



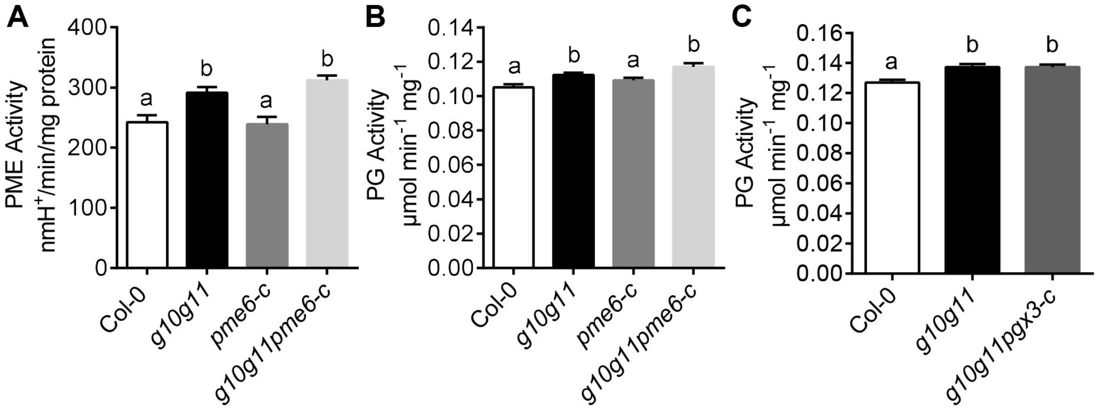
Supplemental Figure S9. *PME6* mutation rescues stomatal dynamic response to CO_2 and light changes in *g10g11*.

A and B, Stomatal conductance of Col-0, *g10g11*, *pme6-c* and *g10g11pme6-c* in response to light intensity or CO_2 concentration changes. Original data of Fig 7E-F. Values are means ± SE, $n \geq 3$ leaves per genotype per experiment. Experiments are repeated three times. C and D, Stomatal conductance of Col-0, *g10g11*, and *g10g11pgx3-c* in response to light intensity or CO_2 concentration changes. Original data of Fig 8E-F. Values are means indicate ± SE, $n \geq 3$ leaves per genotype per experiment. Experiments are repeated three times.



Supplemental Figure S10. *pgx3-c* mutant showed defects in seedling development and cotyledon stomatal development.

A, Light-grown *pgx3-c* seedlings (6-day-old) exhibited shorter primary root length. Bar = 0.5 cm. B, Cotyledon shape of 7-day-old Col-0 and *pgx3-c* seedlings. C, Dark-grown *pgx3-c* seedlings (4-day-old) showed shorter etiolated hypocotyl compared with Col-0. Bar = 0.5 cm. D, Cotyledon stomata in 7-day-old Col-0 and *pgx3-c* seedlings. Bar = 15 µm. E-H, Measurements of cotyledon stomatal complex length (E), pore length (F), pore width (G) and the ratio of pore length to stomatal complex length (H) in 7-day-old Col-0 and *pgx3-c* seedlings. Values are means ± SE ($n \geq 60$ stomata from at least 6 plants per genotype, ***$P < 0.001$; Student's *t*-test).



Supplemental Figure S11. Mutation of *PME6* or *PGX3* does not affect the increased PME or PG activity in the *g10g11* mutant.

A, PME activity in the leaves of 4-week-old Col-0, *g10g11*, *pme6-c* and *g10g11pme6-c* plants. Values are means ± SE, three biological replicates; $P < 0.05$, one-way ANOVA and Tukey's test. B, PG activity in the leaves of 4-week-old Col-0, *g10g11*, *pme6-c* and *g10g11pme6-c* plants. Values are means ± SE, three biological replicates; $P < 0.05$, one-way ANOVA and Tukey's test. C, PG activity in the leaves of 4-week-old Col-0, *g10g11* and *g10g11pgx3-c* plants. Values are means ± SE, three biological replicates; $P < 0.05$, one-way ANOVA and Tukey's test.

Supplemental Table S1. Primers used in this study.

Primer name	Primer sequence (5’-3’)	Method	Vector	Usage
gaut10-3-LP	AGAGTCTTGCAGCTGCTTGAG			Genotyping
gaut10-3-RP	TTTGCAGCGAAGAGAAAGAAG			Genotyping
gaut11-3-LP	CAACCAATTGCCCAAATATTG			Genotyping
gaut11-3-RP	GAAAACCCGAAAGGAGAAAAG			Genotyping
SALK-LBb1.3	ATTTTGCCGATTTCGGAAC			Genotyping
SAIL-LB3	TAGCATCTGAATTTCATAACCAATCTCGATACAC			Genotyping
qGAUT10-F	ACCTAGACCCTCTATTCCTAAGCG			RT-qPCR
qGAUT10-R	TGACGAGCAACGGAAGTAGGAC			RT-qPCR
qGAUT11-F	GCGAGGTAGAAGGAGATTGTCGAG			RT-qPCR
qGAUT11-R	TCGGTTCTCGTGTCTCTCTCAAG			RT-qPCR
qPME6-F	GTTATAAAGACGGTGACAGAG			RT-qPCR
qPME6-R	CCGTAGCCGTAATCCAATAG			RT-qPCR
qPGX3-F	AAGTCCACCGATTCATTTCG			RT-qPCR
qPGX3-R	TCCGGCAATAACTCAACCTC			RT-qPCR
com-GAUT10-F	GG*GGTACC*AGTGTTAGCAAAGATGAT	*Kpn* I	pGreenII 0179	Complementation
com-GAUT10-R	GG*ACTAGT*GTGTGAATATCAGAGAATAT	*Spe* I	(Hellens et al., 2000)	Complementation
com-GAUT11-F	AG*GGGCCC*ATTTGGTTAGTGTTGTAAAT	*Apa* I	pGreenII 0179	Complementation
com-GAUT11-R	GG*ACTAGT*TATTTAAAAACGTAGAACAG	*Spe* I		Complementation
proGAUT10-F	*GGGGACAAGTTTGTACAAAAAAGCAGGCT*TAAGTGTTAGCAAAGATGAT	Gateway cloning	*pMDC163* (Curtis and Grossniklaus, 2003)	GUS staining
proGAUT10-R	*GGGGACCACTTTGTACAAGAAAGCTGGGT*AAGTTACTGTACTCGCAGAGC			GUS staining
proGAUT11-F	*GGGGACAAGTTTGTACAAAAAAGCAGGCT*TAGGTATTTGGTTAGTGTTGT	Gateway Cloning	*pMDC163*	GUS staining
proGAUT11-R	*GGGGACCACTTTGTACAAGAAAGCTGGGT*ACTTCCGCTACAAGTTTACCC			GUS staining
GAUT10-CDS-F	*GGGGACAAGTTTGTACAAAAAAGCAGGCT*TAATGAGAAGGAGAGGAGGG	Gateway cloning	pEarleyGate 101 (Earley et al., 2006)	Overexpression
GAUT10-CDS-R	*GGGGACCACTTTGTACAAGAAAGCTGGGT*AATGAAAATTGCATTGTTGC			Overexpression
GAUT11-CDS-F	*GGGGACAAGTTTGTACAAAAAAGCAGGCT*TAGTAGCGGAAGATGAGGCG	Gateway cloning	pEarleyGate 101	Overexpression
GAUT11-CDS-R	*GGGGACCACTTTGTACAAGAAAGCTGGGT*AAGCTGTGACACAATCTTGT			Overexpression
target1-PME6	AGCTATGGCCGCGCTTAACG	Golden Gate assembly	pYLCRISPR/Cas9P$_{ubi}$-B	Crispr editing
target2-PME6	CCTTGTGGTCAGCTCCACCA		(Ma et al., 2015)	Crispr editing
target1-PGX3	GGACTCGGGTACGGGTCGGA	Golden Gate assembly	pHEE401E	Crispr editing
target2-PGX3	GGCCATTCCTCTGGTCCGTC		(Wang et al., 2015)	Crispr editing

Supplemental Table S2. Measurement of stomatal dimensions in *g10g11* and *GAUT* complementation lines.

Genotype	Avg stomatal complex length (μm)	Avg guard cell width (μm)	Avg stomatal pore length (μm)	Avg stomatal pore width (μm)	Avg pore length/ complex length
Col-0	21.99 ± 0.22^{a}	5.64 ± 0.09^{a}	11.31 ± 0.16^{a}	1.910 ± 0.055^{a}	0.50 ± 0.005^{a}
g10g11	24.33 ± 0.19^{b}	6.41 ± 0.13^{b}	12.97 ± 0.19^{b}	1.596 ± 0.079^{b}	0.52 ± 0.006^{b}
$GAUT10_{com}3^{\#}$	21.94 ± 0.16^{a}	5.75 ± 0.13^{a}	11.07 ± 0.15^{a}	1.999 ± 0.055^{a}	0.50 ± 0.008^{a}
$GAUT10_{com}5^{\#}$	21.88 ± 0.20^{a}	5.76 ± 0.12^{a}	11.15 ± 0.15^{a}	1.897 ± 0.058^{a}	0.49 ± 0.006^{a}
$GAUT11_{com}5^{\#}$	22.27 ± 0.16^{a}	5.84 ± 0.14^{ab}	11.05 ± 0.15^{a}	1.993 ± 0.052^{a}	0.50 ± 0.007^{a}
$GAUT11_{com}6^{\#}$	21.81 ± 0.19^{a}	5.80 ± 0.15^{a}	11.00 ± 0.16^{a}	1.910 ± 0.059^{a}	0.49 ± 0.008^{a}

Stomatal complex length, guard cell width, pore length, pore width, and the ratio of pore length to stomatal complex length in 3- to 4-week-old Col-0, *g10g11*, $G10_{com}3^{\#}$, $G10_{com}5^{\#}$, $G11_{com}5^{\#}$ and $G11_{com}6^{\#}$ plants. Values are means ± SE, ($n \geq 60$ stomata from at least 6 plants per genotype, three independent experiments; $P < 0.05$, one-way ANOVA and Tukey's test).

Supplemental Table S3. Measurement of stomatal dimensions in cotyledons of Col-0, *g10g11* and *g10g11pgx3-c* plants.

Genotype	Avg stomatal complex length (µm)	Avg guard cell width (µm)	Avg stomatal pore length (µm)	Avg stomatal pore width (µm)	Avg pore length/ complex length
Col-0	24.29 ± 0.22^{a}	4.79 ± 0.06^{a}	13.13 ± 0.18^{a}	2.909 ± 0.068^{a}	0.54 ± 0.004^{a}
g10g11	28.53 ± 0.28^{b}	5.52 ± 0.14^{b}	16.49 ± 0.21^{b}	2.820 ± 0.083^{a}	0.58 ± 0.004^{b}
g10g11pgx3-c	28.53 ± 0.24^{b}	5.76 ± 0.08^{b}	16.46 ± 0.17^{b}	2.784 ± 0.073^{a}	0.58 ± 0.004^{b}

Stomatal complex length, guard cell width, pore length, pore width, and the ratio of pore length to stomatal complex length in cotyledons of 7-day-old Col-0, *g10g11* and *g10g11pgx3-c* seedlings. Values are means ± SE, ($n \geq 60$ stomata from at least 6 plants per genotype, two independent experiments; $P < 0.05$, one-way ANOVA and Tukey's test).

Supplemental Table S4. Measurement of stomatal dimensions in Col-0 and *GAUT* overexpression lines.

Genotype	Avg stomatal complex length (µm)	Avg guard cell width (µm)	Avg stomatal pore length (µm)	Avg stomatal pore width (µm)	Avg pore length/ complex length
Col-0	23.68 ± 0.22^{a}	5.03 ± 0.07^{a}	10.66 ± 0.17^{a}	1.880 ± 0.059^{a}	0.44 ± 0.004^{a}
$GAUT10_{OE}4^{\#}$	23.88 ± 0.20^{a}	5.23 ± 0.07^{a}	10.59 ± 0.19^{a}	1.881 ± 0.071^{a}	0.44 ± 0.005^{a}
$GAUT10_{OE}13^{\#}$	23.18 ± 0.16^{a}	5.15 ± 0.07^{a}	10.21 ± 0.15^{a}	1.843 ± 0.067^{a}	0.44 ± 0.006^{a}
$GAUT11_{OE}7^{\#}$	23.39 ± 0.21^{a}	5.16 ± 0.06^{a}	10.23 ± 0.18^{a}	1.826 ± 0.070^{a}	0.43 ± 0.005^{a}
$GAUT11_{OE}13^{\#}$	24.21 ± 0.26^{a}	5.23 ± 0.08^{a}	11.07 ± 0.19^{a}	1.837 ± 0.090^{a}	0.45 ± 0.004^{a}

Stomatal complex length, guard cell width, pore length, pore width, and the ratio of pore length to stomatal complex length in 3- to 4-week-old Col-0, $G10_{OE}4^{\#}$, $G10_{OE}13^{\#}$, $G11_{OE}7^{\#}$ and $G11_{OE}13^{\#}$ plants. Values are means ± SE, ($n \geq 60$ stomata from at least 6 plants per genotype, two independent experiments; $P < 0.05$, one-way ANOVA and Tukey's test).

Supplemental Table S5. Measurement of stomatal dimensions in mature leaves of Col-0, *g10g11*, *pme6-c*, *g10g11pme6-c* and *g10g11pgx3-c* plants.

Genotype	Avg stomatal complex length (µm)	Avg guard cell width (µm)	Avg stomatal pore length (µm)	Avg stomatal pore width (µm)	Avg pore length/ complex length
Col-0	22.52 ± 0.46[a]	4.70 ± 0.07[a]	12.72 ± 0.31[a]	1.926 ± 0.061[a]	0.56 ± 0.006[a]
g10g11	25.67 ± 0.28[b]	5.70 ± 0.08[b]	15.44 ± 0.35[b]	1.612 ± 0.040[b]	0.60 ± 0.010[b]
pme6-c	21.99 ± 0.26[a]	4.53 ± 0.07[a]	12.41 ± 0.27[a]	1.972 ± 0.077[a]	0.55 ± 0.008[a]
g10g11pme6-c	24.73 ± 0.42[b]	5.50 ± 0.07[b]	14.85 ± 0.34[b]	1.574 ± 0.043[b]	0.60 ± 0.007[b]
g10g11pgx3-c	25.90 ± 0.34[b]	5.42 ± 0.07[b]	15.57 ± 0.33[b]	1.662 ± 0.039[b]	0.60 ± 0.007[b]

Stomatal complex length, guard cell width, pore length, pore width, and the ratio of pore length to stomatal complex length in mature leaves of 3- to 4-week-old Col-0, *g10g11*, *pme6-c*, *g10g11pme6-c* and *g10g11pgx3-c* plants. Values are means ± SE, ($n \geq 60$ stomata from at least 6 plants per genotype, three independent experiments; $P < 0.05$, one-way ANOVA and Tukey's test).