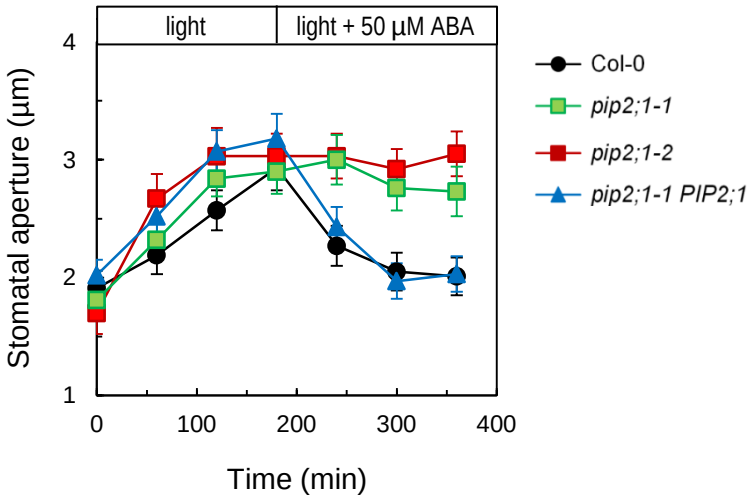
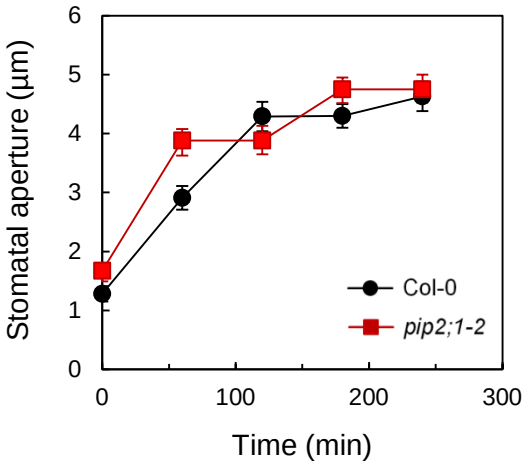


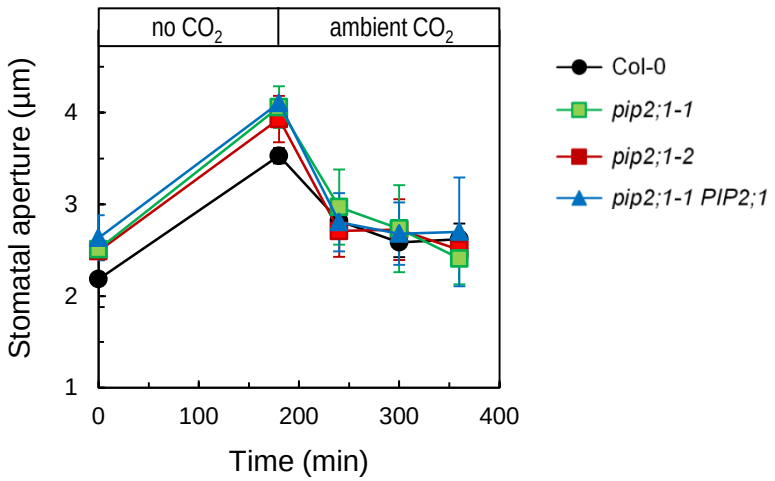
Supplemental Figure 1. Stomatal response of Col-0, *pip2;1* and *PIP2;1*-complemented *pip2;1* plants to 50 µM ABA.

Same conventions and procedures as in Figure 1. In brief, peeled epidermal strips from Col-0 (●), *pip2;1-1* (■), *pip2;1-2* (■), or *pip2;1-1 PIP2;1* (▲) plants were transferred from darkness to light (300 $\mu E.m^{-2}.s^{-1}$) at t = 0. After 180 min, the bathing solution was equilibrated with 50 µM ABA. Averaged data (± SE) from a representative experiment, with ~60 aperture measurements at each time point.



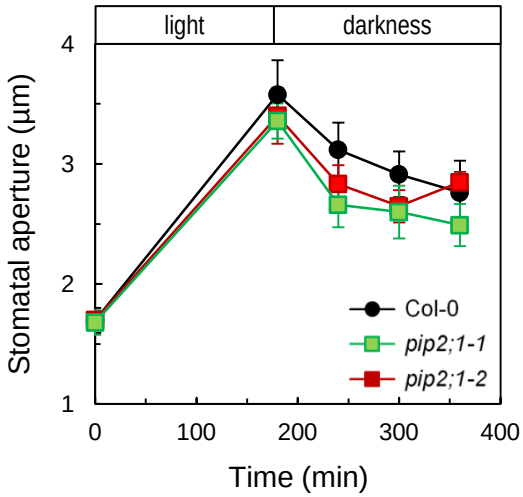
Supplemental Figure 2. Stomatal response of Col-0 and *pip2;1* plants to fusicoccin.

Peeled epidermal strips from Col-0 (●) and *pip2;1-2* (■) plants were incubated in darkness and the bathing solution was equilibrated with 5 µM fusicoccin at t = 0. Averaged data (± SE) from a representative experiment, with ~60 aperture measurements at each time point.



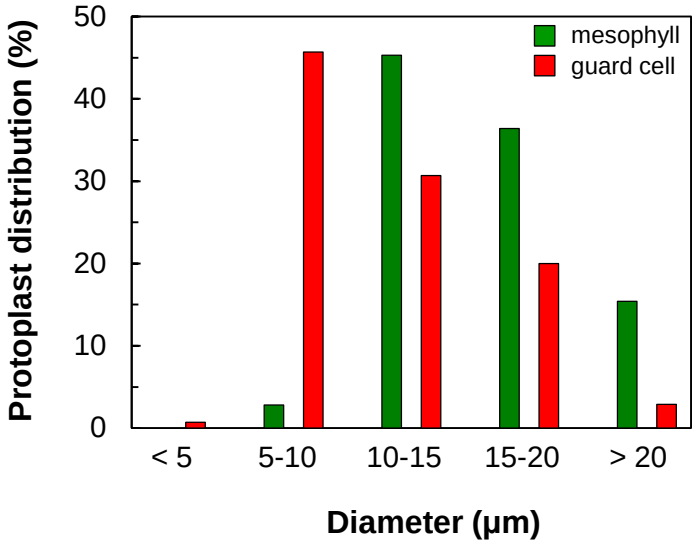
Supplemental Figure 3. Stomatal response of Col-0, *pip2;1* and *PIP2;1*-complemented *pip2;1* plants to various CO_2 concentrations.

Peeled epidermal strips from Col-0 (●), *pip2;1-1* (■), *pip2;1-2* (■), or *pip2;1-1 PIP2;1* (▲) plants were maintained in darkness throughout the whole experiment. The bathing solution was equilibrated by bubbling, first with air deprived of CO_2 (t = 0-180 min) and subsequently with ambient air (t > 180 min). Averaged data (± SE; *n* > 100) from *n*=2 independent experiments, each with ~60 aperture measurements per time point.

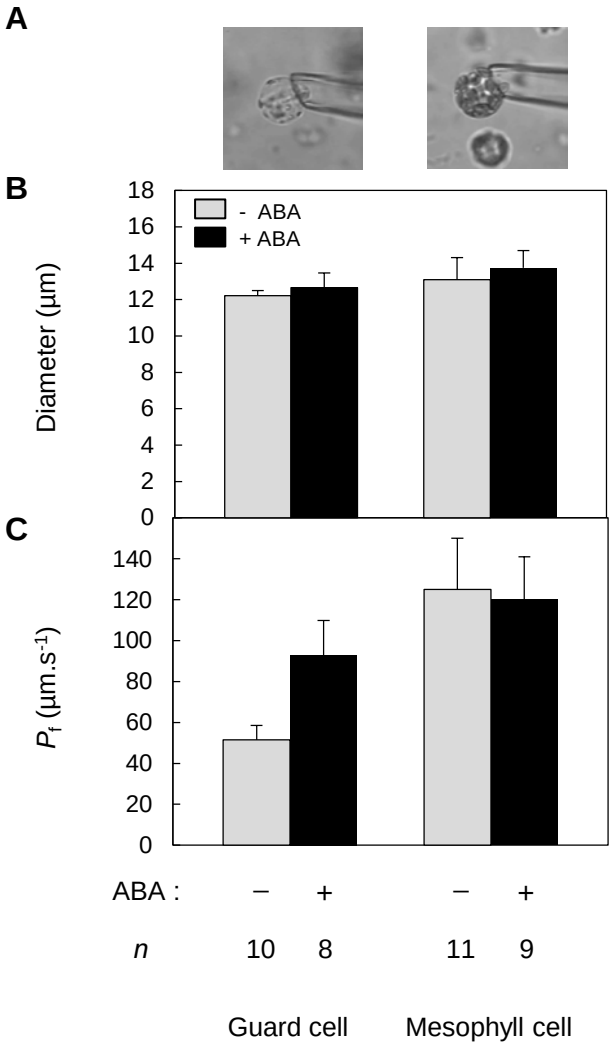


Supplemental Figure 4. Stomatal response of Col-0 and *pip2;1* plants to changing light.

Peeled epidermal strips from Col-0 (●), *pip2;1-1* (■), and *pip2;1-2* (■) plants were transferred from darkness to light (300 µE.m^{-2}.s^{-1}) at t = 0. At t = 180 min, the strips were transferred back to darkness. Averaged data (± SE; *n* > 150) from *n*>3 independent experiments, each with ~60 aperture measurements per time point.



Supplemental Figure 5. Diameter repartition of mesophyll (green bars) and guard cell (red bars) protoplasts isolated from Col-0 plants.



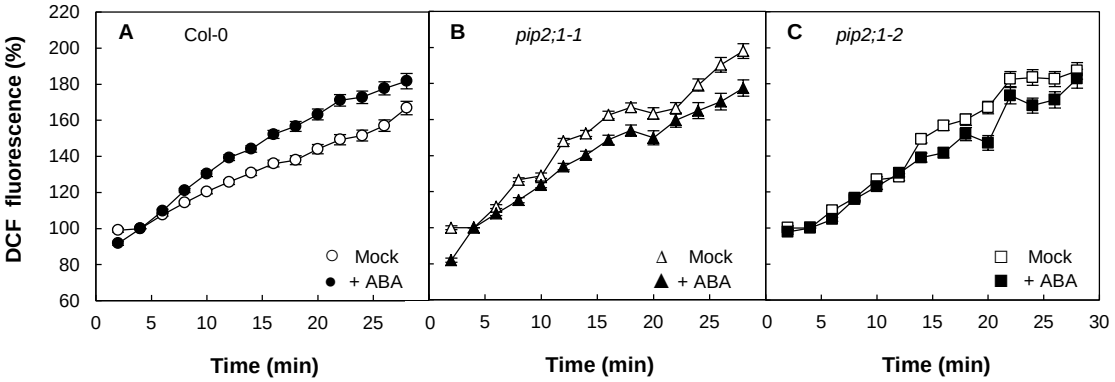
Supplemental Figure 6. Comparative effects of ABA on P_f of guard cell and mesophyll cell protoplasts.

(A) Representative images of guard cell and mesophyll cell protoplasts held by a micropipette. The two types of protoplasts can easily be distinguished based on their chloroplast content.

(B) Mean diameter of protoplasts selected for subsequent characterization of P_f. Protoplasts were prepared in the presence of light (grey bars) or light plus 10 µM ABA (black bars). Guard cell protoplasts are characterized by a mean diameter of ~ 12 µM. Mesophyll protoplasts of similar size were individually selected and their mean diameter was determined.

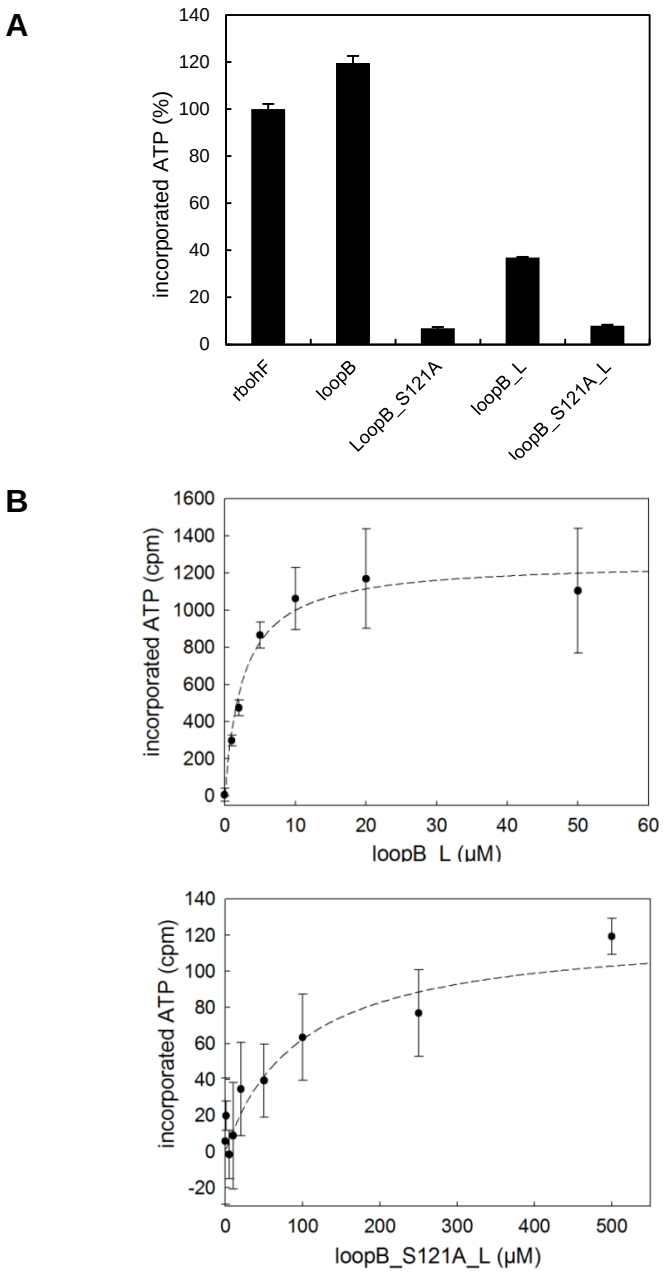
(C) Corresponding P_f values of protoplasts prepared in the absence (grey bars) or presence (black bars) of 10 µM ABA.

Data (± SE) from the indicated number (*n*) of protoplasts.

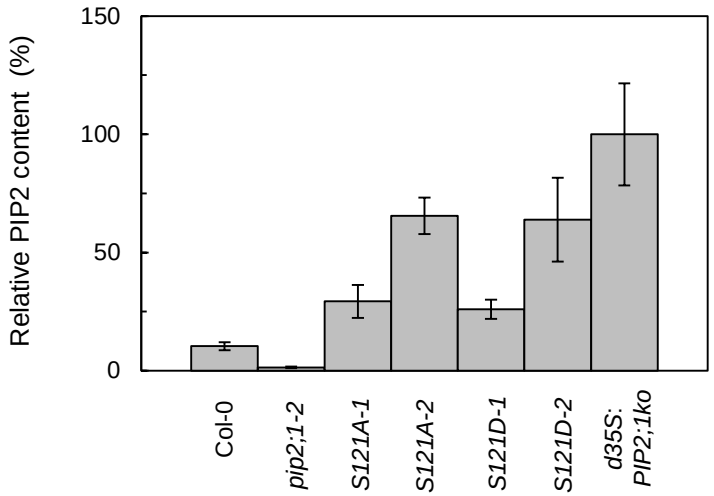


Supplemental Figure 7. Accumulation of ROS in ABA- and mock-treated stomata of Col-0 and *pip2;1* mutant plants.

Peeled epidermal strips from Col-0 (A), *pip2;1-1* (B) or *pip2;1-2* (C) were maintained in a bathing solution under light for 120 min to induce stomatal opening. They were then incubated in the presence of 50 µM H_2DCFDA for 20 min, and extracellular H_2DCFDA was removed by four successive washings, prior to addition (t = 0) of 50 µM ABA (filled symbols) or an equivalent volume of ethanol (Mock; empty symbols). Mean stomatal DCF fluorescence intensity was measured at the indicated time points and normalized to initial fluorescence (t = 4 min). Averaged data (± SE) from *n* = 7 independent experiments, each with 10-20 stomata per time point. When not shown, error bars fall into symbols.

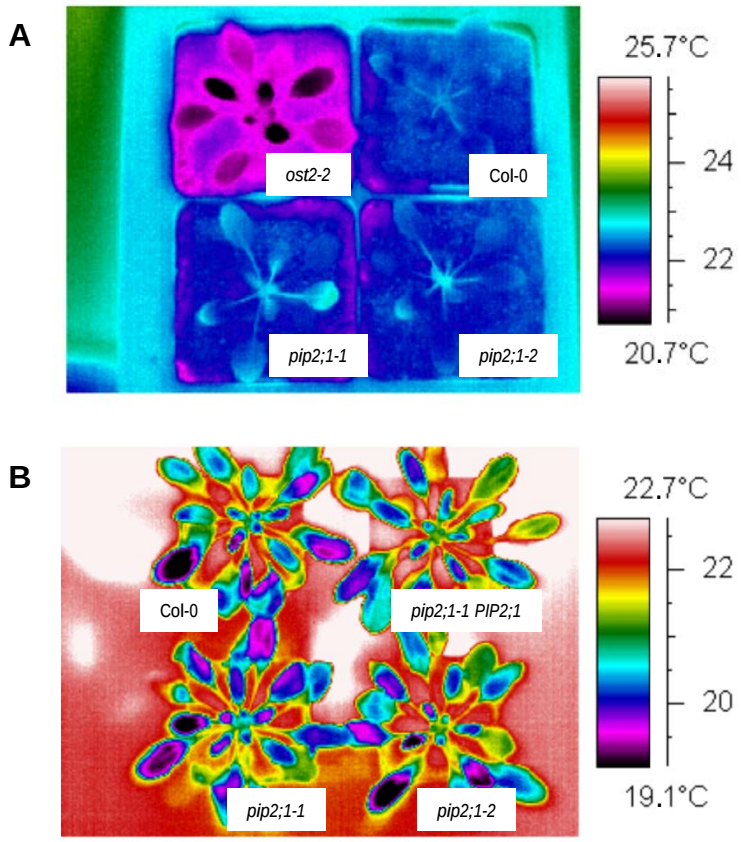


Supplemental Figure 8. *In vitro* phosphorylation of loop *B* PIP2;1 peptides by OST1.
(A) Phosphorylation by purified OST1 of loop *B* peptides either native or carrying a S121A mutation. The loopB and loopB_S121A peptides contain 10 PIP2;1 residues whereas loopB_L and loopB_S121A_L contain 29 PIP2;1 residues. Incorporated ATP (*n* = 2; ± SD) was normalized to the signal observed in a reference rbohF peptide.
(B) The loop B_L (upper panel) and loopB_S121A_L (lower panel) peptides were incubated at the indicated concentrations, in the presence of labeled ATP and purified OST1. The two panels show incorporated ATP (± SE) from *n* = 2 independent experiments, each with 2-3 technical replicates. Note that for clarity, the scales of the *x* and *y* axis are different between the two panels. Calculated affinities are as follows: loop B_L : *K*m = 2.6 ± 0.7 µM ; loopB_S121A_L: *K*m = 96.8 ± 45.1 µM.



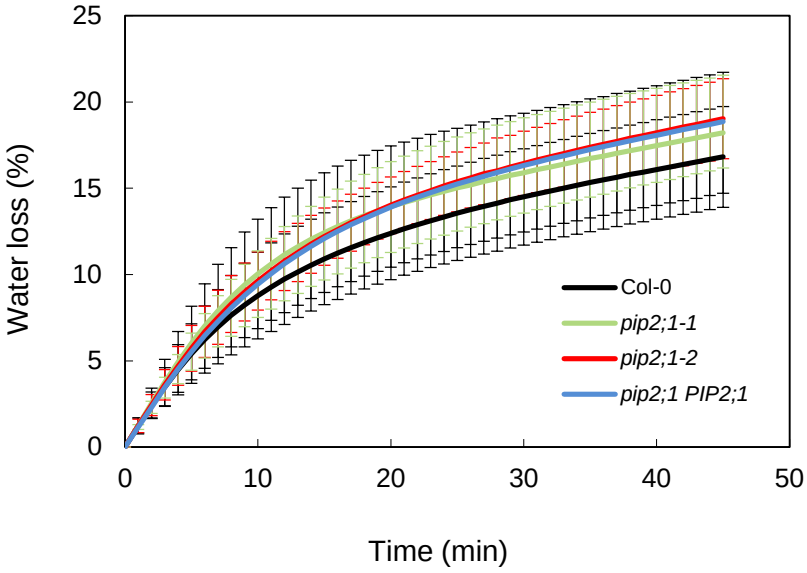
Supplemental Figure 9. PIP2 abundance in leaves of the indicated genotypes.

ELISAs were performed using an anti-PIP2 antibody (Santoni et al., 2003, Biochem. J. 372: 289) on total leaf protein extracts from 3-week-old plants. The antibody recognizes PIP2;1, PIP2;2 and PIP2;3 and reveals by reference to *pip2;1-2* and *d35S:PIP2;1ko* quantitatively distinct abundance of PIP2;1 between genotypes. PIP2 abundance was normalized (in %) to the signal obtained in *d35S:PIP2;1ko.* Cumulated data (mean ± SE) from at least three independent plant cultures, with two repeats per culture.



Supplemental Figure 10. Leaf temperature of Col-0, *pip2;1* and *PIP2;1*-complemented *pip2;1.*

Infra-red images of rosettes of plants grown in individual pots were captured by an infra-red thermography device at the end of the night (**A**) or at midday (**B**). Col-0 and *pip2;1* (**A**) or Col-0, *pip2;1* and *PIP2;1*-complemented *pip2;1-1* (**B**) show similar leaf temperatures. By contrast, the *ost2-2* mutant (Merlot et al., 2007, EMBO J 26: 3216–3226), which exhibits constitutively opened stomata, shows lower leaf temperature than Col-0 and *pip2;1* plants (**A**).



Supplemental Figure 11. Kinetics of water loss from excised rosettes of Col-0, *pip2;1* and *PIP2;1*-complemented *pip2;1*.

Hypocotyls of 4-week-old plants were cut and sealed with silicon grease. Water loss was evaluated by weighing rosette each minute during one hour and expressed as the percentage of initial fresh weight. Values are means (± SE) from *n* = 6 plants per genotype.

Supplemental Table 1. Nucleotide sequences of primers used for *OST1* cDNA amplification and site-directed mutagenesis of PIP2;1

Primer	Sequence (5'→3')
OST1-EcoRI	AAA*GAATTC*GAGAAA**ATG**GATCGACC
BamHI-OST1	AGA*GGATCC*GTA**TCA**CATTGCGTACAC
PIP2;1-S121A:Ps	GGCACGTAAAGTGgctTTACCTAGGG
PIP2;1-S121A:Pa	CCCTAGGTAAagcCACTTTACGTGCC
PIP2;1-S121D:Ps	GGCACGTAAAGTGgatTTACCTAGGG
PIP2;1-S121D:Pa	CCCTAGGTAAatcCACTTTACGTGCC

*Eco*RI and *Bam*H1 restriction sites are indicated in italics. The start (ATG) and stop (TCA) codons of OST1 are indicated in bold. The two Ps and Pa pairs of primers were used for introducing in *PIP2;1* cDNA the indicated mutations at Ser121. The mutagenic codons are shown in small letters.