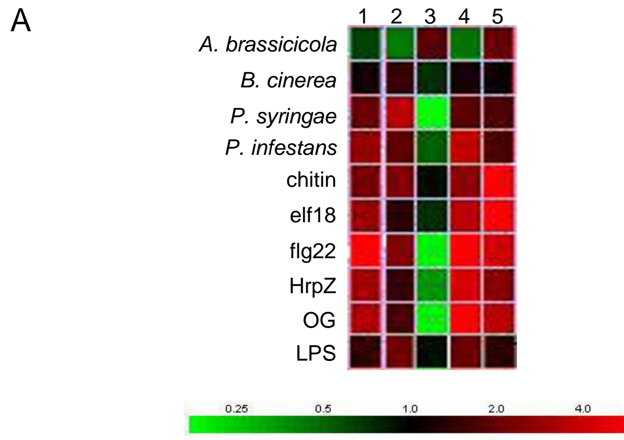
Supplementary Table S1. Primers for RT-PCR and qPCR used in this work.

Gene	AGI Code	Forward primer	Reverse primer
UBQ5	At3g62250	GGAAGAAGAAGACTTACACC	AGTCCACACTTACCACAGTA
PAD3	At3g26830	TCGCTGGCATAACACTATGG	TTGGGAGCAAGAGTGGAGT
PR-1	At2g14610	GTAGGTGCTCTTGTTCTTCCC	CACATAATTCCCACGAGGATC
PDF1.2	At5g44420	CGCACCGGCAATGGTGG	ATCCATGTTTGGCTCCTTCG
RAB18	At5g66400	ACGAGTACGGAAATCCGATG	ACCACCACTTTCCTTGTGGA
AtLYK3[1]	At1g51940	ATCCACCAGCTCCTTCTCCT (L3F)	TGCAAGCACAACTCCAAGAC (L3R)
AtLYK3[2]	At1g51940	GGAGGCGATTGGAACTAAGA (Af)	ATCGGATCATCATCCACACA (Ar)
AtLYK3[2]	At1g51940	GGAGGCGATTGGAACTAAGA (Af)	TCTTCCTTGGACTAGACCACTAAAG (Br)
ABI1	At4g26080	CGGCAAAACTGCACTTCCAT	CACGACGCTCCATTCCACTGAA
PYR1	At4g17870	CGGTTCGAGAAAGAGAATCG	GAGCCATAGCTTCAGCAACC
PYL4	At2g38310	TTCACACACACGAGGTTGGT	CAGAGACGACGTGGACTTGA
AtCERK1	At3g21630	TCGAAGGGTGATTCGTTTTC	CCACCTTGCCCCAATCTTAAA

[1] Primer pairs designed to determine the expression of *AtLYK3* in *atlyk3-1* and for qPCR experiments (see text for details).

[2] Primer pairs designed to identify the location of the T-DNA insertion in *atlyk3-3* (see text for details).

A



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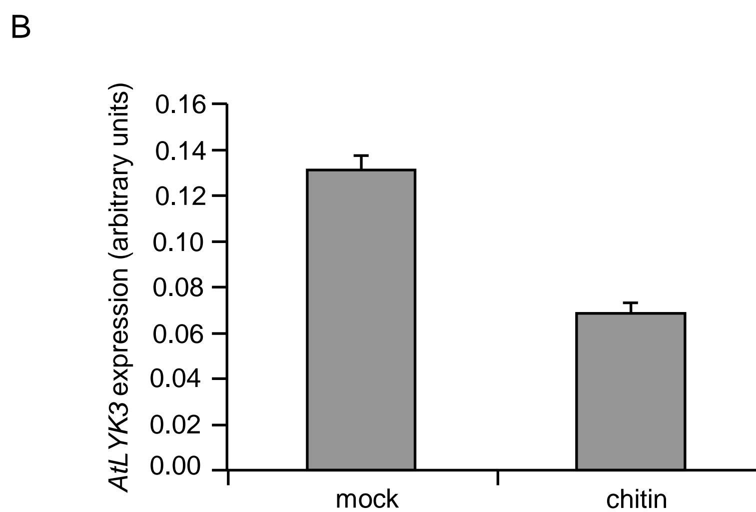

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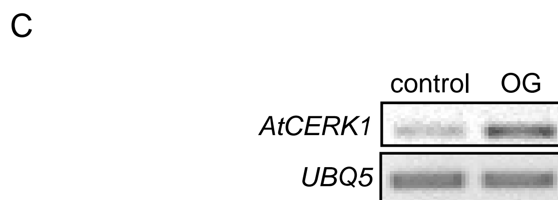
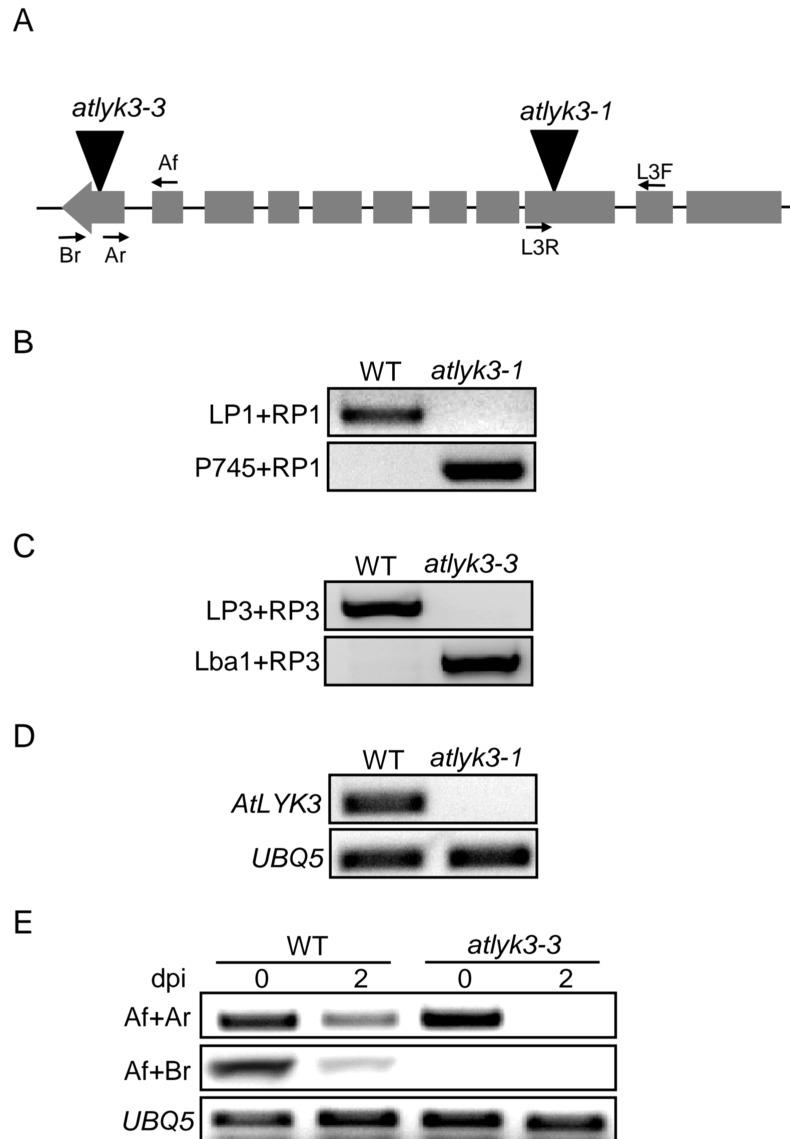
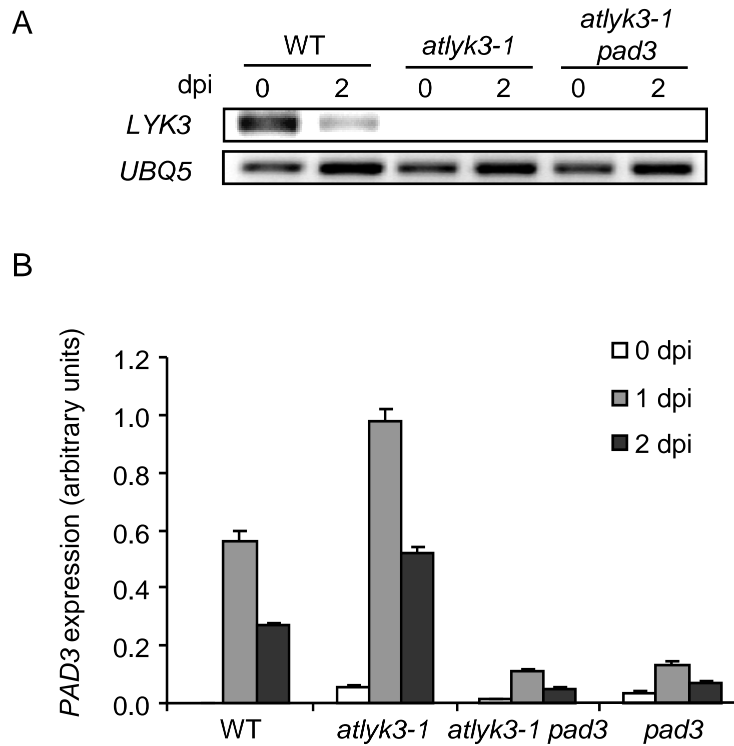


Supplementary Figure S1. Expression of Arabidopsis *AtLYK* genes in response to pathogens and elicitors. A, Heat map of the expression of the five Arabidopsis genes encoding putative LYKs (1 to 5*, AtLYK1* to *AtLYK5*) in response to the indicated biotic stresses, according to publicly available data, generated using Genevestigator (Zimmermann et al., 2004). B, WT seedlings were treated with either 100 µg ml^{-1} chitin or a control solution (mock) for 3 h. Expression of *AtLYK3* was determined by qPCR, using *UBQ5* as reference. This experiment was repeated twice with similar results. C, expression of *AtCERK1* in seedlings treated for three hours with water (control) or 100 µg ml^{-1} OGs was determined by RT-PCR, using *UBQ5* as reference.

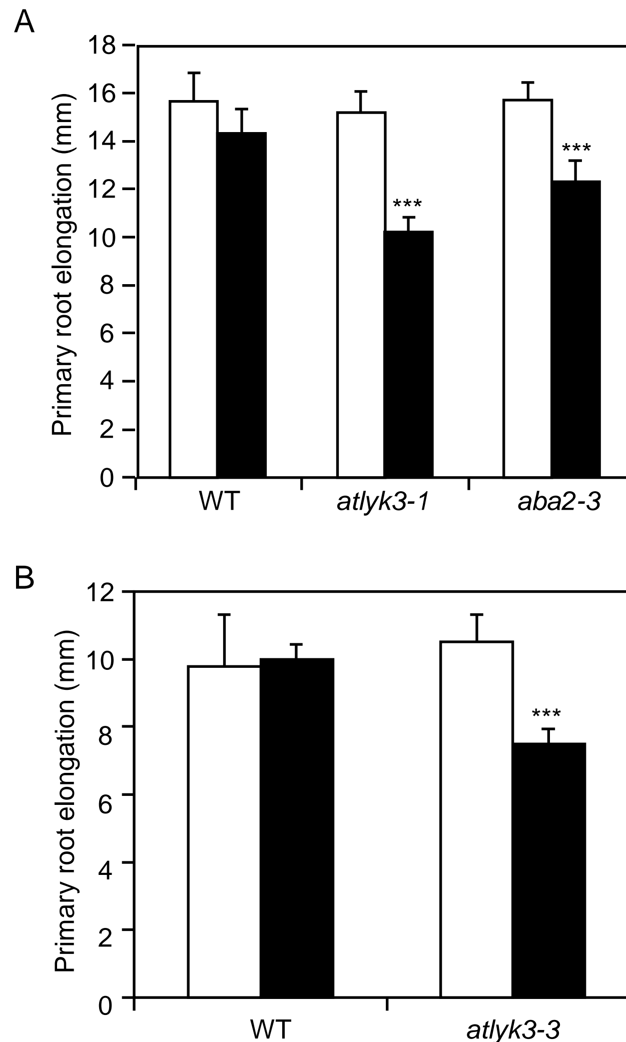


Supplementary Figure S2. Isolation of insertional mutants for *AtLYK3*. A, Schematic representation of the *AtLYK3* locus. Triangles indicate the T-DNA insertion for each allele. Arrows represent the primers used for expression analysis in *atlyk3-1* (L3F and L3R) and *atlyk3-3* (Af + Ar or Af + Br). B and C, genomic DNA from WT and *atlyk3-1* (B) or *atlyk3-3* (C) homozygous plants was subjected to PCR using the indicated primer pairs. LP1+RP1 and LP3+RP3, primer pairs specific for the WT *AtLYK3* sequence used for genotyping *atlyk3-1* and *atlyk3-3*, respectively; P745 and Lba1, primers specific for the T-DNA insertion in *atlyk3-1* and *atlyk3-3*, respectively. D, Total RNA from Arabidopsis WT and *atlyk3-1* rosette leaves was extracted and *AtLYK3* expression was analyzed by RT-PCR, using a forward primer annealing on exon 2 (L3F) and a reverse primer annealing on exon 3 (L3R). E, WT and *atlyk3-3* rosette leaves were inoculated with *B. cinerea*, and total RNA was extracted at 0 and two days post inoculation (dpi). *AtLYK3* expression was analyzed by RT-PCR, using a forward primer annealing on exon 9 (Af) and a reverse primer annealing at the beginning (Ar) or at the end (Br) of exon 11. *UBQ5* was used as a control.

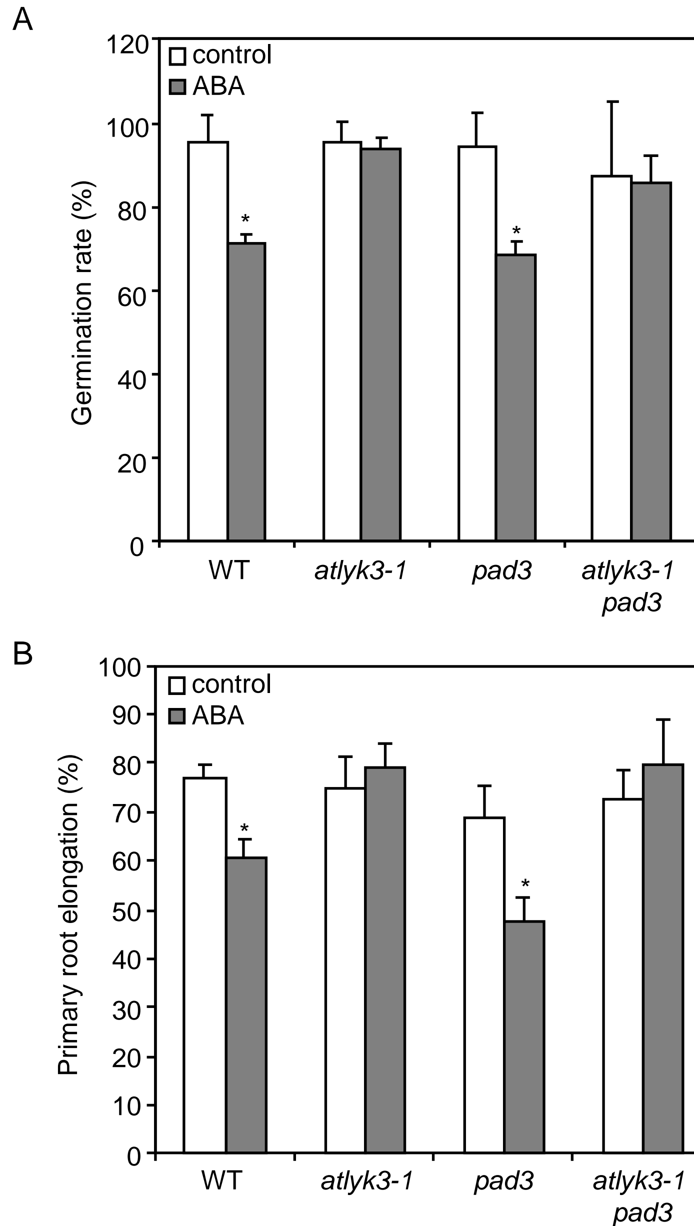
Supplementary Figure S3. Accumulation of reactive oxygen species in response to fungal infection is not affected by *AtLYK3*. WT, *atlyk3-1* and *atlyk3-3* rosette leaves were inoculated with *B. cinerea*; after 48 h leaves showing lesions of comparable size were incubated for 6 h with diaminobenzidine-HCl (2 dpi + DAB) or with a control solution (2 dpi - DAB). Uninfected leaves were also stained for comparison (0 dpi + DAB). After staining, leaves were cleared and pictures of a representative sample for each genotype were taken. Bars, 50 mm.



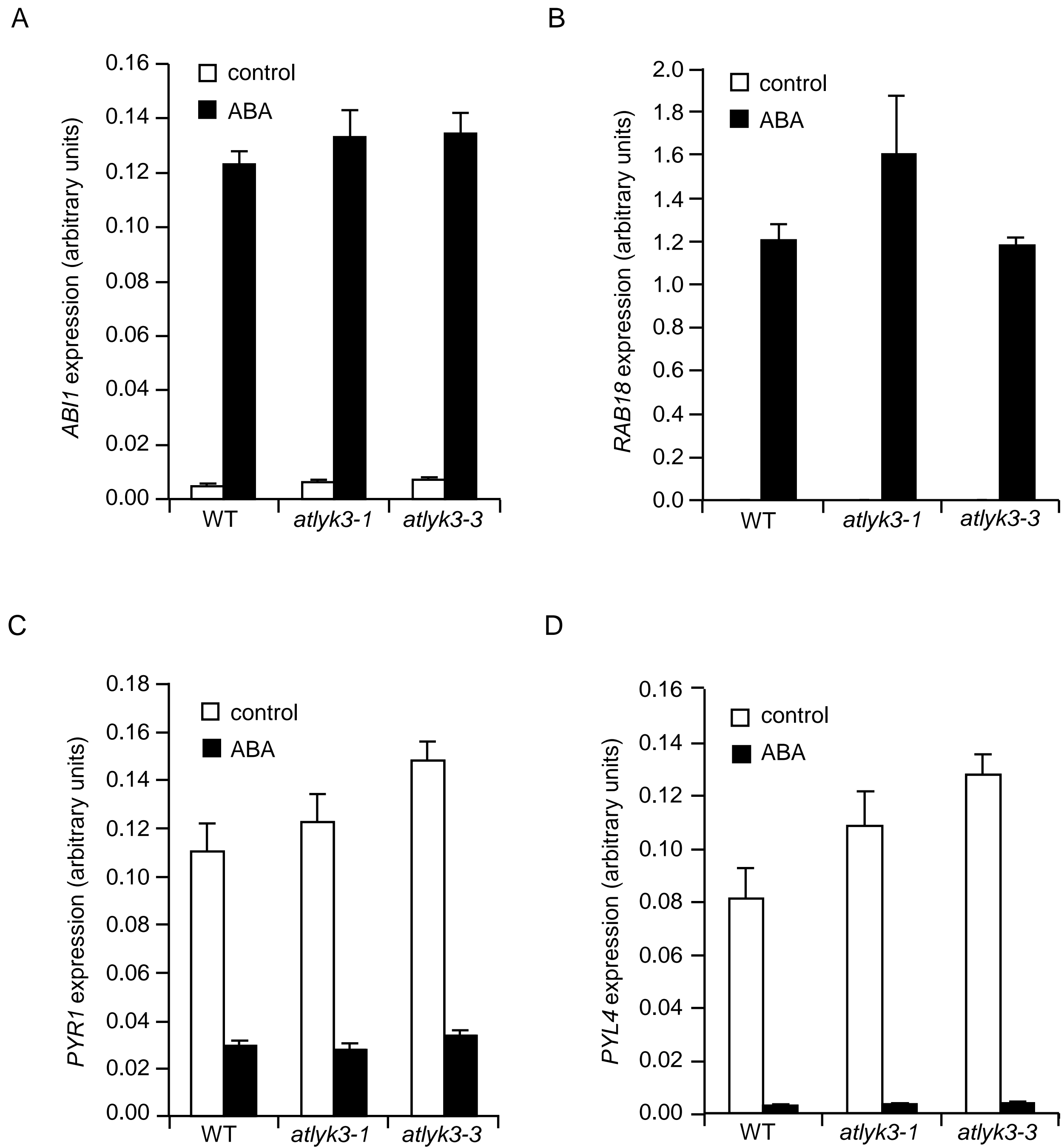
Supplementary Figure S4. Expression of *AtLYK3* and *PAD3* in *atlyk3-1 pad3* double mutants. A, expression of *AtLYK3* in WT, *atlyk3-1* and *atlyk3-1 pad3* rosette leaves inoculated with *B. cinerea* for the indicated times (dpi, days post inoculation) was analyzed by RT-PCR. B, expression of *PAD3* in WT, *atlyk3-1*, *atlyk3-1 pad3* and *pad3* rosette leaves inoculated with *B. cinerea* for the indicated times was analyzed by qPCR. Bars indicate average expression ± SD of three technical replicates. The *UBQ5* gene was used as reference in both experiments.



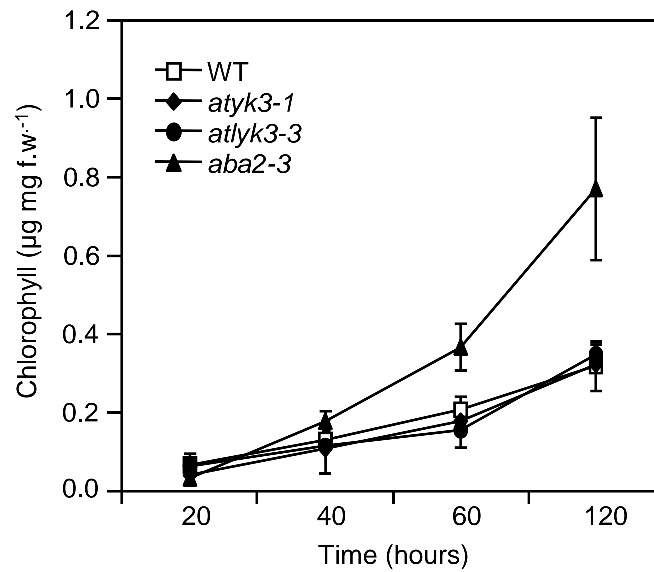
Supplementary Figure S5. *AtLYK3* is required for resistance to salt stress. WT (A, B), *aba2-3*, *atlyk3-1* (A) and *atlyk3-3* (B) seedlings were germinated on solid medium and, after five days, they were transferred to control plates (white bars) or to plates containing solid medium supplemented with 75 mM NaCl (black bars). Elongation of the primary root was measured after two days. Bars indicate the average root growth ± SD (n> 10). For each genotype, asterisks indicate significant differences between control and salt-treated seedlings, according to Student's t-test (***, $P < 0.01$). This experiment was repeated twice with similar results.



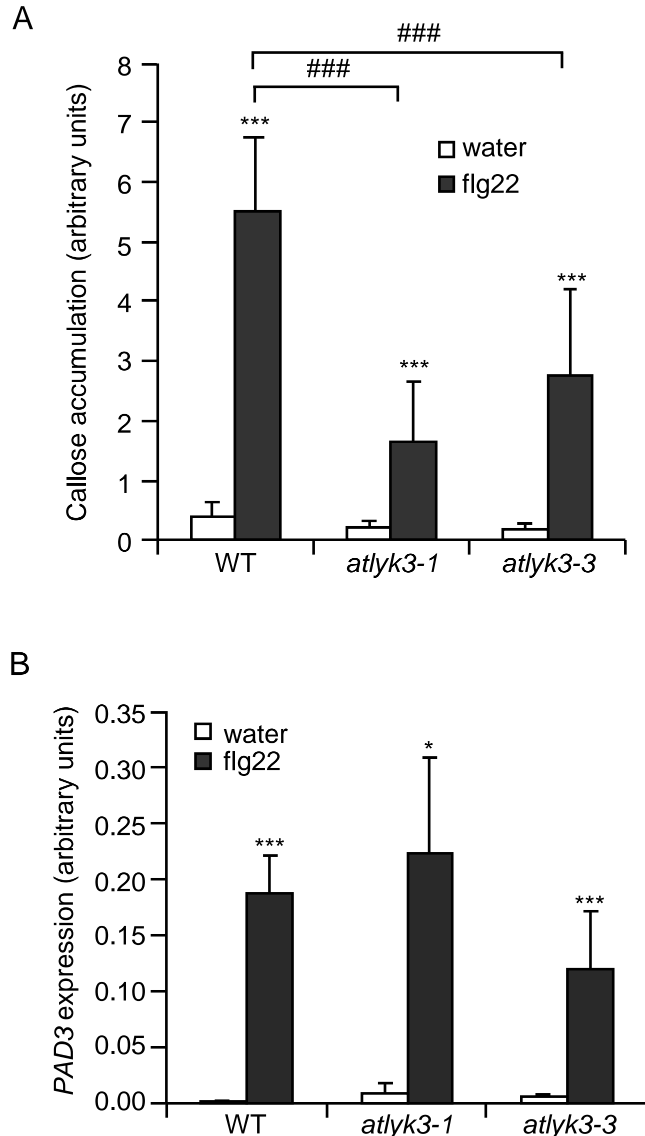
Supplementary Figure S6. ABA-induced physiological responses are independent of *PAD3*. A, WT, *atlyk3-1*, *pad3* and *atlyk3-1 pad3* seeds were stratified for three days at 4°C and sown on solid medium supplemented with 0.05% MeOH (control) or 5 µM ABA. Germination rate was determined after three days. Bars indicate average percentage of germinated seeds in three independent experiments ± SD (n>20 for each experiment). B, WT, *atlyk3-1 pad3* and *atlyk3-1 pad3* seedlings were germinated on solid medium and, after four days, they were transferred to plates containing solid medium supplemented with 0.05% MeOH (control) or 2.5 µM ABA. Elongation of the primary root was measured after two days. Bars indicate the average percentage of root elongation in seedlings grown on ABA compared to seedlings grown on control plates ± SD (n> 10). For each genotype, asterisks indicate significant differences between control- and ABA-treated samples, according to Student's t-test (*, $P < 0.05$).



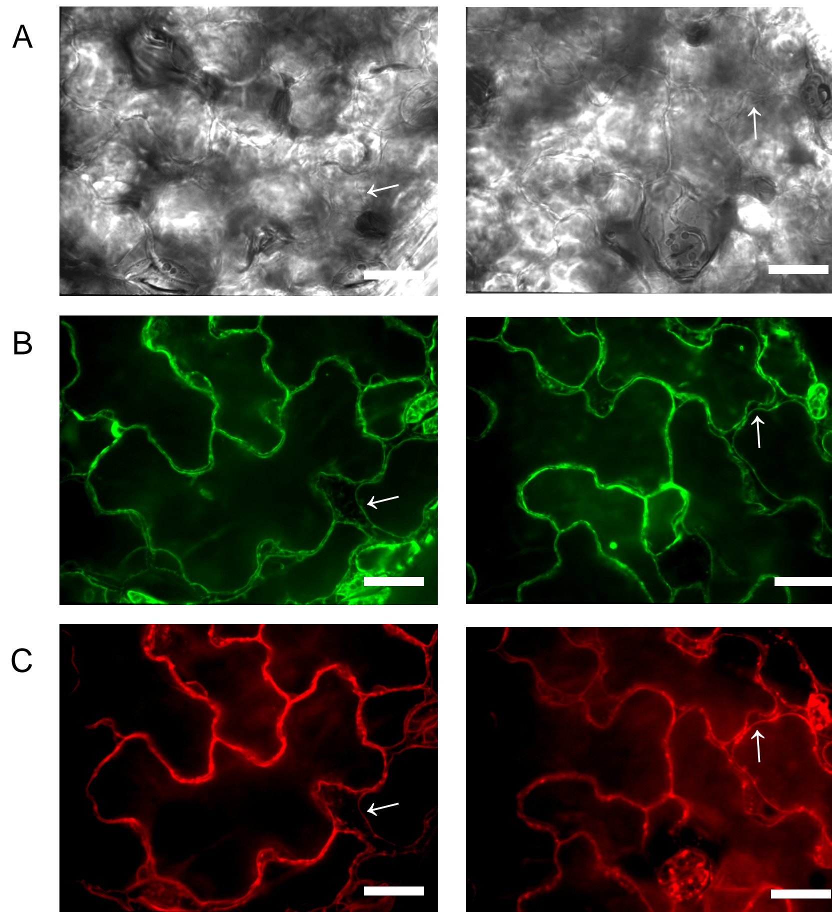
Supplementary Figure S7. AtLYK3 is not required for early responses to ABA. WT, *atlyk3-1* and *atlyk3-3* seedlings were treated for 4 h with 0.01% MeOH (control, white bars) or 1 µM ABA (black bars) and expression of *ABI1* (A), *RAB18* (B), *PYR1* (C) and *PYL4* (D) was analyzed by qPCR, using *UBQ5* as reference. Bars indicate average expression ± SD of three technical replicates. This experiment was repeated twice with similar results.



Supplementary Figure S8. Cuticle permeability is unaffected in *atlyk3-1* and *atlyk3-3* plants. Chlorophyll was extracted with ethanol for the indicated times from rosette leaves of WT (empty squares), *atlyk3-1* (black diamonds), *atlyk3-3* (black circles) and *aba2-3* (black triangles) plants. Chlorophyll concentration in the extracts was determined by spectrophotometry. Each point represents the average of six samples ± SD.



Supplementary Figure S9. Responses to flg22 in *atlyk3* mutants. A, rosette leaves of adult WT, *atlyk3-1* and *atlyk3-3* plants were infiltrated with water or 100 nM flg22. Leaves were harvested after 24 h and callose deposits were stained with aniline blue. Bars represent average fluorescence ± SD of at least six different samples. ***, significant difference between water- and flg22-treated samples, according to Student's t-test (P< 0.01). ###, significant difference between flg22-treated WT and mutants, according to Student's t-test (P< 0.01). This experiment was repeated twice with similar results. B, Expression of *PAD3* was analysed by qPCR in WT, *atlyk3-1* and *atlyk3-3* liquid-grown seedlings treated for 3 h with water or 10 nM flg22. The *UBQ5* gene was used as reference. Bars indicate average expression ± SD of three independent biological replicates. Asterisks indicate significant differences between water- and flg22-treates samples, according to Student's t-test (*, P<0.05; ***, P<0.01). No significant differences between flg22-treated WT and mutant seedlings were observed.



Supplementary Figure S10. AtLYK3-GFP localization in plasmolysed cells. Ten-day-old transgenic seedlings expressing *AtLYK3-GFP* under the control of the CaMV 35S promoter were treated for 20 min with 700 mM mannitol, and then stained with the plasma membrane-specific dye FM4-64. Images of cotyledon epidermal cells were taken by spinning disk laser scanning microscopy. A) bright field; B) GFP; C) FM4-64. Bars, 20 μm. Representative images of two independent transgenic lines are shown. Arrows indicate sites were the plasma membrane detached from the cell wall.