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Strigolactones promote the localisation of the ABA exporter ABCG25 at the plasma membrane in root epidermal cells of Arabidopsis thaliana

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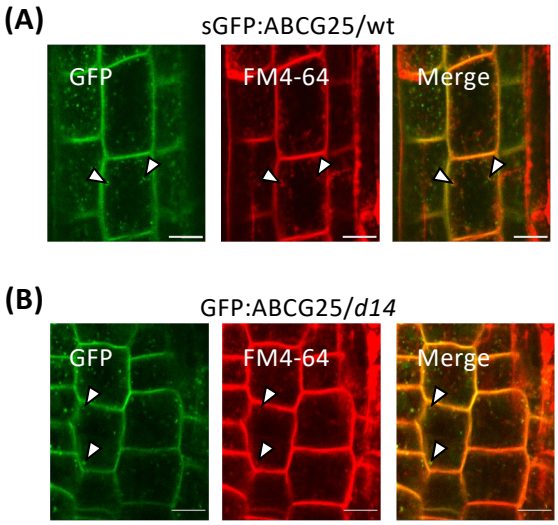
09 November 2026

Supplementary Table S1. List of relevant loci, primers and their sources

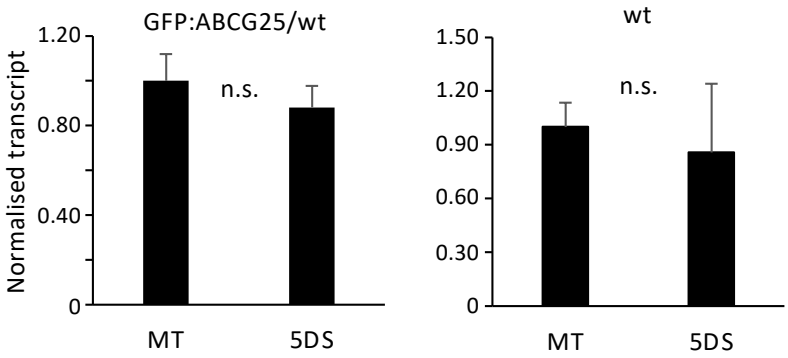
Primers for genotyping			
Primer name	**Sequence 5'->3'**	**Arabidopsis gene ID**	**Source**
Atd14-1-LP Atd14-1-RP Atd14-1-T-DNA	AAGAATATGGCAAGTGCAAC GATGATTCCGATCATAGCG TGATCCATGTAGATTTCCCGGACATGAAG	AT3G03990	This work
Atmax3-11-LP Atmax3-11-RP At-SALK-BP1	TTAGGCGACACCAAAATGAAG TTATGAATCTAAACCGTGGCG ATTTTGCCGATTTCGGAAC	AT2G44990	This work
Atabcg25-5-LP Atabcg25-5-RP At-SALK-BP1	AAGAACACGATTGGCTGATTC TCGTGGAAACGTATTTCATCC ATTTTGCCGATTTCGGAAC	AT1G71960	This work
GFP-F (also for qRT-PCR) GFP-R (also for qRT-PCR)	CACATGAAGCAGCACGACTT TCCTTGAAGTCGATGCCCTT	-	This work
Primers for qRT-PCR			
Primer name	**Sequence 5'->3'**	**Arabidopsis gene ID**	**Source**
AtTUA4-qRT-F AtTUA4-qRT-R	AACCTACACCAACCTCAACC GTGGATTCTTGGGTATGGGAC	AT1G04820	(Chan, 2012)
AtUBQ10-qRT-F AtUBQ10-qRT-R	GGCCTTGTATAATCCCTGATGAATAAG AAAGAGATAACAGGAACGGAAACATAGT	AT4G05320	(Brotman *et al.*, 2009)
AtMAX3-qRT-F AtMAX3-qRT-R	CAACCGAGTCAAGCTTAATCCA AACGCTGATACCATTGGTGACA	AT2G44990	(Booker *et al.*, 2004)
AtMAX4-qRT-F AtMAX4-qRT-R	GAAAGATACCCACTTGGCTGAATG TGTGGAGTAGCCGTCGAAGAG	AT4G32810	(Hayward *et al.*, 2009)
AtRD29b-qRT-F AtRD29b-qRT-R	AAGGGGAAGAGAAAGGTGTG TCTCTCCTCCTCCTCCAAAA	AT5G52300	This work
AtNCED3-qRT-F AtNCED3-qRT-R	CCATCAAAGGAGTGTATGTGC TTAGTCTGAGTAAACCGGCAA	AT3G14440	This work
AtABCG25-qRT-F AtABCG35-qRT-R	GAGACGCCATGGCTTACTTTGA AATACATGTTGTTATTCCACCGCC	AT1G71960	(Kang *et al.*, 2010)

Booker J, Auldridge ME, Wills S, McCarty D, Klee H, Leyser O. 2004. MAX3/CCD7 is a carotenoid cleavage dioxygenase required for the synthesis of a novel plant signaling molecule. Current Biology **14**, 1232-1238.

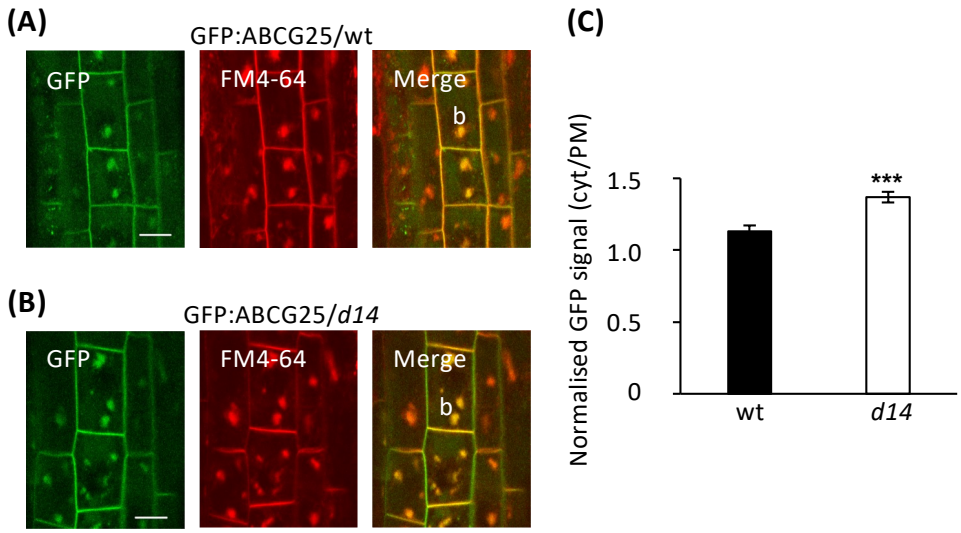
Brotman Y, Makovitzki A, Shai Y, Chet I, Viterbo A. 2009. Synthetic ultrashort cationic lipopeptides induce systemic plant defense responses against bacterial and fungal pathogens. Applied and Environmental Microbiology **75**, 5373–5379.
Chan Z. 2012. Expression profiling of ABA pathway transcripts indicates crosstalk between abiotic and biotic stress responses in Arabidopsis. Genomics **100**, 110-115.
Hayward A, Stirnberg P, Beveridge C, Leyser O. 2009. Interactions between auxin and strigolactone in shoot branching control Plant Physiology **151**, 400-412.
Kang J, Hwang J-U, Lee M, Kim Y-Y, Assmann SM, Martinoia E, Lee Y. 2010. PDR-type ABC transporter mediates cellular uptake of the phytohormone abscisic acid. Proceedings of the National Academy of Sciences, USA **107**, 2355-2360.



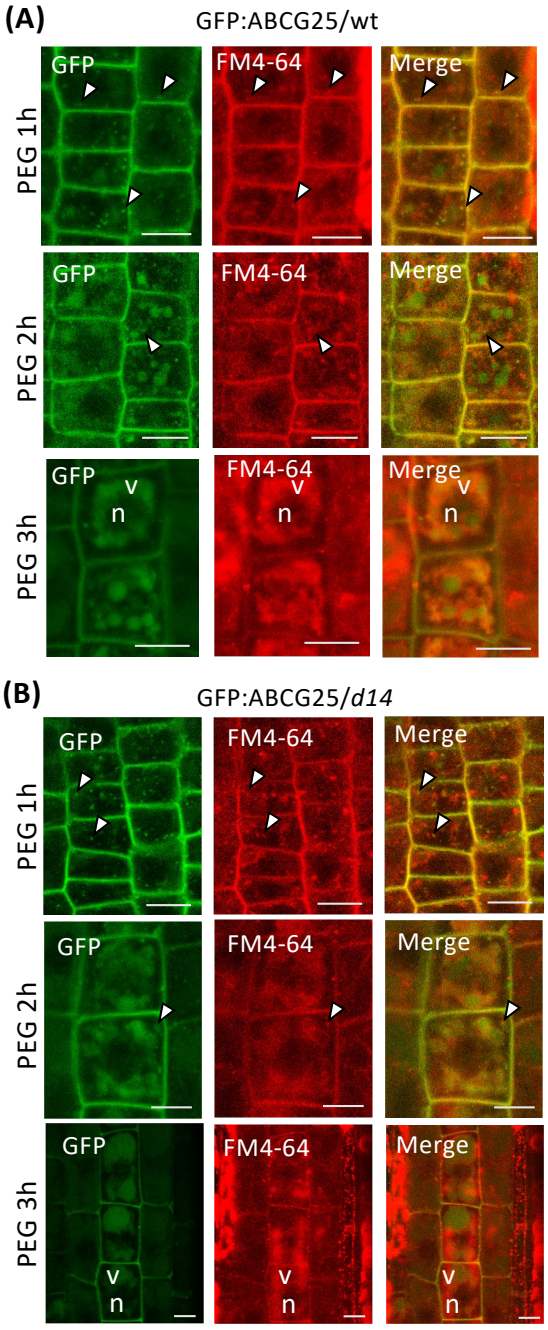
Supplementary Figure S1: Localisation of sGFP:ABCG25 and FM4-64 fluorescent signals. Five-day-old Arabidopsis seedlings expressing GFP:ABCG25 (green) in the wild-type (wt) **(A)** and *d14-1* background **(B)** were counterstained with 4 µM FM4-64 (red) on ice and then observed at the confocal microscope 5 min after endocytosis was restarted by shifting to room temperature. The imaging of root tip epidermal cells showed GFP:ABCG25 labelling of the plasma membrane and vesicle-like compartments in the cytosol. FM4-64 colocalization at the membrane and (partially) at the vesicle structures (arrowheads) was in line with literature data (Park *et al.*, 2016) and confirmed the endosomal nature of the vesicles. Bars = 10 µm.



Supplementary Figure S2: Effect of GR24^{5DS} treatment on *ABCG25* transcripts in the roots of the GFP:ABCG25/wt line **(A)** and in the wild type **(B)**. No significant changes were recorded 4 h after treatment with GR24^{5DS} 10 µM (5DS) with respect to mock-treated controls (MT) in 16-day-old seedlings. Relative expression levels were calculated using the geometric means of *AtTUA4* and *AtUBQ10* transcript concentrations as reference (**Supplementary Table S1**). Data represent the mean ± SE of 4 biological replicates for each condition (each replicate the pool of 20 rootlets) and time point using Student's t-test; P value < 0.05.



Supplementary Figure S3: Localization of sGFP:ABCG25 and FM4-64 fluorescent signals upon BFA incubation. Five-day-old Arabidopsis seedlings expressing GFP:ABCG25 (green) in the wild-type (wt) **(A)** and *d14-1* background **(B)** were stained on ice with 4 µM FM4-64 (red) after 30 min incubation in the BFA solution, and then observed at the confocal microscope 5 min after endocytosis was restarted by shifting to room temperature. The localization of GFP:ABCG25 was examined in epidermal root cells, where both the GFP construct (green) and FM4-64 mark BFA bodies (b) are visible. **(C)** Quantification of the cytosol/plasma membrane (cyt/PM) fluorescence ratio after 30 min BFA incubation. Values were normalised over the corresponding mock-treated samples. GFP:ABCG25 accumulated at BFA bodies more intensely in the *d14-1* mutant than in the wild type. Bars = 10 µm.



Supplementary figure S4: Localization of sGFP:ABCG25 and FM4-64 fluorescent signals upon PEG treatment. Five-day-old Arabidopsis seedlings expressing GFP:ABCG25 (green) in the wild-type (wt) **(A)** and *d14-1* (*d14*) **(B)** background were stained on ice with 4 µM FM4-64 (red) after 1, 2 and 3 h incubation in a 20% PEG solution, and then observed at the confocal microscope 5 min after endocytosis was restarted by shifting to room temperature. PEG treatment caused the endocytosis of GFP:ABCG25, visible at endosomes (arrowheads) at early stages and at vacuole (v) at late stages. n = nucleus; bars = 10 µm.