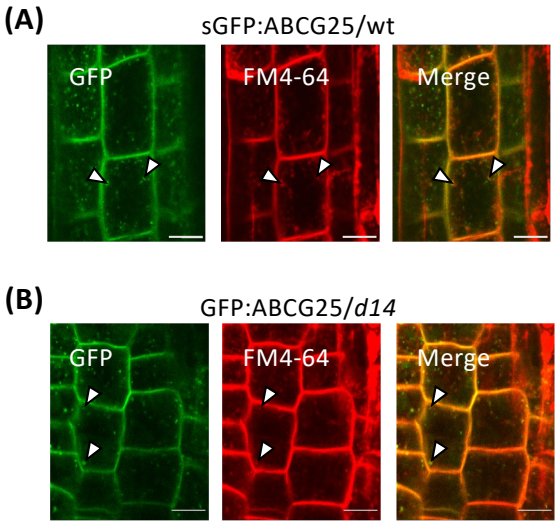
Strigolactones promote the localisation of the ABA exporter ABCG25 at the plasma membrane in root epidermal cells of Arabidopsis thaliana

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

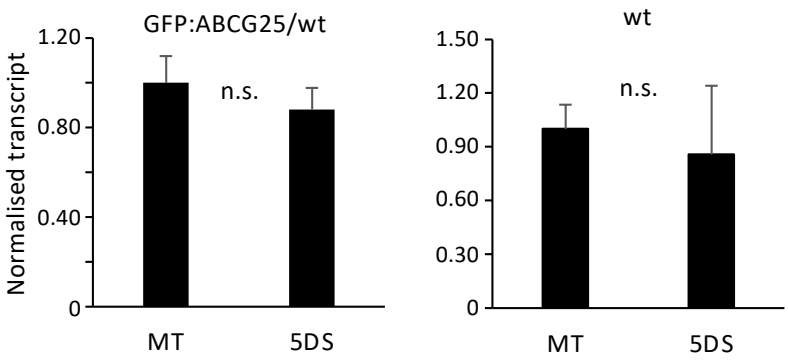
Primers for genotyping			
Primer name	**Sequence 5'->3'**	**Arabidopsis gene ID**	**Source**
Atd14-1-LP Atd14-1-RP Atd14-1-T-DNA	AAGAATATGGCAAGTGCAAC GATGATTCCGATCATAGCG TGATCCATGTAGATTTCCCGGACATGAAG	AT3G03990	This work
Atmax3-11-LP Atmax3-11-RP At-SALK-BP1	TTAGGCGACACCAAAATGAAG TTATGAATCTAAACCGTGGCG 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.

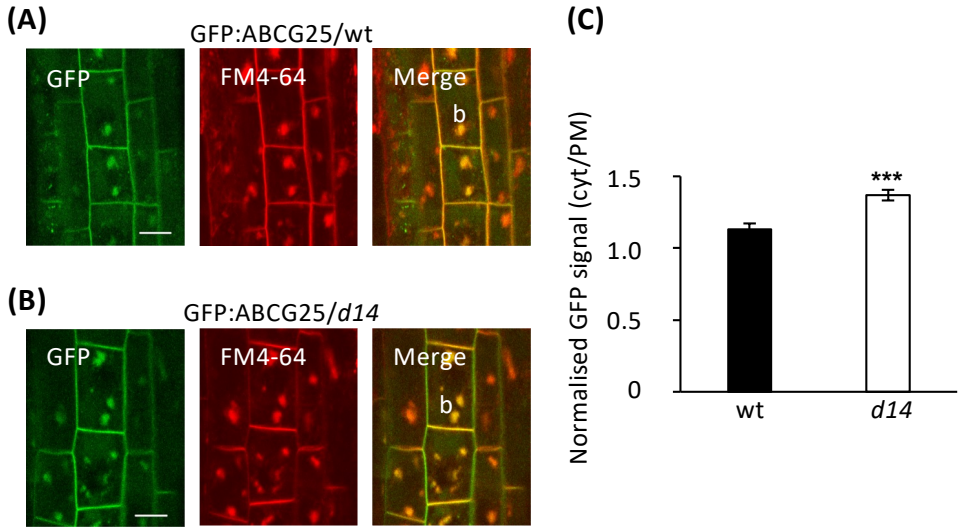
Brotman Y, Makovitzki A, Shai Y, Chet I, Viterbo A. 2009. Synthetic ultrashort cationic lipopeptides induce systemic plant defense responses against bacterial and fungal pathogens. Applied and Environmental Microbiology **75**, 5373–5379.
Chan Z. 2012. Expression profiling of ABA pathway transcripts 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.



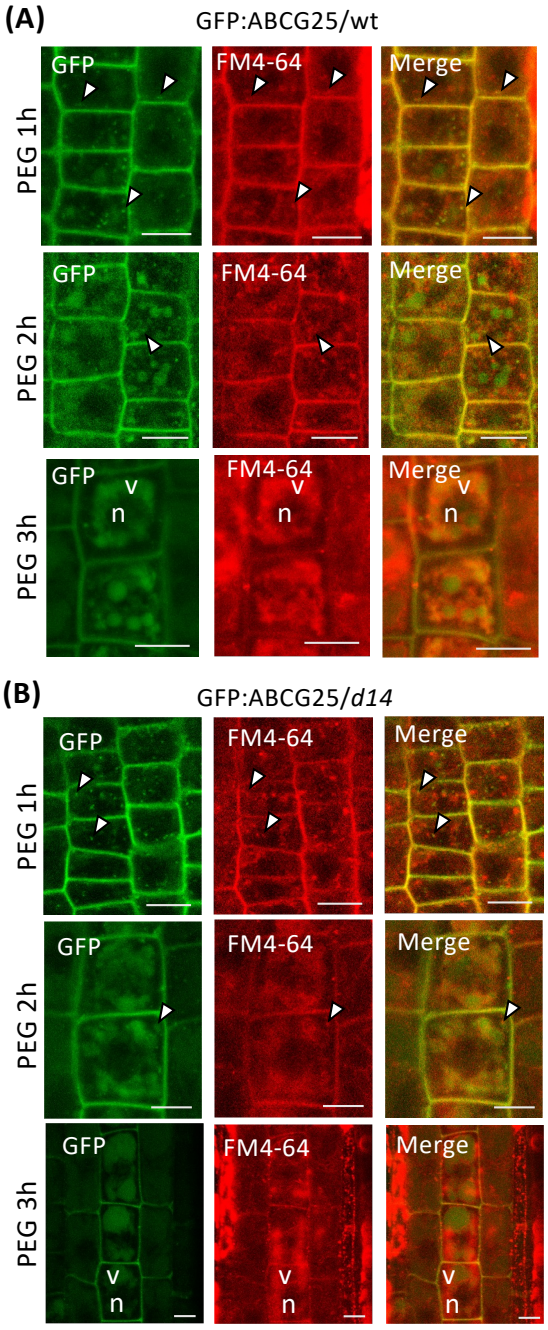
Supplementary Figure S1: Localisation of sGFP:ABCG25 and FM4-64 fluorescent signals. Five-day-old Arabidopsis seedlings expressing GFP:ABCG25 (green) in the wild-type (wt) **(A)** and *d14-1* background **(B)** were counterstained with 4 µM FM4-64 (red) on ice and then observed at the confocal microscope 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.