

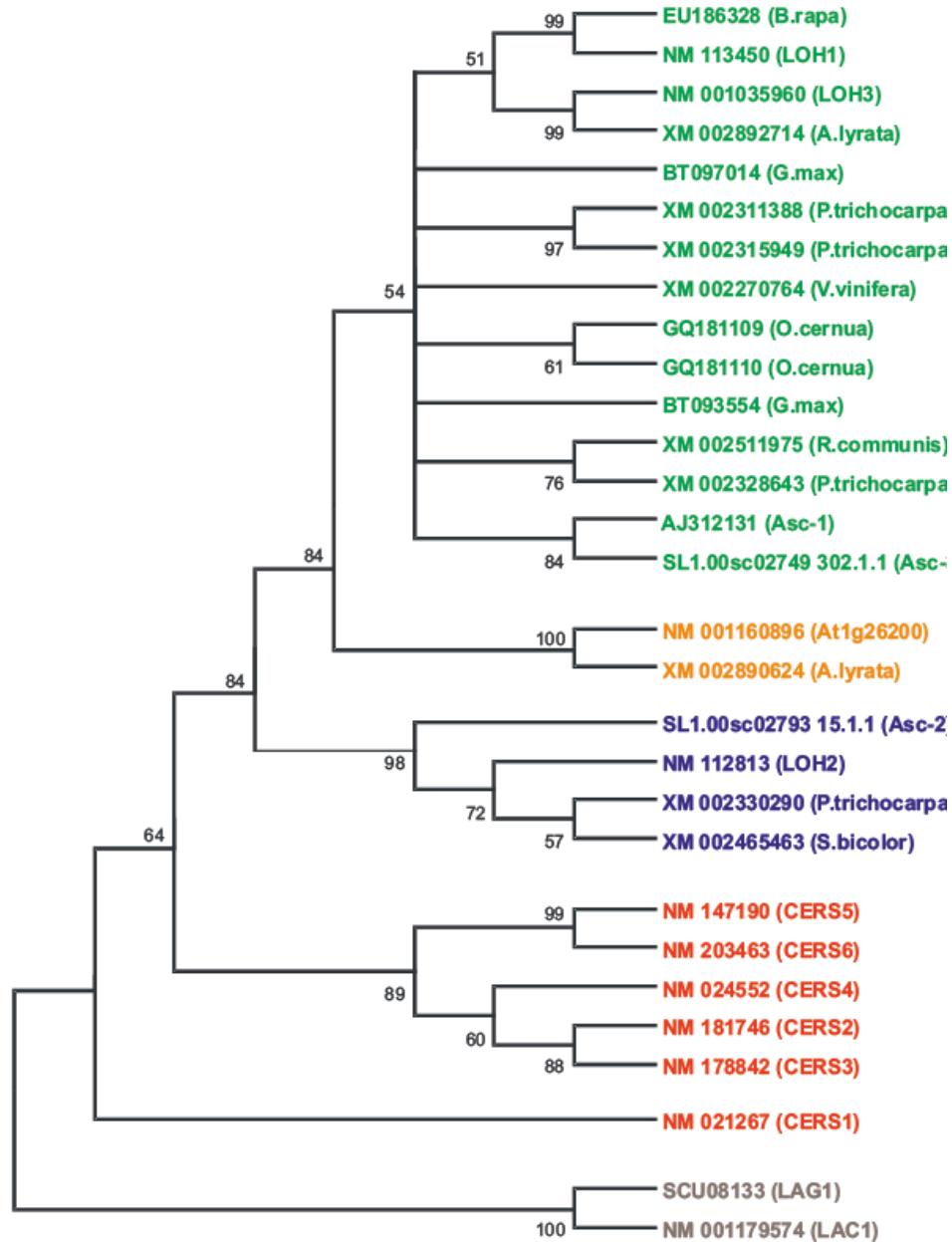

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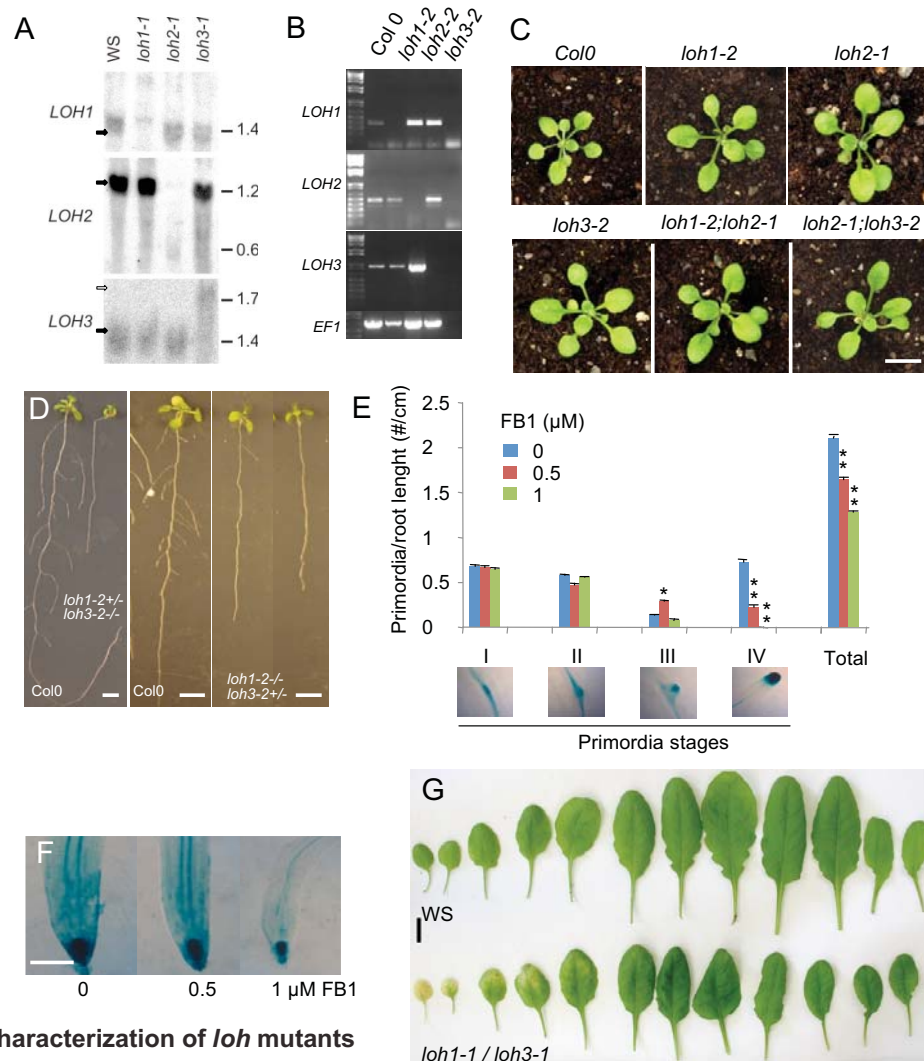
Supp. Fig. 1A. Protein sequence alignment of 29 LAG1 homologs.

Full length protein eudicot sequences are aligned with the CerS homologs from human (HsCerS1-6; #NM_021267, #NM_181746, #NM_178842, #NM_024552, #NM_147190, #NM_203463) and the LAG1 (#SCU08133) / LAC1 (#NM_001179574) sequences from *S. cerevisiae*. The two tomato sequences SIAsc-2 (#SL1.00sc02793_15.1.1) and SIAsc-3 (#SL1.00sc02749_302.1.1) are available from the tomato genome database <http://mips.helmholtz-muenchen.de/plant/tomato/index.jsp>. Other proteins are from *Arabidopsis lyrata*, (AlLOH3 # XM_002892714, AlLOH4 # XM_002890624); *Arabidopsis thaliana*, (AtLOH1-4, #NM_113450, #NM_112813, #NM_001035960 and #NM_001160896); *Brassica rapa* (BrLOH1 #EU186328); *Glycine max* (GmLOH1 #BT097014 and GmLOH3 #BT093554); *Orobanchaceae* (*Orobanchaceae*) (OcLOH1 #GQ181109 and OcLOH3 #GQ181110); *Populus trichocarpa* (PtLOH1a #XM_002311388, PtLOH1b #XM_002315949, PtLOH2 #XM_002330290, PtLOH3 #XM_002328643); *Ricinus communis* (RcLOH3 #XM_002511975); *Solanum lycopersicon* (SIAsc-1 #AJ312131); *Sorghum bicolor* (SbLOH2 #XM_002465463); *Vitis vinifera* (VvLOH1 #XM_002270764) Sequences are listed in Supplemental Dataset 1 online.

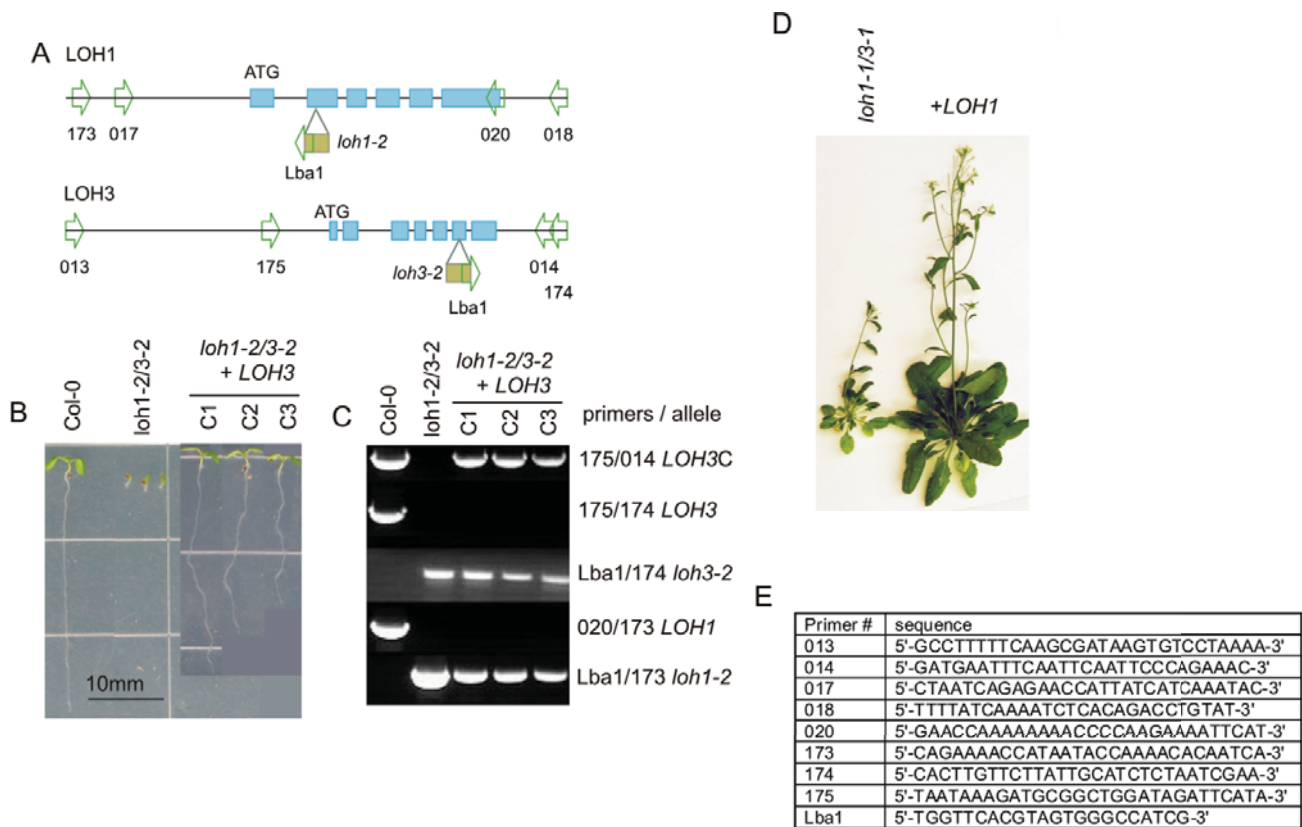


Supp. Fig. 1B. Phylogenetic relationship among LAG1 homologs.

The alignment shown in Fig. S1A was used to examine the phylogenetic relationship between LAG1 protein sequence homologs. Significant groupings are highlighted in colour. The region from 179 to 417 on the alignment ruler was used to establish the tree using Maximum Parsimony methods, gaps were excluded. The bootstrap consensus tree (1000 replicates) is shown, branches where divisions produced less than 50% of the time are collapsed. The percentage of trees where a particular branch occurred is shown next to each branch point. CERS1 is an unique link between the mammalian and fungal sequences and was selected as the root branch. Similar tree arrangements were produced using Maximum Likelihood and Neighbour-Joining algorithms. Alignment is shown in Supplemental Dataset 1 online.

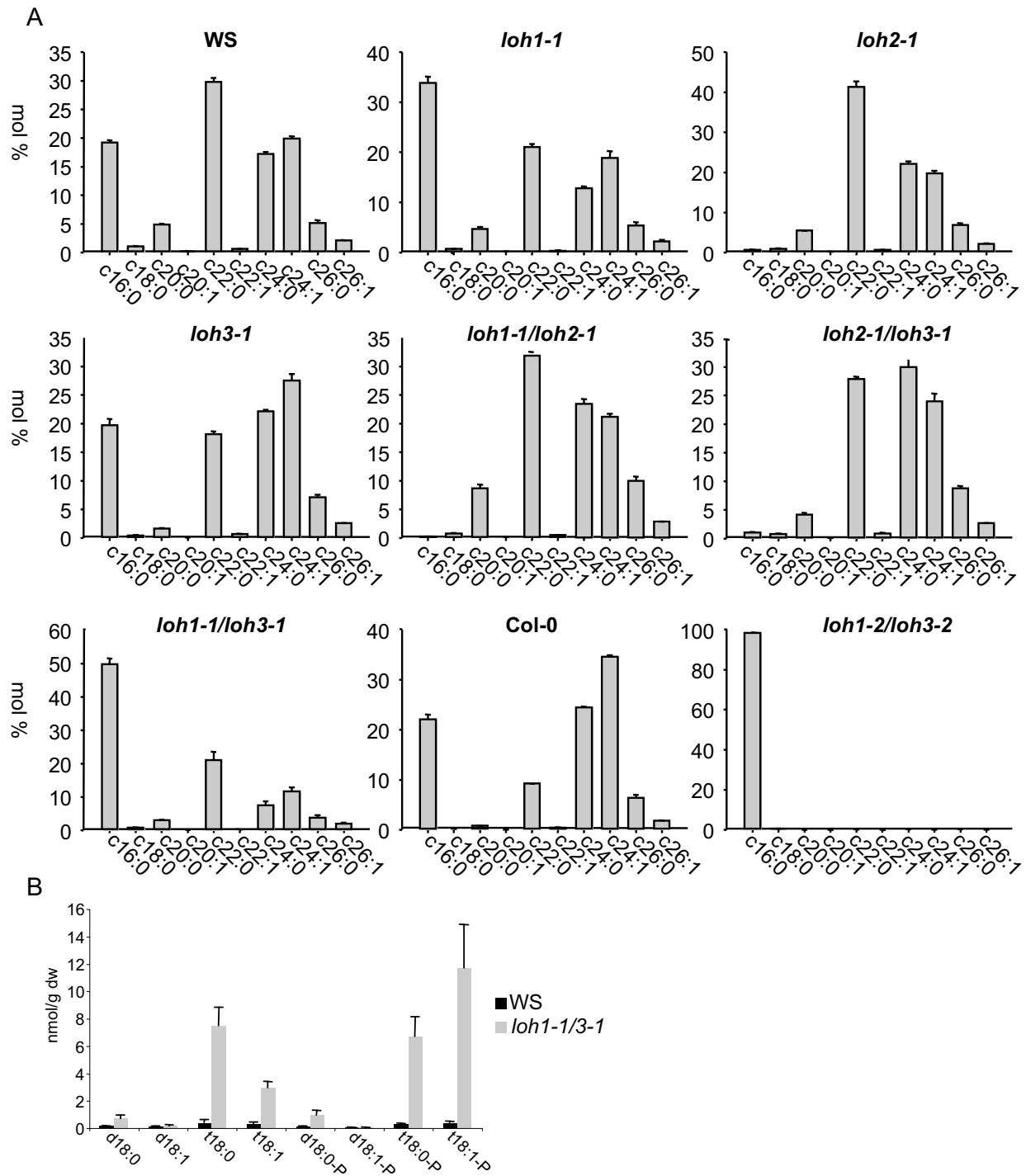
Supp. Fig. 2. Characterization of *loh* mutants

- (A) Northern blot with RNA from *loh1-1*, *loh2-1* and *loh3-1* and hybridized with *LOH1*, *LOH2* and *LOH3* probes. Transcript size (kb) are indicated by solid arrow and the abnormal *LOH3* transcript by an open arrow.
- (B) Molecular analysis of *loh* alleles. RT-PCR of total RNAs from *loh1-2*, *loh2-2*, *loh3-2* with T-DNA flanking primers.
- (C) Phenotype of 3 week-old rosettes of *loh* mutant. Scale bar, 1cm.
- (D) Lateral root development is inhibited in sesqui mutants *loh1-2+/-;loh3-2+/-* and *loh1-2-/-;loh3-2+/-*. Plants were grown *in vitro* for two weeks. Scale bar, 1mm.
- (E) Lateral root outgrowth but not initiation is inhibited by FB1 treatment. Number of lateral roots expressing DR5::GUS were classified according to different developmental stages (defined arbitrarily as I, first pericycle divisions; II, first periclinal divisions; III, primordia formation; IV, primordia outgrowth) in seedlings treated for 9 days with different concentrations of FB1. Data represent the mean of total lateral roots for 30 seedlings as shown in Fig.S2D. Significant differences between means (Student's t-test) are indicated by one ($P \leq 0.05$) or two ($P \leq 0.01$) asterisks.
- (F) GUS staining in wild-type primary root meristems containing the DR5::GUS auxin reporter that were grown for 9 days in the presence of various concentrations (0, 0.5 and 1 μM) of FB1. Scale bar, 100 μm .
- (G) Rosette leaves from wild-type (WS - top) and *loh1-1/loh3-1* mutant plants (bottom). Mutant leaves are smaller, wrinkled and show premature senescence. Scale bar, 10mm.

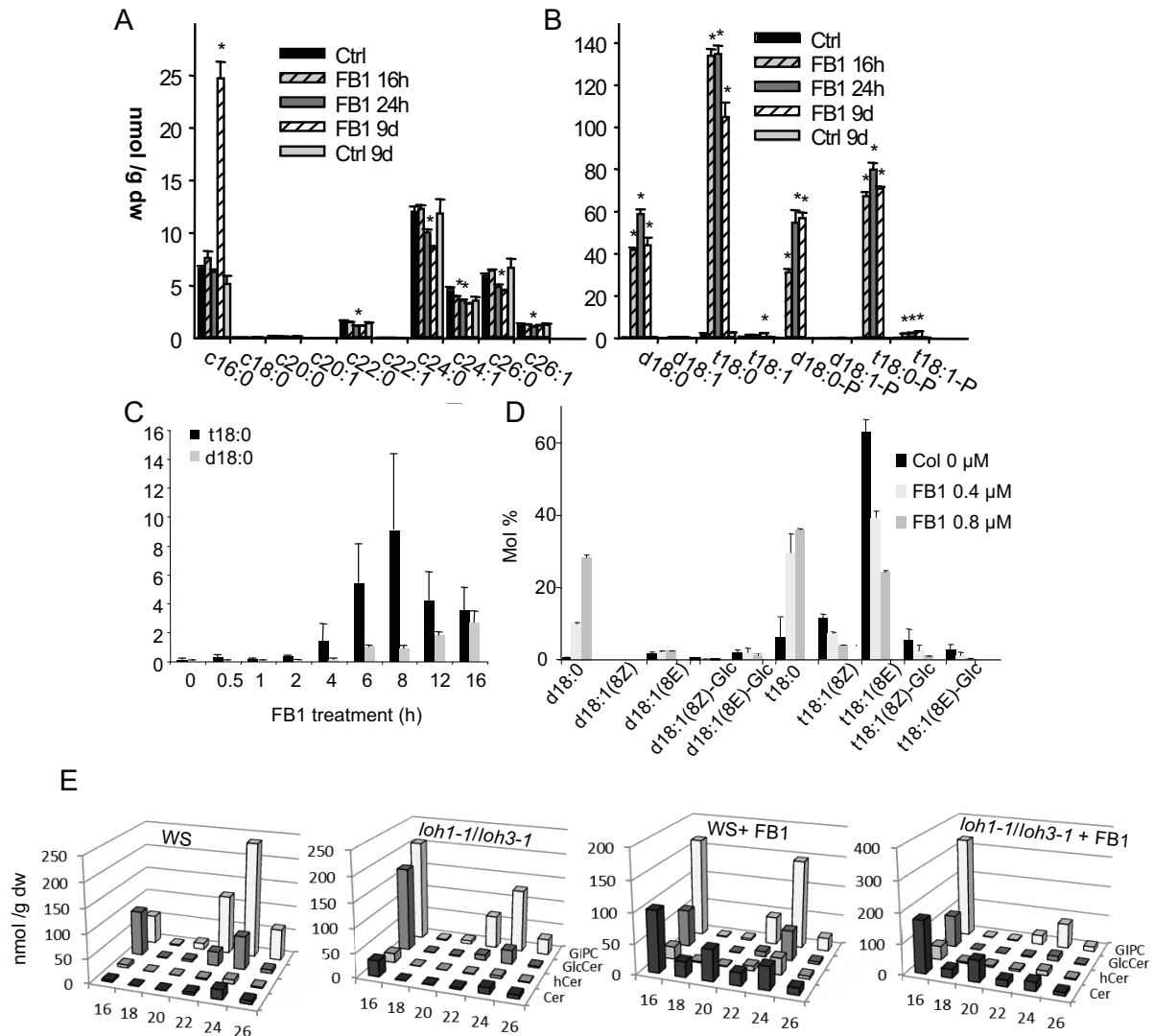


Supp. Fig. 3. Complementation of *loh1/loh3* double mutant.

- Location of primers used to create *LOH* complementation constructs and analyze the complemented plants. Primers are shown as green arrows, the position of exons is indicated by blue boxes, the position of the T-DNA insertions in the *loh1-2* and *loh3-2* allele are indicated.
- Complementation of *loh1-2/loh3-2* by *LOH3*. Shown are a *Col-0* wild-type plant, *loh1-2/loh3-2* seedlings and complemented *loh1-2/loh3-2* plants containing a transgenic copy of the *LOH3* allele.
- PCR analysis of genomic DNA extracted from the plants shown in B. PCR reactions used the primers indicated on the right. Primers 175 and 014 detect the wild-type *LOH3* allele and the complementing *LOH3* containing T-DNA. Primers 175 and 174 detect only the wild-type *LOH3* allele.
- Complementation of *loh1-1/loh3-1* by *LOH1*. A *loh1-1/loh3-1* plant is shown in the left with typical delayed flowering and reduced rosette size. Plants containing the complementing *LOH1* T-DNA do not show these phenotypes.
- Primers used in making and analyzing the complemented plants.

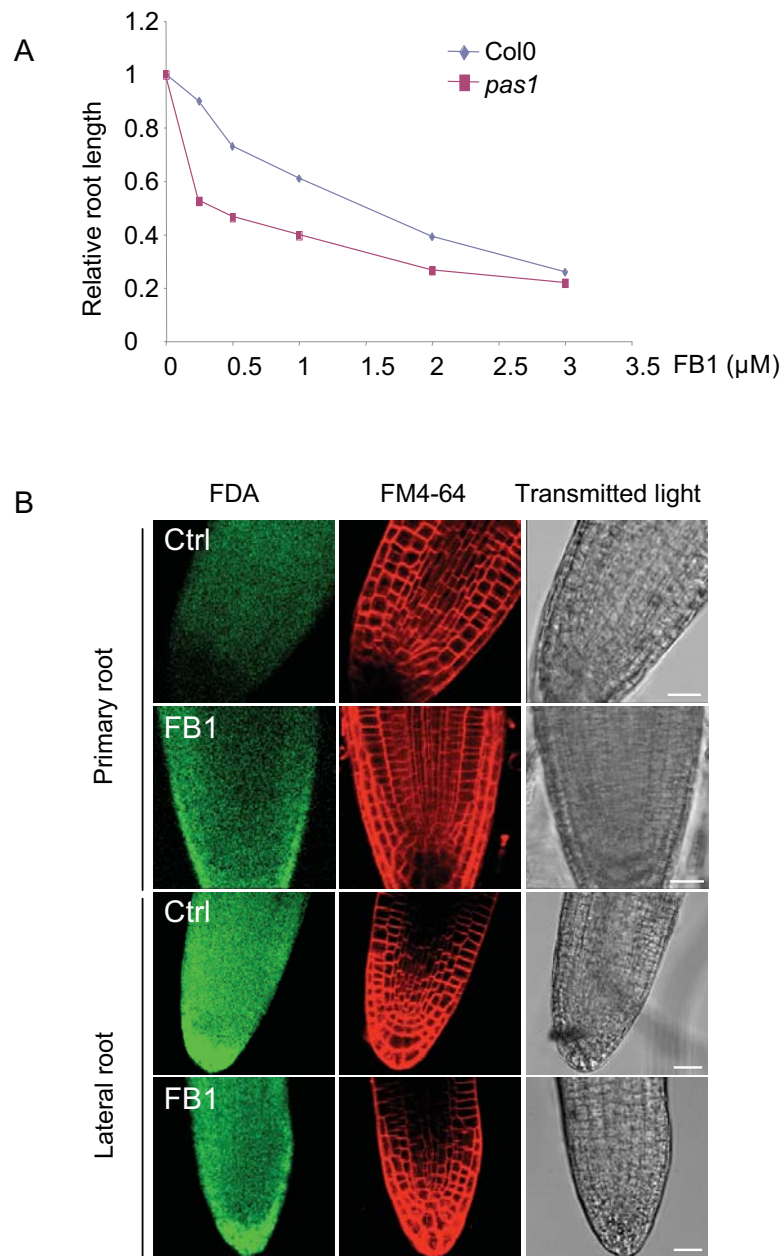


Supp. Fig. 4. Fatty acid content of total sphingolipids and free LCBs from *loh* mutants. (A) Total sphingolipids Total sphingolipid content of *loh* mutants compared to their related control (WS for *loh1-1*, *loh2-1* and *loh3-1*; Col0 for *loh1-2* and *loh3-2*). Sphingolipid content is shown according to the length and saturation of the fatty acid chain. (B) Free LCB content of *loh 1-1/loh3-1* compared to its related control (WS). Analyses were performed on 3-5 biological replicates. Data are the mean and standard error ($n = 5$).



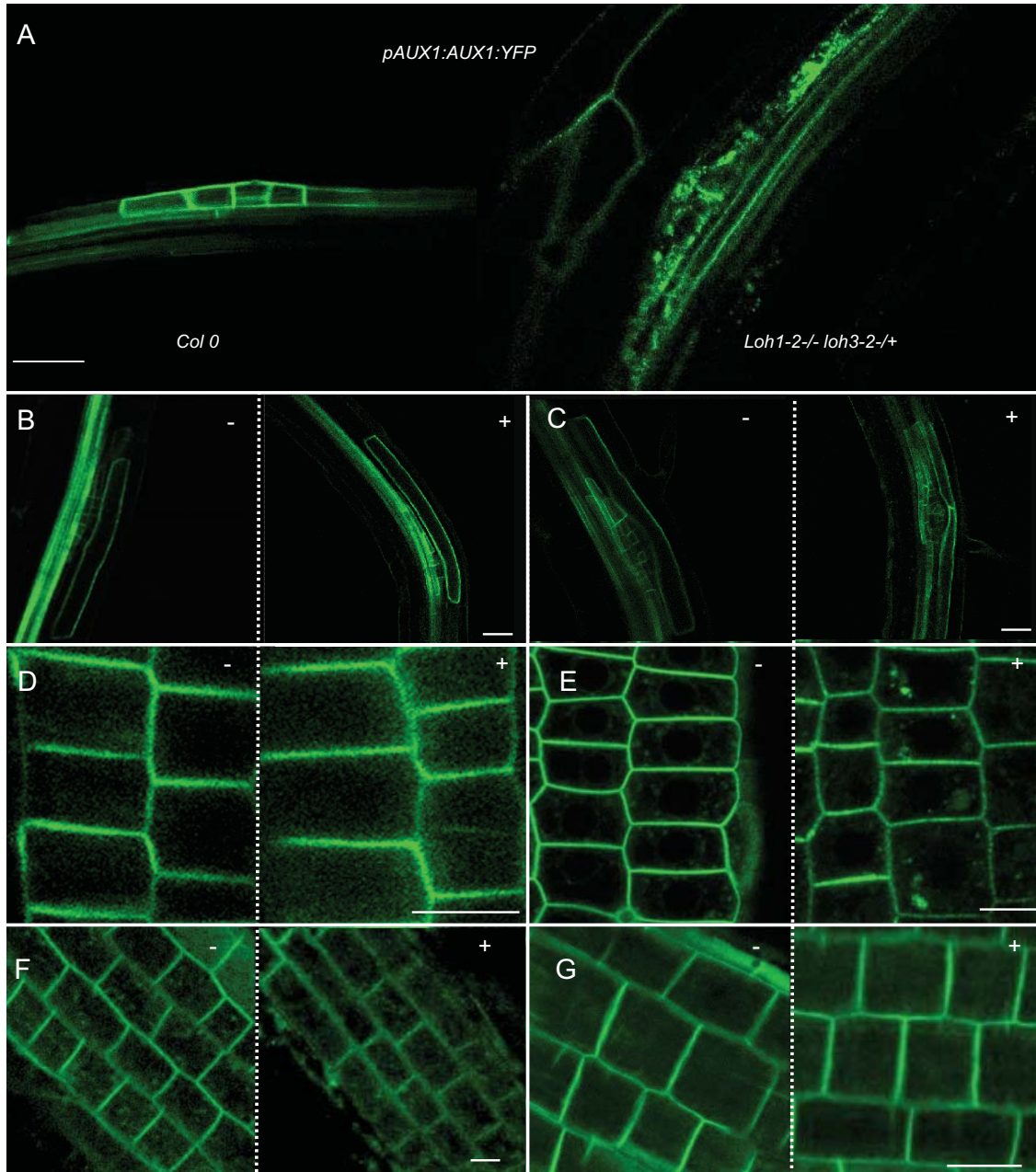
Supp. Fig. 5. Spingolipid analysis of FB1 treated seedlings.

- (A) Ceramide content in seedlings grown for several hours (16h, 24h) or 9 days (9d) on medium supplemented with 0.5 μ M FB1 and compared to untreated seedlings.
- (B) Free LCB content in seedlings grown for several hours (16h, 24h) or 9 days (9d) on medium supplemented with 0.5 μ M FB1 and compared to untreated seedlings.
- (C) Free t18:0 and d18:0 levels during time course treatment with 1 μ M FB1 (h, hours).
- (D) Total LCB from seedlings treated with two FB1 concentrations for 9 days compared to untreated seedlings.
- (E) Total sphingolipid content of WS and loh1-1/loh3-1 double mutant treated (+FB1) or not with 0.5 μ M FB1. Data show mean plus one standard error and significant differences between means ($P \leq 0.05$, Student's t-test) are indicated by an asterisk.



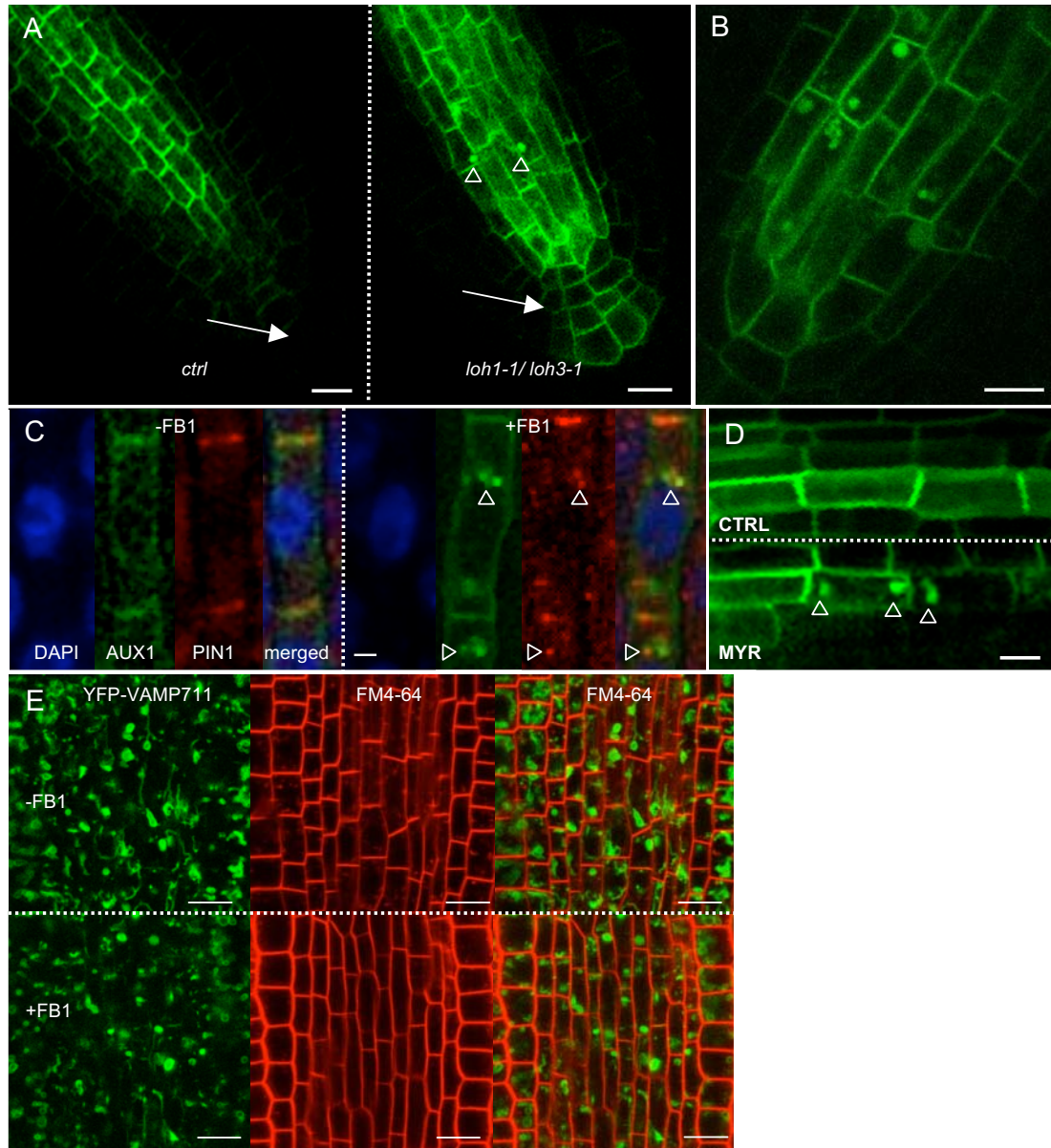
Supp. Fig. 6. Effect of FB1 on root growth and viability.

- (A) *Pasticcino1* mutant root growth is more sensitive to FB1 than wild type. Seedlings were germinated and grown vertically on different concentrations of FB1 for 10 days. Data show root length relative to untreated control seedlings. Data are the mean of 25 to 33 seedling roots.
- (B) Primary and secondary root viability upon FB1 treatment. Root tips treated or not with FB1 were stained with FM4-64 and FDA. Scale bar, 20 μm .



Supp. Fig. 7. FB1 has no effect on several membrane markers.

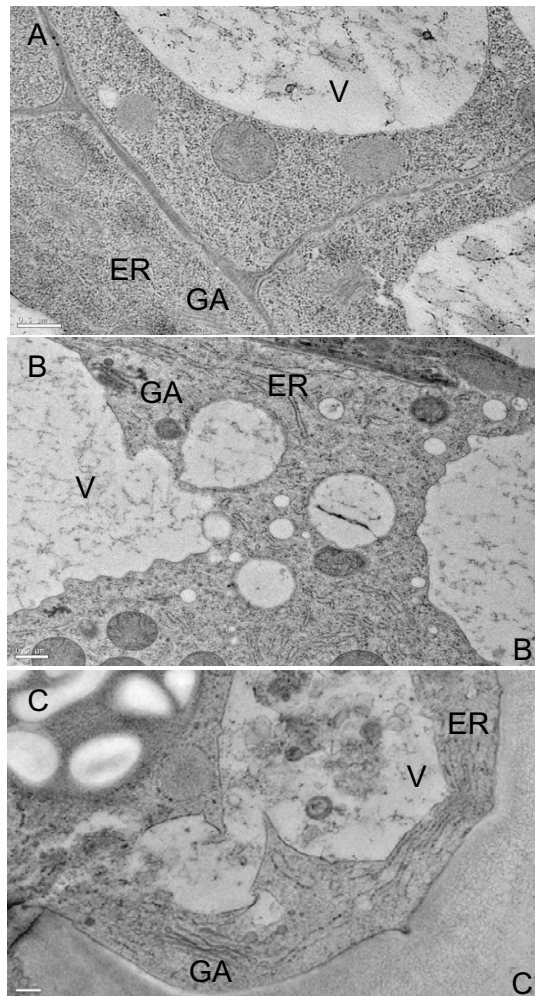
- (A) Expression of *pAUX1:AUX1-YFP* in lateral root primordia of Col0 and *loh1-2^{-/-}/loh3-2^{-/+}* double mutant. Note the absence of membrane localization of AUX1-YFP in *loh1-2^{-/-}/loh3-2^{-/+}*. Scale bar, 10µm.
- (B-C) Expression of *pLAX3:LAX3-GFP* in FB1-16h treated lateral roots (+) compared to untreated roots (-). *LAX3-GFP* expression was observed at two different developmental stages. Scale bar, 10µm. (B) the first anticlinal division of pericycle cells, and (C) the first periclinal cell divisions leading to the lateral root emergence.
- (D-G) Plasma membrane markers in untreated (-) and FB1-16h treated (+) epidermal cells from lateral roots. (D) *pPIN2:PIN2-GFP*, (E) *pPIN2:PIN1-GFP*, (F) *35S:LTi6b-GFP* and (G) *35S:PIP2-GFP*. Scale bar, 10µm.



Supp. Fig. 8. Subcellular Distribution of PIN1-GFP and AUX1-YFP and YFP-VAMP711 upon sphingolipid depletion

- (A) Expression of *pPIN1:PIN1-GFP* in control (*ctrl*) and *loh1-1/loh3-1* double mutant. Arrows indicate the columella and arrows head show the presence of PIN1-GFP aggregates.
- (B) Detail of *pPIN1:PIN1-GFP* in *loh1-1/loh3-1* double mutant.
- (C) Immunolocalization of PIN1 in *pAUX1:AUX1-YFP* secondary root cells treated (+FB1) or not (-FB1) with 2.5 μM FB1 for 16h. Cells were counterstained with DAPI. Note that PIN1 aggregates in presence of FB1 and partially colocalize with AUX1-YFP.
- (C) Myriocin treatment induces *pAUX1:AUX1:YFP* aggregation
- (D) Expression of the vacuolar marker YFP-VAMP711 (Wave 9Y) in the root stele in presence (+FB1) or not of FB1-16h treatment.

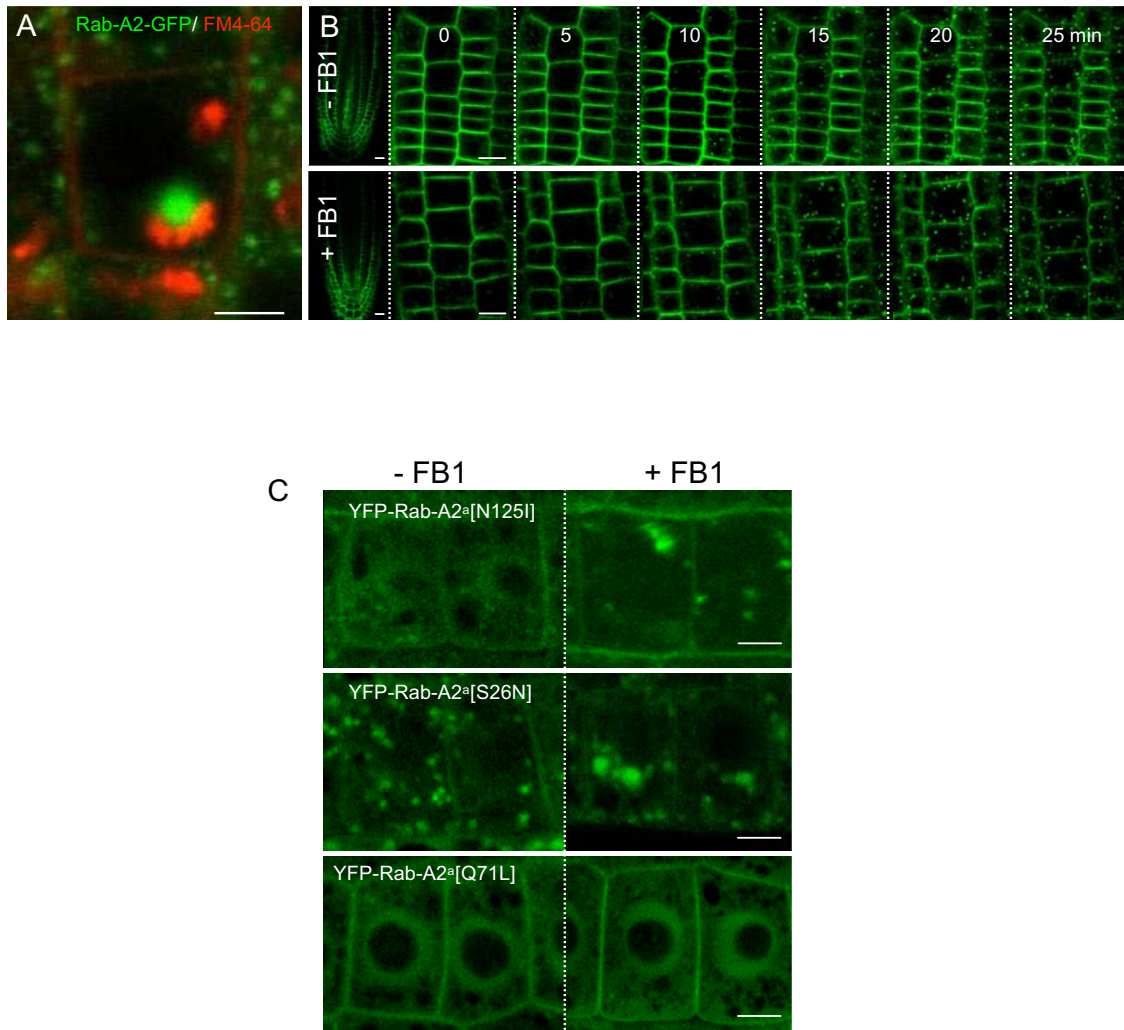
Scale bar. 20 μm in A, 10 μm in B and D, 2 μm in C.



Supp. Fig. 9. Endomembrane ultrastructure is altered in *loh1-1/loh3-1* root cells.

- (A) Control cells exhibiting characteristic tubular endoplasmic reticulum (ER), a Golgi stack (GA) and a large vacuole (V).
- (B-C) In *loh 1* mutant (B) and *loh1-1/3-1* mutant (C), Golgi ultrastructure is not affected, cytoplasm is often filled with small convoluted vacuole-like structures, with modified ER network.

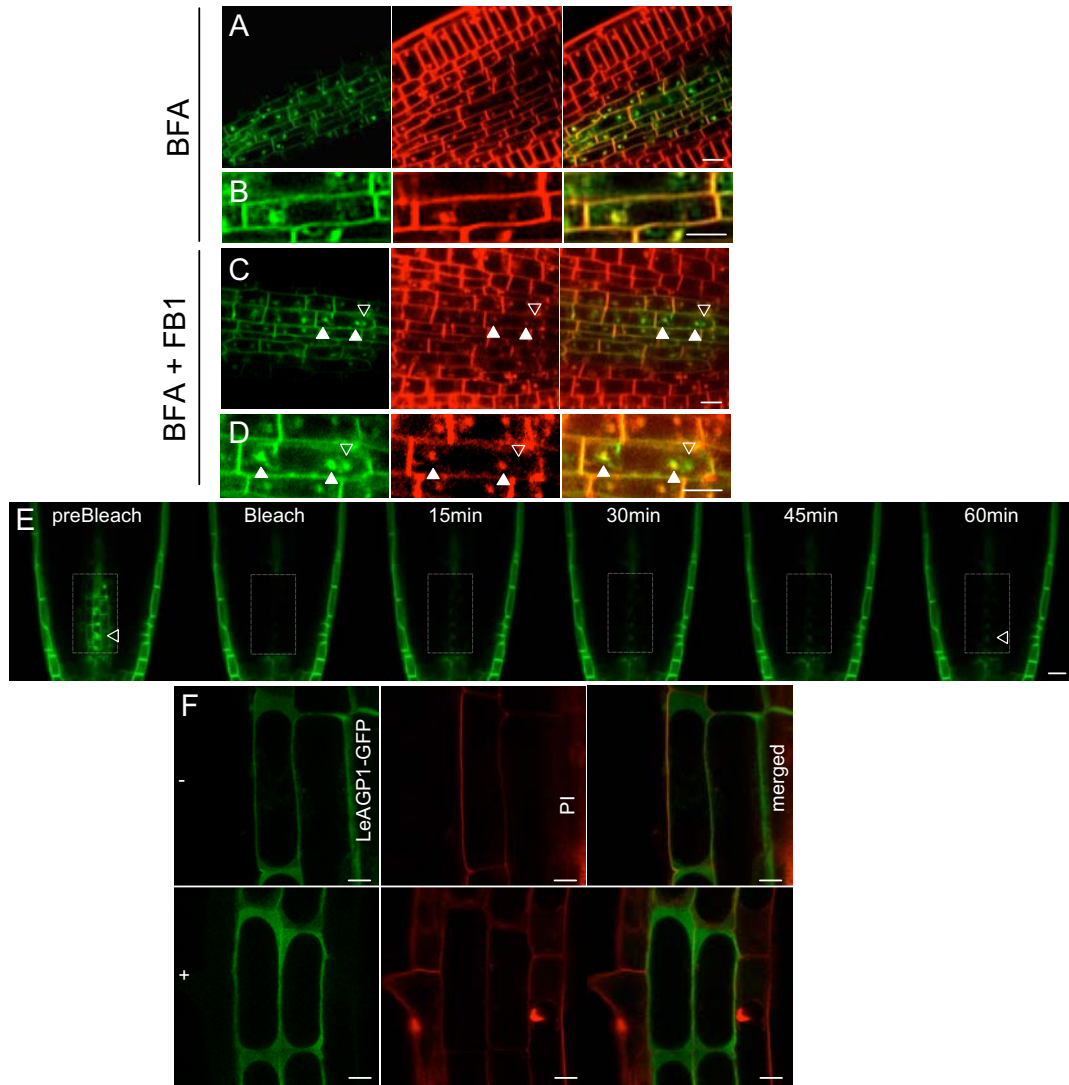
Scale bar, 200nm



Supp. Fig. 10. Involvement of endocytosis in YFP-RabA2^a aggregation

- (A) Proximity but no colocalisation between YFP-Rab-A2^a (green) and FM4-64 (red) in presence of FB1.
- (B) Kinetics of BOR1-GFP endocytosis in the presence of boron. Five day-old BOR1-GFP seedlings were treated with 2.5 μ M FB1 for 16h and incubated in the presence of 100 μ M boric acid (t=0). Internalization of BOR1-GFP was then monitored in FB1 treated seedlings (+FB1) compared to untreated (- FB1). BOR1-GFP are clearly visible in both conditions 10 min after boron application.
- (C) Subcellular distribution of Rab-A2^a mutants. YFP-Rab-A2^a[N125I] is a nucleotide free mutant showing the same subcellular distribution as wild type YFP-Rab-A2^a but with lower protein levels. YFP-Rab-A2^a[S26N] is the GDP-bound inactive isoform localized exclusively in the cytosol and the Golgi. YFP-Rab-A2^a[Q71L] is the GTP-bound constitutively active isoform present in the cytosol and at the plasma membrane.

Scale bar : A and C, 5 μ m; B , 10 μ m.



Supp. Fig. 11. Involvement of endocytic and secretory pathways in YFP-RabA2^a aggregation

- (A) Partial localization of pPIN1:PIN1-GFP (left) and FM4-64 (right) staining in presence of BFA.
- (B) Detail of BFA compartments with presence (open arrow head) or absence (closed arrow head) of colocalization between FM4-64 and PIN1-GFP.
- (C) FM4-64 staining of aggregates in FB1-16h treated pPIN1:PIN1-GFP cells in presence of BFA. Partial colocalization is observed (arrow heads).
- (D) Detail of FM4-64 staining of aggregates in FB1-treated pPIN1:PIN1-GFP cells showing partial colocalisation of PIN1-GFP with FM4-64 in FB1/BFA compartments.
- (E) FRAP analysis of FB1-16h cells expressing pAUX1:AUX1-YFP. AUX1-YFP expressing lateral root (pre-bleach) was bleached and recovery of AUX1-YFP was monitored for 60min.
- (F) LeAGP1-GFP expression in absence (-FB1) or presence of 2.5 μ M FB1 (+ FB1) in plasmolysed epidermal root cells. Cell wall was stained with propidium iodide (PI).

Scale bar is 10 μ m in A, C, E and F and 5 μ m in B and D.