	10	20 30	0 4 0	50	60	70	80	90	100
GmLOH3							1 • • • • 1 • • • • • • •		MG
HsCerS2				MLQTLYDYFWWER	LWLPVNLTWAD	LED-RDGR	VYAKASDLYITL	PLALLFLIVRY	(FF
HsCerS3				MLSSFNEWFWODR	FWLPPNVTWTE	LED - RDGL	VYPHPODLLAAL	PLALVLLAMRI	LAF
HsCerS5			MATAAQO	PLSLLWGWLWSER	FWLPENVSWAD	LEGPADGY	GYPRGRHILSVF	PLAAGIFFVRI	LF
HsCerS6				MAGILAWFWNER	FWLPHNVTWAD	LKN-TEEA	TFPQAEDLYLAF	PLAFCIFMVRI	LIF
HsCerS1		PPPNCCUCKTDI			AT TOD SKKD SP	TIWKUWEC	MAAAGPAAG	PTGPEPMPSYA	AQL
ScLAG1	MSTIKPSPSNNNLKVRSRP	RRK.SSIGKIDLO	DTVPSLGTMFE	TKESKTAAKRRMQ	RLSEATKNDSD	LVKKIWFS	FREISYRHAWIA	PLMILIAVYS	AYF
	110	120 130	140	150	160	170	180	190	200
	···· ··· ···	<u>. .</u>			.		<u> </u> .	· <u>· · · · · · · ·</u>	
P+LOH2	MDSI ANNGA	AVSHLFYAIFY	FGEVIVELEN	RETERRIAVELLE	GTTHLENDER	EAT		TWATUSTCV	IKV
AtLOH2	MESVSSRGGDPVVKPS	MEVWHFQIAVY	FGFFFLRLVII	RYVFORIALWLLS	TGSAPIKLND	ATRA	KIVKCKESI/WKI	LYYAACDFFV	QV
SbLOH2	MAAV	RGGEAVSVALL	LAFFCARLLID	RIVYKPLAVYLFN	TKASKLMNDEA	RQA	KI VKESESSWKI	TYYASVQAWV	MI
SIAsc-1	MKNLDHIAASVD EKDSL	PEYOLIFILFIA REVKOLIFICE	LEEPVLEFING THE PTLEFT	REVEEALAKRMIF	GKTVVNING	STER.	KUNKEKESAWKE	WYFIISADLLA MYFIISADLLA	LSV LVU
PtLOH1a	MGFMEYVKSIEWEHESY	GYEDCIVIPLE	LEEPFVRFFI	REVEOKVAODIIF	GRENQTLDVQS	DIRR	KIRKEKESAWK	IVFLSSEILV	-Cv
PtLOH1b	MGFMEYAKS IQ EHESY	PAYEDYIVUPLES	SLEF <mark>TFVR</mark> FF10)REVE <mark>QKLAQ</mark> RLIF	GREHQMLDAQ	Distration	K I <mark>GKIFKIESAWK</mark>	IYFIISABILV	LУV
VvLOH1	MGLLESAAS IN EHESF	PEYGOFVATPVIN	FILEFSVEFFILE	REVIORLORIUIF	GGGQQLDVG	DKR	KERKISKISAWK	VYFLSABLLA	LSV C
AtLOH1	MGLFESVKS ID EQESF	PTYOLGFUPLE	VEPTICEL	REVEEKLASLVIY	GRMS TNKSDN 1	KDKKNSP	KURKEKESAWK	IYYLSABLLA	LSV
AtLOH3	MGLLESVKS IN WEHESS	PVYQDFRVIPL	VEFPSIRFL)REVE <mark>EKLAKY</mark> LIY	GRHRQDMGDD	TERKK	KTRKEKESAWK	VYYLSABILA	LSV
AlLOH3	MGLLESVKS IN EHESS	PVYQCFRVTPLF	VEEPTICFL	REVEEKLAKHUIY	GOHRODMGDD	TORNE	KURKEKESAWK	VYYLSABILA	LSV
OcLOH1 OcLOH3	MGFLEMVKSVD	SOYEDFIVILE	LO PTV: FFM	IFVEEXVSRPME	GGMOVVANES	ENK	KTRKEKESAWK	VYFLSADFFA	AA
GmLOH1	MTTMSSLSLSLDNHNESY	PAYHOFYLTPIP	LEFPSLRFFM	RFIFERVARDIF	GKGHAALDYQI	Diskrik	KISKEKESAWK	VYYLSABILA	LSV
GmLOH3	TWVLQQVTSIDWNMNHESY	DFRDFSVI PF	LEEPSLRLLM	TELEQVARELIE	GKGHKKMDFQT	LERKK	KI SKEMESAWK	VYFISAEIFA	LAV
PtLOH3	MGVLGINNLID (ESUSY	VAT FIAIPLO	VEFSVEFV	KYVEECSARREIF	GGHVTVDVG	HGNRG	KUNKEKESAWKO	VYFICABILA	LSV LYV
AtLOH4	MDLKLLSRPD	PDSSOFLVTIF	PEFLFLRLIM	CIPERVARRIVV	PGLCADS	Noter	KVVKEKESAWK	LCSFSVDAF	LΥV
AllOH4	MDLKLLSR-DODHESY	PAFSOLWVLIF	PFFLFLRLIM	REIEERVARRIVV	PRGHYGDS	NORRE	KIVKEKESAWK	LCSFSVEALA	ν¥ν
HsCerS2 HsCerS3	ELYVATPLAALLNIKEKTR	KVTP-NTVLENDE	KHSTROPLOT	VELLSRQSGLSGR	OVERWFRRRR	IQUEPS	RLKUDODACORF	A D'A MI TVAG	IAF
HsCerS4	ERFIGLPLSRWLGVRDQTR	ROVKPNATLEKHE	LTEGHRPKEP	LSLLAAQCGLTLQ	QTQRWFRRRR	QDRPQ	LTKKOOBASTRE	LFYLSSFVGG	LSV
HsCerS5	ERFIAKPCALCIGIEDSGP	YQAQPNAILEKVE	ISITKYPDKKR	LEGLSKQLDWNVR	KIQCWFRHRRN	QDKPP	TLTKPOESMORF	TFYICIFCYG	IRF
HSCerS6 HsCerS1	VORGWGSALAAARGCTDCG	WGLARRGLAEHAH	LAPPELLLLAL	GALGWTALRSAAT	ARLFRPLAKRO	CLOPR	DAARMPSMAR	LEY GSWSYS	AYL
ScLAG1	LSGNRTESNPLHMFVAISY	QVDGTDSYAKGIK	DLSFVFFYMIF	FTFLREFLMDVVI	RPFTVYLNVTS	EHRQK	RML QMYAI	FYCGVSGPFG	ΥI
ScLAC1	TSGNTTKTNVLHRFVAVSY	QIGDTNAYGKGIN	DLCFVFYYMIF	FTFLREFLMDVVI	RPFAIRLHVTS	KHRIK	RIM QMYAI	FTGVSGPFG	IYC
	210	220 230	240	250	260	270	280	290	300
SlAsc-2	AVHING LDVKGYFSC		TIMCOC GEAT	-YSLAAT VV DEIVS	RKD DAVMMSC	IVTVFITS	SYILSFRICI	VILLAIHUGSD	VET
PrLOH2	TCN PROPRETARY FRG	OPHOELGFPIN	ILFYNCQCGFYI	-YSTAATLIWERR	RKDESVMMSHI	VINVILIG	YSYSTSFFRIGT	IICAVHDASD	VEL
SbLOH2	IKO SLDMOYFDC	ONOPIASSO	LFXXCOCGEVI		RKDEAVMMSHE	VINSTIG	VSYLTGOFRICT	II	VED
SlAsc-1	TCN SPRETD SRYFT AGEG	VV. IPN LKM (LKM)	LLYNYAGGFYF	-YSIIFATLY MENE	RYDDAAQII	VTTVSLIV	LSYVYGFARIGS	VVLALHDGSD	VEM
SlAsc-3	TYNEPWETN TRYYWTGPGD	OV NPD LKMKLKIK	GWTMYAGGEYI	-YSIFALIYWETR	RSDFAAHMIE	ITSVSLIL	LSYIFGMARAGS	MAALIEDGSD	VLM
PtLOHIA PtLOHIb	TYDEPHEGNAKYFWVGPGS	OVWPDOKMKLKLK	GAMYYAAGEYT	-YSIFALIFWETR	RSDEGVSMSHE RSDEGVSMSHE		LSYILREGRAGS	TVLATHDASD	VEL
VvLOH1	TYDEPWETN TKYFWVGPGN	OVWPDOQIKLKIK	GLYMYCGGFYT	-YSIFALIFWETR	RSDE <mark>GVSM</mark> GHH	VATFILLIV	LSY IFRFARVGS	VVLALHDASD	VEL
BrLOH1	TYNEPWETD TLYFW IGPGD	OIWPNOQMKIKIK	FLYNYTAGEYT	-YSTFALIEWETR	RSDE <mark>GVSM</mark> GHH	ITAVILIV	LSYICRESRAGS	VVLALHDASD	VFL
AtLOHI AtLOH3	TYNEPOPMENAKYPY VGPGD	OTWPDOOTKLKLK	LLYNEVAGEYT	-YSTEALVEWETR	RSDEGVSNGH	IATLILIV	LSYVCSPSRVGS	VVLALHDASD	VEL
AllOH3	TYNERWIPMNTKYFWVGRGD	OTWPDOOTKLKLK	LLYNFVAGFYT	-YSIFALIEWETR	RSDF <mark>GV</mark> SMGHH	IATLILIV	LSYVCS <mark>F</mark> SRVGS	VVLALHDASD	VFL
OcLOH1	TYNEPWETKTKYFWIGPGN	OVWPDOAYKLKLK	GLYNFVGGEYT	-YSIFALIFWETR	RSDF <mark>GVSM</mark> SHH	VATELLIV	LSYVLREARAGS	VVLALHDASD	VEL
GmLOH3	TYDEPOSTNATNANEWCORT	OVWPDOKIKLKLK	AVYAAGEYS	-YSIFALIFWETR	RSDEGVSMSHE	VASSVILLV	LSYIFREARVES	VVLALHDASD	VEL
GmLOH3	TYDERMETDARYEWVGRGN	OIWPDOKIKLKLK	VLW YAAGEYT	-YSILALVEWETK	RSDEVVSMGHH	VIIIIV	LSYIFRFVRVGS	VVLALHDASD	VFI
RcLOH3	SYNEPWETNIKYFWVGPED	OIWPDOKI KLKLK	GHYMFVAGEYI	-YSIFALIFWETR	RSDFAVSMAHH	VATVILLV	MSYILRFARVGS	IVLALHDVOD	GET
AtLOH3	TYKEPARKDARSPALCED	OVWPDOKIKLEM	GMYMFVGCLNV	-YAFEAT FEWER	RSDEKVMLVH	IVASFILI	LSYVFREARIES	VILLALHOISD	VEL
AllOH4	TYKEPWEKDAROFWIGPGD	OIWPDOKIKRLKN	KGMYMFVGGLN	VYAFFAT FFMIST	RSDFKVMLV::::	IVASFLII	LSYVFRFARIGS	VILALHEISD	VFL
HsCerS2	IVDK WEY YDMKKVWEG	YPICSTIPSQY	WYW.IELSOV-	-WSLLFSIASDVK	KDELANT	VANI	FSWFANY IRAGT	LIMATEDSSD	YL.
HsCerS4	LYHESALWAPVMCMDR	YPNOTLOPSOY	WWYLLELGOV-	-LSLLIRLPFDVK	KD KEOVI	FVAV UMT	FSYSANLLETCS	LVLLEDSSD	YL
HsCerS5	LWSSHOPWDIRQCOHN	YPFOPLSSG	HYVIMELANY-	-WSLMFSQ TDIK	KDELIMFV:::	LVTIGLIS	FSYINNMVRVGT	LIMOLEDVSD	FLI
HsCerS6	LKKTPOLWNARHCOYN	YPYOPLTTD H	YYYILELSEV-	-WSLMFSQETDIK	RKDEGIMFL	LVSIFILIT	FSYVNNMARVGT	LVICIEDSAD	AL
ScLAG1	MUHSDLWLFKTKPMYRT	YEVITNPFLD	I FULGOA ADWA	OOACVLVLOLEKP	KOYKELVE	IVALLATW	SSTAFRIHNVCI SSYVFHETKMCL	AIYITMDVSD	
ScLAC1	MUHSDLWFFNTKAMYRT	YPDFTNPFLF	VFYLGQAADWA	QQACILVLQL	KDHNELTF:::	IVALLIAN	SSYVFHFTKMGL	PIYITMDVSD	FLI

	310	320	330	340	350	360	370	380	390	400
012-0						••••••••••				
SIASC-2 P+LOH2	DAARVERYSEKELG	ASVLEGOSAVSW. ASTLECTEATSW	ZTINCIVE FOR	TTKATSVEL	VEFULISLAYD	K		LATIN PERTYNN	TITYS	LR
AtLOH2	TAKIFKYSEKEFG	ASVCIPATIEAVSWI	LLIGRICIYFIPE	TIRATSICI	LDY MMTSA	EC	LMASENTM	LMINAVELLING	YITCALIVRI	LK
SbLOH2	TAKLOXYTEKELG	ASLFFGLFAISW	LLICROTYFIP	II KAS <mark>SY</mark> HS	IAF RKLDEF-	P	ALWYI LNOME	LTINAVERMAN	KHICL MR	LN
SlAsc-1	EIAKMSKYSGFDLI	ADIFISUBALIVE:	ISDRIIC YPH	NI IR <mark>ST</mark> CYBI	LYVI IQKER-	TTG	I I LYFVENAUL	I CHAVIN: LEWE	KIILRAVKNO) IL
SlAsc-3	EIAKMS <mark>IYSG</mark> FHSV	ADISEALEALSW	LLRLIYFPF	FIIYSTSYEV	LFIVDKEKQ	QNING	IILYFVINSML	IONLINHIYWW	THCRVIINI	L-
PtLOHIA PtLOHIb	EVGKMSKYSGAEGT	ASFAETLEVISW		VI WSTSTEV	LLNID KEK	HAVDel		Y G MAY ALL Y	VIIMYBALVIC	ő
VvLOH1	EVGKMSKYKCAETT	ASISFILEVLSW	IV <mark>IARIATYY PE</mark>	TIL <mark>R</mark> STSYEV	ILTIDKEK	HAVE	PI YYYL FNWD	FORMUR HANN	VUMYRMUVKC	VQ
BrLOH1	EVGKMSKYSGREGI	AAFSIFVLEALSW	VL <mark>IARIALYYY</mark> DET	NTT WSTSYQI	IMTVOKEK	HPIE	PT YYYMENYUUT	F <mark>OAAVA:II</mark> Faa	VITIYRMEVKC	VQ
AtLOH1	IGKMSKYCGADSL	ASISFVLEALSW	VVIAR A VALUE	<u>IIII WSTSYQ</u> I	IMTV KEK	HP-NCI	PILYYMENYUU	Y FIAAVA:II FAIA	VITIYRMIVKC	VQ
ALLOH3	EVGKMSKYSCAERT	ASESETTEVIS.		ATT WSTSTEV	VLEINKDK	HPIEC			VINYBALVKO	č
OcLOH1	EVGKMSKYSCAEAI	ASISFVLEVLSW	VVIARIA IVY PE	TILWSTSYEV	IQTVDKEK	HRAD	PITYYY VENSIAL	FSIAAVA SIAAVA	VIMYRMIVKO	10
OcLOH3	EVGKMSKYSCAETL	ASCSEVIEVVSW	VI MRIMIYY PE	NILW <mark>STS</mark> CEI	IPF KDT	HKVD <mark>G</mark> I	PIYYYIFNWIL	F SIMAY MILLAY ()	VUMFROUVD	тк
GmLOH1	EIGKMSKYSCAETM	ASFAFILEVI.SW		NIT WSTSYLEV	LLTIN KEK	HRVDCI	PITY VIN SIM	YOMAMINA	VITYRMVK	20
GmLOH3 RcLOH3	BTAKMSRYSQVEWT	STEPVINVICE.	TT FRT TYY PE	ATTRS TS VEV	VI.TIMMKK			FCTIAVENING	TIMVENVE	K
PtLOH3	IE TAKMSKY SGYIELM	ASVEFILIEVLEW	TILRITYYPF	TIL <mark>R</mark> STSYE	VSALNKEK	QMVDes	STYYYL FNULL	F SIMAVIM: INVOIC	IMVGVMA	Q
AtLOH4	EIGKMOKYSGAETM	TSVSFVLFFLSW	TTURINYYPE	WILL <mark>W</mark> STSYES	IKVKTEYWDKK	HLMETGPPL	LFYYVENDU	Y <mark>OIQI II:IIYiiii</mark>	ILIYRVLISC)IR
AlLOH4	EIGKNOKYSGAEAM	TSVSEVLEFLSW	PAINRINIYYDE	NILW <mark>STSYE</mark> S	IKVKMEYWDKK	NLMETGPNL	VENUE	YOQINELYAN	INTYRVIISC	R
HsCerS2 HsCerS3	SAUVESVACUTOT	CNTLEFIESTIF	TSCHVED	YCALILE	MYHTEPFFS				YYULKUNRO	F
HsCerS4	ACKIVNYMQYQQV	CDALFLISFVF	FYTRUVLFPT	QILYTTYY'S	ISNRGPFFG		YYFFNGLL	MLIQLINIVE	OHILRMUYSE	MK
HsCerS5	BAAKLANYAKYQRL	CDTLEVISAVE	WTRUGI YPS	MILNTALF©S	WEIIGPYAS		WWLLNGW	LTIQLUIVINS	YMARIALKA	LI
HsCerS6	AAKAANAKFOKM	CDLL: VM-AVVF	ITTRIGIFCL	VINTALFOS	WEIVGPYPS			LLVQGINCF	YOVKIAC	VS
ScLAG1	SLSGTLNYLNSVFT	PFVFGLFVFFWI	LRHVVNIRI	LWSVLAEFRH	EGNYVLNFATO	DYKCW	USLPIVEVII.	AAUOLVNIA	FULCIVER	
ScLAC1	SFSUTLNYLDSGLA	FFSFAIFVVAWI	LRHYNLKI	LWSVLTQFRT	EGNYVLNFATQ	QYKCW	SLPIVEV	GAUQLVNLY	FILIFRVUYRI	LW
	410	420	430	440	450	460				
		<u></u>	•••••••••••	•••••••	••••					
P±LOH2	NRCRVGEDTRSDSEL	DE								
AtLOH2	NRGKVGED I RSDSEI	D								
SbLOH2	NKCOVTDDVRSDSBI	DDE								
SlAsc-1	SRCHITDDVREDSES									
PtLOH1a	ARCHLSDDVRSDSE									
PtLOH1b	ARGOLSDDVRSDSE	9HDE-CED								
VvLOH1	ARGOLSDDVRSDSEI									
BrLOH1	DRGKLSEDVRSDSE									
AtLOH3	DRGKI/SEDVRSDSE	DE -HED								
AllOH3	DRGKLSEDVRSDSE	GRIDE - ICED								
OcLOH1	ARGRVSEDVRSDSE									
GmLOH3	ARCKVSEDVRSDSB	A-REHED								
GmLOH3	EKGKVSEDIRSDSEI	CHDE-HKHEE								
RcLOH3	ARGKVSDDVRSDSE									
PtLOH3	AGEOVEDDVRSDSB									
AlLOH4	AKCKVAKDIRSDSE	JEDDENHD								
HsCerS2	CKLVEDERSDREE	TESSEGEEAAAG	GGAKSRPLAN	GHPILNNNH	RKND					
HsCerS3	-MKSIQDVRSDDED	EEEEEEEEAT	KGKEMDCLKN	GLRAERHLII	PNGQHGH					
HsCerS4	-KCOMERDIKSDVB	SSEE E E E E E E E E E E E E E E E E E	PLOLKNGAAG	GPRPAPTDG	CSYWAFF	HTTAT				
HsCerS6	-RGKVSKDDRSDTES	SSSDEEDSEPPGK	NPHTATTTNG	TSGTNGYLL	IGSCSMDD					
HsCerS1	GQVHELKDLREYDT7	AQSLKPSKAEK	PLRNGLVKDK	RF						
ScLAG1	-QG-IQKDERSDSD	SDESAENEESKEK	CE							
SCLACI		SUESSTIPT	DSTFIKEDI							

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*, (AILOH3 # XM_002892714, AILOH4 # 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); *Orobance ceruna* (OcLOH1 #GQ181109 and OcLOH3 #GQ181110); *Populous trichocarpa* (PtLOH1a #XM_002311388, PtLOH1b #XM_002315949, PtLOH2 #XM_002330290, PtLOH3 #XM_002328643); *Riccinus 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) Nothern 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.

loh1-1 / loh3-1

- (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≤0.05) or two (P≤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 1µM) of FB1. Scale bar, 100µm.
- (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.

- A. 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 *loh 3-2* allele are indicated.
- B. 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.
- C. 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.
- D. 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.
- E. 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. Sphingolipid 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 μM 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 μM FB1 and compared to untreated seedlings.
- (C) Free t18:0 and d18:0 levels during time course treatment with 1µM 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 loh1-1/3-1 double mutant treated (+FB1) or not with 0.5µM FB1.

Data show mean plus one standard error and significant differences between means (P ≤ 0.05, Student's t-test) are indicated by an asterisk.





- (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, 20μm.



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 tha 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 2.5µM 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 2.5µM FB1 for 16h and incubated in the presence of 100µM 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]¹ 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]L is the GTP-bound constitutively active isoform present in the cytosol and at the plasma membrane.

Scale bar : A and C, 5µm; B ,10µm.



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 2.5µM FB1 (+ FB1) in plasmolysed epidermal root cells. Cell wall was stained with propidium lodide (PI).

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