Supporting Information

Palmitvaccenic acid (Δ11-cis-hexadecenoic acid) is synthesized by an OLE1-like desaturase in the arbuscular mycorrhiza fungus *Rhizophagus irregularis*

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Table S1. Oligonucleotides used for cloning and RT-PCR

Oligonucleotide	Sequence (5'-3') (restriction sites are underlined)	PCR Target	Reaction conditions
Heterologous expression in *S. cerevisiae*			
FW (bn3075) RW (bn3083)	TATCCCGGGATGGTGGCACAG GCAACATT ATACTCGAGTTATTTGGTTTTC CTATGTT	*RiOLE1* cDNA	98°C-30 s, 10 cycles: (98°C-7 s, 58°C-20 s, 72°C- 32 s), 25 cycles: (98°C-7 sec, 68°C -20 sec, 72°C- 32 s)25x, 72°C-120 sec
FW (bn3077) FW (bn3084)	TATCCCGGGATGGCTGCTGCG CCAGTAAA ATACTCGAGCTATTCTTCCTTC TGACTCT	*RiOLE1 -LIKE* cDNA	98°C-30 s, 10 cycles: (98°C-7 s, 63°C-20 s, 72°C- 32 s), 25 cycles: (98°C-7 sec, 72°C -20 sec, 72°C- 32 s), 72°C-120 sec
Heterologous expression in *N. benthamiana*			
FW (bn3214) RW (bn3076)	TATGGATCCATGGTGGCACAG GCAACATT ATAAAGCTTTTATTTGGTTTTC CTATGTT	*RiOLE1* cDNA	98°C-30 s, 10 cycles: (98°C-7 s, 58°C-20 s, 72°C- 32 s), 25 cycles: (98°C-7 sec, 68°C -20 sec, 72°C- 32 s), 72°C-120 sec
FW (bn3212) RW (bn3213)	TATACGCGTATGGCTGCTGCG CCAGTAAA ATACTCGAGCTATTCTTCCTTC TGACTCT	*RiOLE1 -LIKE* cDNA	98°C-30 s, 10 cycles: (98°C-7 s, 63°C-20 s, 72°C- 32 s), 25 cycles: (98°C-7 sec, 72°C -20 sec, 72°C- 32 s), 72°C-120 sec
Heterologous expression in *C. sativa*			
FW (bn3216) RW (bn3211)	TATGAATTCATGGTGGCACAG GCAACATT ATACTCGAGTTATTTGGTTTTC CTATGTT	*RiOLE1* cDNA	98°C-30 s, 10 cycles: (98°C-7 s, 42°C-20 s, 72°C- 32 s), 25 cycles: (98°C-7 sec, 54°C -20 sec, 72°C- 32 s), 72°C-120 sec
FW (bn3217)[1] RW (bn3213)	TATGGTCTCGAATTCATGGCT GCTGCGCCAGTAAA ATACTCGAGCTATTCTTCCTTC TGACTCT	*RiOLE1 -LIKE* cDNA	98°C-30 s, 10 cycles: (98°C-7 s, 63°C-30 s, 72°C- 32 s), 25 cycles: (98°C-7 sec, 72°C -30 sec, 72°C- 32 s), 72°C-120 sec
Semiquantitative RT-PCR			
FW (bn3551) RW (bn3552)	CCACCACTGCTGAAAGAGA ACCTTCATGGAAGATGGAGC	*ScACT* cDNA	95°C-120 s, 30 cycles: (94°C-30 s, 56°C -40 s, 72°C- 40 s), 72°C-10 min
FW (bn3151) RW (bn3152)	TTATGCGGCTTTACTCCGT ATGATTTACCACCAGGATGTT C	*RiOLE1* cDNA	95°C-120 s, 30 cycles: (94°C-30 s, 56°C -40 s, 72°C- 40 s), 72°C-10 min
FW (bn3153) RW (bn3154)	GGGCTGGCGAACAACTTTA GTAAGTGATGACGGGCAATG A	*RiOLE1 -LIKE* cDNA	95°C-120 s, 30 cycles: (94°C-30 s, 56°C -40 s, 72°C- 40 s), 72°C-10 min
FW (bn1942) RW (bn1943)	TGTCCAACCGGTTTTAAAGT AAAGCACGTTTGGCGTACAT	*RiαTUB ULIN*	95°C-120 s, 30 cycles: (94°C-30 s, 56°C -40 s, 72°C- 40 s), 72°C-10 min

[1]= For site-directed ligation of *RiOLE1-LIKE* into pBinGlyBar1 for expression in *C. sativa*, a *Bsa*I restriction site was added upstream of the *Eco*RI site and the *Eco*RI sticky-overhang created by *Bsa*I restriction.

Table S2. Quantification of fatty acids in transgenic Camelina seeds expressing the *R irregularis* desaturase *RiOLE1-LIKE*

The table shows fatty acid contents (in mol%) for Camelina CAM139 or CpuFatB1 backgrounds. Values are means ± SD for seeds of the non-transgenic lines CAM 139 and CpuFatB1. For the *Ri*OLE1-LIKE expressing lines, the results of single seed analysis from three independent lines is presented.

Genotype	14:0	16:1Δ9	**16:1Δ11 (myc)**	16:0	18:1Δ9	18:1	18:2	18:3	18:0	20:1Δ11	20:1	20:2	20:0
	Fatty Acids (mol%)												
CAM139	0.21± 0.04	0.20± 0.02	**0.00± 0.00**	11.33± 0.60	8.49± 1.03	1.14± 0.36	22.55± 0.93	36.43± 5.77	2.98± 0.43	12.73± 3.06	0.86± 0.30	1.51± 0.49	1.57± 0.75
CAM139 + *Ri*OLE1-LIKE 1	0.25	0.32	**0.03**	16.78	6.94	1.87	33.42	11.08	4.70	17.48	1.13	4.20	1.80
CAM139 + *Ri*OLE1-LIKE 2	0.24	0.47	**0.05**	17.47	3.19	2.27	37.44	6.65	4.91	20.46	1.93	2.43	2.47
CAM139 + *Ri*OLE1-LIKE 3	0.32	0.49	**0.08**	17.68	5.96	3.24	34.59	7.68	4.36	19.39	1.85	2.37	1.98
CpuFatB1	1.86± 0.90	0.15± 0.09	**0.00± 0.00**	42.34± 0.16	4.36± 0.44	1.06± 0.18	23.43± 0.39	16.48± 3.54	5.04± 1.33	2.21± 0.67	0.40± 0.01	0.56± 0.12	2.10± 0.89
CpuFatB1 + *Ri*OLE1-LIKE 1	1.93	0.08	**0.82**	42.32	7.95	0.66	22.04	9.13	6.74	3.23	0.38	0.51	4.21
CpuFatB1 + *Ri*OLE1-LIKE 2	3.35	0.13	**0.57**	41.27	7.95	0.90	23.31	7.68	6.67	3.04	0.51	0.71	3.92
CpuFatB1 + *Ri*OLE1-LIKE 3	3.48	0.14	**0.24**	42.86	4.90	1.19	22.10	9.45	6.25	4.16	0.48	0.83	3.94

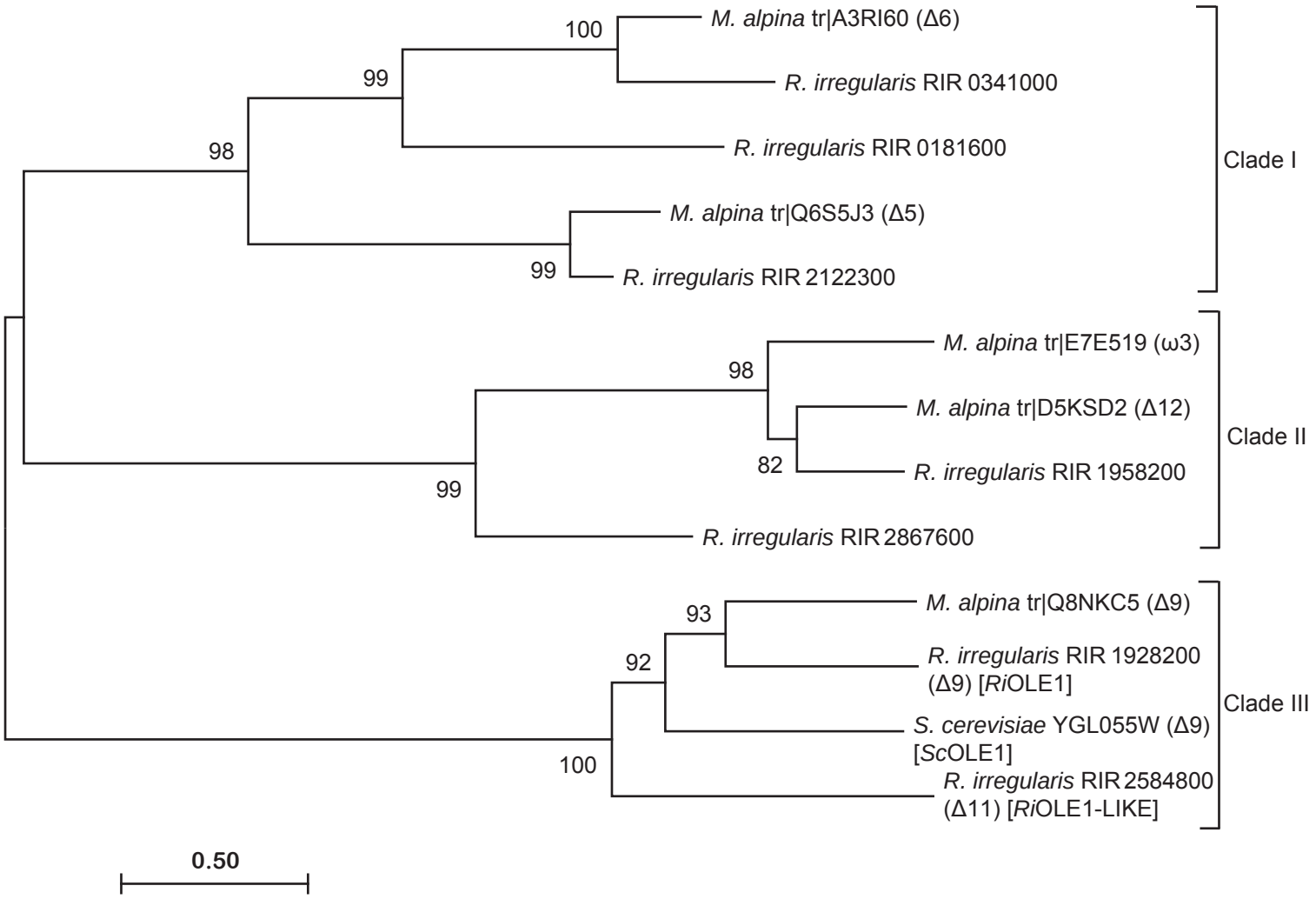

Figure S1. Phylogenetic tree of desaturases from R*. irregularis, S. cerevisae* and *M. alpina*.
Amino acids sequences were aligned using MUSCLE implemented in MEGA 7.0 and a maximum likelihood tree constructed. The branch numbers show bootstrap values (1000 iterations). The fatty acid position of desaturation by the respective desaturase is indicated in brackets. The *M. alpina* desaturase annotation was taken from [37].
M. alpina, *Mortierella alpina*; *R. irregularis*, *Rhizophagus irregularis*; *S. cerevisiae*, *Saccharomyces cerevisiae*.

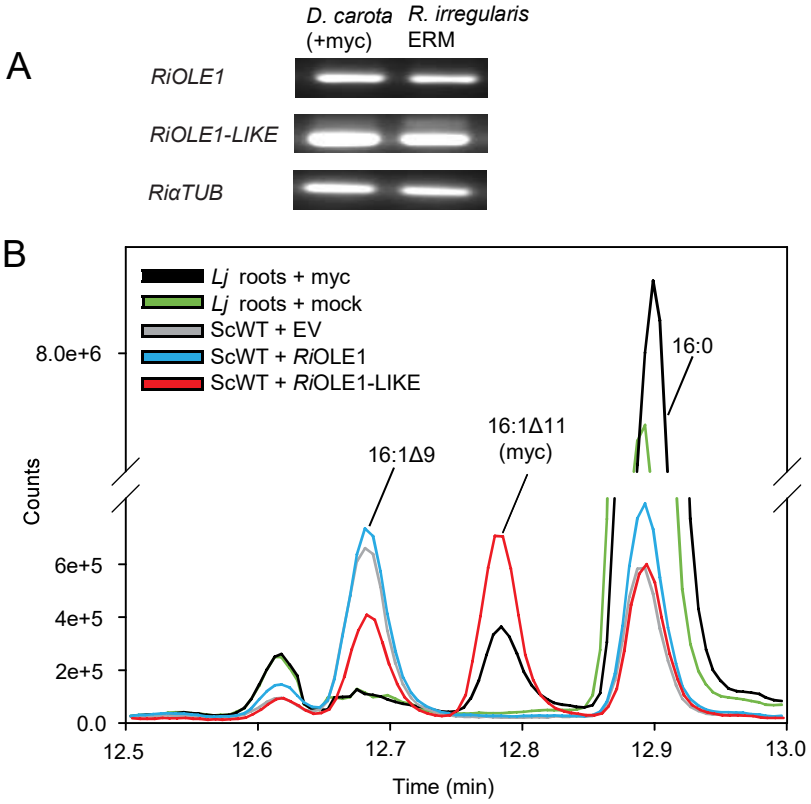

Figure S2. Expression of RiOLE1 and RiOLE1-LIKE and accumulation of palmitvaccenic acid (16:1Δ11cis) in plant roots colonized with *R. irregularis.*
(**A**) Semiquantitative RT-PCR of *Daucus carota* roots colonized by *Rhizophagus irregularis* (+myc) and the connected extraradical mycelium (ERM). RNA from roots (+myc) and ERM was isolated from a single split-petri dish harboring an axenic, infected *D. carota* root-culture on one side and outgrowing ERM on the other side. (**B**) Overlay of GC-MS chromatograms of fatty acids methyl esters from mycorrhiza-colonized and non-colonized *Lotus japonicus* roots, and yeast cells (WT) expressing RiOLE1 or RiOLE1-LIKE. The mycorrhiza-signature fatty acid 16:1Δ11cis is only present in mycorrhiza colonized roots and yeast expressing RiOLE1-LIKE. Lj, *Lotus japonicus*, Sc, *Saccharomyces cervisiae*.

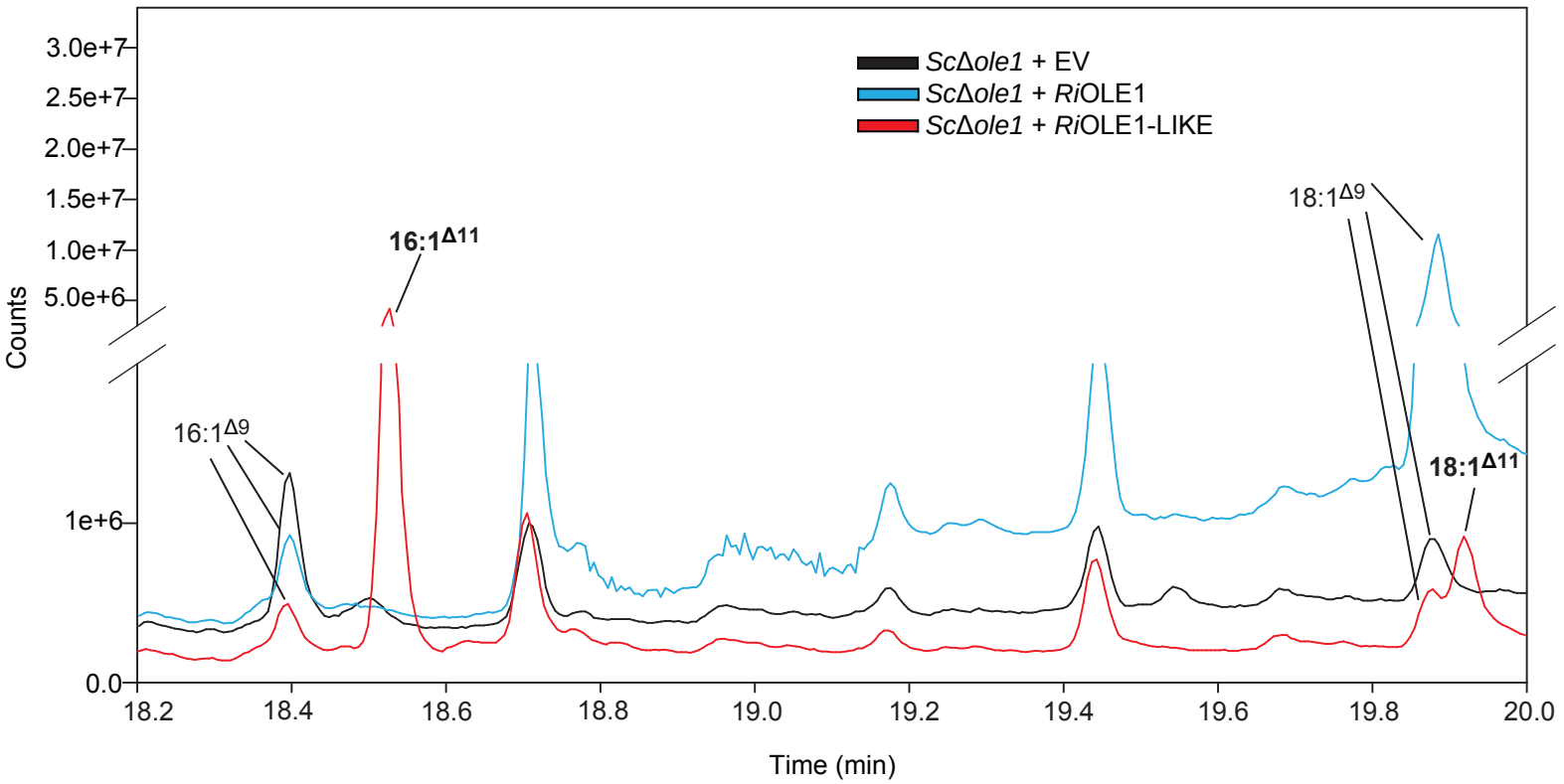

Figure S3. Determination of double bond positions in monounsaturated fatty acids from yeast cells expressing RiOLE1 or RiOLE1-LIKE.
Overlay of GC-MS chromatograms of dimethyldisulfide adducts of fatty acid methyl esters derived from the yeast Δole1 mutant transformed with RiOLE1 or RiOLE1-LIKE. Mass spectra and structures of the four labeled peaks are shown in Figure 3. The mycorrhiza-signature fatty acids 16:1Δ11cis and 18:1Δ11cis are highlighted in bold.

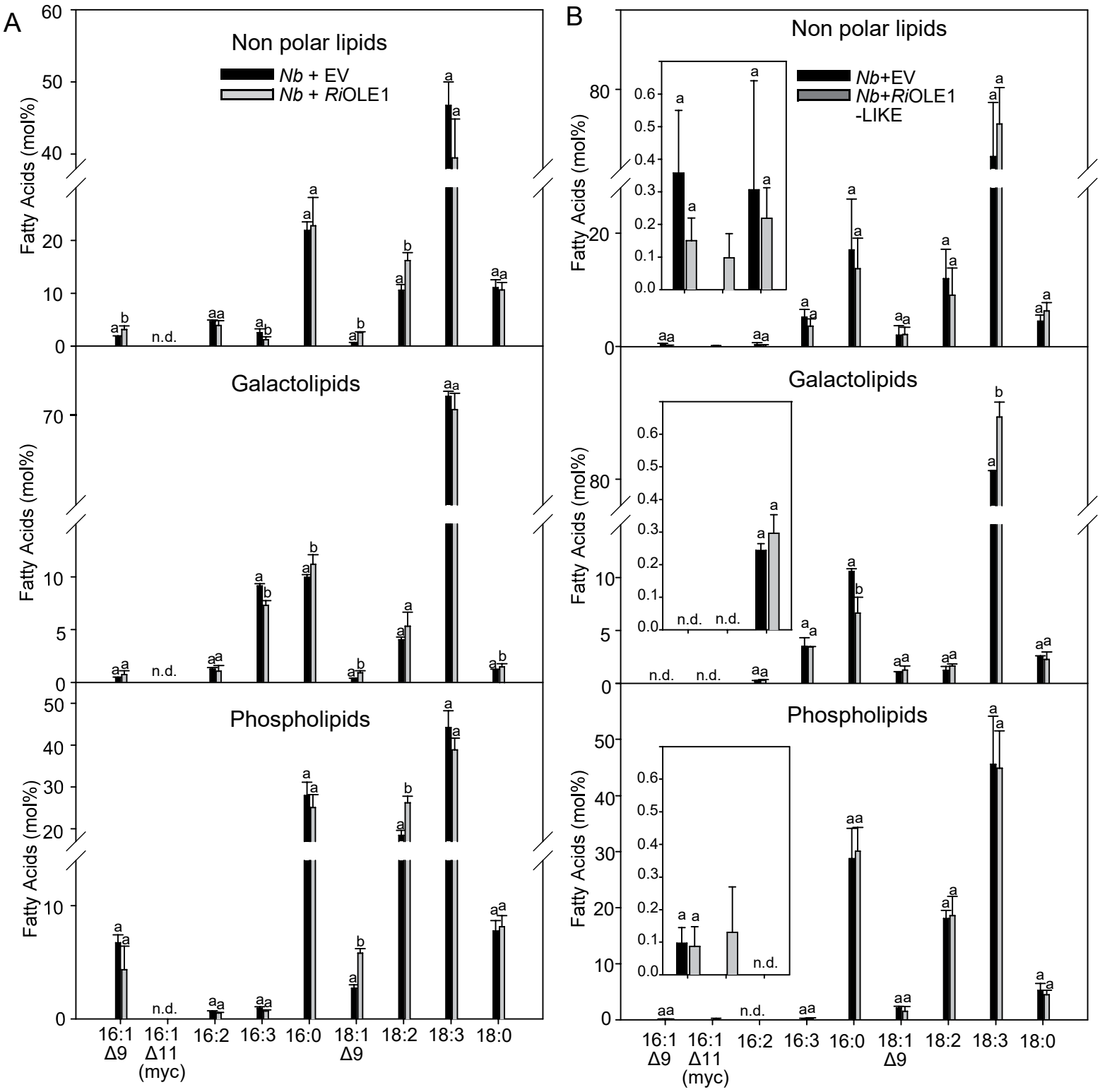

Figure S4. Fatty acid composition of lipid fractions from *N. benthamiana* leaves transiently expressing *Rhiozphagus* desaturases.
Lipids isolated from *N. benthamiana* control leaves or leaves expressing RiOLE1-LIKE were separated into different fractions (non polar lipids, galactolipids, phospholipids) by solid phase extraction. Fatty acid methyl esters were produced and quantified by GC-MS. (**A**) Distribution fatty acids after expression of RiOLE1. Letters indicate significant differences among treatments (ANOVA; posthoc Tukey; $p \leq 0.05$; n=4). (**B**) Distribution fatty acids after expression of RiOLE1-LIKE. Letters indicate significant differences among treatments (ANOVA; posthoc Tukey; $p \leq 0.05$; EV, n=3; RiOLE1-LIKE, n=4). The insets show the amounts of 16:1Δ9, 16:1Δ11(myc) and 16:2 at higher scale. Bars represent means ± SD. Nb, *Nicotiana benthamiana*; n.d., not detected; EV, empty vector.