

Table S1. Fatty acid composition of glycerolipids in leaves of WT plants (mol%)

	16:0	16:1 ³	16:2 ^{7,10}	16:3 ^{7,10,13}	18:0	18:1 ⁹	18:2 ^{9,12}	18:3 ^{9,12,15}
Time (min) after wounding								
MGDG								
0	5.1 ± 0.2	nd	1.3 ± 0.3	30.6 ± 1.7	1.0 ± 0.1	0.4 ± 0.1	1.9 ± 0.1	59.5 ± 2.2
2.5	4.2 ± 0.8	nd	0.7 ± 0.2	29.3 ± 4.5	0.8 ± 0.2	0.3 ± 0.1	1.8 ± 0.0	62.9 ± 8.3
5	3.7 ± 0.1	nd	0.8 ± 0.3	29.4 ± 4.5	0.5 ± 0.1	0.3 ± 0.1	1.9 ± 0.1	63.4 ± 3.0
10	3.6 ± 0.4	nd	0.6 ± 0.1	27.8 ± 2.0	0.5 ± 0.0	0.2 ± 0.0	2.0 ± 0.3	65.3 ± 4.1
20	3.7 ± 0.1	nd	1.1 ± 0.0	25.0 ± 2.4	0.7 ± 0.2	0.4 ± 0.0	1.7 ± 0.2	67.4 ± 0.8
DGDG								
0	32.0 ± 2.2	nd	nd	1.9 ± 0.2	6.0 ± 0.3	1.3 ± 0.2	4.9 ± 0.3	51.2 ± 1.7
2.5	29.4 ± 1.7	nd	nd	2.1 ± 0.1	5.4 ± 0.3	0.9 ± 0.2	4.6 ± 1.0	56.3 ± 1.2
5	27.5 ± 1.1	nd	nd	2.0 ± 0.3	4.9 ± 0.6	0.8 ± 0.1	4.2 ± 0.7	60.6 ± 2.7
10	26.5 ± 0.3	nd	nd	1.7 ± 0.1	4.7 ± 0.3	1.0 ± 0.1	4.3 ± 0.4	59.6 ± 4.3
20	26.8 ± 2.5	nd	nd	1.7 ± 0.2	5.6 ± 1.3	0.9 ± 0.2	3.5 ± 0.1	61.1 ± 4.3
PE								
0	35.0 ± 2.6	nd	nd	0.5 ± 0.2	8.0 ± 1.5	1.4 ± 0.3	29.9 ± 3.7	25.2 ± 2.2
2.5	34.4 ± 9.5	nd	nd	nd	8.5 ± 2.3	2.5 ± 1.2	23.1 ± 6.5	31.6 ± 6.0
5	34.1 ± 5.1	nd	nd	0.8 ± 0.2	5.1 ± 0.6	1.1 ± 0.2	29.9 ± 3.9	29.0 ± 3.4
10	33.4 ± 4.3	nd	nd	0.7 ± 0.2	5.3 ± 1.1	1.0 ± 0.2	30.1 ± 5.5	29.5 ± 2.3
20	29.6 ± 2.7	nd	nd	0.7 ± 0.2	6.5 ± 1.6	1.6 ± 0.4	32.4 ± 5.7	29.2 ± 4.3
PC								
0	34.4 ± 5.2	nd	nd	0.3 ± 0.3	9.8 ± 1.1	3.3 ± 1.0	25.3 ± 5.5	26.9 ± 4.1
2.5	34.2 ± 3.3	nd	nd	nd	8.7 ± 1.6	nd	26.4 ± 4.8	30.7 ± 2.9
5	31.8 ± 2.6	nd	nd	0.2 ± 0.2	7.4 ± 0.1	2.5 ± 0.9	24.9 ± 3.4	33.2 ± 3.4
10	32.1 ± 1.7	nd	nd	0.2 ± 0.2	7.1 ± 0.7	1.6 ± 0.4	24.8 ± 5.6	34.2 ± 2.7
20	30.1 ± 3.8	nd	nd	0.2 ± 0.2	9.2 ± 1.2	3.0 ± 0.4	27.8 ± 2.9	29.7 ± 0.5
Time (min) after FAC elicitation								
MGDG								
0	5.3 ± 0.4	nd	1.3 ± 0.1	31.0 ± 4.5	0.8 ± 0.1	0.5 ± 0.1	3.1 ± 0.5	58.0 ± 6.4
2.5	3.9 ± 0.3	nd	0.6 ± 0.1	28.8 ± 3.3	0.6 ± 0.1	0.3 ± 0.0	1.8 ± 0.1	64.0 ± 5.4
5	3.4 ± 0.4	nd	0.6 ± 0.1	28.5 ± 6.5	0.4 ± 0.1	0.3 ± 0.0	1.7 ± 0.2	65.1 ± 10.3
10	3.7 ± 0.8	nd	0.6 ± 0.3	27.8 ± 5.1	0.7 ± 0.1	0.3 ± 0.1	1.7 ± 0.3	65.2 ± 11.0
20	3.6 ± 0.1	nd	0.7 ± 0.2	29.8 ± 2.5	0.6 ± 0.1	0.3 ± 0.0	1.8 ± 0.2	63.2 ± 3.2
DGDG								
0	32.2 ± 1.5	nd	nd	2.0 ± 0.3	4.8 ± 0.3	1.3 ± 0.1	6.9 ± 0.3	49.5 ± 1.3
2.5	28.1 ± 5.1	nd	nd	2.0 ± 0.2	5.0 ± 0.7	0.7 ± 0.2	4.0 ± 0.8	60.1 ± 8.1
5	28.6 ± 1.8	nd	nd	2.0 ± 0.3	4.4 ± 0.3	0.7 ± 0.1	3.7 ± 0.1	59.9 ± 4.8
10	28.8 ± 2.5	nd	nd	1.8 ± 0.4	4.7 ± 1.0	0.7 ± 0.1	3.6 ± 0.3	60.3 ± 2.4
20	29.1 ± 3.8	nd	nd	1.9 ± 0.3	4.9 ± 0.9	0.7 ± 0.1	3.4 ± 0.6	59.8 ± 4.5
PE								
0	32.9 ± 3.2	nd	nd	0.5 ± 0.1	5.8 ± 0.8	1.4 ± 0.2	36.9 ± 4.0	22.5 ± 2.2
2.5	33.1 ± 4.6	nd	nd	0.7 ± 0.2	6.3 ± 1.7	1.2 ± 0.3	29.4 ± 4.5	29.3 ± 3.7
5	33.9 ± 3.4	nd	nd	0.7 ± 0.1	4.9 ± 0.9	0.9 ± 0.2	30.4 ± 5.5	29.2 ± 3.8
10	32.8 ± 6.1	nd	nd	0.3 ± 0.2	7.9 ± 1.1	2.0 ± 1.0	22.5 ± 6.6	34.5 ± 5.5
20	33.0 ± 1.8	nd	nd	0.6 ± 0.2	5.6 ± 0.7	1.3 ± 0.1	31.2 ± 3.9	28.3 ± 3.6
PC								
0	30.7 ± 5.0	nd	nd	2.3 ± 2.3	7.2 ± 1.1	3.2 ± 1.1	31.8 ± 6.7	24.8 ± 4.3
2.5	33.5 ± 5.2	nd	nd	0.5 ± 0.2	8.0 ± 1.0	2.4 ± 0.5	22.2 ± 2.4	33.4 ± 3.5
5	33.1 ± 0.8	nd	nd	0.5 ± 0.2	6.4 ± 0.4	2.2 ± 0.4	23.9 ± 2.4	33.9 ± 2.7
10	32.6 ± 3.1	nd	nd	nd	7.8 ± 0.6	2.1 ± 0.2	28.2 ± 1.9	29.3 ± 4.9
20	33.6 ± 2.8	nd	nd	0.3 ± 0.1	6.9 ± 0.6	2.4 ± 0.5	24.4 ± 3.0	32.4 ± 2.0

Table S2. Sequences of primers used for cloning and RT-qPCR

Primers used for RT-qPCR		
	Forward	Reverse
NaAOC*	CTATATACCGGAGACCTAAAGAAGA	AGTATCCTCGTAAGTCAAGTACGAT
NaAOS*	GACGGCAAGAGTTTTCCAC	TAACCGCCGGTGAGTTCAGT
NaEF1a	ACACTTCCCACATTGCTGTCA	AAACGACCCAATGGAGGGTAC
NaGLA1	AGTAGCAGATGATGTTAGTACATGTA	ACATGTGAATATGCCCATGGCATACT
NaGLA2	CGAGATTAAGTGCTAGAGCACAGCT	GCTTTGTTCCCTACTTGTGGACTAC
NaGLA3	TAGCCTAGGTGCATCACTTGCAAC	TATTCGGAACAATGTCCAGTAGGT
NaLOX2*	TTAGTAGAAAATGAGCACCACAA	TTGCACTGGTGTGTTGAGATGGT
NaLOX3*	GGCAGTGAAATTCAAAGTAAGAG	CCCAAATTGAATCCACAACA

*: Described in Paschold et al (2008), Halitschke et al (2004).

Primers used to clone the homologues of Arabidopsis DAD1 and DGL in <i>N. attenuata</i>		
	Forward	Reverse
NaGLA1	CAAGCTTAGCTGAATCTGTG	ACATGTGAATATGCCCATGG
NaGLA2	TCGTGGTACAACGAGAAATTATGA	TAATGAGTTATAAGATCAATCTTG
NaGLA3	GCGAGGAACGATTCAGACACTGGA	TATTCGGAACAATGTCCAGTAGG

Primers used for construction of VIGS vectors		
	Forward	Reverse
NaGLA1	GCGGCGGTTCGACACAGGACATAGTCTTGGTGC	GCGGCGGGATCCGCCCATGGCATACTATTGTC
NaGLA2	GCGGCGGTTCGACGCGCCTAAAGTAATGAATGG	GCGGCGGGATCCGAAACTCCTTTAGCCTTTCG
NaGLA3	GCGGCGGTTCGACTAATCAGGCTAGTGCCAGAG	GCGGCGGGATCCTCGGAACAATGTCCAGTAGG

Primers used to evaluate gene silencing in VIGS-silenced plants		
	Forward	Reverse
NaGLA1	GTGGGTTTTTGTAGCTTATACCAAAC	ACATGTGAATATGCCCATGGCATACT
NaGLA2	GATCCATTAAACCTCCATCTCCGG	TTGTAATCTGAAGCCGATTCTGAACA
NaGLA3	ACTTGACCCACTCAAGTATAAAGTA	TCCAGTGTCTGAATCGTTCCTCGC

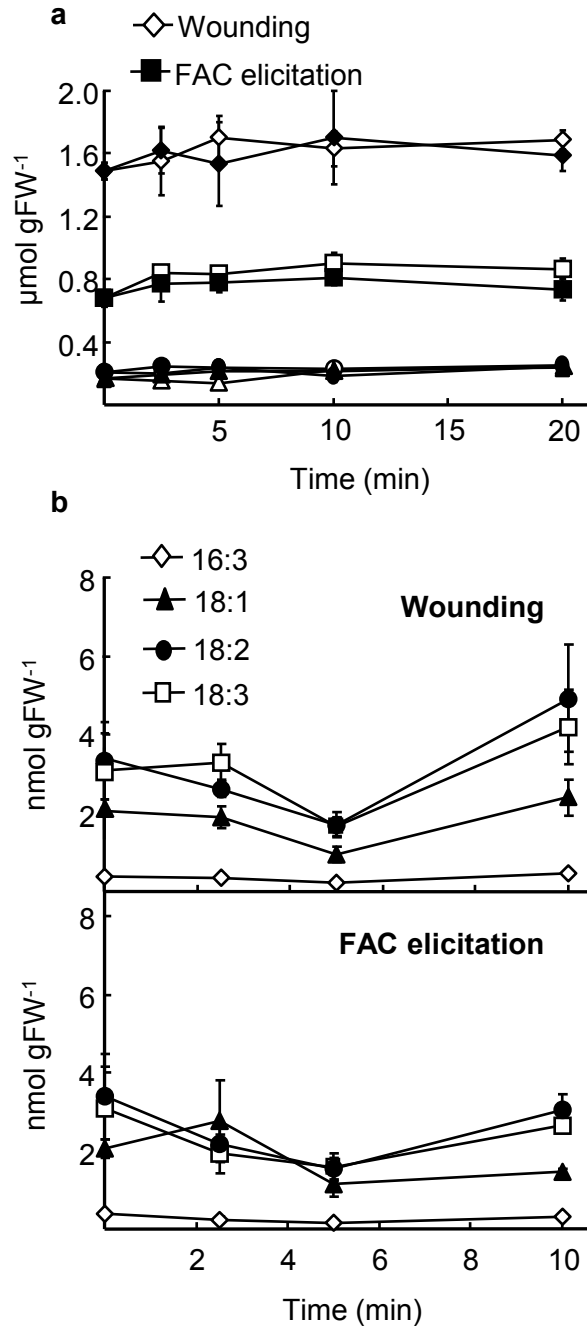


Figure S1. Membrane glycerolipid and unsaturated FFA levels in WT plants

(a) Leaves of rosette stage WT plants were wounded or FAC elicited, glycerolipid classes isolated and quantified at different times. Lipid classes: MGDG, DGDG, PC, PE. Bars (SE, n=4). **(b)** Leaves of rosette stage WT plants were wounded or FAC elicited, FFA isolated and quantified at different times. Bars (SE, n=4). **Note:** The data corresponding to free 18:3 levels in FAC elicited leaves has been used in Fig. 1 and it has been included here for comparison with the other FFAs.

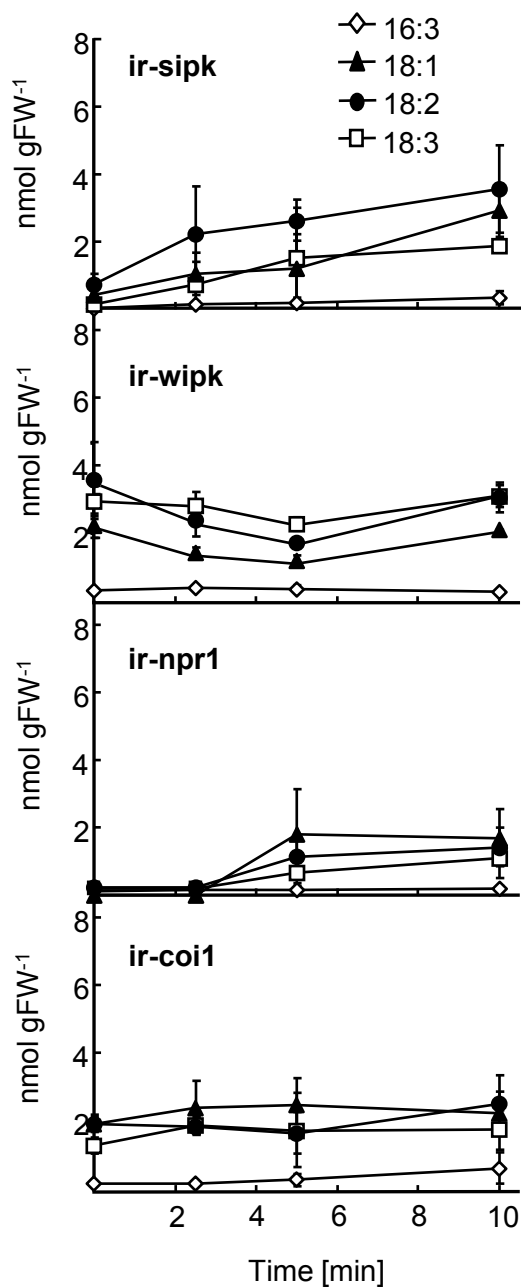


Figure S2. Unsaturated FFA levels in RNAi-silenced plants

Leaves of rosette stage *ir-sipk*, *ir-wipk*, *ir-npr1* and *ir-coi1* plants were wounded and FAC elicited for different times, FFA isolated and quantified. Bars (SE, n=4). **Note:** The data corresponding to free 18:3 levels has been used in Fig. 1 and it has been included here for comparison with the other FFAs.

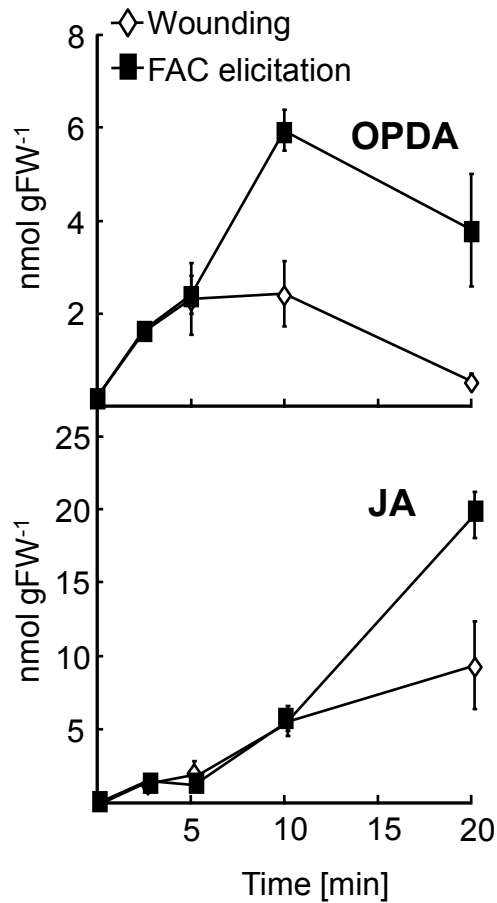


Figure S3. OPDA and JA levels in WT plants within 20 min of induction

Leaves of rosette stage WT plants were wounded or FAC elicited for different times and OPDA and JA quantified. Bars (SE, n=5). **Note:** The data between time 0 and 10 min is presented in Fig. 3 and it has been included here for comparison with time 20 min.

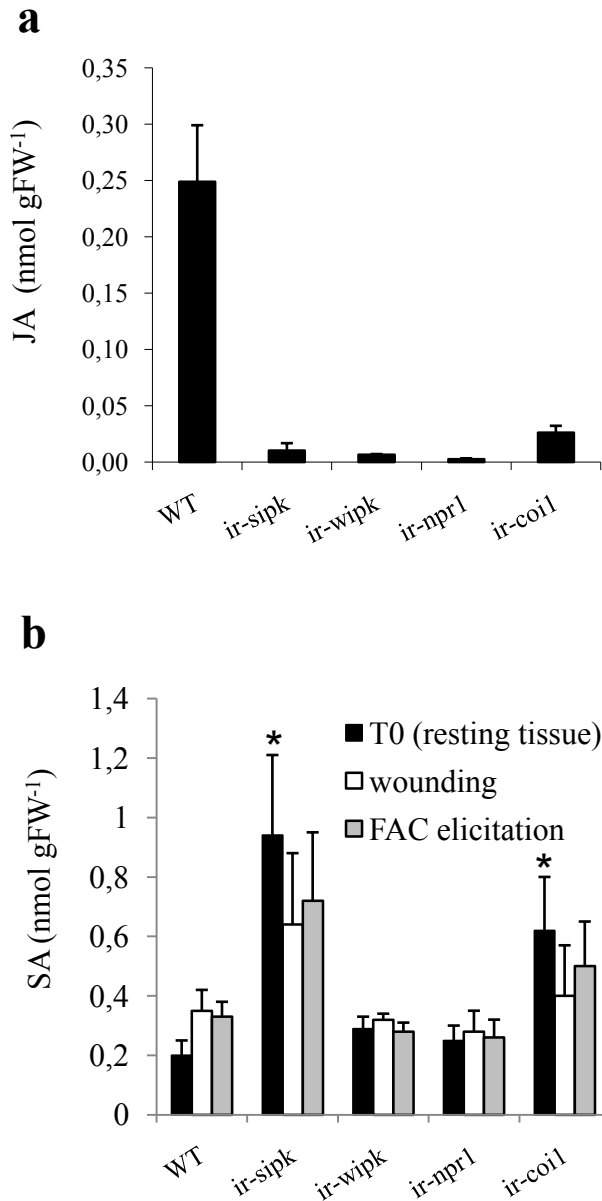


Figure S4. Basal levels of JA and basal and induced levels of SA in leaves

(a) Basal levels of JA in unelicited leaves of WT and RNAi-silenced genotypes. **(b)** Levels of SA in unelicited, wounded and FAC elicited (10 min after the treatments) leaves from WT and RNAi-silenced genotypes. Bars (SD, n=5). *see text.

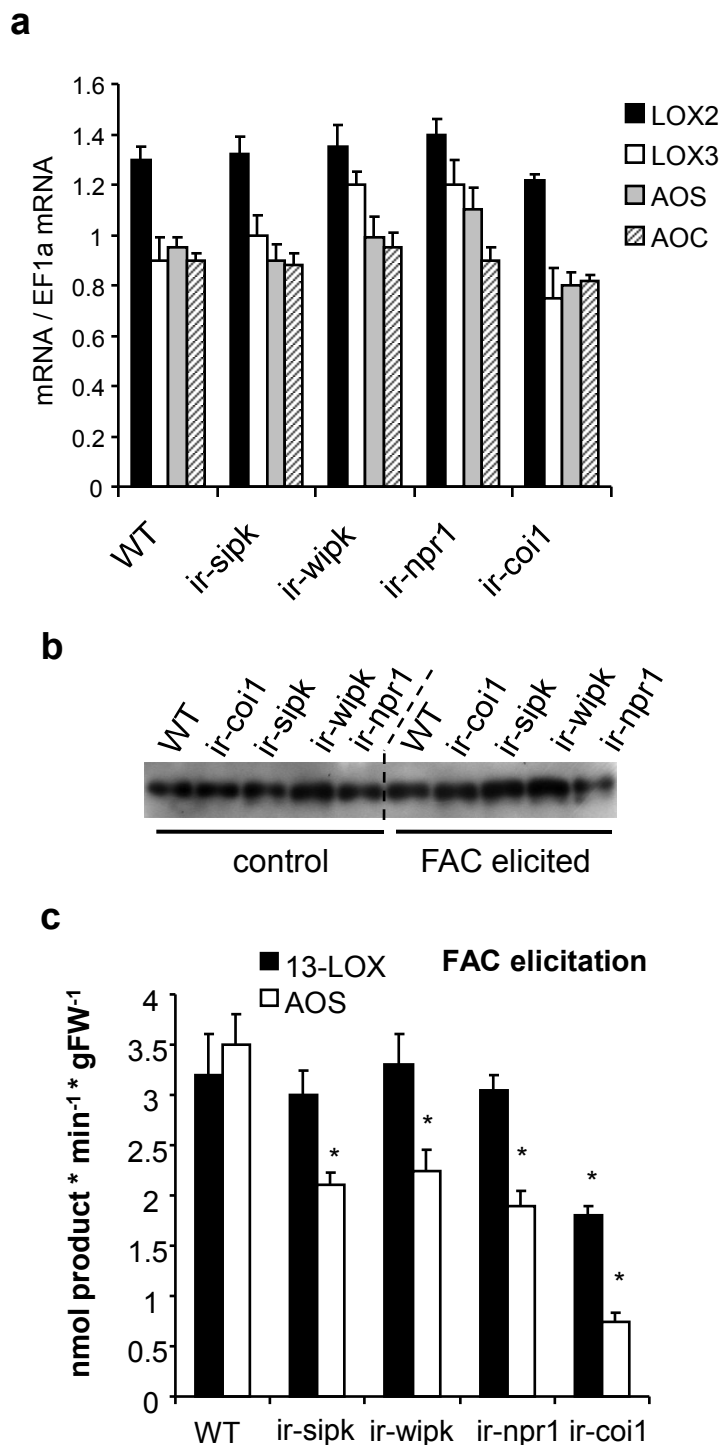


Figure S5. RT-qPCR, LOX3 protein levels and protein activities in elicited leaves

(a) Basal transcript levels were quantified in resting leaves of WT, *ir-sipk*, *ir-wipk*, *ir-npr1* and *ir-coi1* plants. Relative transcript abundance was quantified by RT-qPCR and expressed as the ratio of abundance of the queried mRNA over the standard (EF1a). Bars (±SD, n=3). **(b)** LOX3 protein levels were analyzed by immuno-blotting using a purified anti-NaLOX3 antibody. Total proteins were extracted from resting (control) and FAC elicited (20 min), separated by SDS-PAGE and NaLOX3 detected by immuno-chemoluminescence. Gel loading was evaluated by coomassie-blue staining. **(c)** 13-LOX and AOS activities were quantified 20 min after FAC elicitation in leaves of WT, *ir-sipk*, *ir-wipk*, *ir-npr1* and *ir-coi1* plants using [1-¹⁴C]-18:3 or [1-¹⁴C]-13-OOH-18:3 as substrates, respectively. Assays were performed in the linear phase of the reactions and ¹⁴C products were extracted, separated by thin layer chromatography and quantified by densitometric scanning. Bars (±SD, n=3). *see text.

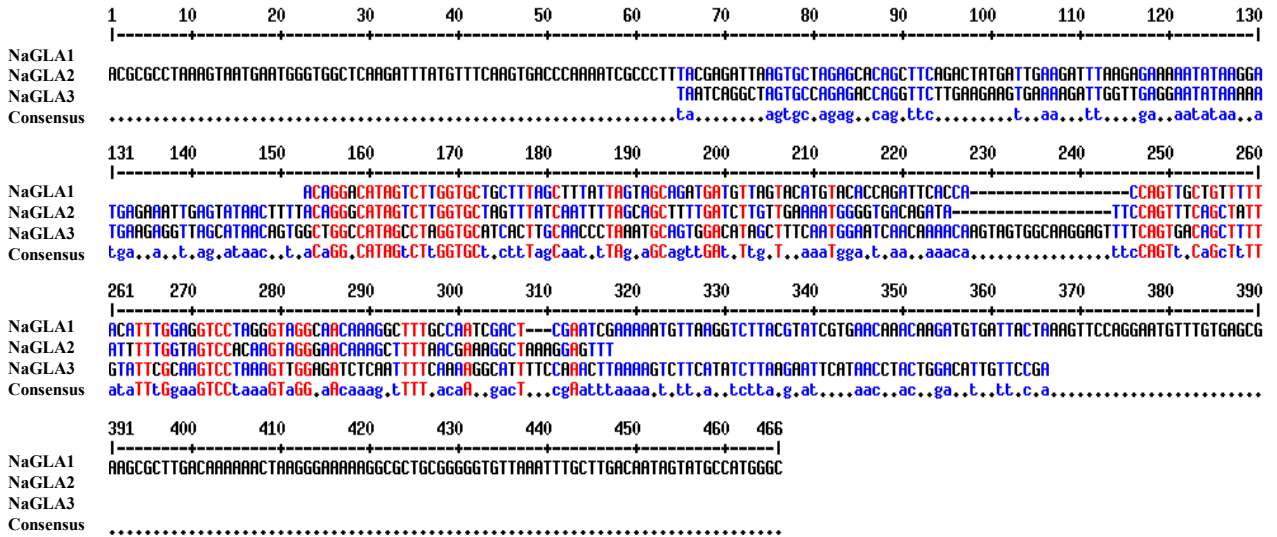


Figure S6. Alignment of GLA sequences used for VIGS

DNA alignments were performed using the MultiAlign software (Symbol comparison table: dna Gap weight: 5 Gap length weight: 0) as described (F. Corpet (1988) Multiple sequence alignment with hierarchical clustering. Nucl. Acids Res., 16, 10881-10890).

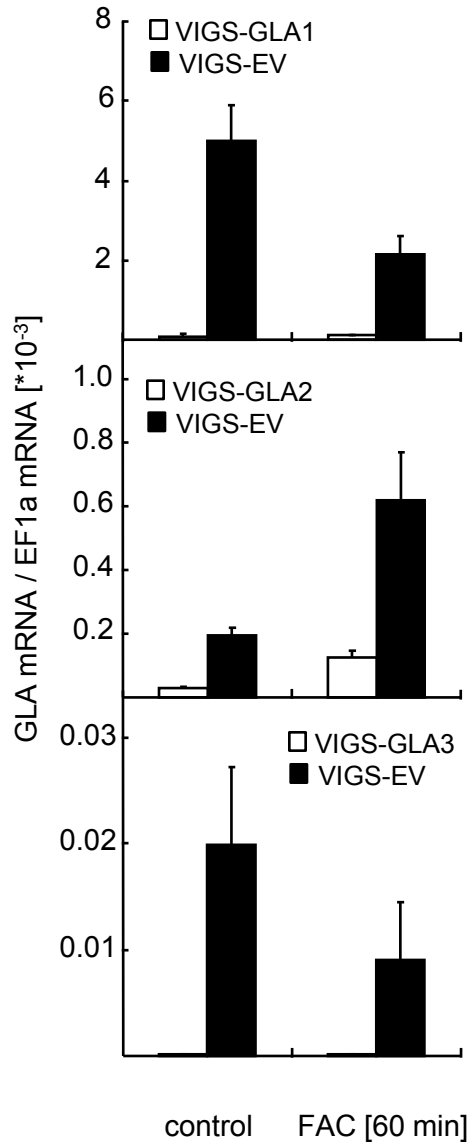


Figure S7. Quantification of GLA mRNA levels in VIGS-silenced plants

VIGS vectors carrying specific sequences in antisense orientation of GLA1, GLA2 and GLA3 cDNAs were generated and used for specific gene silencing. The efficiency of gene silencing was evaluated by quantification of GLA1, GLA2 and GLA3 transcript levels by RT-qPCR in unelicited leaves and 60 min after elicitation. mRNA levels are expressed as the ratio of abundance of the queried mRNA over the standard (EF1a). Control plants were empty vector (EV) plants. Bars (SD, n=4).

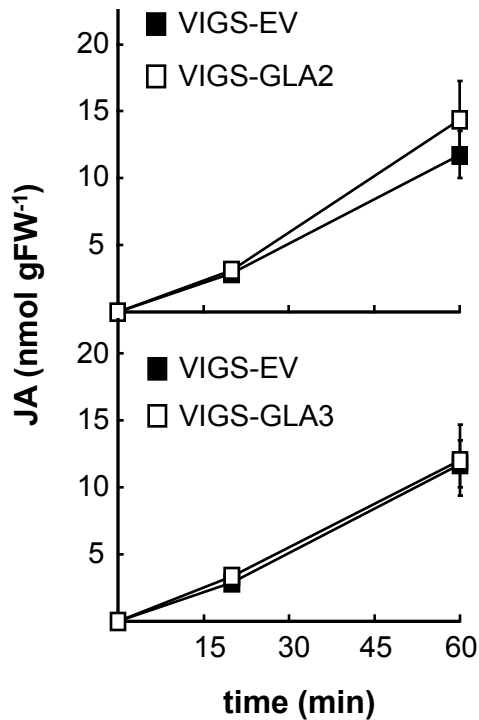
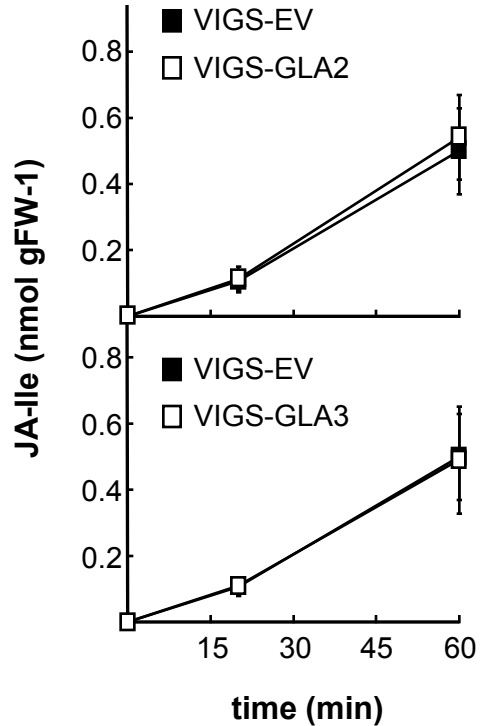
a**b**

Figure S8. Quantification of JA and JA-Ile levels in GLA2 and GLA3 VIGS-silenced plants

JA and JA-Ile levels were quantified in unelicited leaves and at 20 and 60 min after FAC elicitation. VIGS-GLA silenced plants (white), VIGS-EV plants (black). Bars (SE, n=8).

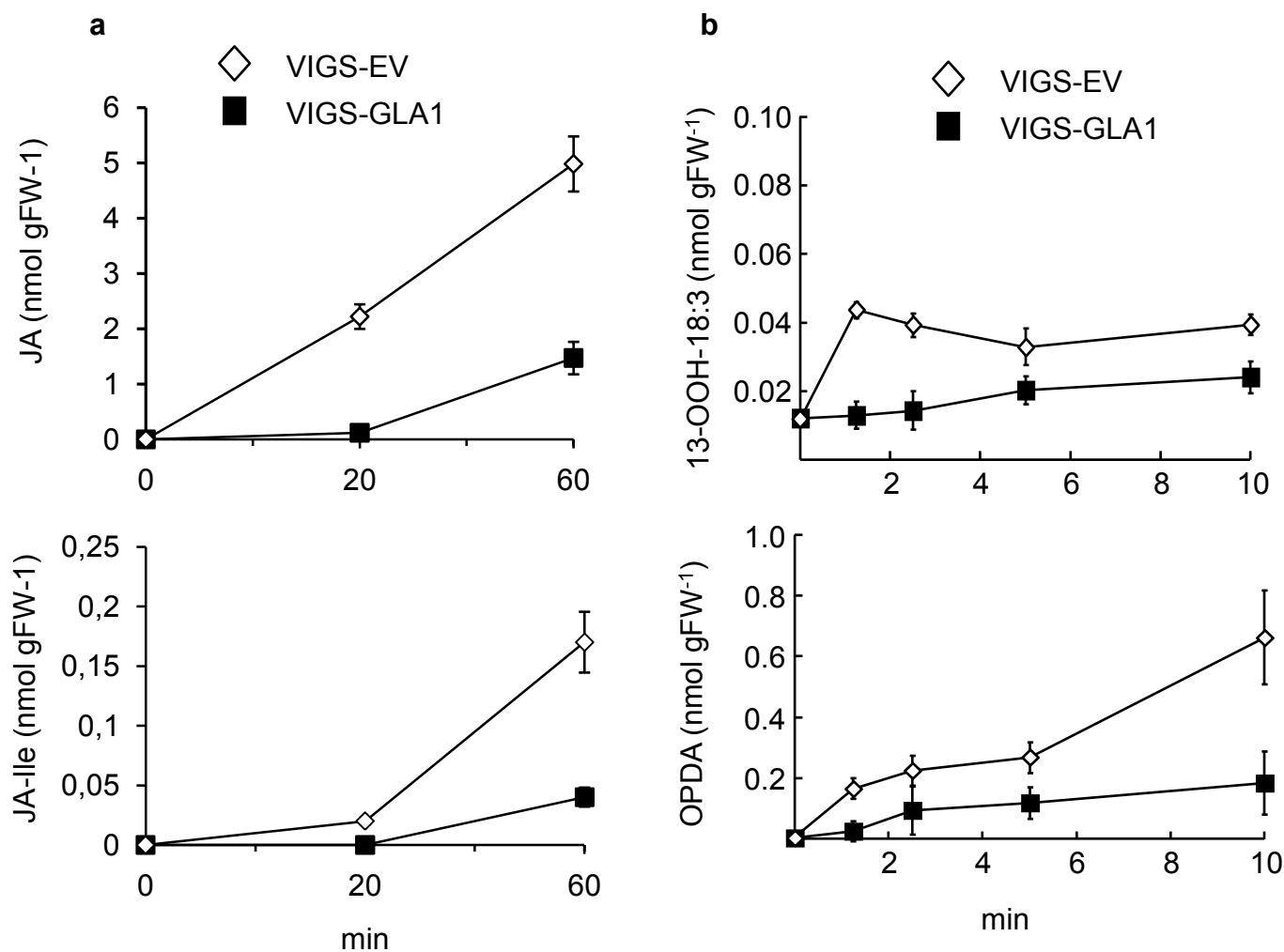


Figure S9 . Quantification of 13-OOH-18:3, OPDA , JA and JA-Ile levels in plants silenced in GLA1 expression.

(a) JA and JA-Ile levels were quantified at different times after wounding in plants silenced in GLA1 expression by VIGS . (b) 13-OOH-18:3 and OPDA levels were quantified at different times after FAC elicitation in plants silenced in GLA1 expression by VIGS. VIGS-EV (empty vector plants), VIGS-GLA1 (GLA1-silenced plants). Bars (\pm SE, n=5).

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Cloning of the full length GLA1 cDNA and construction of MBP and EGFP fusion proteins

For cloning of full length GLA1 cDNA, total RNA was extracted from 0.1 g of leaf material with TRIzol[®] (Invitrogen, Karlsruhe, Germany) and DNase-I treated according to commercial instructions. 5'RACE was performed using 5'RACE System for Rapid Amplification of cDNA Ends (Invitrogen, Karlsruhe, Germany). 5 µg of total RNA were reverse-transcribed with SuperScript-II reverse transcriptase and gene specific primer CCGCAGCGCCTTTTCCCT according to commercial instructions. PCR amplification was performed using the AUAP primer and the gene specific primer TCGAGTCGATTGGCAAAGCCTT. 3'RACE was performed using the 3'RACE System for Rapid Amplification of cDNA Ends (Invitrogen, Karlsruhe, Germany). Five µg of total RNA were reverse-transcribed with SuperScript-II reverse transcriptase and primer AP according to commercial instructions. PCR amplification was performed using the AUAP primer and the gene specific primer ACTCGAATCGAAAAATGTTAAGGTC. The PCR products were purified, subcloned into pGEM-T easy vector (Promega, Madison, WI) and sequenced. Analysis of the full length amino acid sequence of GLA1 by ChloroP gave a score of 0.557 cTP/Y/CS-score 2.553 and by TargetP of cTP 0.655 for plastid localization.

The GLA1 cDNA was cloned in frame to the maltose binding protein (MBP) in the vector pMAL-c4X (New England Biolabs, Beverly, MA). In this case, the putative plastid signal peptide was excluded to avoid activity inhibition (Fig. S4). For PCR amplification, the primers GGCCGAATTCATGAAAGCAGCTGAAGAATA and CCGGCTGCAGTTATCAAGCTGAAGGACTAGGCA were used and the amplicon was digested with EcoRI and PstI for subcloning into pMAL-c4X. *E.coli* BL21(DE3) cells were transformed and used for recombinant protein expression. MBP-GLA1 and MBP (control) were induced with 1mM of IPTG for 16 h at 25°C in LB media in the presence of ampicillin (100 µg/mL). The proteins were purified by amylose column chromatography (New England Biolabs, Beverly, MA) and washed and concentrated with Microcon YM-3 centrifugal filter units (Millipore, Schwalbach/Ts, Germany) according to commercial instructions. Protein amounts were quantified using the Bio-Rad Protein Assay kit (Bio-Rad, München, Germany) and BSA as a

standard. SDS-PAGE and staining with Bio-Safe™ Commasie (Bio-Rad, München, Germany) were used to evaluate protein purification.

For generation of EGFP fusion proteins, the full length GLA1 was PCR amplified from total cDNA using the primers GGCCCTGCAGATGCAGGTGGCAGTGGCAAC and CCGGGGTACCGCAGCTGAAGGACTAGGCAAGA. After purification, the PCR product was digested with PstI and KpnI and subcloned into the pEGFP vector (Clontech, Mountain View, CA). The GLA1-EGFP fusion construct was PCR amplified using the primers GGCCCTCGAGATGCAGGTGGCAGTGGCAAC and CCGGGAGCTCTCATTACTTGTACAGCTCGTCCAT and cloned into the pCAMBIA-1201 downstream of CaMV35S. The first 273 bp of the LOX3 coding region were PCR amplified from total cDNA with the primers GGCCCTGCAGATGGCACTAGCTAAAGAAATTAT and CCGGGGTACCGCTTCCTTGTCTTGTTCCTCACTG. After purification, the PCR product was digested with PstI and KpnI and subcloned into pEGFP. The pLOX3-EGFP fusion product was amplified using the primers GGCCCTCGAGATGGCACTAGCTAAAGAAATTAT and CCGGGAGCTCTCATTACTTGTACAGCTCGTCCAT and subcloned into pCAMBIA-1201 downstream of CaMV35S. EGFP gene was PCR amplified from the pEGFP vector with primers GGCCCTCGAGATGGTGAGCAAGGGCGAGGA and CCGGGAGCTCTCATTACTTGTACAGCTCGTCCAT and subcloned into the pCAMBIA-1201 downstream of CaMV35S.