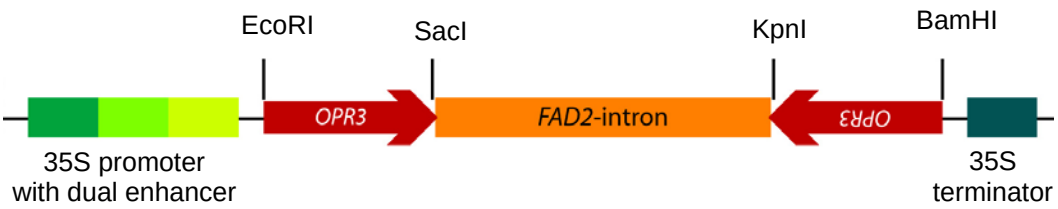
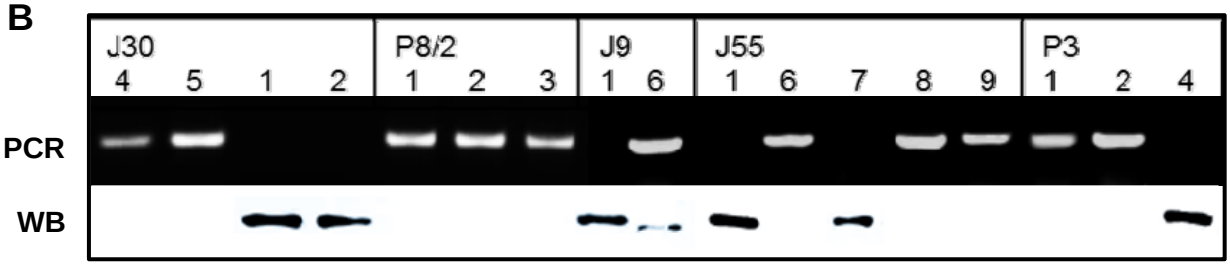
A



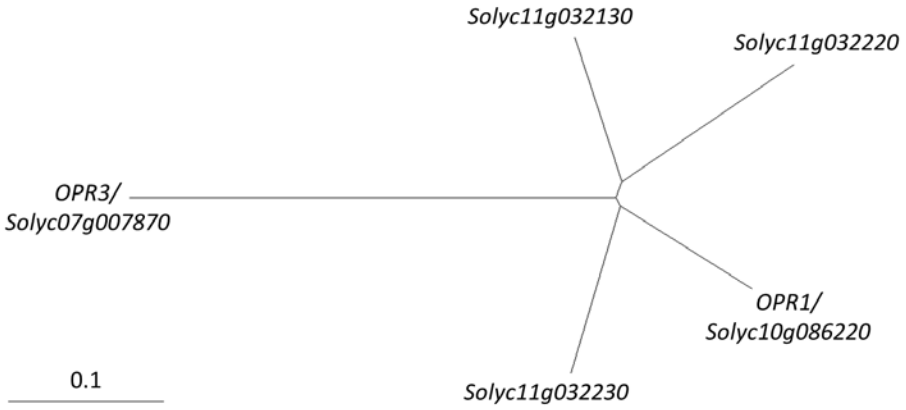
B



Supplemental Figure S1. Silencing of *OPR3* expression by RNAi. A, The hairpin construct used to generate transgenic *OPR3-RNAi* plants. 408 bp of the tomato *OPR3* cDNA in sense in antisense orientations separated by the first intron of the *FAD2* gene were cloned into the vector pRTL2 under control of the 35S promoter with dual enhancer and the 35S terminator. B, Confirmation of *OPR3* gene silencing. The segregating T1 progeny was tested by PCR for the presence of the hairpin construct (top, PCR). Representative results are shown for lines J30, P8/2, J9, J55, and P3, out of more than ten independently silenced lines. PCR products were separated on a 1% (w/v) agarose gel and visualized by ethidium bromide staining. Leaf extracts were prepared from the same plants that were wounded two hours before to induce the expression of *OPR3*. 30 µg total leaf protein were separated by SDS-PAGE and analyzed on western blots (bottom, WB) for the presence of the OPR3 protein using a polyclonal antiserum directed against OPR3 expressed in *E. coli* and affinity purified against the recombinant protein. OPR3 was undetectable in plants carrying the silencing construct.

.

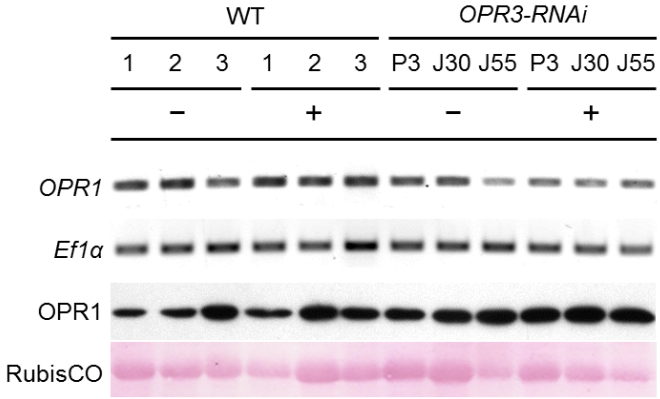
A



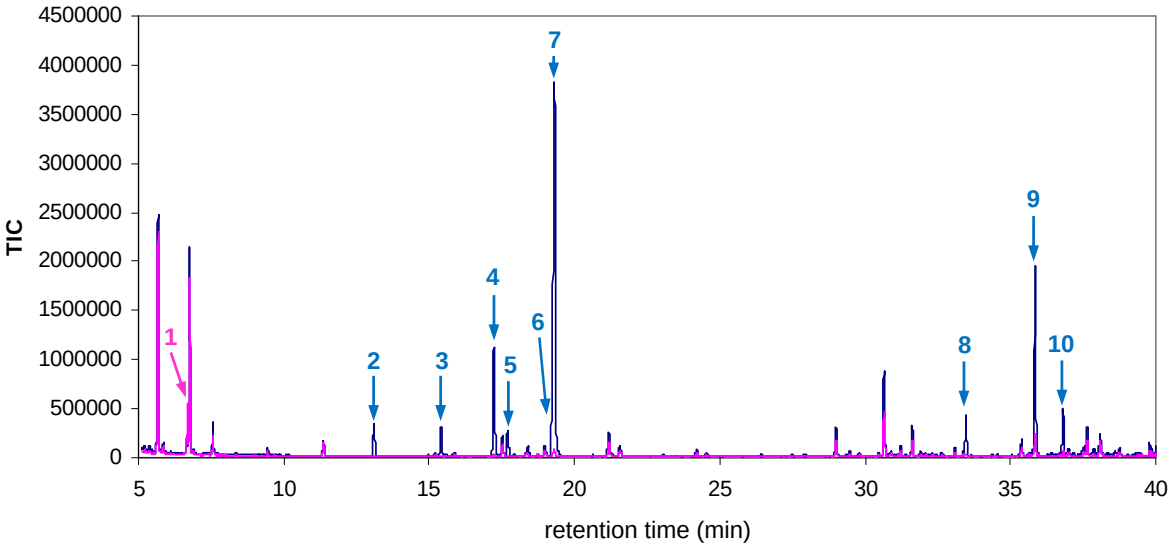
B

	OPR3/ Solyc07g007870	OPR1/ Solyc10g086220	Solyc11g032130	Solyc11g032220	Solyc11g032230
OPR3/ Solyc07g007870	100%	57%	54%	48%	51%
OPR1/ Solyc10g086220		100%	82%	75%	80%
Solyc11g0032130			100%	80%	81%
Solyc11g0032220				100%	81%
Solyc11g0032230					100%

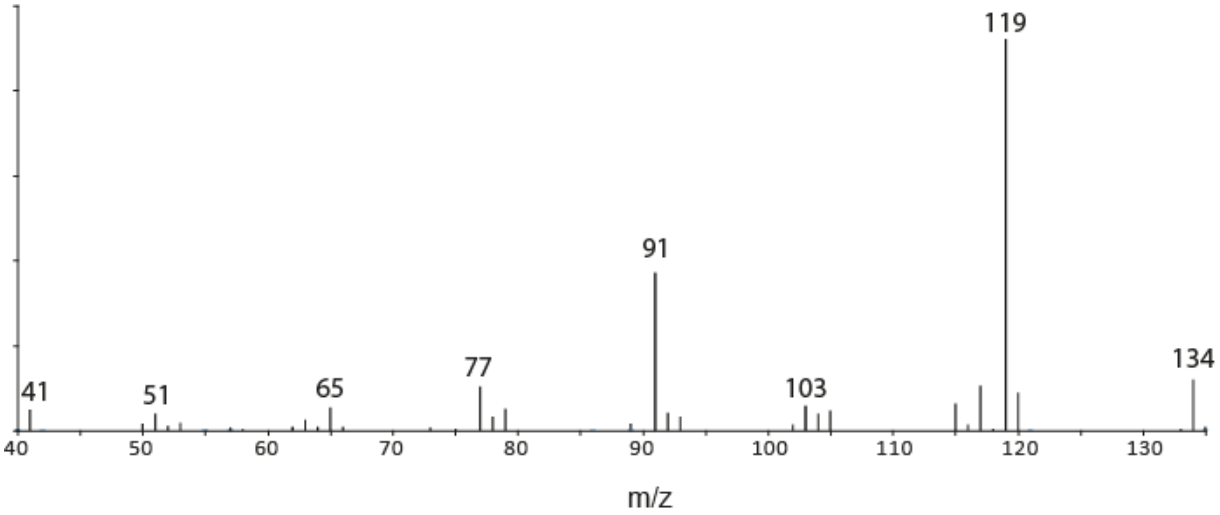
C



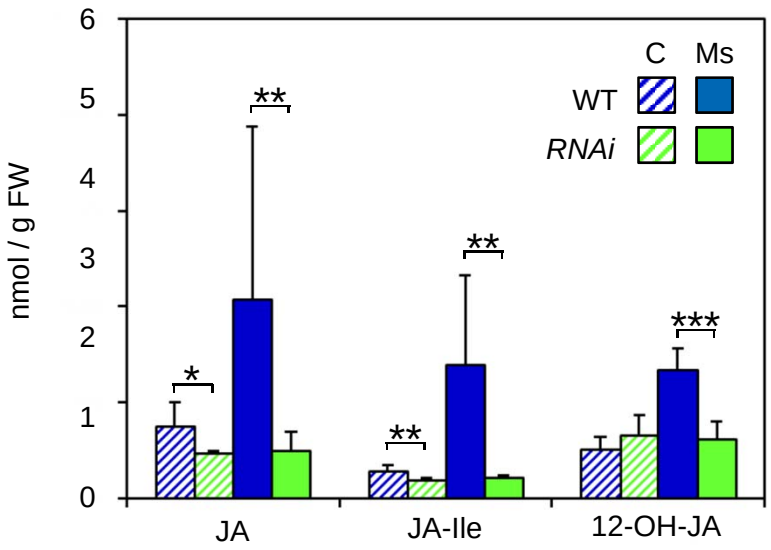
Supplemental Figure S2. Specificity of silencing. A, Phylogenetic tree showing the relationship of *OPR3*, *OPR1* and three putative *OPR* genes retrieved from the tomato genome database at http://solgenomics.net. The tree was generated from a multiple sequence alignment in ClustalX. B, Pairwise sequence comparison indicating sequence identity between *OPR3*, *OPR1* and three putative *OPR* genes in %. C, Expression of OPR1 analyzed in three wild-type plants and three independent RNAi lines before (-) and 24 hours after wounding (+). *OPR1* transcript abundance was analyzed by semiquantitative RT-PCR using the *EF1α* transcript as a control. OPR1 protein abundance was analyzed by western blot with the Ponceau Red-stained RubisCO band as a loading control.



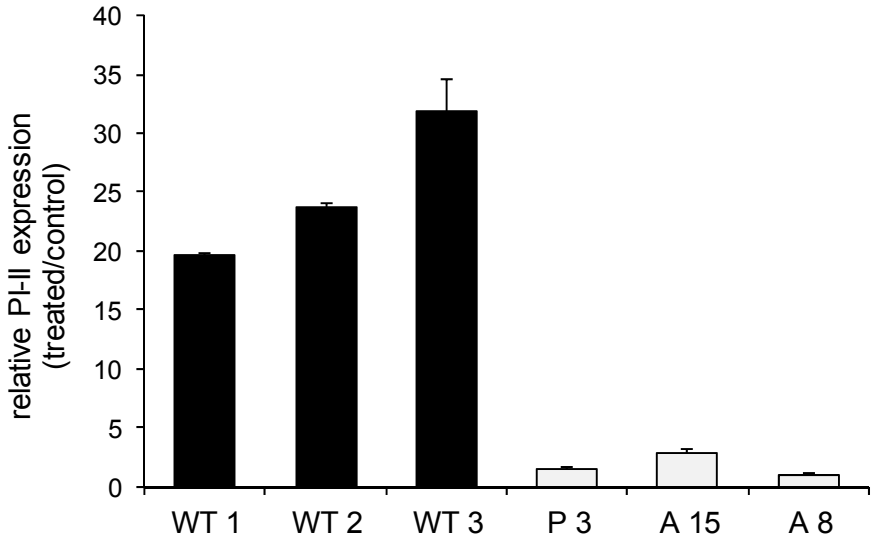
Supplemental Figure S3. Total ion chromatograms for trichome extracts from wild type (WT, blue) and *OPR3-RNAi* (RNAi, magenta) plants. The samples were analyzed on a Hewlett-Packard 6890 series gas chromatograph connected to a Hewlett-Packard 5973 quadrupole mass selective detector. Ten peaks showed pronounced differences in height between the WT and RNAi samples. The compounds were identified as *cis*-3-hexenal (1), α-pinene (2), unknown (3), 2-carene (4), α-phellandrene (5), limonene (6), β-phellandrene (7), δ-elemene (8), β-caryophyllene (9), α-humulene (10). The mass spectrum for the unknown compound (3) is shown in supplemental figure S4. The identification of δ-elemene (8) may be an artifact as it may have formed from germacrene C in the injector during gas chromatography (Quintana et al., 2003).



Supplemental Figure S4. Fragmentation spectrum of the unknown compound detected in *OPR3-RNAi* trichome extracts. The mass spectrum is shown for the compound detected as peak three (RT = 15.42 min) in the total ion chromatogram of trichome extracts (Suppl. Fig. S3).



Supplemental Figure S5. Induction of jasmonates by *M. sexta* feeding in wild-type and *OPR3-RNAi* plants. JA, JA-Ile, and 12-OH-JA were quantified by LC-MS/MS in wild-type (WT, dark blue) and *OPR3-RNAi* leaf tissue (*RNAi*, green) 40 minutes after the onset of *M. sexta* (forth-instar larvae) feeding (Ms, solid bars) and compared to untreated controls (C, hatched bars). Jasmonate levels are given in nmol/g fresh weight as the mean +/- SD of six biological replicates for wild-type plants. For *OPR3-RNAi* plants, three biological replicates were performed on each of three independent transgenic lines. Asterisks indicate significant differences between *OPR3-RNAi* and wild-type plants (*P<0.05, **P<0.01, ***P<0.001).



Supplemental Figure S6. qPCR analysis of *cis*-3-hexenal-induced *PI-II* expression in *OPR3-RNAi* and wild-type plants. Experimental plants (three independent *OPR3-RNAi* lines (P3, A15, A8; gray bars) and the corresponding tomato wild type (WT1-3; black bars) were exposed to 10 µl 0.1 M *cis*-3-hexenal on a cotton swab. After 24 hours, plants were harvested and RNA was extracted from pooled leaf material of three plants for each data point. RevertAid M-MuLV reverserse transcriptase (Fermentas) was used for cDNA synthesis and qPCR was performed on a MX3000P System (Stratagene) using the SYBR® Green technology. The *PI-II* expression levels were quantified relative to solvent (methanol)-exposed controls by the $2^{-\Delta\Delta CT}$ method using the Stratagene software with elongation factor 1α as the reference gene. For each of the three biological replicates, fold-induction values are shown as the mean +/- SD of two and three technical replicates for WT and *OPR3-RNAi* plants, respectively.

Suplemental Table S1. Volatile blends used in dual choice feeding assays. The volatile blends were prepared to reflect the composition of trichomes from either wild type (WT) or *OPR3-RNAi* leaves (*RNAi*). The terpene blend was prepared in hexane and contained commercially available terpenes in 10 times the concentration found in WT or *RNAi* trichome extracts (cf. Fig. 4C and Supplemental Table S3). *cis*-3-hexenal was diluted in water from a 50% stock in triacetin to a concentration 10 times higher than that in WT or *RNAi* trichome extracts.

compound	WT blend [µg / ml]	*RNAi* blend [µg / ml]
cis-3-hexenal	35.0	84.0
α-pinene	12.6	0.45
2-carene	58.8	3.2
α-phellandrene	740.8	34.0
β-caryophyllene	1183.0	191.5
α-humulene	20.2	1.8
limonene	50.8	1.9

Supplemental Table S2. Identification of trichome volatiles. Peaks were selected on basis of differences in height between extracts from wild-type and *OPR3-RNAi* trichomes. Compounds were identified by comparison of mass spectrum, retention times (RT) and the co-chromatography of pure standards where available.

Compound	RT	ID[a]	KI[b]	AI[c]	KI values from literature		
cis-3-hexenal	6.7	A	?	?			
α-pinene	13.12	A	933	928	933[d]	934[e]	939[f]
unknown*	15.42	B	970	966			
2-carene	17.24	A	996	995	1001[g]	1002[f]	
α-phellandrene	17.72	A	1003	1003	1032[e]	1002[f]	
limonene	19.26	A	1029	1026	1036[e]	1039[d]	1029[f]
β-phellandrene	19.33	C	1030	1027	1053[e]	1029[f]	
δ-elemene**	33.48	C	1335	1334	1053[f]	1340[i]	1338[f]
β-caryophyllene	35.87	A	1419	1418	1053[h]	1467[e]	1419[f]
α-humulene	36.82	A	1456	1455	1053[d]	1454[f]	

[a] method of identification: A, Identification based on mass spectrum, retention times and co-chromatography of external standard; B, unknown compound; C, Identification based on mass spectrum and retention times.

[b] Kovats index

[c] Arithmetic index

? Kovats index could not be determined due to co-elution of the relevant alkane standard

[d] Choi HS (2003) J. Agric. Food Chem. 51:2687-2692.

[e] Hognadottir A. and Rouseff R.L. (2003) J. Chromatogr. A. 998:201-211.

[f] Adams RP (2007) Identification of essential oil components by gas chromatography/ mass spectrometry. Allured Publishing Corporation, Carol Stream, IL.

[g] Karioti A, et al. (2003) J. Agric. Food Chem. 51:6505-6508.

[h] Kobaisy M, et al. (2002) Phytochem. 61:37-40.

[i] Priestap HA, et al. (2003) Phytochem. 63:221-225.

* the mass spectrum for this compound is shown in supplemental figure S4

** possibly formed from germacrene C in the injector during gas chromatography

Supplemental Table S3. Quantification of trichome volatiles. Compounds were selected on basis of differences in abundance between extracts from wild type (WT) and *OPR3-RNAi* (*RNAi*) trichomes of two independent transgenic lines. Values are given as ng per ul of trichome extract (1 µl corresponding to 2 mg of leaf tissue) with the standard deviation obtained for two runs.

Compound	WT #1	WT #1	*RNAi* #1	*RNAi* #2
cis-3-hexenal	2.92 ± 0.24	4.07 ± 0.10	8.99 ± 0.32	7.83 ± 0.14
α-pinene	1.75 ± 0.01	0.77 ± < 0.00	0.06 ± 0.01	0.03 ± < 0.00
unknown[a]	2.38 ± 0.01	1.24 ± 0.01	0.12 ± < 0.00	0.06 ± < 0.00
2-carene	8.19 ± 0.13	3.56 ± 0.02	0.41 ± 0.01	0.23 ± 0.01
α-phellandrene[b]	99.71 ± 3.19	48.44 ± 2.12	4.37 ± 0.34	2.42 ± 0.04
limonene	6.85 ± 0.24	3.31 ± < 0.00	0.25 ± 0.01	0.13 ± 0.01
β-phellandrene	1461.8 ± 19.9	692.83 ± 2.78	63.51 ± 0.42	31.87 ± 0.97
δ-elemene[c]	49.06 ± 5.32	32.00 ± 1.58	4.04 ± 0.01	3.29 ± 0.05
β-caryophyllene	132.98 ± 1.69	103.62 ± 0.31	22.19 ± 1.17	16.04 ± 0.64
α-humulene	2.28 ± 0.04	1.76 ± 0.02	0.36 ± 0.01	0.26 ± < 0.00

[a] quantified as α-pinene equivalents

[b] quantified as α-phellandrene equivalents

[c] quantified as β-caryophyllene equivalents

Supplemental Methods: Oligonucleotide primer sequences.

all primers were obtained from Operon, Köln, Germany

cloning of the tomato *OPR3* hairpin construct for gene silencing

(BamHI/EcoRI and KpnI/SacI restriction sites underlined)

OPR3-HP, forward: 5'-CCCGGGGATCCGAATTCTAATGCCTGATGGAACTCATGG-3'
OPR3-HP, reverse: 5'- CCCGGGGTACCGAGCTCGCTTGGAACCAGAATGGAGTTGGATTTTG -3'

genotyping of *OPR3*-RNAi lines

sense part of the hairpin construct
$OPR3_{421}$: 5'-ATGCCTGATGGAACTCATGGGA-3'
$FAD2_{rev}$: 5'-AGCGGAGAAATTCACAGAGCAGGA-3'

antisense part of the hairpin construct
$OPR3_{421}$: 5'-ATGCCTGATGGAACTCATGGGA-3'
$FAD2_{fw}$: 5'-TGTGGCAATCCCTTTCACAACCTG-3'

genotyping of *jai1-1*

JAI-1-F: 5'-GTGGAGACGATATGTTGAGACTAA-3'
JAI -1-R: 5'-CCATGGAGTCCATCACCTAACAGT-3'
Jai-1-R: 5'-GTGGTCAGATCAGAGCCCTCTATT-3'

semi-quantitative RT-PCR analysis

ef1α-F: 5'-ACACCTCCCACATTGCTGTCAAGT-3'
ef1α-R: 5'-TTTGGGCAGCCTTGGTGACTTTAG-3'

PI-II-F: 5'-CCATCATGGCTGTTCACAAGGAAG-3'
PI-II-R: 5'-ACGTGGTAACATCCGGTGGGATAAA-3'

qPCR analysis

ef1α-F: 5'-AGCCCATGGTTGTTGAGACCTTTG-3'
ef1α-R: 5'- TTCGAAACACCAGCATCACACTGC-3'

PI-II-F: 5'-GGATATGCCCACGTTCAGAAGGAA-3'
PI-II-R: 5'-AATAGCAACCCTTGTACCCTGTGC-3'