

Supporting Information for

Sex pheromone communication in an insect parasitoid, *Campoletis chlorideae* Uchida

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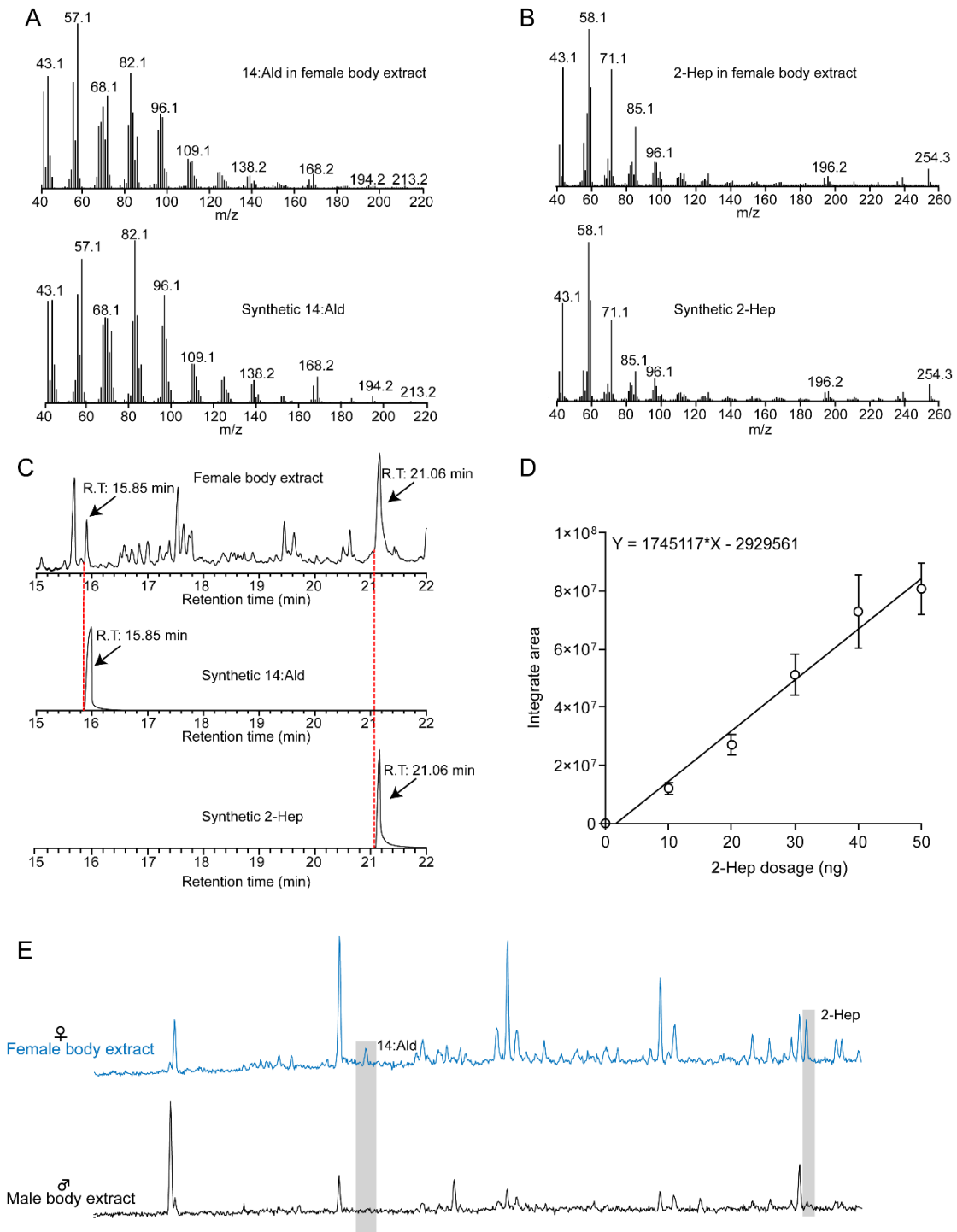


Figure S1. Gas Chromatography-Mass Spectrometer analysis of male and female body extract. (A-B) Mass spectra of candidate sex pheromones in female body extract and synthetic compounds. **(C)** Total ion chromatogram (TIC) of the candidate sex pheromone components and synthetic compounds. **(D)** The regression of synthetic 2-Hep in GC-MS. The X-axis represents sampling dosages and the Y-axis indicates the integrated area. **(E)** Comparison of TIC of female (upper) and male (lower) body extracts.

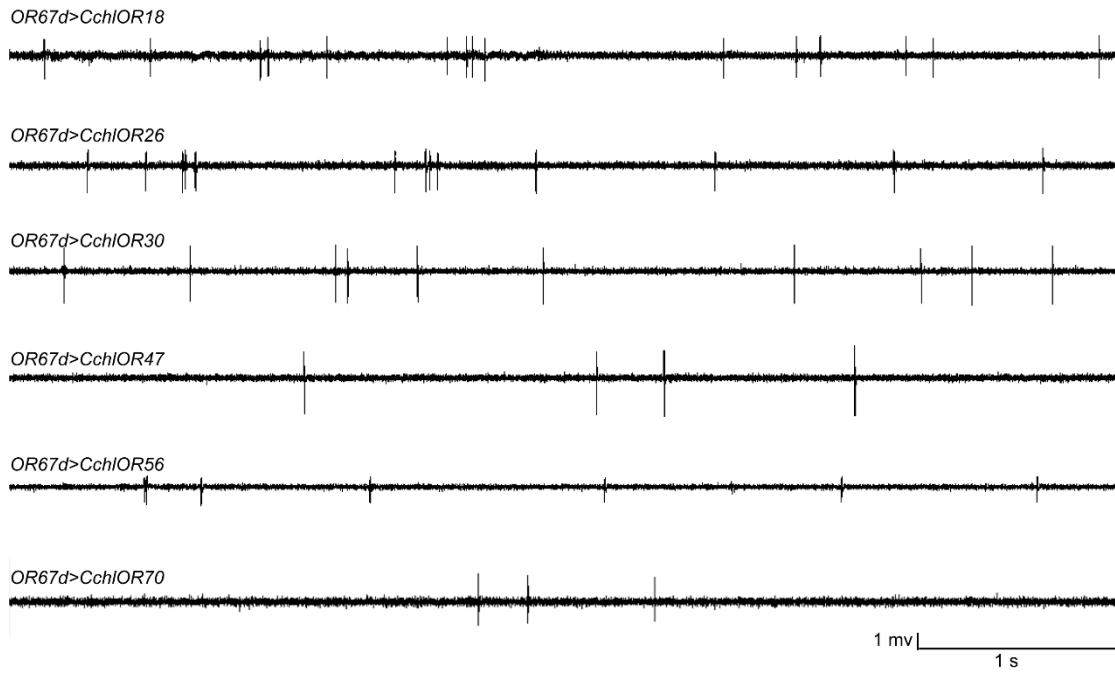


Figure S2. Spontaneous spiking of the *Drosophila* T1 neurons expressing CchIORs.

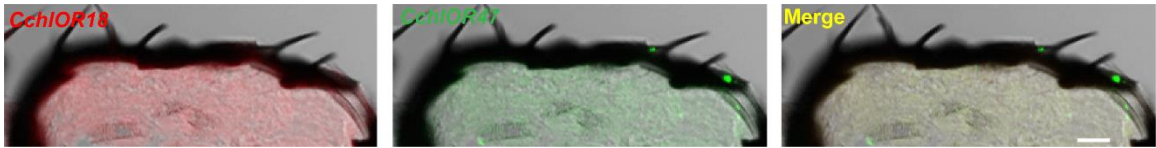


Figure S3. Staining of the micro-sections of virgin antennae with the sense-probes of CchIOR18 (red) and CchIOR47 (green). Scale bar represents 10 μ m.

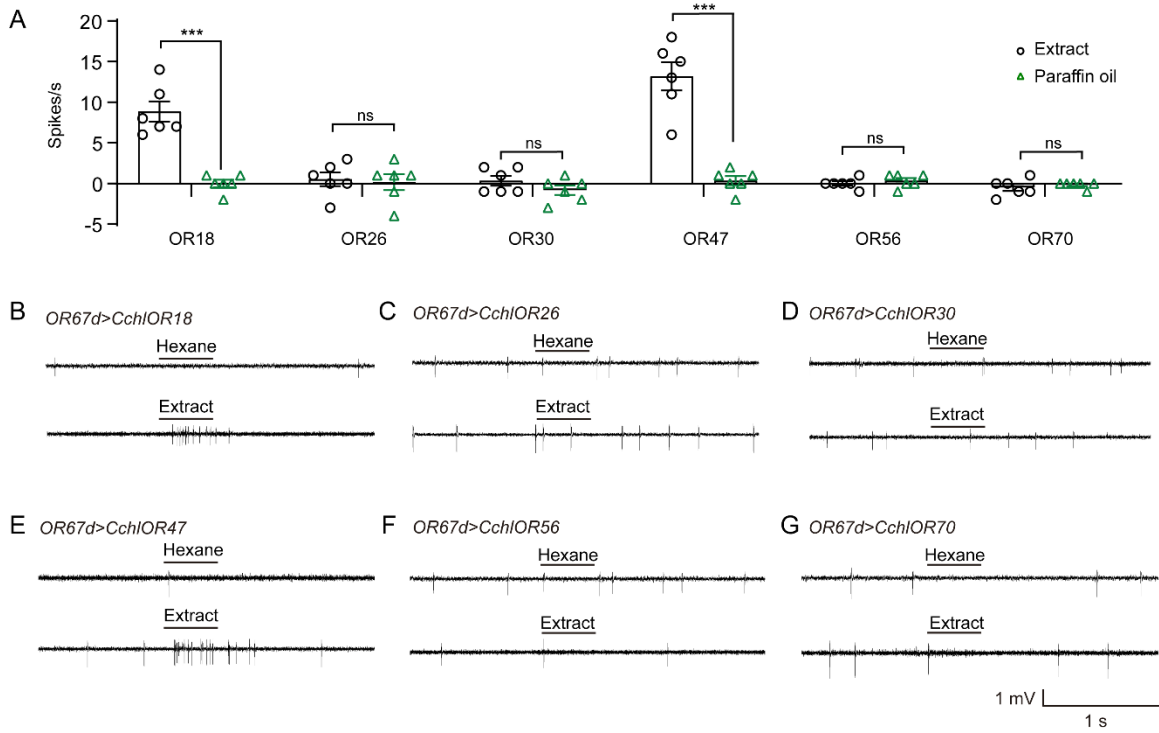


Figure S4. The responses of CchIOR-expressing T1 neurons to hexane extracts of female bodies. (A) Response spikes of CchIOR-expressing T1 neurons to 10 μ L of concentrated hexane extracts (equivalent to 5 females) and to 10 μ L of hexane. ($n = 6$ flies for each genotype). **(B-G)** Representative traces of the responses of CchIOR18-, CchIOR26-, CchIOR30-, CchIOR47-, CchIOR56-, and CchIOR70-expressing neurons to body extracts and hexane, respectively. $P < 0.001$ by two-tailed student t -test.

Table S1. Chemicals used for single sensillum recordings

	Order	Chemicals	Vendor	Purity	CAS#
Green leaf volatiles	1	1-hexanol	Fluka	99%	111-27-3
	2	2-hexanol	Aldrich	99%	626-93-7
	3	3-hexanol	Fluka	99%	623-37-0
	4	4-hexanol	Aldrich	97%	928-92-7
	5	5-hexanol	Aldrich	99%	821-41-0
	6	(Z)-2-hexenol	Fluka	95%	928-94-9
	7	(Z)-3-hexenol	TCI	96%	928-96-1
	8	(E)-2-hexenol	Fluka	95%	928-95-0
	9	(E)-3-hexenol	Roth	98%	928-97-2
	10	Hexanal	TCI	99%	66-25-1
	11	(Z)-3-hexenal	Sigma-Aldrich	95%	6789-80-6
	12	(E)-2-hexenal	Sigma-Aldrich	95%	6728-26-3
	13	Hexyl acetate	Fluka	99%	142-92-7
	14	(Z)-3-hexenyl acetate	Roth	97%	3681-71-8
	15	(E)-2-hexenyl acetate	Sigma-Aldrich	98%	2497-18-9
	16	(Z)-2-hexenyl acetate	Sigma-Aldrich	95%	56922-75-9
Terpenoids	17	2-carene	Sigma-Aldrich	97%	4497-92-1
	18	3-carene	Sigma-Aldrich	99%	498-15-7
	19	(E)- β -caryophyllene	Sigma-Aldrich	98%	87-44-5
	20	α -caryophyllene	TCI	93%	6753-98-6
	21	E- β -farnesene	Sigma-Aldrich	90%	18794-84-8
	22	farnesene (α & β mixture)	Sigma-Aldrich		
	23	indole	TCI	99%	120-72-9
	24	linalool	Sigma-Aldrich	97%	78-70-6
	25	linalool oxide	TCI	97%	60047-17-8
	26	S-(-)-limonene	Sigma-Aldrich	96%	5989-54-8
	27	R-(+)-limonene	Fluka	98%	5989-27-5
	28	β -mycene	Sigma-Aldrich	90%	123-35-3
	29	E- β -ocimene	Sigma-Aldrich	90%	3779-61-1
	30	α -pinene	Aldrich	98%	7785-26-4
	31	β -pienne	Macklin	98%	18172-67-3
	32	α -terpinene	Fluka	99%	99-83-2
	33	γ -terpinene	TCI	95%	99-85-4
	34	DMNT	Kunbo	92%	19945-61-0
	35	TMTT	Kunbo	92%	62235-06-7
	36	α -phellandrene	Sigma-Aldrich	95%	99-83-2
	37	β -phellandrene	TRC	96%	555-10-2
	38	α -humulene	Sigma-Aldrich	96%	6753-98-6
	39	geraniol	Sigma-Aldrich	98%	106-24-1
	40	guaiene	Sigma	92%	88-84-6
	41	sabinene	Yuanye	98%	3387-41-5
	42	citronellal	Roth	96%	106-23-0
	43	citronellol	Fluka	95%	106-22-9
	44	terpinol	Roth	97%	8000-41-7
	45	(+,-)- α -terpinyl acetate	Aldrich	90%	80-26-2
	46	citral	Sigma-Aldrich	96%	5392-40-5
	47	nerol	Fluka	95%	106-25-2
	48	farnesol	Sigma-Aldrich	95%	4602-84-0
	49	farnesyl acetate	Sigma-Aldrich	95%	29548-30-9

Terpenoids	50	δ -decanolactone	TCI	97%	705-86-2
	51	(-)-bornyl acetate	Sigma-Aldrich	97%	5655-61-8
	52	(Z)-9-tricosene	Sigma-Aldrich	97%	27519-02-4
	53	piperitone	TCI	94%	89-81-6
	54	(+,-)-nerolidol	TCI	97%	7212-44-4
	55	zingiberene	TRC	95%	495-60-3
	56	α -curcumene	TRC	95%	644-30-4
	57	β -curcumene	TRC	95%	451-56-9
	58	1,8-cineole	TCI	98%	470-82-6
	59	(+,-)-longifolene	Sigma-Aldrich	99%	475-20-7
	60	p-cymene	TCI	95%	99-87-6
	61	β -elemene	APEXBIO	95%	515-13-9
	62	camphene	BDH	98%	79-92-5
	63	(S)-cis-verbenol	Sigma-Aldrich	95%	18881-04-4
	64	citronellyl acetate	Sigma-Aldrich	95%	150-84-5
65	(+)-limonene oxide	Sigma-Aldrich	97%	203719-54-4	
Aliphatic	66	propanol	Fluka	99.5%	71-23-8
	67	1-butanol	Sigma-Aldrich	99.8%	71-36-3
	68	2-butanol	Sigma-Aldrich	99.5%	78-92-2
	69	1-pentanol	Fluka	99%	71-41-0
	70	2-pentanol	Sigma-Aldrich	98%	6032-29-7
	71	1-heptanol	Fluka	99.5%	22543-790
	72	2-heptanol	TCI	98%	543-49-7
	73	1-octen-3-ol	Fluka	98%	3391-86-4
	74	nonanol	Fluka	98%	143-08-8
	75	(Z)-6-nonen-1-ol	Sigma-Aldrich	95%	35854-86-5
	76	decanol	Fluka	97%	112-30-1
	77	2-methyl-1-butanol	Sigma-Aldrich	99%	137-32-6
	78	3-methyl-1-butanol	Sigma-Aldrich	99%	123-51-3
	79	2-Butyl-1-octanol	Sigma-Aldrich	95%	3913-02-8
	80	4-methylphenol	Sigma-Aldrich	99%	106-44-5
	81	1-heptaldehyde	Fluka	95%	111-71-7
	82	octanal	Sigma-Aldrich	99%	124-13-0
	83	nonanal	Fluka	97%	124-19-6
	84	decanal	Fluka	97%	112-31-2
	85	undecanal	Fluka	97%	112-44-7
	86	dodecyl aldehyde	Sigma-Aldrich	92%	112-54-9
	87	2-methylbutyl acetate	Sigma-Aldrich	99%	624-41-9
	88	pentyl acetate	Fluka	99%	628-63-7
	89	ethyl acetate	TCI	99.5%	141-78-6
	90	ethyl butyrate	Acros	99%	105-54-4
	91	butyl butyrate	Sigma-Aldrich	98%	109-21-7
	92	ethyl valerate	Sigma-Aldrich	98%	539-82-2
	93	ethyl hexanoate	Sigma-Aldrich	99%	123-66-0
	94	ethyl laurate	TCI	99%	106-33-2
	95	butyl hexanoate	Sigma-Aldrich	98%	626-82-4
	96	butyl laurate	Sigma-Aldrich	99%	628-63-7
	97	propyl hexanoate	TCI	98%	626-77-7
	98	hexyl hexanoate	TCI	98%	6378-65-0
	99	hexyl butyrate	TCI	98%	2639-63-6
	100	hexyl propionate	TCI	98%	2445-76-3
	101	(Z)-3-hexenyl butyrate	Sigma-Aldrich	98%	16491-36-4
	102	(E)-2-hexenyl butyrate	TCI	93%	53398-83-7

Aliphatic	103	(Z)-3-hexenyl salicylate	Sigma-Aldrich	97%	65405-77-8
	104	(Z)-3-hexenyl-2-methyl butanoate	Sigma-Aldrich	97%	53398-85-9
	105	(Z)-3-hexenyl isovalerate	TCI	98%	35154-45-1
	106	dodecane	Aladdin	99.5%	112-40-3
	107	(Z)-3-hexenyl (Z)-3 hexenoate	TCI	95%	61444-38-0
	108	Ethyl 2-ethylhexanoate	TCI	95%	2983-37-1
	109	isoamyl butyrate	TCI	98%	106-27-4
	110	isoamyl propionate	TCI	98%	105-68-0
	111	isoamyl Isovalerate	Macklin	98%	659-70-1
	112	6-methyl-5-hepten-2-one	Sigma-Aldrich	99%	110-93-0
	113	geranyl acetone	TCI	96%	689-67-8
	114	geranyl acetate	Sigma-Aldrich	97%	105-87-3
	115	2-acetylcyclohexanone	Sigma-Aldrich	97%	874-23-7
	116	2,3-butanedione	Sigma-Aldrich	97%	431-03-8
	117	2,3-butanediol	TCI	97%	513-85-9
	118	(Z)-jasnone	TCI	92%	488-10-8
	119	methyl jasmonate	Sigma-Aldrich	95%	39924-52-2
	120	jasmonic acid	Sigma-Aldrich	90%	77026-92-7
	121	formate	TCI	99%	71-47-6
	122	acetic acid	Sigma-Aldrich	99%	64-19-7
	123	butyl acid	Sigma-Aldrich	99%	107-92-6
	124	linoleic acid	Sigma-Aldrich	95%	60-33-3
	125	hexanoic acid	Sigma-Aldrich	99%	142-62-1
	126	valeric acid	Sigma-Aldrich	99%	109-52-4
	127	propionic acid	Sigma-Aldrich	99%	79-09-4
	128	(-)-ethyl-L-lactate	Sigma-Aldrich	98%	687-47-8
	129	4-methylcyclohexanol	TCI	98%	589-91-3
	130	pentadecane	Sigma-Aldrich	99%	629-62-9
	131	hexadecene	Sigma-Aldrich	98.5%	629-73-2
	132	2-heptanone	Sigma-Aldrich	98%	110-43-0
	133	butyl aldehyde	TCI	95%	78-84-2
	134	3-heptanol	Fluka	99%	589-82-2
	135	ethyl caproate	Fluka	98%	123-66-0
	136	2-tridecanone	Sigma-Aldrich	99%	593-08-8
	137	allyl isothiocyanate	Sigma-Aldrich	95%	57-06-7
	138	(Z)-3-hexenyl propionate	Macklin	97%	33467-74-2
	139	pentyl valerate	Sigma-Aldrich	97%	2173-56-0
	140	octadecane	Sigma-Aldrich	99%	593-45-3
	141	nonadecane	Sigma-Aldrich	99%	129-92-5
	142	17-pentatriacontene	Sigma-Aldrich	99%	6971-40-0
	143	1-heptadecene	TCI	99.5%	6795-39-5
	144	tridecanal	TCI	95%	10486-19-8
	145	2-heptadecanone	Sigma-Aldrich	99%	2922-51-2
	146	hexacosane	Sigma-Aldrich	99%	630-01-3
	147	1-nonadecene	TCI	95%	18435-45-5
	148	2-hexadecanol	Sigma-Aldrich	99%	14852-31-4
	149	ethyl (2E,4Z)-deca-2,4-dienoate	Acros	97%	3025-30-7
	150	undecanoic y lactone	Sigma-Aldrich	99%	104-67-6

Aliphatic	151	pelargonic acid	Fluka	95%	112-05-0
	152	lauric acid	Fluka	99%	143-07-7
	153	(D,L)-lactic acid	TCI	85%	50-21-5
	154	octoic acid	BDH	98%	124-07-2
	155	1-undecanol	Sigma-Aldrich	99%	112-42-5
	156	n-valeraldehyde	Fluka	98%	110-82-0
	157	methyl laurate	TCI	98%	111-82-0
	158	1-dodecanol	Sigma-Aldrich	98%	113-53-8
	159	capric acid	Fluka	99%	334-48-5
	160	undecylenic acid	Beijing	97%	112-38-9
	161	octyl acetate	Macklin	98%	112-14-1
	162	2-methyl-1-propanol	Macklin	99%	78-83-1
	163	2-acetylthiazole	Macklin	98%	24295-03-2
	164	3-pentanone	TCI	98%	96-22-0
	165	3-heptanone	TCI	98%	106-35-4
	166	2-octanone	TCI	98%	111-13-7
	167	3-octanone	TCI	98%	106-68-3
	168	3-nonanone	TCI	97%	925-78-0
	169	ethyl propionate	TCI	99%	105-37-3
	170	isoamyl acetate	TCI	98%	123-92-2
	171	nonyl acetate	TCI	99%	143-13-5
172	pyrazine	TCI	98%	290-37-9	
173	1,4-diaminobutane	AlfaAesar	98%	110-60-1	
Aromatic	174	benzaldehyde	Fluka	99%	100-52-7
	175	4-ethyl benzaldehyde	Sigma-Aldrich	97%	4748-78-1
	176	benzyl alcohol	Sigma-Aldrich	98%	100-51-6
	177	benzyl acetate	Fluka	99%	140-11-4
	178	methyl benzoate	Sigma-Aldrich	99%	93-58-3
	179	ethyl benzoate	Sigma-Aldrich	99%	93-89-0
	180	butyl benzoate	TCI	99%	136-60-7
	181	methyl salicylate	Sigma-Aldrich	99%	119-36-8
	182	butyl salicylate	TCI	99%	2052-14-4
	183	ethyl salicylate	Sigma-Aldrich	99%	119-36-8
	184	phenylacetaldehyde	Sigma-Aldrich	90%	122-78-1
	185	salicylaldehyde	Sigma-Aldrich	98%	90-02-8
	186	2-phenylethyl acetate	Fluka	99%	103-45-7
	187	linalyl acetate	Sigma-Aldrich	96%	115-95-7
	188	methyl phenylacetate	Sigma-Aldrich	98%	101-41-7
	189	2-phenylethanol	Sigma-Aldrich	99%	60-12-8
	190	eugenol	Sigma-Aldrich	98%	97-53-0
	191	anethol	Sigma-Aldrich	99%	4180-23-8
	192	acetophenone	TCI	98.5%	98-86-2
	193	β -ionone	Sigma-Aldrich	96%	14901-07-6
	194	(+,-)-nicotine	Sigma-Aldrich	90%	22083-74-5
	195	(-)-nicotine	Sigma-Aldrich	99%	54-11-5
	196	cinnamaldehyde	TCI	98%	14371-10-9
	197	2-phenylethyl propionate	TCI	98%	122-70-3
	198	3-ethyltoluene	Maclin	98%	620-14-4
	199	benzonitrile	Sigma-Aldrich	99%	100-47-0
	200	3,4-dimethyl acetophenone	Sigma-Aldrich	98%	3637-01-2
	201	(Z)-3-hexenyl benzoate	Sigma-Aldrich	97%	25152-85-6

Aromatic	202	DEET	Sigma-Aldrich	99%	134-62-3
	203	dibutyl phthalate	Sigma-Aldrich	99%	95-48-7
	204	O-cresol	Fluka	98%	611-06-31
	205	2,4-dichloro-1-nitrobenzene	Fluka	97%	100-02-7
	206	4-nitrophenol	Fluka	97%	100-02-7
	207	benzyl benzoate	TCI	99%	120-51-4
	208	benzylamine	TCI	99%	100-46-9
Insect sex pheromones and analogs	209	Z11-16:Ald	Shin-Etsu	98%	53939-28-9
	210	Z9-16:Ald	Shin-Etsu	98%	56219-04-6
	211	Z9-14:Ald	Shin-Etsu	98%	53939-27-8
	212	Z7-16:Ald	Kunbo	92%	56797-40-1
	213	14:Ald	Ark Pham	96%	124-25-4
	214	16:Ald	TCI	98%	629-80-1
	215	18:Ald	TCI	98%	638-66-4
	216	Z11-16:OH	Shin-Etsu	98%	56683-54-6
	217	Z9-16:OH	Shin-Etsu	98%	10378-01-5
	218	Z9-14:OH	Kunbo	92%	35153-15-2
	219	Z11-16:OAc	Shin-Etsu	98%	34010-21-4
	220	Z9-16:OAc	Shin-Etsu	98%	34010-20-3
	221	Z9-14:OAc	Kunbo	96%	16725-53-4
	222	Z7-16:OAc	Kunbo	95%	23192-42-9
	223	Z9-E11-14:OAc	Kunbo	92%	50767-79-8
	224	Z9-E12-14:OAc	Kunbo	92%	51937-00-9
	225	14:OAc	Ark Pham	96%	638-59-5
	226	16:OAc	TRC	99.5%	620-70-9
	227	18:OAc	TCI	92%	822-23-1
	228	Z11-16:COOH	Abcam	98%	34010-21-4
	229	Z11-18:OAc	Cayman	98%	4273-95-4
	230	E11-16:OAc	Kunbo	92%	57491-33-5
	231	Z11-14:OAc	Kunbo	92%	20711-10-8
	232	E11-16:Ald	Kunbo	92%	57491-33-5
	233	Z7-12:OAc	Kunbo	92%	14959-86-5
	234	Z9-12:OAc	Kunbo	92%	16974-11-1
	235	Z11-14:Ald	Kunbo	92%	35237-64-0
	236	Z11-18:Ald	Kunbo	92%	56554-95-1
	237	E7-12:OAc	Kunbo	92%	16695-41-3

CAS: Chemical Abstracts Service; TMTT: (E,E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene; DMNT: (3E)-4,8-dimethyl-1,3,7-nonatriene; DEET: N,N-Diethyl-meta-toluamide; TRC: Toronto Research Chemicals INC; TCI: Tokyo Chemical Industry CO., LTD; BDH: The British Drug Houses LTD; Fluka: Fluka Chemika; Acros: Acros organics; Kunbo: Kunbo technology, Kunming, China; Shin-Etsu: Shin-Etsu Chemicals; Beijing: Beijing Chemicals.

Table S2. Primers for gene cloning and real-time quantitative RT-PCR.

	Forward primers	Reverse primers
Cloning		
<i>OR18</i>	5'- <u>ctcgag</u> c caaat ggcattgtccgatttaag-3'	5'- <u>tctag</u> a ctaat cttcgacttgccg-3'
<i>OR26</i>	5'- <u>ctcgag</u> c caaat gaattccaacaattcc-3'	5'- <u>tctag</u> atcatgccattgtcctcaac-3'
<i>OR30</i>	5'- <u>gaatt</u> c caaat gtctctcgagg tcaaac g-3'	5'- <u>tctag</u> atc atac attgaacgttctaag-3'
<i>OR47</i>	5'- <u>gaatt</u> c caaat ggagaaggatattttcc-3'	5'- <u>ctcgag</u> tcattcacgaagtgaagaaaac-3'
<i>OR56</i>	5'- <u>gaatt</u> c caaat ggagttgcagaaagtc-3'	5'- <u>ctagt</u> ctagattatcgtagcgatgctagaac-3'
<i>OR70</i>	5'- <u>gaatt</u> c caaat ggcatgcttttcg atc -3'	5'- <u>ctcgag</u> ttaacgcactgatgacagg-3'
qRT-PCR		
<i>OR18</i>	5'-ctgcaataaggttgacgcgt-3'	5'-cgcaacgatcgtataacccg-3'
<i>OR47</i>	5'-actgggaatcctggccaat-3'	5'-tgcctccaccgtacatcatt-3'
<i>Actin</i>	5'-ctaagctcaacgtaacggcc-3'	5'-cctccctcccttcgtttct-3'
DsRNA		
<i>OR18</i>	5'-atggcattgtccgatttaag-3'	5'-gaagcagaagtagcatattc-3'
<i>OR18T7</i>	5'- <u>taatac</u> gactcactataggatggcattgtccgatttaag-3'	
<i>OR47</i>	5'-atggagaaggatattttcc-3'	5'-cacgtataagtaacgatgcac-3'
<i>OR47T7</i>	5'- <u>taatac</u> gactcactataggatggagaaggatattttcc-3'	
<i>GFP</i>	5'-cgaggacaacaacatggc-3'	5'-cttcaatgtgtctaat-3'
<i>GFPT7</i>	5'- <u>taatac</u> gactcactataggcaggacaacaacatggc-3'	

The restriction enzyme sites and T7 promoter sequence are underlined. CAAC was used as the Kozak sequence.