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Supplementary Table 1 | Quantification of IR84a agonists present in food sources and *Drosophila* cuticle extracts. (± s.e.m.; n=6 for all extracts, except for prickly pear, where n=3). “N.D.”, not detected. “Clean” *Drosophila* extracts were those from flies cultured on minimal medium (see Methods).

Sample extract	Phenylacetic acid	Phenylacetaldehyde
banana	601 ± 229 ng/g	338 ± 50 ng/g
prickly pear	347 ± 52 ng/g	10 ± 1 ng/g
standard fly food	3958 ± 316 ng/g	208 ± 22 ng/g
male *D. melanogaster*	9.8 ± 0.3 ng/fly	0.14 ± 0.03 ng/fly
virgin female *D. melanogaster*	9.2 ± 0.3 ng/fly	0.16 ± 0.01 ng/fly
"clean" male *D. melanogaster*	N.D.	N.D.
"clean" virgin female *D. melanogaster*	N.D.	N.D.

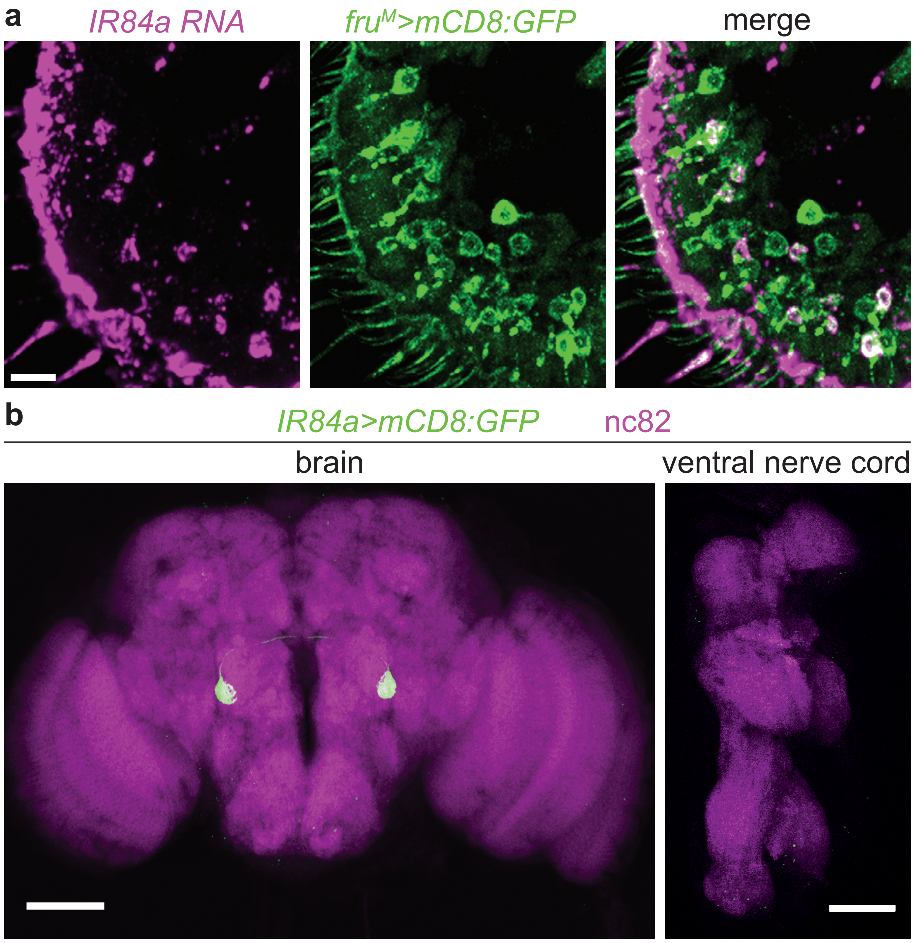
Supplementary Table 2 | Details of odours used in the screen of ac4 sensilla responses.

Odour name	CAS No.	Solvent	Purity
(-)-beta-citronellol	7540-51-4	paraffin oil	>98.5%
(-)-beta-pinene	18172-67-3	paraffin oil	≥99.0%
(-)-linalool	126-91-0	paraffin oil	>95%
(R)-(+)-limonene	5989-27-5	paraffin oil	97%
(S)-(-)-limonene	5989-54-8	paraffin oil	>95%
(S)-(+)-carvone	2244-16-8	paraffin oil	96%
(Z)-11-octadecenyl acetate	1775-43-5	paraffin oil	>99%
1,3-octenol	3391-86-4	paraffin oil	>98%
1,4-diaminobutane	110-60-1	water	>99%
1-methylcyclohexanol	590-67-0	paraffin oil	96%
2,3-butanediol	513-85-9	paraffin oil	98%
2,3-butanedione	431-03-8	paraffin oil	>99%
2-butanone	78-93-3	paraffin oil	>99%
2-ethylfenchol	18368-91-7	paraffin oil	>97%
2-ethylhexanoic acid	149-57-5	paraffin oil	>99%
2-heptanone	110-43-0	paraffin oil	99%
2-hexanol	626-93-7	paraffin oil	99%
2-hexanone	591-78-6	paraffin oil	98%
2-hexenal	6728-26-3	paraffin oil	>97%
2-methylphenol	95-48-7	paraffin oil	>99%
2-nonanone	821-55-6	paraffin oil	>99%
2-octanone	111-13-7	paraffin oil	98%
2-oxobutyric acid	600-18-0	water	≥97.0%
2-oxopentanoic acid	1821-02-9	paraffin oil	>97%
2-pentanol	6032-29-7	paraffin oil	>98%
2-pentanone	107-87-9	paraffin oil	99.50%
2-phenylethanol	60-12-8	paraffin oil	>98%
3-(methylthio)-1-propanol	505-10-2	paraffin oil	98%
3-acetylpyridine	350-03-8	water	≥99.5%
3-carene	13466-78-9	paraffin oil	90%
3-hexanol	623-37-0	paraffin oil	>98%
3-methyl-1-butanol	123-51-3	paraffin oil	98%
3-methylphenol	108-39-4	paraffin oil	≥99.0%
3-octanol	589-98-0	paraffin oil	>95%
3-octanone	106-68-3	paraffin oil	>97%
4-ethylguaiacol	2785-89-9	paraffin oil	>98%
4-methylcyclohexanol	589-91-3	paraffin oil	> 98%
4-methylphenol	106-44-5	paraffin oil	99%
6-methyl-5-hepten-2-one	110-93-0	paraffin oil	>96%
acetaldehyde	75-07-0	water	>99%
acetic acid	64-19-7	water	>99.5%
acetoin	513-86-0	water	>92%
acetone	67-64-1	paraffin oil	>99.5%
acetophenone	98-86-2	paraffin oil	99%
alpha-terpinene	99-86-5	paraffin oil	>89%
ammonium hydroxide	1336-21-6	water	28-30% as NH_3

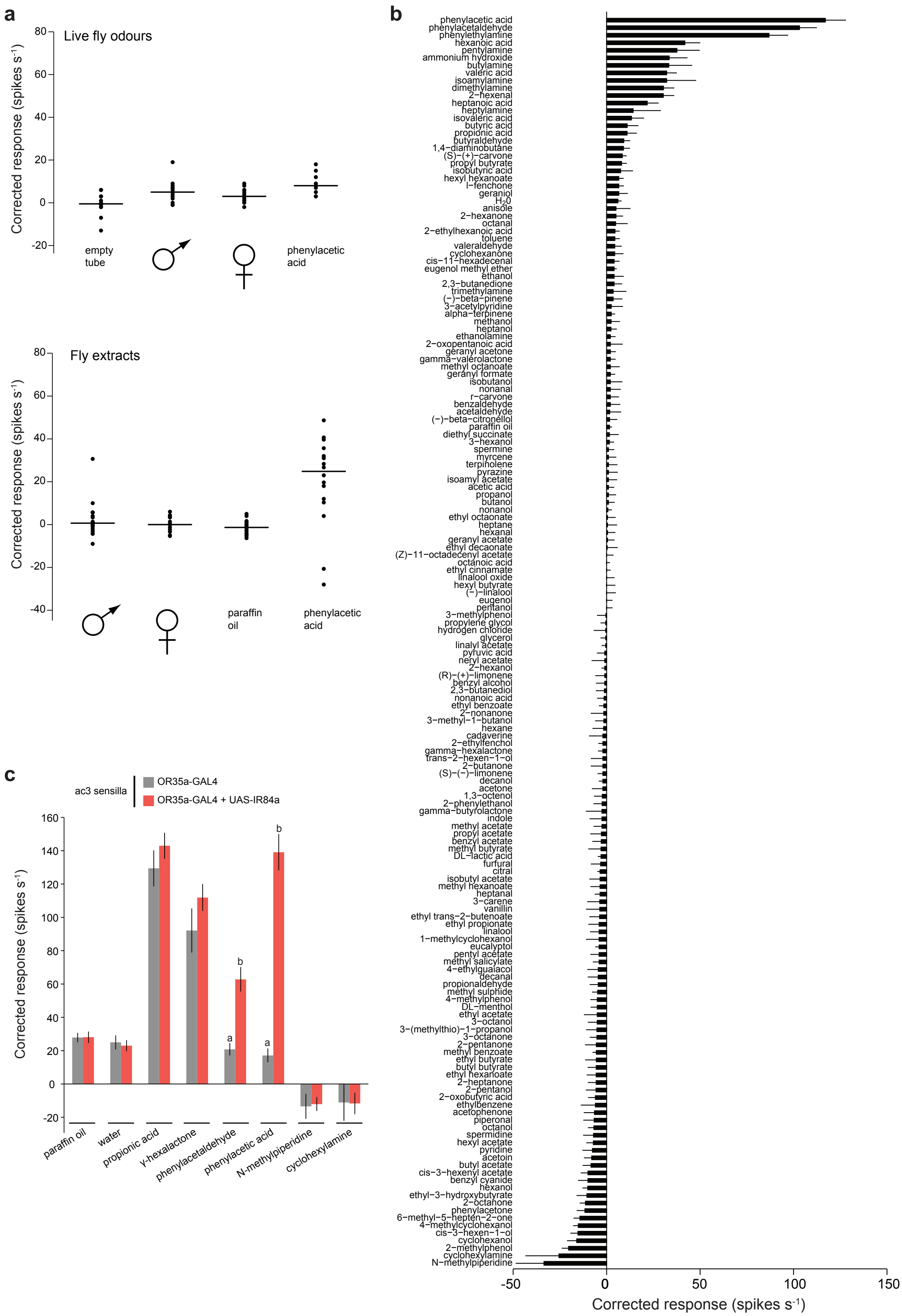
Odour name	CAS No.	Solvent	Purity
anisole	100-66-3	paraffin oil	99%
benzaldehyde	100-52-7	paraffin oil	>99%
benzyl acetate	140-11-4	paraffin oil	>99%
benzyl alcohol	100-51-6	paraffin oil	>99%
benzyl cyanide	140-29-4	paraffin oil	>98%
butanol	71-36-3	paraffin oil	>99%
butyl acetate	123-86-4	paraffin oil	>99.7%
butyl butyrate	109-21-7	paraffin oil	>99%
butylamine	109-73-9	paraffin oil	99.5%
butyraldehyde	123-72-8	paraffin oil	>98%
butyric acid	107-92-6	water	>99%
cadaverine	462-94-2	water	≥97.0%
cis-11-hexadecenal	53939-28-9	paraffin oil	≥95.0%
neryl acetate	141-12-8	paraffin oil	98%
cis-3-hexen-1-ol	928-96-1	paraffin oil	98%
cis-3-hexenyl acetate	3681-71-8	paraffin oil	>98%
citral	5392-40-5	paraffin oil	>96%
cyclohexanol	108-93-0	paraffin oil	>99%
cyclohexanone	108-94-1	paraffin oil	>99%
cyclohexylamine	108-91-8	water	≥99%
decanal	112-31-2	paraffin oil	>98%
decanol	112-30-1	paraffin oil	>99%
diethyl succinate	123-25-1	paraffin oil	>99%
dimethylamine	124-40-3	water	33% in absolute ethanol
DL-lactic acid	598-82-3	water	~90%
DL-menthol	89-78-1	propylene glycol	NA
ethanol	64-17-5	water	200 proof (absolute)
ethanolamine	141-43-5	water	≥99.5%
ethyl acetate	141-78-6	paraffin oil	>99.5%
ethyl benzoate	93-89-0	paraffin oil	>99%
ethyl butyrate	105-54-4	paraffin oil	99%
ethyl cinnamate	103-36-6	paraffin oil	>98%
ethyl decaonate	110-38-3	paraffin oil	>99%
ethyl hexanoate	123-66-0	paraffin oil	>99%
ethyl octaonate	106-32-1	paraffin oil	>98%
ethyl propionate	105-37-3	paraffin oil	99%
ethyl trans-2-butenoate	623-70-1	paraffin oil	>96%
ethyl-3-hydroxybutyrate	5405-41-4	paraffin oil	>98%
ethylbenzene	100-41-4	paraffin oil	>99%
eucalyptol	470-82-6	paraffin oil	99%
eugenol	97-53-0	paraffin oil	>99%
eugenol methyl ether	93-15-2	paraffin oil	>96%
furfural	98-01-1	paraffin oil	99%
gamma-butyrolactone	86-48-0	paraffin oil	>99%
gamma-hexalactone	695-06-7	paraffin oil	>98%
gamma-valerolactone	108-29-2	paraffin oil	99%
geraniol	106-24-1	paraffin oil	98%
geranyl acetate	105-87-3	paraffin oil	98%
geranyl acetone	3796-70-1	paraffin oil	≥98.0%
geranyl formate	105-86-2	paraffin oil	NA

Odour name	CAS No.	Solvent	Purity
glycerol	56-81-5	paraffin oil	>99%
heptanal	111-71-7	paraffin oil	>95%
heptane	142-82-5	paraffin oil	>99%
heptanoic acid	111-14-8	paraffin oil	>99%
heptanol	111-70-6	paraffin oil	>99%
heptylamine	111-68-2	paraffin oil	>98%
hexanal	66-25-1	paraffin oil	98%
hexane	110-54-3	paraffin oil	95%
hexanoic acid	142-62-1	water	>99.5%
hexanol	111-27-3	paraffin oil	>99%
hexyl acetate	142-92-7	paraffin oil	>98.5%
hexyl butyrate	2639-63-6	paraffin oil	>98%
hexyl hexanoate	6378-65-0	paraffin oil	>97%
hydrogen chloride	7647-01-0	water	37%
indole	120-72-9	paraffin oil	2000 µg/ml in methanol
isoamyl acetate	123-92-2	paraffin oil	98%
isoamylamine	107-85-7	water	99%
isobutanol	78-83-1	paraffin oil	>99%
isobutyl acetate	109-19-0	paraffin oil	99%
isobutyric acid	79-31-2	water	>99.5%
isovaleric acid	503-74-2	water	>98.5%
l-fenchone	7787-20-4	paraffin oil	>98%
linalool	78-70-6	paraffin oil	>95%
linalool oxide	60047-17-8	paraffin oil	>97%
linalyl acetate	115-95-7	paraffin oil	>95%
methanol	67-56-1	water	>99.9%
methyl acetate	79-20-9	paraffin oil	99.50%
methyl benzoate	93-58-3	paraffin oil	99%
methyl butyrate	623-42-7	paraffin oil	>99%
methyl hexanoate	106-70-7	paraffin oil	>99%
methyl octanoate	111-11-5	paraffin oil	99%
methyl salicylate	119-36-8	paraffin oil	>99%
methyl sulphide	75-18-3	paraffin oil	>99%
myrcene	123-35-3	paraffin oil	~90%
N-methylpiperidine	626-67-5	water	99%
nonanal	124-19-6	paraffin oil	>95%
nonanoic acid	112-05-0	paraffin oil	>97%
nonanol	143-08-8	paraffin oil	98%
octanal	124-13-0	paraffin oil	99%
octanoic acid	124-07-2	paraffin oil	>99%
octanol	111-87-5	paraffin oil	>99.5%
pentanol	71-41-0	paraffin oil	>99%
pentyl acetate	628-63-7	paraffin oil	99%
pentylamine	110-58-7	paraffin oil	≥98.5%
phenylacetaldehyde	122-78-1	paraffin oil	>90%
phenylacetic acid	103-82-2	water	99%
phenylacetone	103-79-7	paraffin oil	99%
phenylethylamine	64-04-0	paraffin oil	≥99.5%
piperonal	120-57-0	paraffin oil	>99%
propanol	71-23-8	paraffin oil	>99.5%

Odour name	CAS No.	Solvent	Purity
propionaldehyde	123-38-6	paraffin oil	97%
propionic acid	79-09-4	water	99%
propyl acetate	109-60-4	paraffin oil	>96%
propyl butyrate	105-66-8	paraffin oil	99%
pyrazine	290-37-9	water	≥99%
pyridine	110-86-1	paraffin oil	>99.8%
pyruvic acid	127-17-3	water	>98%
r-carvone	6485-40-1	paraffin oil	98%
spermidine	124-20-9	water	≥99.5%
spermine	71-44-3	water	≥96%
terpinolene	586-62-9	paraffin oil	>85%
toluene	108-88-3	paraffin oil	99.90%
trans-2-hexen-1-ol	928-95-0	paraffin oil	96%
trimethylamine	75-50-3	water	~45 wt. % in water
valeraldehyde	110-62-3	paraffin oil	>97%
valeric acid	109-52-4	water	>99%
vanillin	121-33-5	propylene glycol	99%

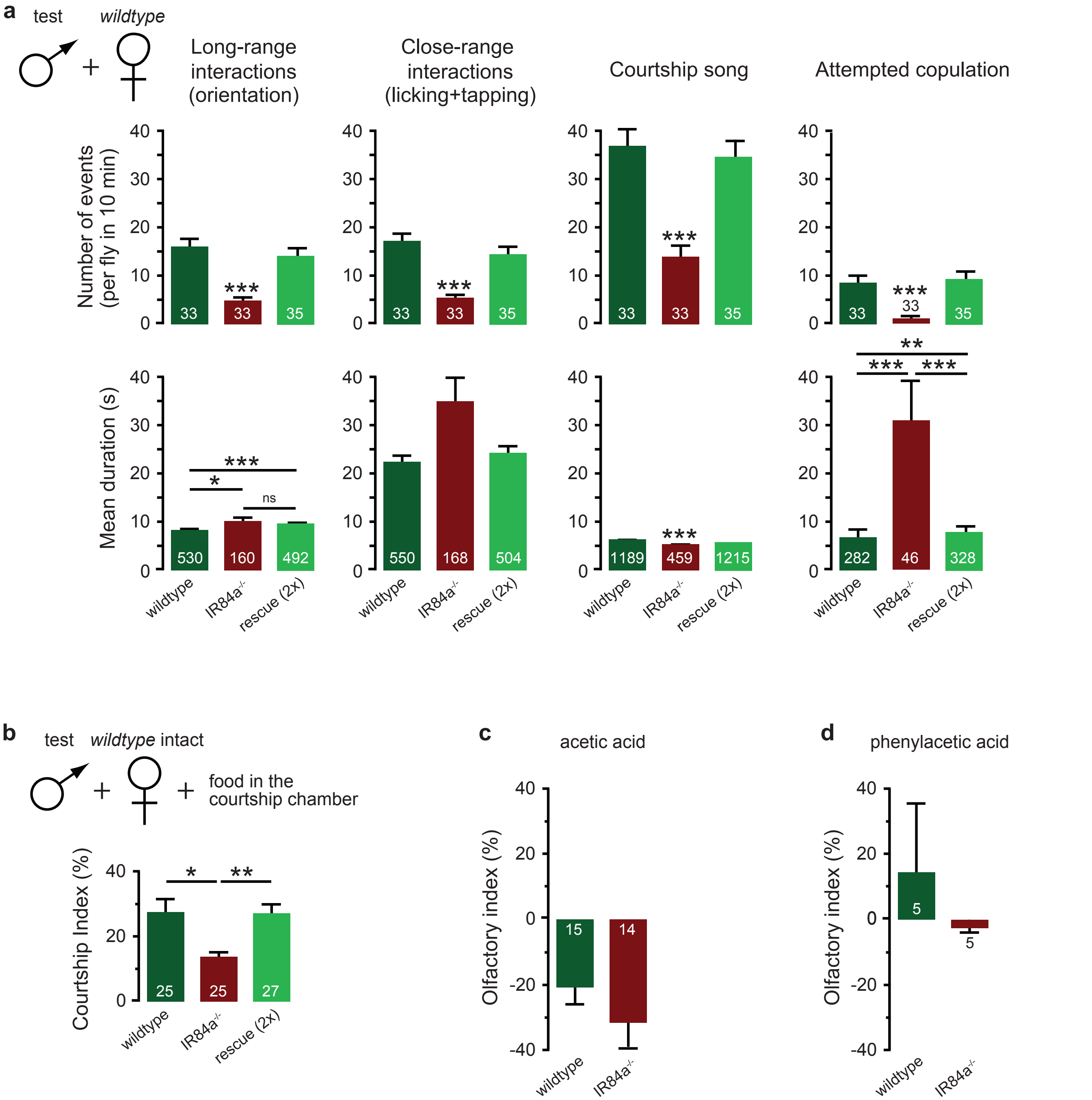


Supplementary Figure 1 | *Ir84a* is expressed only in *fru*^{M} positive olfactory sensory neurons innervating the VL2a glomerulus.
a, Combined RNA fluorescent *in situ* hybridization (FISH) using an *Ir84a* RNA probe (magenta) and anti-GFP immunofluorescence (green) on antennal sections expressing a *UAS-mCD8:GFP* reporter under the control of the *fru*^{GAL4} driver[5]. The scale bar represents 10 μm. The *fru*^{GAL4} driver labels many *Ir84a*-negative neurons, which presumably corresponding to those expressing *Or67d* and *Or47b*[18,20]. Conversely, we found that the *fru*^{M} reporter is not detectable in all *Ir84a*-positive cells; while we cannot exclude the possibility that these represent functionally-distinct populations of IR84a neurons, variation in *Ir84a/fru*^{M} overlap in analyses with two independently-generated *fru*^{M} reporters[4,5] suggests this may in part be due to incomplete labelling of *fru*^{M} neurons in adult flies by these reporter transgenes (*fru*^{M} reporter-positive *Ir84a*-expressing neurons: males 49% (n=96), females 91% (n=78)[4]; males 51% (n=37), females 57% (n=21)[5]. **b**, Immunofluorescence using anti-GFP (green) and neuropil marker nc82 (magenta) antibodies on a whole mount brain (left) and ventral nerve cord (right) from *UAS-mCD8:GFP/UAS-mCD8:GFP;Ir84a*^{GAL4}*/Ir84a*^{GAL4} animals. The scale bars represent 70 μm.

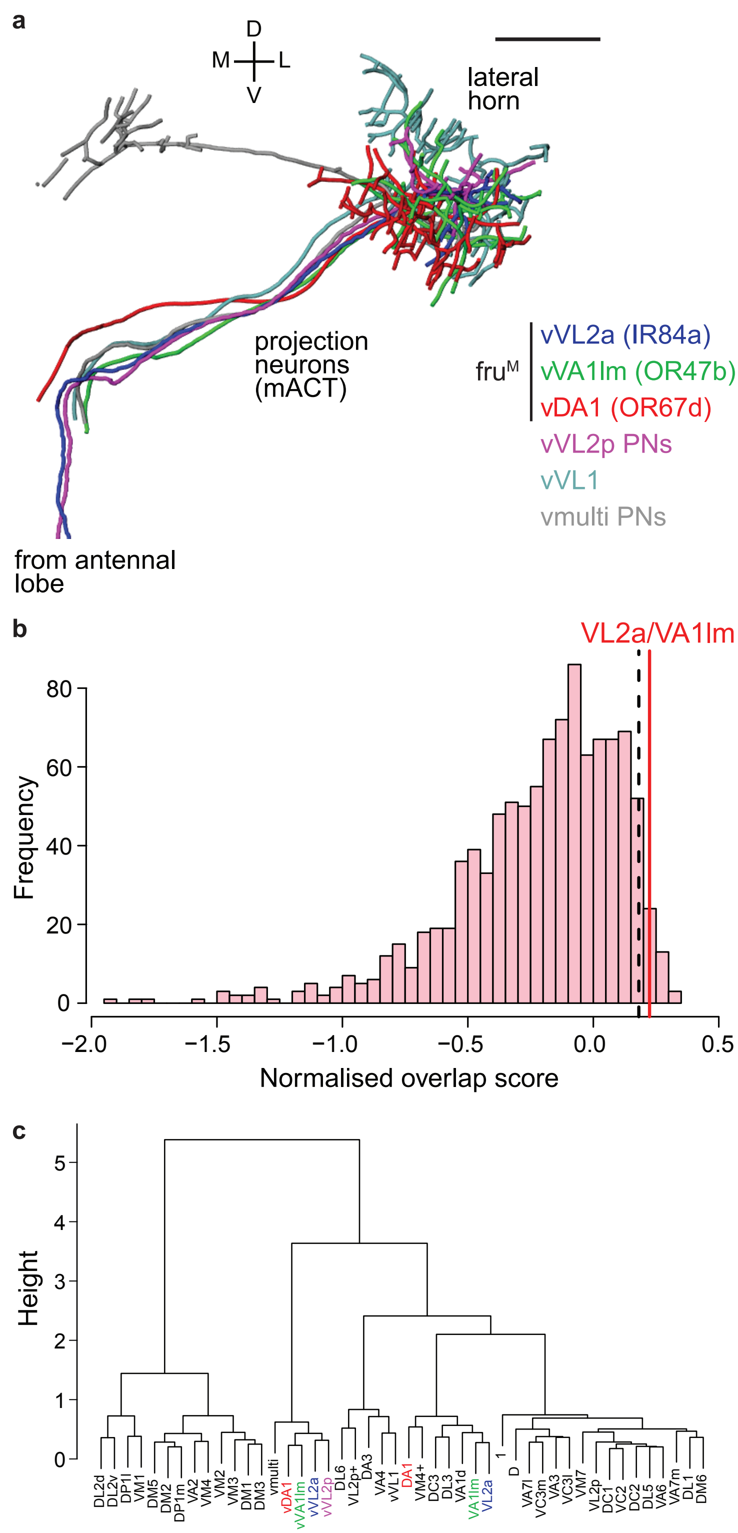


Supplementary Figure 2 | Electrophysiological characterisation of IR84a neurons.

a, Top: Responses of ac4 sensilla to odours of living flies. Approximately 100-120 male or virgin female flies or 10 µl phenylacetic acid (0.1 µg/µl in paraffin oil) were placed in a glass pipette through which an airstream was blown. An empty pipette was used as a negative control. Horizontal bars represent the median (n=10-13). The responses to male odour, female odour and phenylacetic acid were extremely small (median ≤ 8 spikes/s), although significantly different from zero (two sided t-test, $p<0.05$, Bonferroni corrected). Bottom: Responses of ac4 sensilla to cuticular extracts from virgin female and male flies (see Methods), paraffin oil or phenylacetic acid (0.1 μg/μl in paraffin oil). For these recordings, 1 μl of odour solution on the tip of a glass capillary was brought close (~5 mm) to the antenna of a fly. Horizontal bars represent the median (n=13-18). Only the response to phenylacetic acid is significantly different from zero (two sided t-test, $p<0.05$, Bonferroni corrected). **b**, Odour ligand screen in ac4 sensilla: mean evoked responses of ac4 sensilla neurons (representing the summed activities of the IR84a, IR75d and IR76a+IR76b neurons) to a panel of 163 odours (± s.e.m.; n≥4, mixed genders), and paraffin oil and water solvent controls. **c**, Mis-expression of IR84a is sufficient to confer phenylacetic acid and phenylacetaldehyde responsiveness on ac3 sensilla OR35a neurons. Mean evoked responses of ac3 neurons (± s.e.m.; n=18, male flies) to the indicated odour stimuli (10 μl phenylacetic acid (10 μg/μl) or 10 μl 1% v/v for other odours). Propionic acid and γ-hexlactone are control diagnostic odours that activate native ac3 sensilla neurons[13]. N-methylpiperidine and cyclohexylamine appear to be general antagonists of ac3 and ac4 (see **b**) neurons. For responses of the two different genotypes to the same odour stimulus, bars labelled with different letters are significantly different (ANOVA, $p<0.0001$). Genotypes: *Or35a-GAL4/+* (grey bars) and *UAS-Ir84a/+; Or35a-GAL4/+* (red bars).

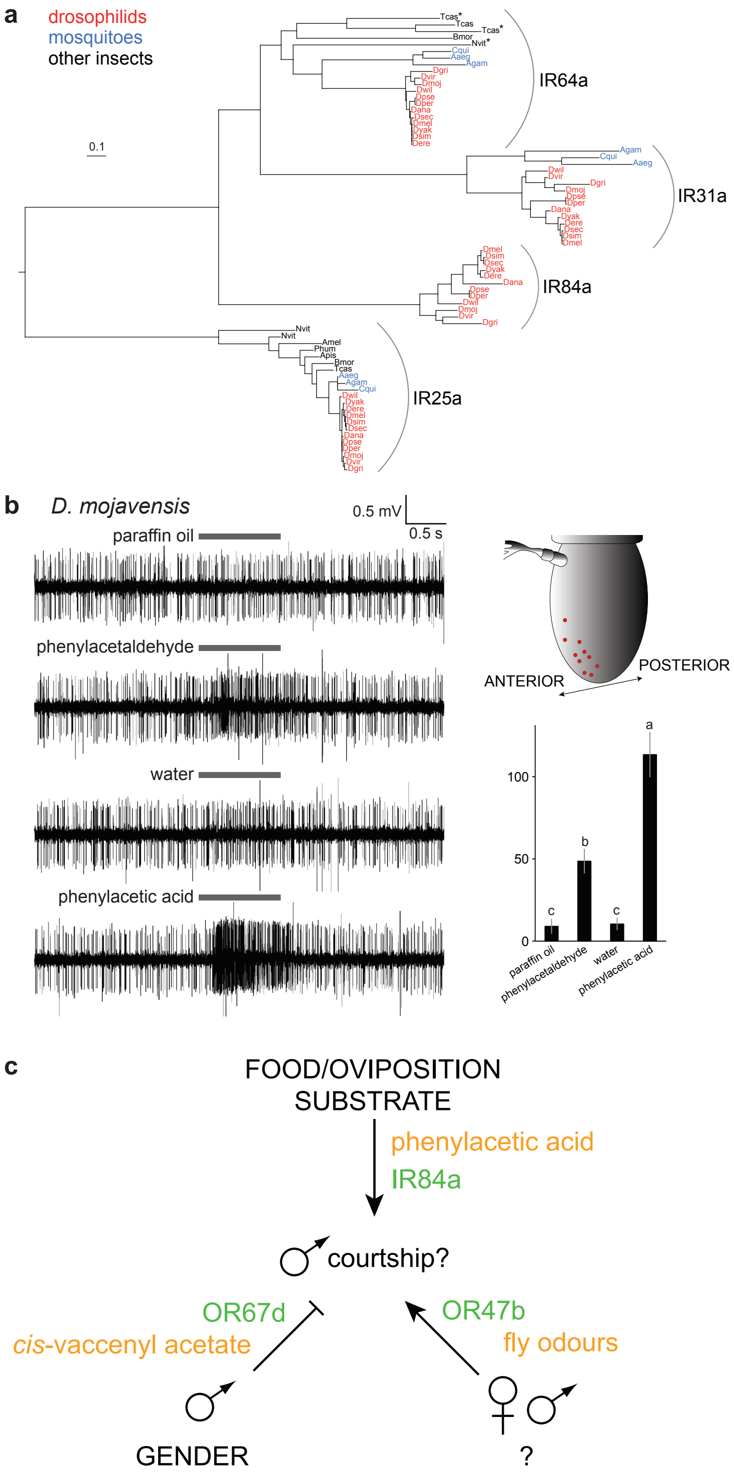


Supplementary Figure 3 | Behavioural characterisation of *Ir84a* mutant flies. **a**, Quantification of the frequency (top histograms) and duration (bottom histograms) of individual components of the courtship routine for heterosexual courtship by wildtype (w^{1118}), IR84a$^{-/-}$ mutant (*Ir84a*^{GAL4}/*Ir84a*^{GAL4}) and IR84a rescue (*UAS-Ir84a/UAS-Ir84a;Ir84a*^{GAL4}/*Ir84a*^{GAL4}) males of decapitated virgin wildtype females, for the data in Figure 3a (± s.e.m.; n is shown in the corresponding bar in this and other histograms): long-range interactions correspond to the orientation step; close-range interactions are those occurring between flies separated by less than a head diameter and encompass licking and tapping behaviours; courtship song reflects periods of unilateral wing extension; attempted copulations. Statistical analysis was performed using a one-way ANOVA (Kruskal-Wallis, P<0.0001 for all data sets except for close range mean durations (P=0.0795) and courtship song duration (P=0.0052)) followed by Dunn's multiple comparison post-hoc test where all genotypes are individually compared to each other (* p<0.05, ** p<0.01, and *** p<0.001). **b**, Mean courtship indices for male flies of the indicated genotypes paired with wildtype virgin females in the presence of food (see Methods) (± s.e.m.). Statistical analysis was performed using a one-way ANOVA (Kruskal-Wallis P=0.0042) followed by Dunn's multiple comparison post-hoc test as in **a**. **c-d**, Olfactory responses of male flies of the indicated genotypes to **c**, acetic acid or **d**, phenylacetic acid, in a Y-maze assay (see Methods). Negative index indicates avoidance behaviour. Statistical analysis was performed using Mann Whitney t-test giving no statistical difference between genotypes.



Supplementary Figure 4 | Anatomical analysis of VL2a (IR84a) projection neurons.

a, Three-dimensional rendering of registered axonal projections of the minor class of GABAergic "vPNs", which have ventral cell bodies and axons that travel directly to the lateral horn via the middle antennocerebral tract (mACT)[22]. Five uniglomerular vPN classes have been defined[9,22]; notably, these include the three *fru*^{M} glomeruli, VL2a (IR84a), VA1lm (OR47b) and DA1 (OR67d) – as well as two non-*fru*^{M} glomeruli (VL2p and VL1) and certain multiglomerular PNs ("vmulti PNs"). vVL2a and vVL2p vPNs have very similar axon projections, and these interdigitate with vVA1lm and vDA1 in the anterior-ventral lateral horn. D = dorsal, V = ventral, M = medial, L = lateral; the scale bar represents 25 µm. **b**, Histogram of median overlap scores (see Methods) between the lateral horn axon terminal fields of all pairwise comparisons of all 46 classes of mapped PNs (including vPNs). The 95% percentile is marked by a dotted black line and the actual value for VL2a and VA1lm overlap is marked by a red line. **c**, Cluster analysis of all 46 PN classes, confirming that VL2a (IR84a) and VA1lm (OR47b) co-cluster. vDA1/vVA1lm and vVL2a/vVL2p vPN classes also co-cluster (coloured as in **a**).



Supplementary Figure 5 | Drosophilid-specific evolution of *Ir84a*: a mechanism to couple food substrate recognition and male courtship.
a, Phylogenetic tree of selected antennal-expressed IR orthologous groups rooted using *Ir25a* (see Methods). The scale bar indicates the number of substitutions per site. Putative pseudogenes are marked with asterisks. Drosophilid species are highlighted in red and mosquitoes in blue. Species

abbreviations: *Tribolium castaneum* (Tcas), *Apis mellifera* (Amel), *Nasonia vitripennis* (Nvit), *Bombyx mori* (Bmor), *Anopheles gambiae* (Agam), *Aedes aegypti* (Aaeg), *Culex quinquefasciatus* (Cqui), *D. melanogaster* (Dmel), *D. simulans* (Dsim), *D. sechellia* (Dsec), *D. erecta* (Dere), *D. yakuba* (Dyak), *D. ananassae* (Dana), *D. pseudoobscura* (Dpse), *D. persimilis* (Dper), *D. willistoni* (Dwil), *D. virilis* (Dvir), *D. mojavensis* (Dmoj), *D. grimshawi* (Dgri). **b**, Left: Representative traces of recordings from coeloconic sensilla on *D. mojavensis* antennae containing a neuron responsive to 10 µl solvent, phenylacetaldehyde (1% v/v) or phenylacetic acid (10 µg/µl). Grey bars above traces mark the stimulus time (1 s). Top right: Schematic of the topological distribution (red dots) of these sensilla mapped on the anterior antennal surface. Bottom right: Quantification of mean responses (± s.e.m.; n=10, mixed genders) to the indicated stimuli. Bars labelled with different letters are significantly different (ANOVA, p<0.0001). **c**, Model for the role of volatile ligands and receptors in regulating male courtship in drosophilids. While *Or47b* mutants do not display defects in male courtship in the presence of food and light[20], genetic perturbation of their synaptic function affects female localisation in a larger assay arena in the dark[19], suggesting that this receptor has a redundant courtship-promoting function.