

DEET Olfactory Reception in the Yellow Fever Mosquito

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Abstract

Developed almost 80 years ago, DEET is the first line of defense against the transmission of vector-borne diseases, and yet its mode of action is still a matter of controversy. Here we identified a dedicated odorant receptor that underlies DEET's detection as a spatial repellency for the yellow fever mosquito *Aedes aegypti*. Using heterologous expression systems, we show that this receptor is finely tuned to DEET. CRISPR-mediated disruption impaired DEET-evoked neural activity in a defined olfactory sensillum and markedly reduced spatial avoidance at behaviorally relevant concentrations. Fluorescence-guided single-sensillum recordings revealed that DEET selectively activates a distinct olfactory sensory neuron population that projects to a single, defined glomerulus in the antennal lobe, establishing a direct receptor-to-circuit link for repellency coding. These findings uncover a dedicated sensory pathway for volatile repellent detection, revealing an unexpected specialization for a manmade chemical and providing a mechanistic framework for receptor-guided development of next-generation spatial repellents.

Keywords: *Aedes aegypti*; DEET; Odorant Receptor, *AaegOr54*; Olfaction; Spatial Repellency

45

46 **Introduction**

47 Spatial repellents are a cornerstone of personal protection against insect-borne
48 diseases, yet how volatile repellents are detected by the nervous system of the yellow
49 fever mosquito, *Aedes aegypti*, and translated into avoidance behavior remains a
50 matter of intense debate. In insects, host-seeking and avoidance behaviors are
51 mediated primarily by the olfactory system, in which chemical cues are detected by
52 three major families of ligand-gated ion channels¹, odorant receptors (Ors), ionotropic
53 receptors (Irs), and gustatory receptors (Grs), expressed in peripheral sensory
54 appendages²⁻⁵. Ors function as heteromeric complexes composed of a highly
55 conserved co-receptor (Orco) and a ligand-selective tuning receptor (Or), with Or
56 subunits conferring odor specificity and Orco acting as a non-ligand-binding scaffold
57 ^{6,7}. Identifying the specific tuning receptors that encode ecologically relevant cues is
58 therefore essential for understanding how olfactory systems drive adaptive behavior.
59 N,N-diethyl-meta-toluamide (DEET) is the most widely used insect repellent
60 worldwide and has remained the gold standard for more than 70 years^{8,9}.

61

62 Formulations containing 10–35% DEET provide substantial and durable protection,
63 with efficacy increasing and plateauing near 50% DEET^{10,11}. Despite occasional
64 adverse reactions¹²⁻¹⁴, DEET continues to be recommended by public health agencies
65 as a primary tool for preventing vector-borne disease¹⁵⁻¹⁷. Despite its widespread use,
66 the molecular basis of DEET's volatile (spatial) repellency remains unresolved.
67 Competing hypotheses propose that DEET may directly activate specific odorant
68 receptors, suppress host-odor detection, and/or disrupt the spatial structure of human
69 odor plumes¹⁸⁻²⁰. Volatile DEET elicits antennal responses in some mosquito lineages
70 but not others²¹⁻²³, highlighting fundamental differences in chemosensory physiology.
71 In *Culex quinquefasciatus*, the odorant receptor *CquiOr136* responds to DEET²⁴;
72 however, this receptor is restricted to the *Culex* lineage and is absent in *Aedes*. Thus,
73 the molecular receptor responsible for volatile DEET detection in *Aedes aegypti* has
74 remained unknown. Moreover, *Aedes* Orco mutants lack antennal responses to
75 volatile DEET²², implicating the Or pathway while leaving the identity of the tuning
76 receptor unresolved. This gap has limited the mechanistic understanding of spatial
77 repellency and constrained rational repellent design.

78

79 Here, we identified the molecular basis of volatile DEET detection in the mosquito
80 *Aedes aegypti*, a globally invasive species and major vector of dengue, Zika,
81 chikungunya, and yellow fever viruses²⁵. Using single-sensillum recordings, ultra-
82 low-input RNA transcriptomics, CRISPR-mediated gene disruption, and behavioral
83 assays, we mapped DEET-sensitive olfactory sensory neurons to a defined trichoid
84 sensillum subtype and identify the tuning receptor they express, *AaegOr54*. This
85 receptor defines a dedicated DEET-sensing pathway that links peripheral detection to
86 a single antennal-lobe glomerulus and to spatial avoidance behavior. Together, these
87 findings resolve a long-standing question in insect chemosensation and establish a

88 receptor-to-circuit framework for receptor-guided development of next-generation
89 spatial repellents.

90

91 **Results**

92

93 **Identification of DEET-sensitive OSN in *Ae. aegypti***

94

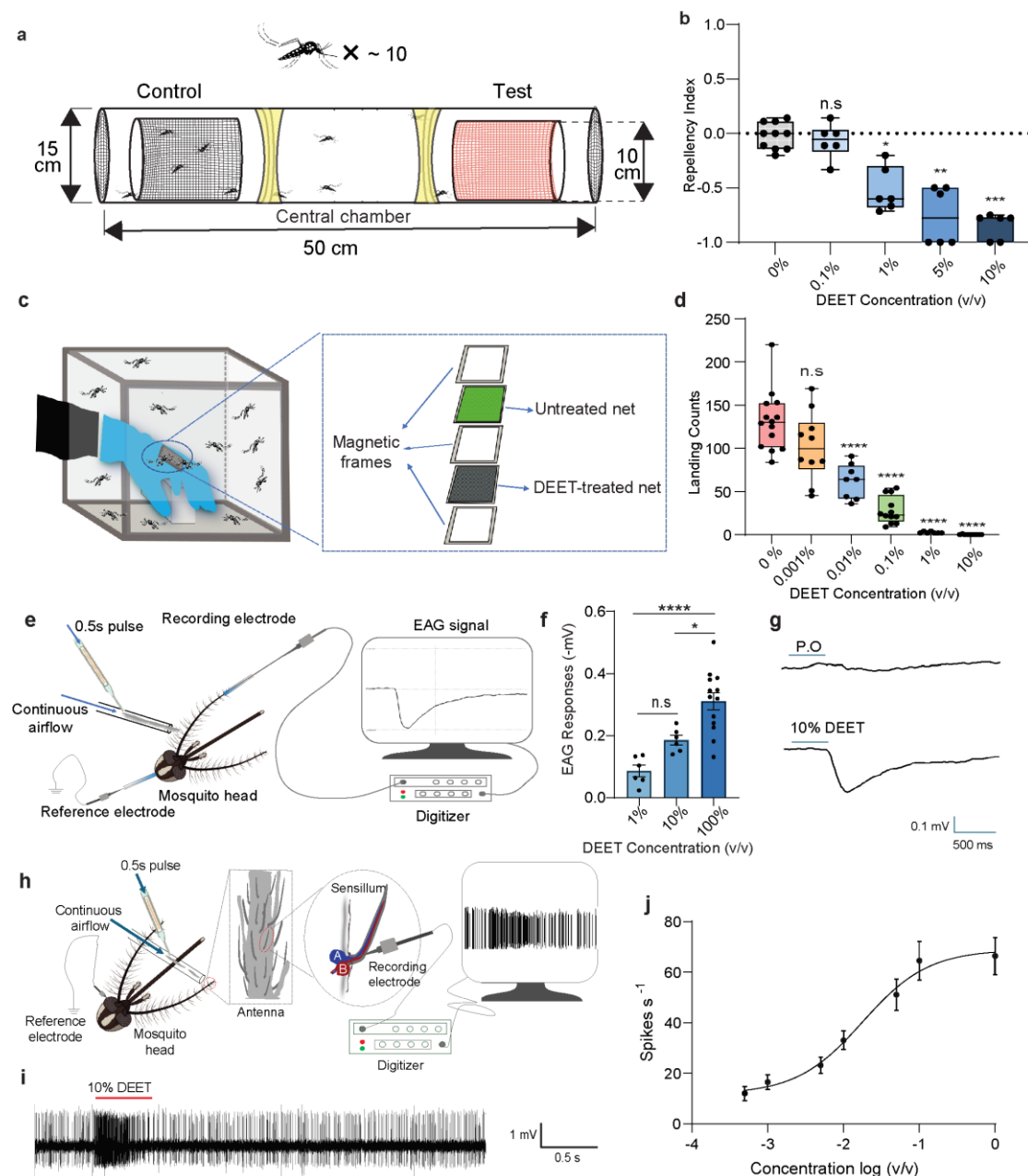
95 To evaluate the spatial repellency of DEET, we first performed the WHO-
96 recommended assay²⁶ (Fig. 1a). Low concentration of DEET (0.1%) elicited minimal
97 repellency, whereas concentrations $\geq 1\%$ produced robust and dose-dependent
98 avoidance (Fig. 1b). We next assessed behavioral repellency in the presence of human
99 odor cues using the hand-in-cage assay (Fig. 1c). Quantification of mosquito landing
100 events revealed a strong dose-dependent inhibition, with higher DEET concentrations
101 producing near-complete suppression of landings (Fig. 1d). At concentrations above
102 1%, DEET eliminated $>99\%$ of landing events (Extended Data Fig. S1a), confirming
103 potent spatial repellency in *Ae. aegypti*.

104

105 To determine whether mosquito antenna detected volatile DEET, we recorded
106 antennal responses using electroantennography (EAG) (Fig. 1e). DEET elicited clear,
107 concentration-dependent EAG signals, with higher doses producing significantly
108 stronger responses (Fig. 1f-1g). These findings indicated that DEET is detected by the
109 olfactory sensilla on the mosquito antenna, which comprise several morphologically
110 distinct types, such as trichoid, basiconic, and ceoloconic sensilla²⁷. Because previous
111 studies suggested that trichoid sensilla were preferentially activated by DEET^{23,28-30},
112 we systematically screened trichoid sensilla across all antennal flagella to identify
113 DEET-sensitive neurons using single sensillum recording (SSR) (Fig. 1h).

114

115 Across >200 individual recordings spanning all 13 antennal flagellomeres, we
116 identified a DEET-sensitive short sharp-tipped (SST) trichoid sensillum, which
117 contains two olfactory sensory neurons (OSNs) distinguished by spike amplitude (Fig.
118 1i and Extended Data Fig. S1b). Spike-sorting analysis demonstrated that DEET
119 exclusively activated the larger-amplitude “A” neuron. Notably, the DEET-sensitive
120 SST sensilla were restricted to the distal four flagellomeres (10th-13th) and none were
121 detected on proximal segments (1st -9th), indicating a spatially biased distribution
122 along the antenna. A subsequent survey of SST sensilla within the distal flagellomeres
123 using a panel of 28 odorants spanning multiple chemical classes revealed that SST
124 sensilla can be classified into five functional subtypes, of which one subtype (SST-1)
125 exhibited strong and dose-dependent activation by DEET (Fig. 1j and Extended Data
126 Fig. S1c-d, S2). Together, these results demonstrate that DEET is detected by a
127 dedicated OSN, the “A” neuron of the SST-1 trichoid sensillum, located specifically
128 within the distal portion of the *Ae. aegypti* antenna.



129

130 **Fig. 1. DEET elicits robust spatial repellency and activates antennal olfactory**
 131 **neurons in *Ae. aegypti*.** **a**, Schematic of the uniport olfactometer used to measure
 132 non-contact spatial repellency. The inner cylinders were covered with nylon treated
 133 with solvent or varying concentrations of DEET to facilitate odor release. **b**, Dose-
 134 dependent DEET repellency index in wild-type (WT) mosquitoes ($n = 6-10$; Kruskal-
 135 Wallis test followed by Dunn's multiple comparisons; ns, not significant; $*0.01 < P <$
 136 0.05 ; $**0.001 < P < 0.01$; $***0.0001 < P < 0.001$). **c**, Schematic of the hand-in-cage
 137 assay. **d**, Effect of DEET concentration on mosquito landing counts ($n = 8-14$;
 138 Kruskal-Wallis test with Dunn's multiple comparisons; ns, not significant; $****P <$
 139 0.0001). **e**, Schematic of electroantennogram (EAG) setup. **f**, Concentration-
 140 dependent EAG responses to DEET ($n = 6-12$; Kruskal-Wallis test with Dunn's
 141 multiple comparisons; ns, not significant; $****P < 0.0001$). **g**, Representative EAG
 142 traces from WT antennae stimulated with solvent (paraffin oil, P.O.) and DEET

143 (10%). **h**, Schematic of the single sensillum recording (SSR) setup. **i**, Representative
144 SSR traces from an SST-1 sensillum showing strong activation of the “A” neuron by
145 DEET (10^{-1} v/v). **j**, Dose-dependent SSR responses to DEET in WT mosquitoes (n =
146 10-12).

147

148 **Identification of odorant receptors expressed in the DEET-sensitive neurons**

149

150 Previous studies demonstrated that DEET reception in mosquitoes is mediated by the
151 Or/Orco complex³¹, and loss of the *Orco* gene abolishes DEET-evoked behavioral
152 repellency in *Ae. aegypti*²². We therefore sought to identify the specific odorant
153 receptor(s) expressed in the DEET-sensitive SST-1 “A” neurons of *Ae. aegypti*. After
154 confirming that DEET-responsive SST-1 sensilla are restricted to the distal four
155 flagellomeres (10th–13th) of the antenna (Fig. 2a), we microdissected individual
156 antennae across 3 biological replicates into proximal (1st–9th) and distal (10th–13th)
157 regions for comparative transcriptomic profiling using ultra-low-input RNA
158 sequencing, a method capable of detecting transcripts from only a few antennal
159 flagellomeres.

160

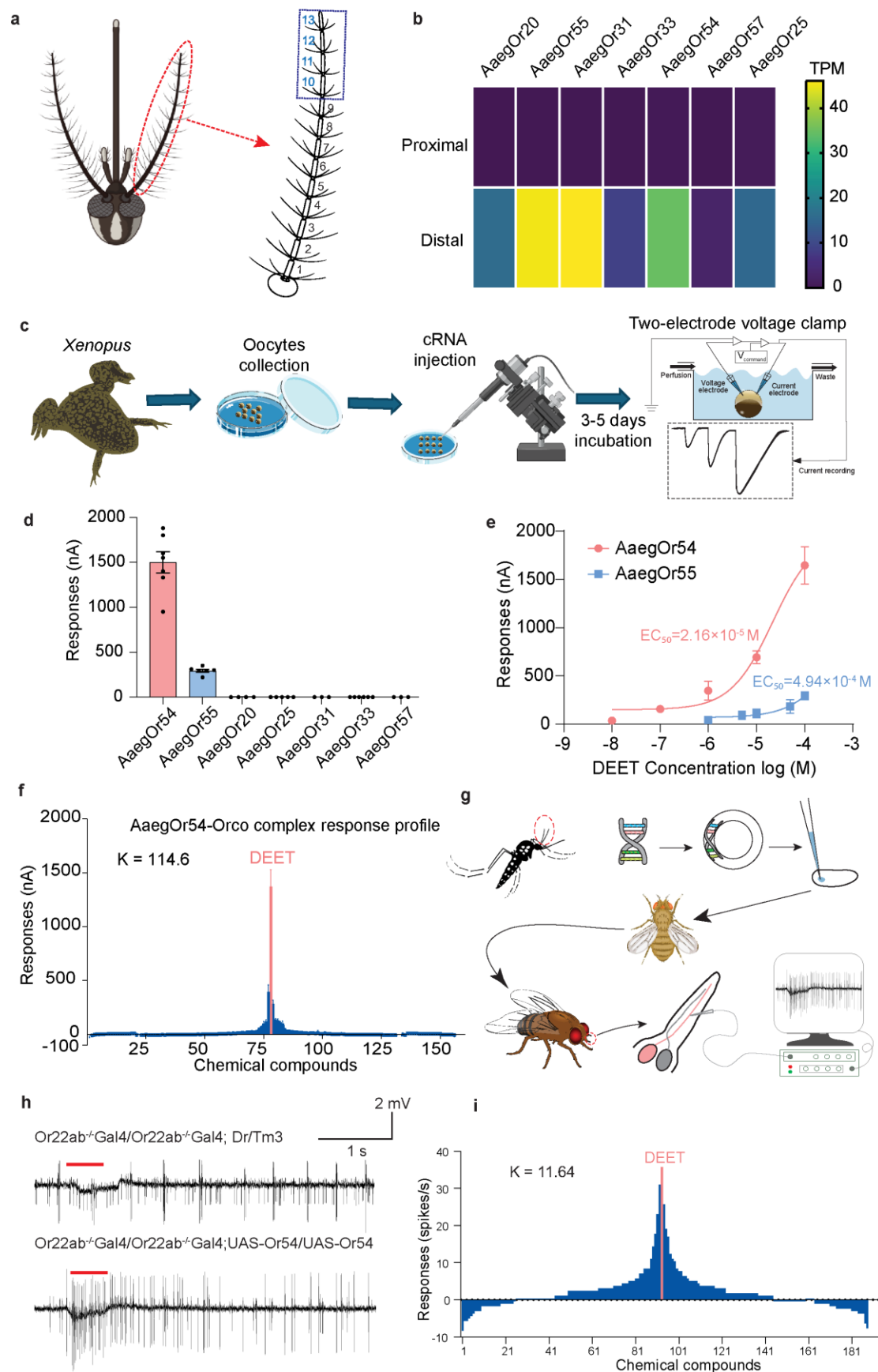
161 Because electrophysiological mapping localized DEET-responsive OSNs exclusively
162 to the distal region, we reasoned that candidate DEET receptors would be highly
163 enriched distally but minimally expressed proximally. Candidate genes were therefore
164 selected using two stringent criteria: (1) very low expression levels (TPM < 1) in the
165 proximal region, and (2) significantly higher expression in the distal region. Of the 91
166 *Ae. aegypti* Or genes detected, seven (*AaegOr20*, *AaegOr25*, *AaegOr31*, *AaegOr33*,
167 *AaegOr54*, *AaegOr55*, and *AaegOr57*) met both criteria (Fig. 2b and Extended Data
168 Fig. S3a, Table S1). Among these, *AaegOr54* and *AaegOr55* share 87.66% amino acid
169 sequence identity.

170

171 To test whether any of these receptors respond to DEET, we amplified their coding
172 sequences from antennal cDNA and co-expressed each with *Ae. aegypti* Orco
173 (*AaegOrco*) in *Xenopus laevis* oocytes (Fig. 2c). Two-electrode voltage clamp
174 recordings revealed that only *AaegOr54/Orco* and *AaegOr55/Orco* heteromeric
175 complexes generated significant inward currents in response to DEET, with
176 amplitudes of $1,462 \pm 155$ nA (n = 5) and 345 ± 28 nA (n = 6), respectively (Fig. 2d
177 and Extended Data Fig. S3b-c). Dose-response analyses showed that *AaegOr54* is
178 substantially more sensitive to DEET ($EC_{50} = 2.16 \times 10^{-5}$ M) than *AaegOr55* ($EC_{50} =$
179 4.94×10^{-4} M) (Fig. 2e), indicating a predominant role for *AaegOr54* in detecting
180 DEET.

181

182 We next focused on *AaegOr54* and examined its tuning breadth using a panel of
183 structurally diverse odorants. In *the Xenopus* oocyte expression system,
184 *AaegOr54/Orco* displayed striking selectivity, responding almost strongly to DEET,
185 with a high kurtosis value ($K = 114.6$) (Fig. 2f and Extended Data Table S2). To
186 evaluate *AaegOr54* function in a neuronal context, we expressed *AaegOr54* using the
187 *Drosophila melanogaster* 'empty' neuron system (Fig. 2g), in which the *Or22a/b*
188 genes are deleted³². Expression of *AaegOr54* conferred strong and dose-dependent
189 responses to DEET across a panel of 186 compounds, and DEET elicited the strongest
190 activation among all tested odorants (Fig. 2h-i and Extended Data S3d-e, Table S3).
191 Although its tuning profile was broader in this neuronal platform than in *the Xenopus*
192 *oocyte* expression system, the magnitude and specificity of DEET activation remained
193 highest. Together with TEVC recordings, these results identify *AaegOr54* and
194 *AaegOr55* as DEET-responsive receptors, with *AaegOr54* exhibiting exceptionally
195 high sensitivity, making it the strongest mediator of volatile DEET detection in *Ae.*
196 *aegypti*.



197

198

199

Fig. 2. Identification of *AaegOr54* as the DEET receptor in *Ae. aegypti*. **a**, Image of the *Ae. aegypti* antenna. The numbers indicate flagellomeres from the antennal base

200 (1st) to the tip (13th). Flagellomeres 1-9 constitute the proximal region, whereas 10th-
201 13th form the distal region. **b**, Heatmap showing the average transcript levels of seven
202 candidate *AeegOrs* that met the selection criteria ($n = 3$). The dark blue-green-yellow
203 color gradient indicates expression levels, ranging from low (dark blue) to high
204 (yellow) **c**, Schematic of the *Xenopus laevis* oocyte expression system. **d**, Responses
205 of the seven candidate *AeegOrs* to DEET (10^{-4} M). **e**, Dose-response curves of
206 *AeegOr54* and *AeegOr55* to DEET. EC_{50} of *AeegOr54* = 2.16×10^{-5} M; EC_{50} of
207 *AeegOr55* = 4.94×10^{-4} M. **f**, Tuning curve of *AeegOr54* ($n = 3-9$). Tested
208 compounds are arranged along the x-axis, with those eliciting the strongest responses
209 near the center and weaker ligands toward the edges. The kurtosis (k), indicating
210 tuning “peakedness” is shown. **g**, Schematic of the *Drosophila* empty neuron
211 expression system. **h**, Representative SSR traces from control flies (*Or22ab*^{+/+}-
212 *Gal4/Or22ab*^{+/+}-*Gal4*; *Dr/Tm3*) and transgenic flies (*Or22ab*^{+/+}-*Gal4/Or22ab*^{+/+}-*Gal4*;
213 *UAS-AeegOr54/UAS-AeegOr54*) in the ab3A neuron in response to DEET (10^{-2} v/v).
214 **i**, Tuning curve of *AeegOr54* expressed in the *Drosophila* empty neuron system ($n =$
215 3). Tested compounds are arranged along the x-axis, with those eliciting the strongest
216 responses near the center and weaker ligands toward the edges. The kurtosis (k) is
217 shown.

218

219 ***AeegOr54* knockout significantly reduces electrophysiological responses to DEET**

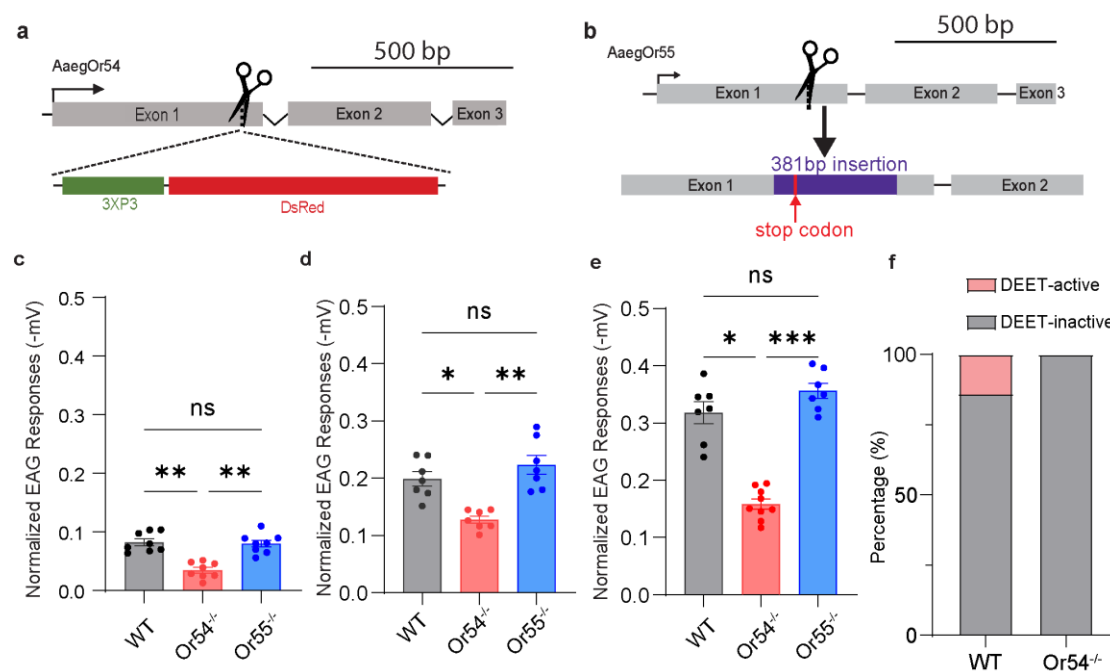
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221 Because *AeegOr54* and *AeegOr55* showed strong and moderate DEET responses,
222 respectively, in heterologous expression systems, we next investigated their functional
223 roles *in vivo*. Using CRISPR/Cas9 genome editing, we generated independent loss-of-
224 function mutants for both receptors (Fig. 3a-b and Extended Data Table S4).
225 Homozygous *AeegOr54*^{-/-} and *AeegOr55*^{-/-} lines were validated by PCR amplification
226 of the target locus followed by Sanger sequencing. Electroantennogram (EAG)
227 recordings revealed a pronounced reduction in DEET-evoked antennal responses in
228 *AeegOr54*^{-/-} mutants across all tested concentrations (Fig. 3c-e). In contrast,
229 *AeegOr55*^{-/-} lines showed EAG responses indistinguishable from wild-type
230 mosquitoes (Fig. 3c-e). These results indicate that *AeegOr54*, but not *AeegOr55*, is
231 required for DEET-evoked antennal activation.

232

233 To determine whether *AeegOr54* knockout eliminates DEET-sensitive sensilla, we
234 performed a comprehensive SSR survey with individual recordings of SST sensillum
235 across the distal four flagellomeres. In wild-type mosquitoes, 10 of 70 (14.3%) SST
236 sensilla exhibited strong DEET-evoked OSN activation (Fig. 3f, Extended Data Fig.
237 S4). In striking contrast, none of the 88 SST sensilla recorded from *AeegOr54*^{-/-}
238 mutants showed any response to DEET (Fig. 3f, Extended Data Fig. S4). This
239 complete loss of sensillum responsiveness confirms that the DEET-activated SST-1
240 “A” neuron depends entirely on functional *AeegOr54*. Together, these data
241 demonstrate that *AeegOr54*, but not *AeegOr55*, is the essential odorant receptor
242 mediating DEET-evoked neuronal activation in *Ae. aegypti*.

243



244

245 **Fig. 3. *AegOr54* mutation attenuates electrophysiological responses to DEET. a,**
 246 Schematic of CRISPR/Cas9-mediated *AegOr54* mutagenesis. **b,** Schematic of
 247 CRISPR/Cas9-mediated *AegOr55* mutagenesis. Non-homologous end joining
 248 (NHEJ) resulted in a 381-bp insertion, the 5' end of which contained a premature
 249 termination codon. **c-e,** DEET responses of WT, *AegOr54* knockout (*AegOr54*^{-/-}),
 250 and *AegOr55* knockout (*AegOr55*^{-/-}) mosquitoes to DEET at 1% (c), 10% (d), and
 251 100% (e) (n = 7-9). The data shown in this figure represent DEET response values
 252 after subtraction of the P.O. (solvent) response. **f,** Activation rate of sensilla in WT
 253 and *AegOr54*^{-/-} antennae.

254

255 Anatomical and functional validation of *AegOr54*-expressing neurons

256

257 To further validate the role of *AegOr54*-expressing olfactory sensory neurons
 258 (OSNs) in DEET detection, we performed fluorescence-guided single sensillum
 259 recording (FgSSR), which integrates SSR with a binary Q-system³³ enabling visual
 260 localization of target sensilla (Fig. 4a). We generated an *AegOr54*-QF2 driver line by
 261 inserting a *T2A-QF2-3×P3-DsRed* cassette into the first exon of *AegOr54* (Fig. 4b
 262 and Extended Data Table S4). This knock-in produced DsRed fluorescence in the
 263 compound eyes (Extended Data Fig. S5a). When crossed to a *QUAS-mCD8:GFP*³³
 264 reporter strain with ECFP fluorescence in the compound eyes (gift from Dr. Leslie
 265 Vosshall, Rockefeller University), the progeny (*AegOr54*^{+QF2} > *QUAS-mCD8:GFP*)
 266 exhibited bright GFP labeling in all *AegOr54*-expressing OSNs along with
 267 DsRed/ECFP in the eyes (Extended Data Fig. S5b). This dual-reporter system allowed
 268 unambiguous identification of sensilla housing *AegOr54*-positive neurons.

269

270 Consistent with our ultra-low-input RNA transcript profiling, all GFP-labeled sensilla
 271 belonged to the SST subtype and were restricted to the distal four flagellomeres (10th

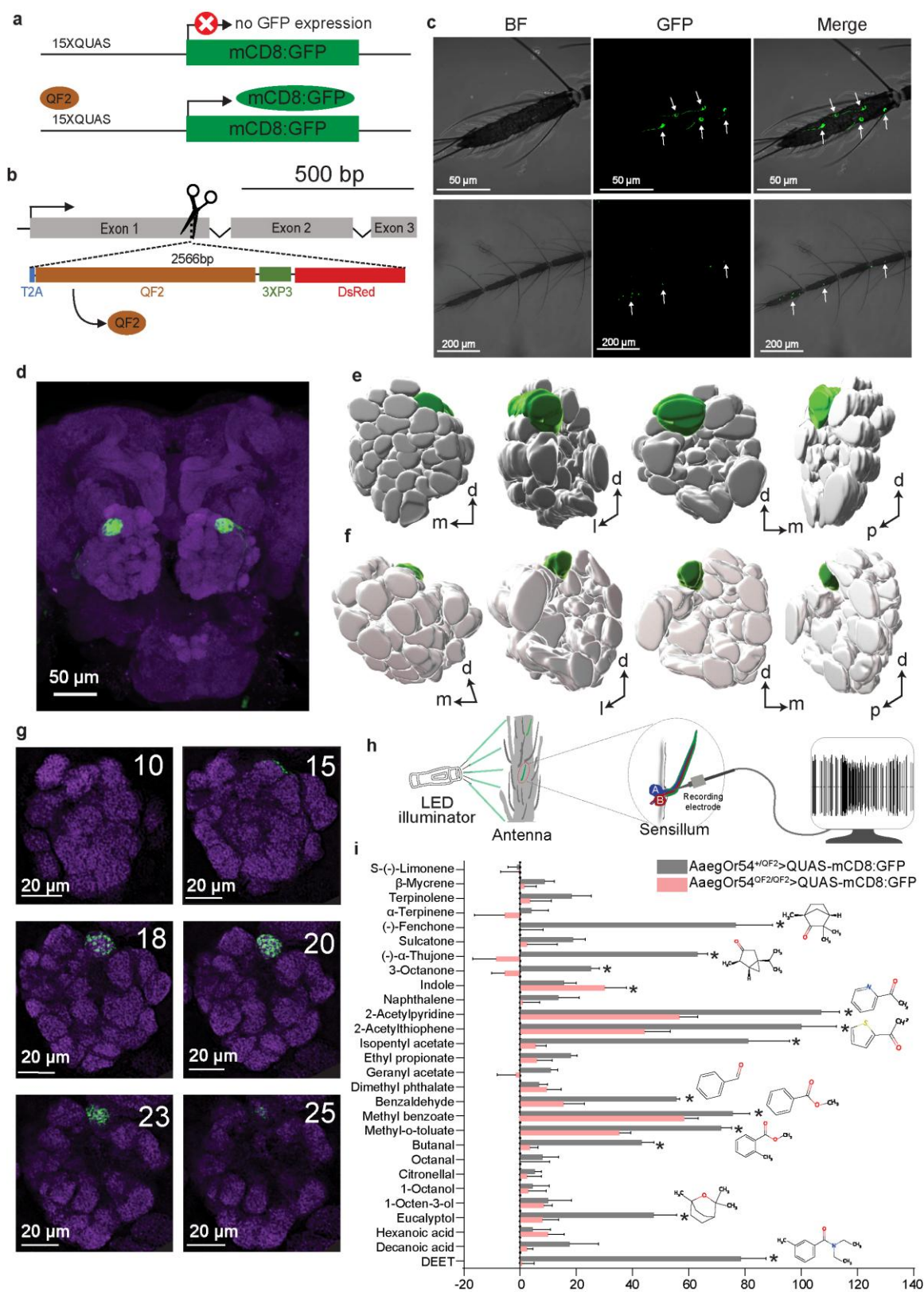
272 –13th) in females, with the highest density on the 13th flagellomere (Fig. 4c) which
273 contains a significantly higher number of *AaegOr54*-expressing neurons (~6 neurons)
274 compared with the remaining distal flagellomeres, which each contains approximately
275 one neuron (Extended Data Fig. S5c). In males, GFP-labeled sensilla were restricted
276 to the 13th flagellomere (Extended Data Fig. S5d). Immunofluorescence of the brains
277 further revealed that all *AaegOr54*-expressing OSNs projected to a single, well-
278 defined glomerulus in the anterior antennal lobe (Fig. 4d and Extended Data Fig.
279 S5e). Three-dimensional reconstruction confirmed that these neurons target the AL3
280 glomerulus, a member of the anterolateral glomerular group (Fig. 4e-g)^{34,35}. These
281 anatomical data demonstrate that *AaegOr54* exhibits a highly localized distal antennal
282 expression and funnels its sensory input into a single glomerulus, forming a dedicated
283 olfactory channel.

284

285 FgSSR recordings showed that *AaegOr54*-positive SST sensilla
286 (*AaegOr54*^{+/*QF2*}>*QUAS-mCD8:GFP*) responded strongly to DEET, with robust
287 responses to an additional subset of compounds (Fig. 4h-i), which is well aligned with
288 the response profile of the DEET-sensitive SST-1 sensillum in the wildtype mosquito
289 ($r = 0.9567$) (Extended Data Fig. S5f). To compare responses between the
290 heterologous *Drosophila* empty-neuron (*ab3A*) expression system and endogenous
291 mosquito neurons, we performed a correlation analysis using response profiles
292 obtained from both systems with the same odorant panel (FgSSR panel), which
293 revealed a moderate correlation coefficient ($r = 0.62$) (Extended Data Fig. S5g). .
294

295 Because insertion of the *T2A-QF2-3×P3-DsRed* cassette disrupts the endogenous
296 *AaegOr54* coding sequence, homozygous knockout mosquitoes (*AaegOr54*^{QF2/QF2} >
297 *QUAS-mCD8:GFP*), selected by PCR verification, served simultaneously as
298 fluorescent reporter animals and loss-of-function mutants. Recordings from these
299 mutants showed a complete loss of DEET-evoked activity, together with strongly
300 reduced or abolished responses to several terpenoid compounds (e.g., (-)-fenchone, α -
301 (-)-thujone, eucalyptol) and heterocyclic/benzoic compounds (e.g., 2-acetylthiophene,
302 2-acetylpyridine, methyl benzoate, methyl o-toluate, benzaldehyde) (Fig. 4i and
303 Extended Data Fig. S6). A small residual response persisted in mutant neurons,
304 suggesting that additional chemoreceptors may be co-expressed within these OSNs.
305 Together, these *in vivo* recordings demonstrate that *AaegOr54*-expressing SST-1 “A”
306 neurons on the distal antenna are the primary detectors of volatile DEET in *Ae.*
307 *aegypti*.

308



309

310 **Fig. 4. Organization of *Ae. aegypti* antennal lobe glomeruli and projections of**
 311 ***AaegOr54*-*QF2*-expressing neurons.** **a**, Schematic of the binary Q-system used to
 312 label *AaegOr54*-expressing olfactory sensory neurons (OSNs). **b**, Schematic of
 313 CRISPR-mediated insertion of the *T2A-QF2-3XP3-DsRed* cassette into the *AaegOr54*
 314 locus. **c**, Representative maximum-intensity projections of confocal z-stacks showing

315 GFP-labeled SST sensilla on the 13th flagellomere (top) and across the 10th-13th
316 flagellomeres (bottom) of female antennae. Arrowheads indicate *AaegOr54*-
317 expressing neurons. **d**, Immunofluorescent staining of the adult female antennal lobe
318 showing GFP-positive axons of *AaegOr54*-expressing OSNs. **e-f**, Three-dimensional
319 reconstruction of a representative right antennal lobe viewed from four angles; the
320 *AaegOr54*-innervated glomerulus is highlighted in green. **g**, Single optical sections
321 from the confocal z-stack shown in (**d**), with immunolabeling for GFP (green) and
322 Brp (synaptic marker, magenta). Numbers in the upper right indicate depth relative to
323 the glomerulus surface, illustrating spatial localization of the *AaegOr54*-targeted
324 glomerulus. **h**, Schematic of fluorescence-guided single sensillum recording (FgSSR).
325 **i**, Odorant response profiles of *AaegOr54*-expressing SST sensilla in heterozygous
326 (*AaegOr54*^{/QF2} > *QUAS-mCD8:GFP*; n = 5-6) and homozygous (*AaegOr54*^{QF2/QF2} >
327 *QUAS-mCD8:GFP*; n = 6-7) lines. Responses to each compound were compared
328 between genotypes using the nonparametric Mann–Whitney test (*P < 0.05).
329 Representative compounds that showed significantly different responses are labeled
330 with their chemical structures.

331

332 **Reduced repellency of DEET in *AaegOr54* knockout mosquitoes**

333

334 To determine whether *AaegOr54* is required for DEET-mediated spatial repellency,
335 we evaluated avoidance behavior in *AaegOr54* knockout (*AaegOr54*^{-/-}) mosquitoes
336 using two complementary paradigms: the hand-in-cage assay, which incorporates
337 human odor cues, and the WHO spatial-repellency assay performed in the absence of
338 human volatiles.

339

340 ***WHO spatial-repellency assay (odor-free conditions)***. To isolate the olfactory
341 component of DEET repellency and eliminate interference from human odor plumes,
342 we used the WHO spatial-repellency assay under odor-free conditions. Solvent
343 controls revealed no differences between WT and *AaegOr54*^{-/-} mosquitoes (Fig. 5a),
344 indicating that the knockout exhibits normal baseline movement and decision-making
345 in this arena. At 0.1% DEET, both genotypes showed comparable levels of repellency
346 (Fig. 5b). In contrast, at higher concentrations (1%, 5%, and 10%), repellency was
347 significantly reduced in *AaegOr54*^{-/-} mosquitoes relative to WT (Fig. 5c-e). These
348 results indicate that *AaegOr54* is required for robust volatile DEET avoidance under
349 odor-free conditions. Notably, residual repellency persisted in *AaegOr54*^{-/-} mosquitoes
350 even at 10% DEET, suggesting that additional sensory pathways—such as modulation
351 of other odorant receptors, activation of non-Or olfactory channels, or Or-independent
352 contact chemoreception—may contribute to DEET detection at higher concentrations.

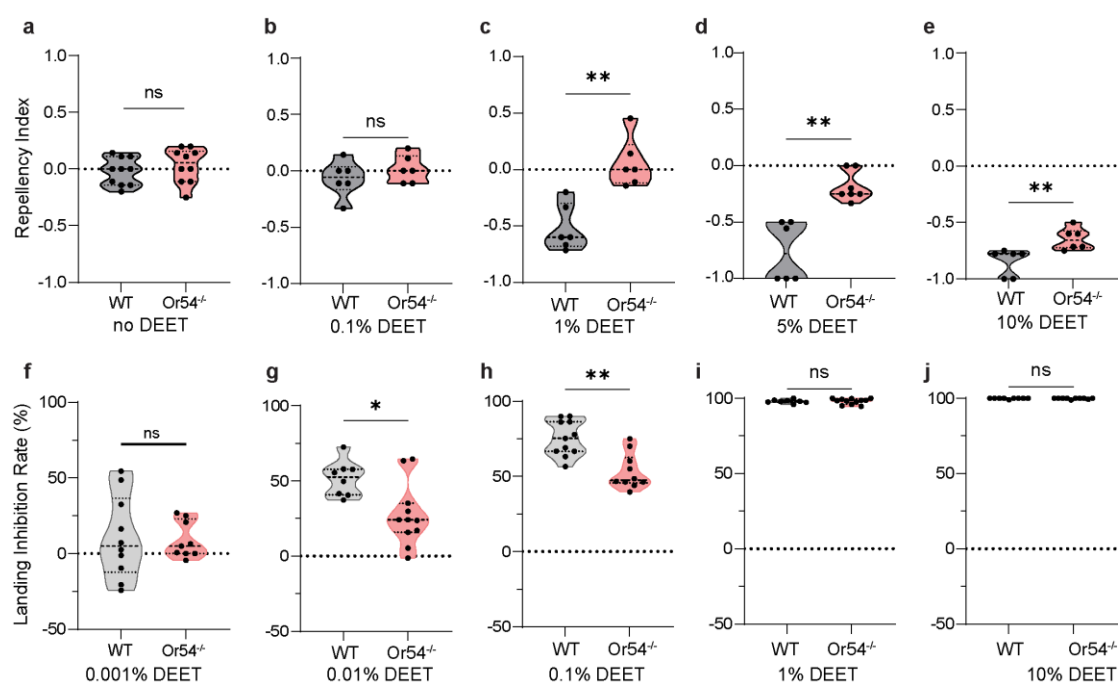
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354 ***Hand-in-cage assay (with human odor cues)***. We examined whether deletion of
355 *AaegOr54* affects baseline attraction to human odor. *AaegOr54*^{-/-} mutants exhibited
356 normal attraction to a human hand, comparable to wild-type (WT) controls (Extended
357 Data Fig. S7a), confirming that *AaegOr54* does not participate in host-seeking
358 behavior itself. DEET produced a clear dose-dependent inhibition of landing behavior

359 in *AeegOr54* mutants (Extended Data Fig. S7b). At very low concentrations
 360 (0.001%), neither genotype exhibited detectable repellency (Fig. 5f). However, at
 361 0.01% and 0.1% DEET, *AeegOr54*^{-/-} mutants showed significantly weaker repellency
 362 than WT (Fig. 5g-h), demonstrating that *AeegOr54* is required for detecting low
 363 concentrations of volatile DEET under realistic conditions in which host odors are
 364 present. At higher concentrations (1–10%), DEET still produced strong inhibition in
 365 both strains (Fig. 5i-j). The persistence of avoidance in the knockout strain is likely
 366 attributable to DEET’s well-described non-specific masking and/or “confusant”
 367 effects on host-odor perception³⁶⁻³⁸, which can dominate behavioral output in hand-
 368 in-cage assays and obscure the contribution of specific olfactory receptors when
 369 DEET is presented at saturating doses.

370

371 Together, these behavioral analyses establish that *AeegOr54* is essential for low- and
 372 intermediate-dose spatial repellency and is a major determinant of volatile DEET
 373 detection in *Ae. aegypti*. At higher concentrations, additional olfactory or non-
 374 olfactory pathways potentially compensate for the loss of *AeegOr54*, explaining the
 375 incomplete abolishment of avoidance behavior observed in the knockout strain.



376

377 **Fig. 5. *AeegOr54* knockout reduce DEET repellency in *Ae. aegypti*.** a-e,
 378 Repellency indices of WT and *AeegOr54*^{-/-} mosquitoes assessed using the
 379 unidirectional olfactometer at DEET concentrations of 0% (a, no DEET), 0.1% (b),
 380 1% (c), 5% (d), and 10% (e) (n = 6-7). Statistical comparisons between genotypes at
 381 each concentration were performed using the Mann–Whitney test (ns, not significant;
 382 **0.001 < P < 0.01). f-j, Landing inhibition rates of WT and *AeegOr54*^{-/-} mutant
 383 mosquitoes exposed to DEET in the hand-in-cage assay at 0.001% (f), 0.01% (g),
 384 0.1% (h), 1% (i), and 10% (j) (n = 8-11). Statistical comparisons between WT and
 385 *AeegOr54*^{-/-} groups were performed using the Mann–Whitney test (ns, not significant;
 386 *0.01 < P < 0.05; **0.001 < P < 0.01).

387

388 Discussion

389

390 The molecular basis of DEET repellency has been debated for decades, particularly
391 the contribution of odorant receptors (Ors) to spatial avoidance behavior^{22,28,31,39}.
392 Previous work in *Culex quinquefasciatus* identified the receptor CquiOr136 as
393 contributing to antennal responses and behavioral repellency to DEET and related
394 compounds²⁴. In contrast, the present study establishes a direct causal link between a
395 single odorant receptor and spatial avoidance behavior through CRISPR-mediated
396 disruption of *AaegOr54*, demonstrating that loss of receptor function abolishes DEET-
397 evoked neural activity and significantly reduces repellency. Beyond this causal
398 relationship, we define a multi-level mechanistic framework linking odor detection to
399 behavior, integrating receptor function, in vivo neural activity, defined olfactory
400 sensilla, and behavioral responses. We further identify the neural circuitry underlying
401 DEET detection in *Aedes aegypti*, showing that DEET-responsive *AaegOr54*-
402 expressing neurons arise from a specific sensillum type and project to a single,
403 anatomically defined glomerulus. While spatial organization of olfactory sensory
404 neurons and glomerular convergence are well-established principles, their functional
405 implementation in a behaviorally relevant repellent pathway has not been previously
406 resolved in mosquitoes.

407

408 Notably, the DEET-sensitive pathway identified here quite differs from that described
409 in *Culex*. Whereas *CquiOr136* responds broadly to multiple commercial mosquito
410 repellents, including DEET, IR3535, Picaridin, and PMD, *AaegOr54* exhibits
411 selective sensitivity to DEET, certain plant-derived terpenoids, aromatics and
412 heterocyclics. These differences in ligand tuning, together with the observation that
413 residual repellency persists in *AaegOr54* mutants, indicate that DEET detection likely
414 involves multiple sensory pathways and that *AaegOr54* represents a key, but not
415 exclusive, contributor to repellency. Recent single-nucleus transcriptomic studies
416 indicate that *AaegOr54*-expressing neurons may co-express additional olfactory
417 receptors^{40,41}, which could contribute to the residual odor responses observed in Or54
418 mutants. Collectively, these findings support the idea that DEET-sensitive odorant
419 receptors have evolved distinct functional properties across mosquito lineages,
420 suggesting convergent evolution in volatile repellent detection. While our findings
421 establish a role for *AaegOr54* in DEET detection, we did not directly test whether
422 activation of this pathway is sufficient to drive repellency, nor whether it represents
423 the sole olfactory pathway responsive to DEET. In addition, EAG responses still can
424 be detected in the absence of *AaegOr54*, suggesting that other receptors on the
425 antennae are likely involved in DEET reception.

426

427

428 Consistent with these observations, because DEET is a compound synthesized in 1946
429 (about 80 years ago), it is unlikely that mosquito receptors evolved specifically for its
430 detection. Rather, DEET may activate receptors that naturally respond to structurally

431 or functionally related compounds. Indeed, *in vivo* FgSSR recordings showed that
432 *AaegOr54* is activated by a subset of naturally occurring ligands that share a
433 conserved 'polar–apolar' structural motif, typically consisting of a carbonyl or
434 oxygen-containing group linked to a hydrophobic ring (Fig. 4i). Many of these
435 compounds, including (-)-fenchone, α -(-)-thujone, and eucalyptol, are major
436 constituents of essential oils with documented repellent activity against mosquitoes
437 ^{42,43}. Others, such as 2-acetylthiophene and benzaldehyde, have also been shown to
438 elicit mosquito repellency in behavioral assays ^{44,45}. Although methyl benzoate has
439 not been reported as a mosquito repellent, it is a strong repellent to other
440 hematophagous or agricultural pests, such as bed bugs and whiteflies ^{46,47}. Together,
441 these findings support the hypothesis that *AaegOr54* may have evolved to detect
442 structurally related natural repellents, with DEET acting as a fortuitous synthetic
443 mimic that engages a pre-existing avoidance pathway. Given the deep evolutionary
444 divergence among mosquito genera, *Anopheles* (~150-200 million years ago) and the
445 *Aedes-Culex* split (~50-100 million years ago), distinct mosquito lineages appear to
446 have independently recruited different Ors to detect DEET and other structurally
447 related repellents. For example, *CquiOr136* is restricted to *Culex*, and *AaegOr54*
448 appears specific to *Aedes* mosquito, with no clear orthologs in *Anopheles*. This
449 represents a striking case of convergent evolution in repellent detection pathways
450 driven by ecological pressures resulting in spatial avoidance.

451

452 Although multiple mechanisms of DEET action have been proposed, work using *orco*
453 mutants in *Ae. aegypti* has led to two principal hypotheses: (1) DEET activates
454 specific odorant receptors that drive avoidance, and (2) DEET broadly modulates Or
455 activity, producing a sensory “confusant” effect that disrupts host-odor perception
456 ^{23,28,29,36-39}. Our findings support the specific-activation model by demonstrating that
457 *AaegOr54* is both necessary and sufficient for detection of volatile DEET. *AaegOr54*-
458 expressing OSNs are confined to a distinct SST-1 trichoid sensillum subtype in the
459 distal antennal flagellomeres and project exclusively to the AL3 glomerulus,
460 consistent with observed DEET-evoked activity in this region ⁴⁸. A previous study
461 reported a different DEET-responsive glomerulus (AM2) in *Ae. Aegypti* ⁴⁹. The
462 discrepancy between our identified glomerulus (AL3) and AM2 may reflect the
463 involvement of different DEET-responsive receptors or differences in glomerular
464 nomenclature. Moreover, DEET has been shown to activate additional antennal lobe
465 regions (MD and PD)⁴⁸, which are likely innervated by IR76b-expressing neurons²,
466 suggesting that non-Or pathways may also contribute to DEET detection. It is
467 important to note that our survey of DEET-sensitive sensilla primarily focused on SST
468 sensilla based on prior studies, and therefore represents only a subset of the sensilla
469 present on the mosquito antenna. Other sensillum types, such as grooved peg and
470 coeloconic sensilla—which are known to house olfactory sensory neurons expressing
471 ionotropic receptors (IRs)—may also contribute to the spatial detection of DEET.

472

473 The *AaegOr54* pathway, therefore, may represent a labeled-line architecture for
474 DEET detection, distinct from the combinatorial glomerular coding typical of host-

475 associated odorants⁴⁸. This organization suggests that the mosquito olfactory system
476 treats DEET as a dedicated avoidance cue, such that activation of the AL3 glomerulus
477 may contribute to repellency. The distal enrichment of *AaegOr54* OSNs mirrors other
478 spatially organized chemosensory microdomains, such as the uneven distribution of
479 ionotropic receptors in *Anopheles antennae*⁵⁰, suggesting a compartmentation-based
480 odor coding mode in mosquito olfaction.

481

482 Of note, in heterologous expression assays, DEET was consistently the most potent
483 agonist; however, secondary tuning profiles varied across platforms, reflecting
484 limitations inherent to non-native receptor contexts^{51,52}. In contrast, endogenous
485 *Aedes Or54*-expressing OSNs displayed high sensitivity to DEET as well as other
486 compounds that closely paralleled behavioral repellency, underscoring the importance
487 of in vivo receptor context, including native Orco, accessory proteins, and membrane
488 physiology, in defining true ligand specificity.

489

490 Genetic ablation of *AaegOr54* eliminated DEET-evoked responses in SST-1 sensilla
491 and markedly reduced spatial repellency across low to intermediate concentrations
492 (0.01–10%). At higher doses, DEET continued to elicit strong avoidance in both wild-
493 type and knockout mosquitoes, consistent with masking and/or “confusant” effects on
494 host-odor perception^{21,28,31,36,38}. The use of a standardized spatial-repellency assay
495 was critical for eliminating interference from human odor, previously shown to
496 obscure responses to compounds such as nootkatone²⁶, thereby isolating the specific
497 contribution of *AaegOr54* to volatile DEET detection. Residual repellency at 10%
498 DEET suggests recruitment of additional mechanisms, including activation of
499 additional ORs and/or broad OR modulation^{36,53,54}. Notably, *AaegOr55* shares highly
500 conserved amino acid sequence similarity with *AaegOr54* and exhibits weak
501 responses to high concentrations of DEET. This similarity in amino acid sequence and
502 DEET-evoked activation may provide useful framework for future structure-function
503 studies to identify the regions of Ors, particularly *AaegOr54*, that mediate DEET
504 binding. In addition, there are several additional Ors, including *AaegOr55*, found to
505 be co-expressed within some *AaegOr54*-expressing OSNs based on the single-cell
506 atlases generated by Goldman et al.⁴⁰ and Adavi et al.⁴¹, which may explain the
507 residual spikes of Or54 knockout OSNs in response to certain compounds (e.g. 2-
508 acetylpyridine and 2-acetylthiophene), however, no residual responses elicited by
509 DEET was observed, suggesting the predominant role of *AaegOr54* (but not other co-
510 expressed Ors) in detecting DEET.

511

512

513 **Conclusions and implications**

514

515 Our integrated genetic, electrophysiological, and behavioral analyses establish
516 *AaegOr54* as the central molecular determinant of volatile DEET reception in *Ae.*
517 *aegypti*. To our knowledge, this is the first identification of a bona fide DEET receptor
518 in *Aedes* mosquitoes, revealing a chemical repellent sensory pathway that links

519 peripheral OSN activation to glomerular projection and avoidance behavior.
520 *AaegOr54*'s exceptional sensitivity provide a mechanistic foundation for rational
521 design of next-generation spatial repellents, including safer and more durable
522 compounds tailored to mosquito chemosensory biology. Receptor-guided strategies
523 offer strong potential to transform chemical vector management and reduce the global
524 burden of mosquito-borne disease.
525
526

527 **Method**

528 **Mosquito rearing**

529 The *Ae. aegypti* mosquitoes were maintained under controlled conditions at $27 \pm 2^\circ\text{C}$,
530 $75 \pm 5\%$ relative humidity, and a 12:12 h light:dark photoperiod. Larvae were reared
531 in deionized water and fed TeraMin Tropical Fish Flakes according to their needs.
532 After pupation, male and female pupae were collected and placed together in the
533 rearing cages, where adults were provided with a 10% sucrose solution. Adult females
534 were blood-fed using cow blood obtained from the Large Animal Teaching Hospital at
535 Auburn University.

536

537 **Hand-in-cage assay**

538 Spatial (non-contact) repellency was evaluated using a modified hand-in-cage assay
539 based on previous study⁵⁵. Assays were conducted in a 30 cm × 30 cm × 30 cm
540 mosquito cage equipped with a top-mounted digital camera connected to the computer
541 for video recording and quantitative analysis. A nitrile glove was modified by cutting
542 a 6 × 5 cm window on the dorsal surface. A magnetic frame, slightly larger than the
543 opening, was affixed around the cutout to increase a stable base for stacking
544 additional magnetic frames to a total height of approximately 10.0 mm. Two layers of
545 polyester netting were used: a lower “chemical treated” net positioned about 3 mm
546 above the glove surface, and an upper “untreated” net positioned roughly 8 mm above
547 the glove. This configuration ensured that mosquitoes could land on the untreated net
548 without physically contacting the treated one. The two nets were separated and
549 secured by stacked magnetic frames held together with a binder clip.

550

551 Female mosquitoes (4-9 day-old, non-blood fed) were aspirated into cages in groups
552 of approximately 40 individuals 24 hours prior to testing. A water-soaked cotton pad
553 was placed on the cage top to maintain hydration. All assays were performed at 27-28
554 °C and ~50% relative humidity. Immediately before testing, the lower net was placed
555 in a Petri dish and treated with 500 μL of either acetone (solvent control) or a DEET
556 solution at the desired concentration. Nets were prepared in an adjacent room, and
557 acetone was allowed to evaporate completely (~ 7 min) before the net was assembled
558 onto the glove. The tester then inserted gloved hand into the cage and mosquito
559 behavior was recorded for 5 minutes. Landing events on the upper, untreated net were
560 quantified from minute 2 through minute 5 of each trial.

561

562 Each cage was first treated with the solvent control and subsequently with the DEET
563 treatment. A minimum interval of 90 minutes was allowed between trials to ensure
564 full recovery of mosquitoes landing activity and to ventilate volatiles from the room.
565 Control trials in which mosquitoes exhibited abnormally low landing activity were
566 excluded from analysis. Percent repellency was calculated using the formula:
567 Landing inhibition rate (%) = $(1 - N_{\text{DEET}} / N_{\text{control}}) \times 100$, where N_{DEET} is the
568 cumulative number of landings under DEET treatment, and N_{control} is the cumulative
569 number under solvent control.

570

571 Immediately after each assay, cages were sprayed with 70% ethanol, rinsed with
572 distilled water, sprayed again with ethanol, and allowed to air-dry. The modified glove
573 and magnetic frames were similarly decontaminated by soaking in 70% ethanol,
574 rinsing with distilled water, and applying a final ethanol rinse before air-drying.

575

576 **Uniport olfactometer assay**

577 A modified spatial-repellency assay was used to quantify mosquito avoidance of
578 volatile stimuli (DEET or solvent control) without physical contact. The apparatus
579 consisted of three interconnected plexiglass chambers, each cylindrical in shape (15
580 cm long × 15 cm diameter) and arranged linearly. Mosquitoes were released into the
581 central chamber, and each of the two outer chambers contained a smaller nylon-
582 covered inner cylinder (10 cm long × 10 cm in diameter) that served as the odor
583 source. Immediately before each test, 500 µL of DEET solution was applied to a
584 nylon sock, allowed to air-dry for 5 minutes, and then fitted over the smaller inner
585 cylinder before being placed into one of the designated outer chambers. Control odor
586 sources were prepared identically using acetone alone. The DEET-treated and solvent-
587 control odor cylinders were positioned symmetrically in the two outer chambers
588 flanking the central release chamber.

589

590 For each assay, ten adult female mosquitoes of the specified strain were introduced
591 into the central chamber via a small port in the middle of the central chamber, which
592 had both ends sealed with plastic wrap, and acclimated for 20 minutes. The side
593 chambers were connected to the central chamber, and the plastic wrap was gently
594 removed from the connection part, exposing the mosquitoes to both odor chambers.
595 To promote movement by positive phototaxis, the apparatus was surrounded with
596 black cloth except for clear openings at both ends that allowed external light to enter.
597 Mosquito movement was recorded for 8 minutes, after which the number of
598 individuals in each chamber was counted. The apparatus was thoroughly washed with
599 70% ethanol and air-dried between replicates to eliminate residual volatiles. Each
600 assay was repeated at least six times. The repellency index (RI) was calculated as: RI
601 $= (N_c - N_t) / (N_c + N_t)$, where N_c is the number of mosquitoes in the control chamber
602 and N_t is the number in the DEET-treated chamber.

603

604 **Electroantennogram (EAG)**

605 The electroantennogram (EAG) procedures were performed following the methods
606 described by Chen et al⁵⁶. The head of a female *Ae. aegypti* was excised and mounted
607 onto an EAG holder equipped with two glass capillary electrodes. Each electrode was
608 filled with 0.1% potassium chloride (KCl) and 0.5% polyvinylpyrrolidone (PVP), and
609 chlorinated silver wires were inserted to establish electric contact. The excised head
610 was positioned such that the base of the head connected to the reference electrode,
611 while one antenna, with only an extremely small portion of the distal tip of the 13th
612 antennal segment clipped to improve electrical continuity, was inserted into the
613 recording electrode. The recording electrode was connected to a high-impedance
614 AC/DC preamplifier (IDAC-2, Syntech, Germany).

615

616 During recording, the antenna was continuously exposed to a humidified airstream
617 (20 ml/s). Odor stimuli pulses were delivered as 500-ms pulses injected into the
618 airstream at the flow rate of 2 ml/s. DEET was formulated at the desired test
619 concentration(s) using paraffin oil as the solvent. For each stimulus, 10 μ L of the
620 DEET solution was applied onto a 3 \times 40 mm filter paper, inserted into a Pasteur
621 pipette, and allowed to equilibrate for 30 seconds prior to use. Antennal responses
622 were recorded for a total of 11 seconds, including a 1-second baseline period before
623 the stimulus delivery. Consecutive stimuli were presented at a 30-second interval to
624 minimize sensory adaptation.

625

626 **Single sensillum recording (SSR)**

627 Single sensillum recordings were performed as Liu et al with minor modifications⁵⁵.
628 An adult female *Ae. aegypti* (7-14 days old) was anesthetized on ice (~2 min) and
629 immobilized inside a 200- μ L pipette tip. The mosquito was then secured onto a block
630 of dental wax using a coverslip mounted with double-sided tape. The tungsten
631 reference electrode was inserted into the compound eye, and a tungsten recording
632 electrode was advanced into an individual antennal sensillum under high
633 magnification (\times 720) of a microscope (Leica Z6 Apo). Action potentials were
634 preamplified using a Universal AC/DC Probe (10X Gain, Syntech) and digitized via
635 an IDAC 4 interface (Syntech).

636

637 Test odorants were diluted to 1% (v/v) in paraffin oil. For each stimulus, 10 μ L of
638 odorant solution was applied to a 3 \times 40 mm filter paper inserted into a glass Pasteur
639 pipette to create a stimulus cartridge. After 30-s equilibration period, a 500-ms odor
640 pulse was delivered using a Syntech CS-55 Stimulus Controller into a continuously
641 humidified airstream (20 mL/s) directed at the mosquito antenna. Action
642 potential was recorded for a total of 11 seconds, including a 1-second per-stimulation
643 baseline. Spike frequency was quantified as the differences in spike counts between
644 500 ms pre-stimulus period and 500 ms post-stimulus period, multiplied by 2 to
645 convert to spikes/s.

646

647 The hierarchical cluster analysis of the response profile was conducted by PAST
648 (4.17C) based on Euclidean distances and Complete linkage classification.

649

650 *Fluorescence-guided SSR (FgSSR)*

651 For fluorescence-guided single sensillum recording, a LED fluorescent illuminator
652 (BGIMAGING BGI-P300-LG1.0) was used to visualize GFP-labeled sensilla prior to
653 electrode insertion. After confirming fluorescence, electrophysiological recording
654 proceeded identically to the conventional SSR protocol described above.

655

656 **Two-electrode voltage clamp (TEVC)**

657 cRNA was synthesized followed the procedure described in previous study with
658 minor modifications⁵⁶. Total RNA was isolated from approximately 500 *Ae. aegypti*

659 antennae using the acid guanidinium thiocyanate-phenol-chloroform method and
660 treated with a TURBO DNase (Invitrogen) to remove genomic DNA. First-strand
661 cDNA was synthesized from 1.5 µg of DNA-free RNA using SuperScript™ IV with
662 oligo(dT)₂₀ primers. Full-length coding sequences of target odorant receptors (Ors)
663 were amplified by high-fidelity PCR (Q5 Polymerase, NEB) with gene-specific
664 primers (Table S5), gel-purified, restriction-digested, and cloned into the pT7Ts
665 vector. Plasmids were transformed into TOP10 *E. coli*, extracted, and sequencing
666 verified by Sanger sequencing. The verified plasmids were linearized, and capped
667 cRNA was generated using the mMMESSAGE mMACHINE T7 Kit (Ambion)
668 following the manufacturer's protocol.

669
670 TEVC recordings were performed as described⁵⁸. Stage V-VI *Xenopus laevis* oocytes
671 (Nasco, Salina, CA) were treated with collagenase I (GIBCO, Carlsbad, CA) in
672 washing buffer (96 mM NaCl, 2 mM KCl, 5 mM MgCl₂•6H₂O, 5 mM HEPES sodium
673 salt, and 10 µg/mL gentamycin, pH = 7.6) for ~1 hour at room temperature. Oocytes
674 were then rinsed sequentially with 1× Ringer's solution, washing buffer, and modified
675 Barth's saline (78 mM NaCl, 1 mM KCl, 0.33 mM Ca(NO₃)₂•4H₂O, 0.41 mM
676 CaCl₂•2H₂O, 0.82 mM MgSO₄•7H₂O, 2.4 mM NaHCO₃, 10 µg/mL gentamycin, 10
677 µg/mL streptomycin, and 10 mM HEPES sodium salt, pH = 7.6) and incubated
678 overnight at 18°C.

679
680 For expression of odorant receptors, each oocyte was injected with 20 nL of a
681 premixed solution containing *AeegOr* and *AeegOrco* (both at 250 ng/µL) and
682 incubated at 18°C for 4-7 days. Whole-cell odorant-evoked currents were recorded
683 under voltage clamp (TEVC) (holding potential of -80 mV) using an OC-725C
684 amplifier (Warner Instruments, Hamden, CT). Data were acquired and analyzed with
685 Digidata 1440A and pCLAMP 10.2 (Axon Instruments Inc., CA). Odorants were
686 initially dissolved in DMSO at 1:10 M and diluted to the desired concentrations (10⁻⁴
687 M) in ND96 solution (91 mM NaCl, 2 mM KCl, 1.8 mM CaCl₂•2H₂O, 2 mM
688 MgCl₂•6H₂O, 5 mM HEPES sodium salt, pH = 7.6) immediately before application.

689
690 **Ultra-low-input RNA sequencing of mosquito antennal flagellomeres**
691 RNA-seq data for antennal flagellomeres of *Ae. aegypti* were generated using an
692 optimized ultra-low-input RNA sequencing workflow. Adult mosquitoes were
693 anesthetized on ice and proximal (flagellomeres 1-9) and distal (flagellomeres 10-13)
694 antennal segments were microdissected into low-binding PCR tubes containing lysis
695 buffer (Vazyme N712) supplemented with 0.2% Triton X-100 and 2 U/µL RNase-
696 Inhibitor (Thermo Fisher Scientific). Three replicates were collected for each antennal
697 region. Samples were immediately flash-frozen in liquid nitrogen to ensure rapid and
698 complete lysis.

699
700 cDNA synthesis and whole transcriptome amplification were performed using the
701 Vazyme WTA kit (N712). Amplified cDNA was purified with AMPure XP beads
702 (Beckman Coulter, Cat. No. A63881) at a bead-to-sample ratio of 0.8:1, and assessed

703 using a Qubit 3.0 Fluorometer and Agilent Bioanalyzer 2100 to verify expected
704 fragment sizes (~1000-2000 bp). Sequencing libraries were prepared using the
705 TruePrep Flexible DNA Library Prep Kit for Illumina (Vazyme, TD504) and
706 sequenced (paired-end 150 bp) on an Illumina NovaSeq X Plus platform. Raw reads
707 were processed by adapter trimming and removal of low-quality reads. Clean reads
708 were aligned to the *Ae. aegypti* genome (VectorBase) using Salmon, and transcript
709 abundance was quantified as Transcripts Per Million (TPM).

710

711 **CRISPR/CAS9 transgenesis**

712 Single-guide RNAs (sgRNAs) were synthesized following previously described by Li
713 ⁵⁹ with minor modifications. sgRNAs targeting *AaegOr54* (AAEL015286) and
714 *AaegOr55* (AAEL010415) were designed using the CHOPCHOP web tool
715 (<http://chopchop.cbu.uib.no/>), scanning both sense and antisense strands for NGG
716 protospacer-adjacent motifs (PAMs). Target sequences and primers were listed in
717 Table S5. Linear double-stranded DNA (dsDNA) templates for each sgRNAs were
718 generated by template-free PCR using Q5 High-Fidelity DNA Polymerase (NEB
719 #M0491S). PCR amplification conditions were: 98 °C for 30 s, followed by 35 cycles
720 of 98 °C for 10 s, 58 °C for 10 s, and 72 °C for 10 s, with a final extension at 72 °C for
721 2 min. PCR products were purified using the Wizard SV Gel and PCR Clean-Up
722 System (Promega #A9281). sgRNAs were transcribed using the MEGAscript T7 Kit
723 (Ambion #AM1334), with 300 ng of purified DNA template per reaction. Resulting
724 sgRNAs were purified using the MegaClear™ Kit (Ambion #AM1908), resuspended
725 at 1000 ng/μL in nuclease-free water, and stored at -80 °C in single-use aliquots.
726 Recombinant *Streptococcus pyogenes* Cas9 protein (PNA Bio #CP01) was diluted to
727 1000 ng/μL, aliquoted, and stored at -80 °C.

728

729 To generate the *AaegOr54*-QF2 driver line for the Q-system, *T2A-QF2-3xP3-*
730 *DsRed* element was inserted into the *AaegOr54* coding region through CRISPR-
731 mediated homologous recombination. The homologous template (donor plasmid) that
732 contains *T2A-QF2-3xP3-DsRed* element, which was amplified from the *ppk301-T2A-*
733 *QF2* HDR plasmid (Addgene plasmid# 130667)³³, flanked by ~1kb homologous arms
734 was constructed using NEBuilder HiFi DNA Assembly kit (New England Biolabs).
735 The left homologous arm was amplified with primer pair of HDR-*AaegOr54*LAF and
736 HDR-*AaegOr54*LAR. The right homologous arm was amplified with primer pair of
737 HDR-*AaegOr54*RAF and HDR-*AaegOr54*RAR. The primer pair for confirming the
738 knock-in element was *AaegOr54*ExamF and *AaegOr54*ExamR. From the left
739 homologous arm immediately preceding the *T2A*, 1 bp was removed to keep the *T2A*
740 sequence in-frame. Red-eyed F1 mosquitoes were backcrossed for five generations
741 and then crossed to the effector line to acquire progeny for *AaegOr54* localization
742 studies. All primer sequences are listed in Table S5. Embryo collection and
743 microinjection were performed according to Li et al⁵⁸ with minor adaptations. Adult
744 females were blood fed five days prior to egg collection. A blackened oviposition cup
745 lined with filter paper was placed in the cage under dark conditions. After 15-30
746 minutes, the cup was removed, and freshly laid (non-melanized) eggs were transferred

747 onto a glass slide and aligned on a moistened filter paper strip. Injection needles were
748 pulled from the aluminosilicate capillaries using a Sutter P-1000 puller and beveled
749 on a Sutter BV-10 microbeveler.

750

751 Microinjections were carried out under a compound microscope (100× magnification)
752 using an Eppendorf FemtoJet injector. Approximately 50 embryos were injected per
753 session. For generating the *AaegOr54*-QF2 driver line, the injection mixture contained
754 Cas9 protein (300 ng/μL) premix with sgRNA (40 ng/μL) and donor plasmid (300
755 ng/μL). Injected embryos were transferred to water and maintained under standard
756 rearing conditions until adulthood. The first generation (G0) of injected adults were
757 separated based on sex and crossed to 5X wild-type counterparts. Their offspring (F1)
758 were manually screened for DsRed-derived red eye fluorescence using an Compound
759 Fluorescent Microscope (DP74, Olympus, Japan). Red-eyed F1 males were
760 individually backcrossed to 5-fold females to establish a stable mutant line. DNA
761 extraction was performed using FastPure Gel DNA Extraction (Vazyme Biotech,
762 Nanjing) protocols and genomic DNA templates for PCR analyses of all individuals
763 were performed (after mating) to validate the fluorescence marker insertion using
764 primers that cover DSB sites. PCR products were sequenced to confirm the accuracy
765 of the genomic insertion. Heterozygous mutant lines were thereafter backcrossed to
766 wild-type *Ae. aegypti* for five generations before self-crossing and the progenies were
767 used for screening homozygous individuals according to their DsRed-derived red eye
768 fluorescence intensity. Putative homozygous mutant individuals were mated to each
769 other and female adults laid eggs before being sacrificed for genomic DNA extraction
770 and PCR analyses (as above) to confirm their homozygosity.

771 For generating the *AaegOr54*^{-/-} and *AaegOr55*^{-/-} lines, the injection mixture contained
772 Cas9 protein (300 ng/μL) premix with sgRNA (40 ng/μL). Injected embryos were
773 transferred to water and maintained under standard rearing conditions until adulthood.
774 The first generation (G0) of injected adults were separated based on sex and crossed
775 to 5X wild-type counterparts. Their offspring (F1) were screened by clipping one hind
776 leg from each adult for genomic DNA extraction using the DNeasy Blood & Tissue
777 Kit (QIAGEN) and PCR amplification of the target locus was performed using
778 *AaegOr54*- or *AaegOr55*-specific primers, *AaegOr54ExamF/AaegOr54ExamR* and
779 *AaegOr55ExamF/AaegOr55ExamR*, respectively (Table S5). PCR amplicons were
780 Sanger-sequenced to identify indel mutations. Detailed sequencing results of the
781 mutant mosquitoes are provided in Table S4.

782

783 ***Drosophila* empty neuron expression system**

784 To generate the *UAS-AaegOr54* construct for transgenic *Drosophila melanogaster*, we
785 employed a seamless homologous recombination strategy³². Total RNA was extracted
786 from approximately 100 dissected *Ae. aegypti* antennae preserved in Trizol
787 (Invitrogen, Carlsbad, CA, USA). The full-length coding sequence of *AaegOr54* was
788 amplified with primers (Table S5) according to flanking homology arms designed
789 from VectorBase reference sequence (<https://www.vectorbase.org>). The pUASTattB
790 vector (Addgene #96148) was linearized by PCR using Phusion High-Fidelity DNA

791 Polymerase (New England Biolabs, Ipswich, MA, USA) and primers listed in Table
792 S5. The *AaegOr54* insert was assembled into the vector using the pEASY-Basic
793 Seamless Cloning and Assembly Kit (TransGen Biotech, Beijing, China).
794 Recombinant constructs were transformed into Trans1-T1 Phage Resistant Chemically
795 Competent Cells (TransGen Biotech) and positive clones were verified and purified
796 using the Plasmid Mini Kit (Omega Bio-Tek, Norcross, GA, USA) prior to
797 microinjection.

798

799 Heterologous expression of *AaegOr54* in the *Drosophila* ab3A “empty neuron”
800 system followed two-stage genetic crossing as described by Chahda et al³². The *UAS-*
801 *AaegOr54* plasmid was microinjected into a *Drosophila* strain containing a white-eye
802 rescue marker, generating transgenic flies with red eyes (genotype: w; +; *UAS-*
803 *AaegOr54*(w+)/+). The ab3A empty neuron driving line (*Or22ab^{-/-}-Gal4/Cyo*) and the
804 balancer line (*sp/Cyo; Dr/Tm3*) were gifts from Dr. John Carlson (Yale University).

805

806 To generate the expression line, we first generated an intermediate genotype (*Or22ab⁻*
807 *^{-/-}-Gal4/Cyo; Dr/Tm3*), identified by curly wings and stubble bristles, and then self-
808 crossed it to obtain a homozygous empty neuron line (*Or22ab^{-/-}-Gal4/Or22ab^{-/-}-*
809 *Gal4; Dr/Tm3*) with straight wings and stubble bristles. In parallel, the *UAS-*
810 *AaegOr54* insertion line was balanced to produce *Sp/Cyo; UAS-AaegOr54/Tm3*.

811 Crossing the homozygous empty neuron driver to the balanced *UAS-AaegOr54* line
812 produced progeny of genotype *Or22ab^{-/-}-Gal4/Cyo; UAS-AaegOr54/Tm3*, recognized
813 by orange eyes, curly wings, and stubble bristles. Self-crossed these flies yielded the
814 stable homozygous expression line (*Or22ab^{-/-}-Gal4/Or22ab^{-/-}-Gal4; UAS-*
815 *AaegOr54/UAS-AaegOr54*), which expresses the mosquito receptor exclusively in the
816 ab3A empty neuron. Adult flies of this final genotype were used for single sensillum
817 recordings.

818

819 **Brain immunofluorescence**

820 Brain dissection and immunofluorescence staining were performed as previously
821 described³³ with minor modification. Adult female mosquitoes (6- to 14-day-old)
822 were anesthetized on ice, and heads were removed by gently pinching the cervix with
823 fine forceps. Excised heads were fixed in phosphate-buffered saline (PBS) containing
824 4% paraformaldehyde, and 0.25% Triton X-100 for 3 hours on a nutator. Brains were
825 then dissected from the head capsules in ice-cold PBS and transferred to a 48-well
826 plate.

827

828 All subsequent steps were conducted with gentle agitation on a low-speed orbital
829 shaker. Brains were washed six times for 15 minutes each in PBS containing 0.25%
830 Triton X-100 (PBT) at room temperature, followed by permeabilization ~48 hours at
831 4 °C in PBS supplemented with 4% Triton X-100 and 2% normal goat serum (NGS).
832 After permeabilization, brains were washed again six times in PBT and incubated for
833 ~48 hours at 4 °C with primary antibodies diluted in PBT containing 2% NGS. The
834 primary antibodies included mouse anti-dmBrp (NC82, DSHB; 1:50) to label synaptic

835 neuropil, and rabbit anti-GFP (A11122, Life Technologies; 1:10,000).

836

837 Following primary antibody incubation, brains were washed six times in PBT and
838 then incubated for an additional ~48 hours at 4 °C with secondary antibodies - goat
839 anti-mouse Cy5 (115-605-003, Jackson ImmunoResearch; 1:250) and goat anti-rabbit-
840 Alexa Fluor 488 (111-545-003, Jackson ImmunoResearch; 1:500) - diluted in PBT
841 with 2% NGS. Afterward, brains received a final series of six 15-minute washes in
842 PBT. Prior to imaging, brains were equilibrated overnight in mounting medium
843 (P0126, Beyotime) and subsequently mounted in the same medium.

844

845 **Female antennal lobe confocal imaging**

846 Confocal imaging of mosquito brains was performed using an Andor Dragonfly
847 confocal microscope system (CR-DFLY-202-2540) equipped with a 20X objective.
848 Antennal lobes were acquired at resolutions of 1024 × 1024 pixels in the xy-plane
849 with a z-step size of 1 μm. Laser power and detector gain were incrementally adjusted
850 along the z-axis to compensate for signal attenuation with imaging depth. Care was
851 taken to avoid signal saturation while ensuring sufficient contrast to visualize the
852 deepest glomeruli for subsequent segmentation. All imaging acquisition was
853 optimized for reliable visualization of glomerular boundaries and for determining the
854 presence or absence of specific fluorescence labeling, rather than for quantitative
855 intensity measurements.

856

857 To distinguish specific neuronal expression patterns, we used the 3xP3 promoter-
858 driven fluorescent reporters in both knock-in and QUAS transgenic lines. The 3xP3
859 promoter drives expression primarily in the optic lobes and in a subset of dorsal brain
860 neurons, regions that do not overlap with the antennal lobe. Consistent with prior
861 reports (33), no 3xP3-derived fluorescence was detected in the antennal lobes of
862 reporter-only controls (data not shown). For clarity in the representative figures,
863 representative images of antennal lobes were cropped to remove 3xP3-derived
864 fluorescence signal originating in other brain regions.

865

866 **Antennal lobe 3D reconstruction**

867 Three-dimensional reconstruction and glomerular segmentation were performed using
868 Imaris 10.1.0 (Bitplane, UK). Confocal image stacks were imported into Imaris, and
869 each glomerulus was manually delineated by tracing its boundary in every optical
870 section based on immunohistochemical staining. Surface rendered 3D models were
871 generated from these segmented contours. Glomeruli were identified and named
872 following the neuroaxis-based nomenclature^{27, 35}, using two-letter codes denoting
873 relative positions: anterior (A), posterior (P), ventral (V), dorsal (D), lateral (L),
874 medial (M).

875

876 **Data analysis and statistics**

877 All statistical analyses were performed using Prism 10 (GraphPad Software). Data are
878 presented as mean ± s.e.m. Statistical significance was defined as P < 0.05. The

879 specific statistical tests, sample sizes, and replication details used for each experiment
880 are provided in figure legends.

881

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1036

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1044 X.W., Q.Q., and Y.W. conducted SSR and EAG experiments. R.Y. and M.C.
1045 performed FgSSR. D.J.B., X.W., and Y.W. generated the *AaegOr54-QF2* lines. X.F.
1046 and D.J.B. generated the *AaegOr55* knockout lines. X.W. and D.J.B. performed hand-
1047 in-cage and uniport olfactometer assay experiments. X.W., R.Y, Z.C., S.L., and Y.W.
1048 performed plasmid construction and TEVC experiments. Y.W. and Q.Q. performed
1049 ultra-low-input RNA sequencing and analysis. X.W. and H.Z. performed brain
1050 immunofluorescence and antennal lobe 3D reconstruction. N.L., F.L., X.W., and
1051 W.S.L. wrote and edited the manuscript. All authors read and approved the
1052 manuscript.

1053

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1055

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Extended Data for

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DEET Olfactory Reception in the Yellow Fever Mosquito

1069

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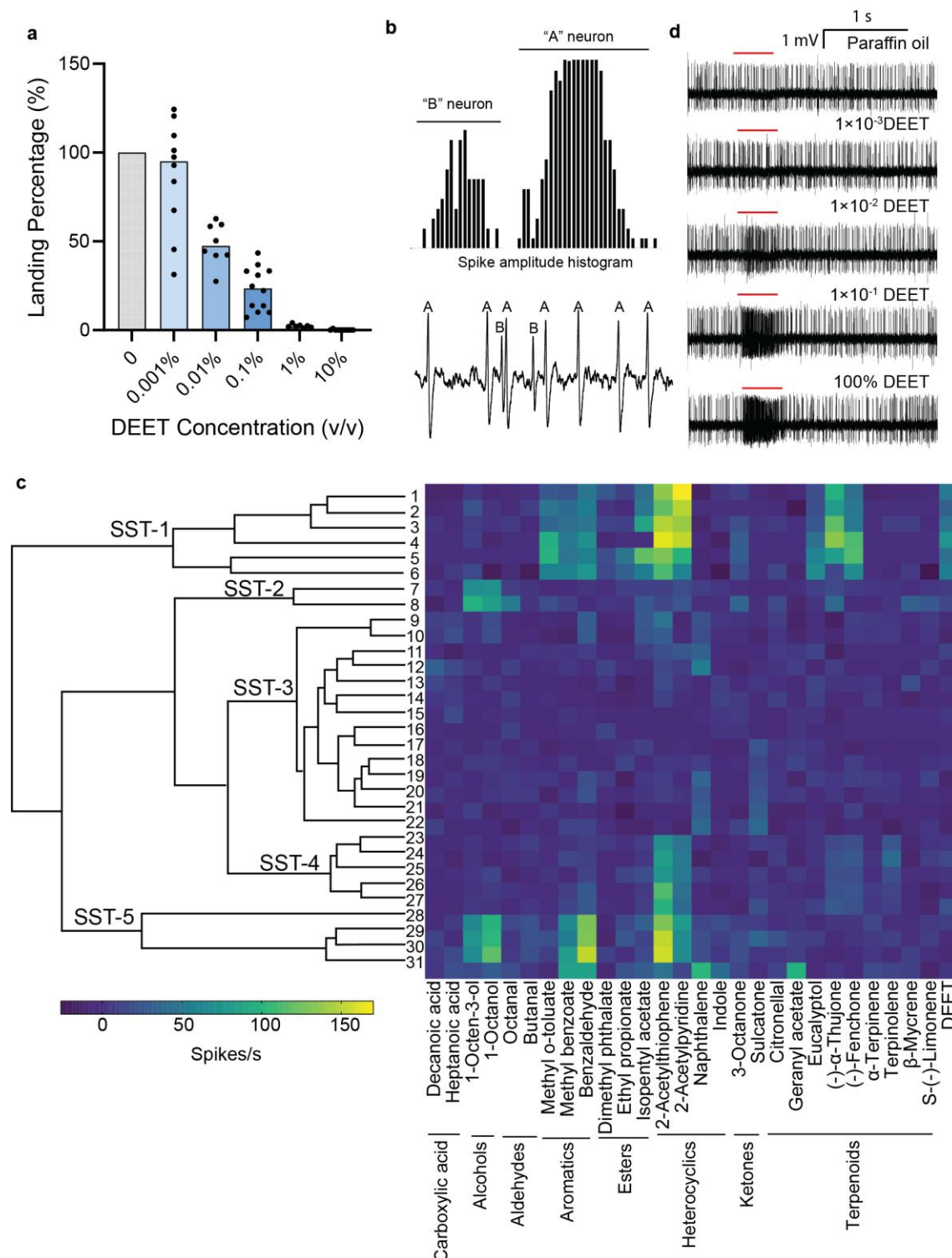
1096 Figs S1 to S7

1097 Tables S1 to S5

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1099 **Extended Data**

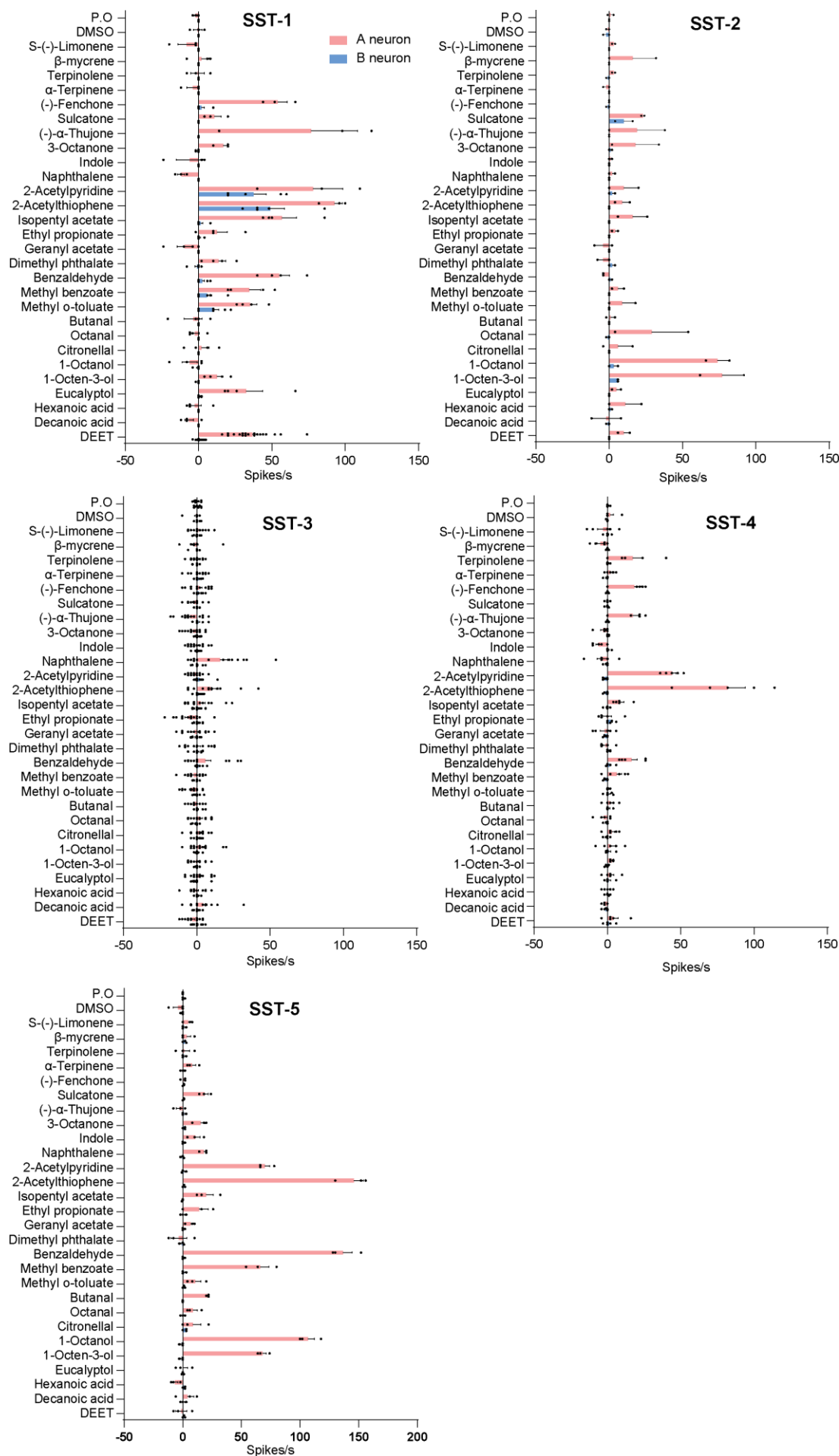
1100 **Figs. S1 to S6**



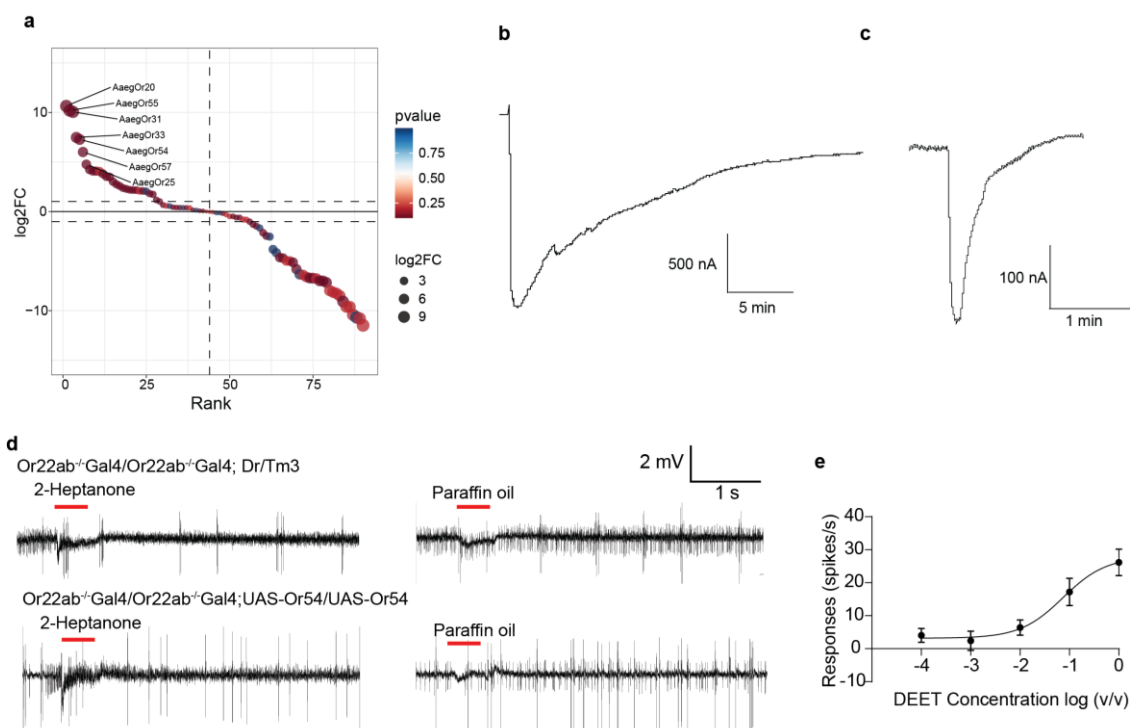
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1102 **Fig. S1. Behavioral and electrophysiological responses of *Ae. aegypti* to DEET. a,**
 1103 **Effect of DEET concentration on landing percentage in WT *Ae. aegypti* females. b,**
 1104 **Distinct spontaneous neuronal activities were distinguishable based on spike**
 1105 **amplitude, with larger amplitudes corresponding to "A" neurons and the smaller**

1106 amplitudes to “B” neurons (top). The distribution of spike amplitudes is also shown
1107 (bottom). **c**, Dendrogram from complete linkage cluster analysis of 31 ORNs housed
1108 in SST sensilla, based on their response profiles to 28 tested compounds. Five major
1109 clusters were identified. **d**, Representative SSR traces from WT mosquitoes exposed
1110 to increasing concentrations of DEET (1×10^{-3} v/v to 100%; paraffin oil served as
1111 solvent).



1113 **Fig. S2. Response profiles of SST sensilla identified by the single sensillum**
 1114 **recording.** Data are presented as mean \pm s.e.m., with individual data points shown for
 1115 each biological replicate. Solvents used were paraffin oil (P.O) or DMSO.
 1116

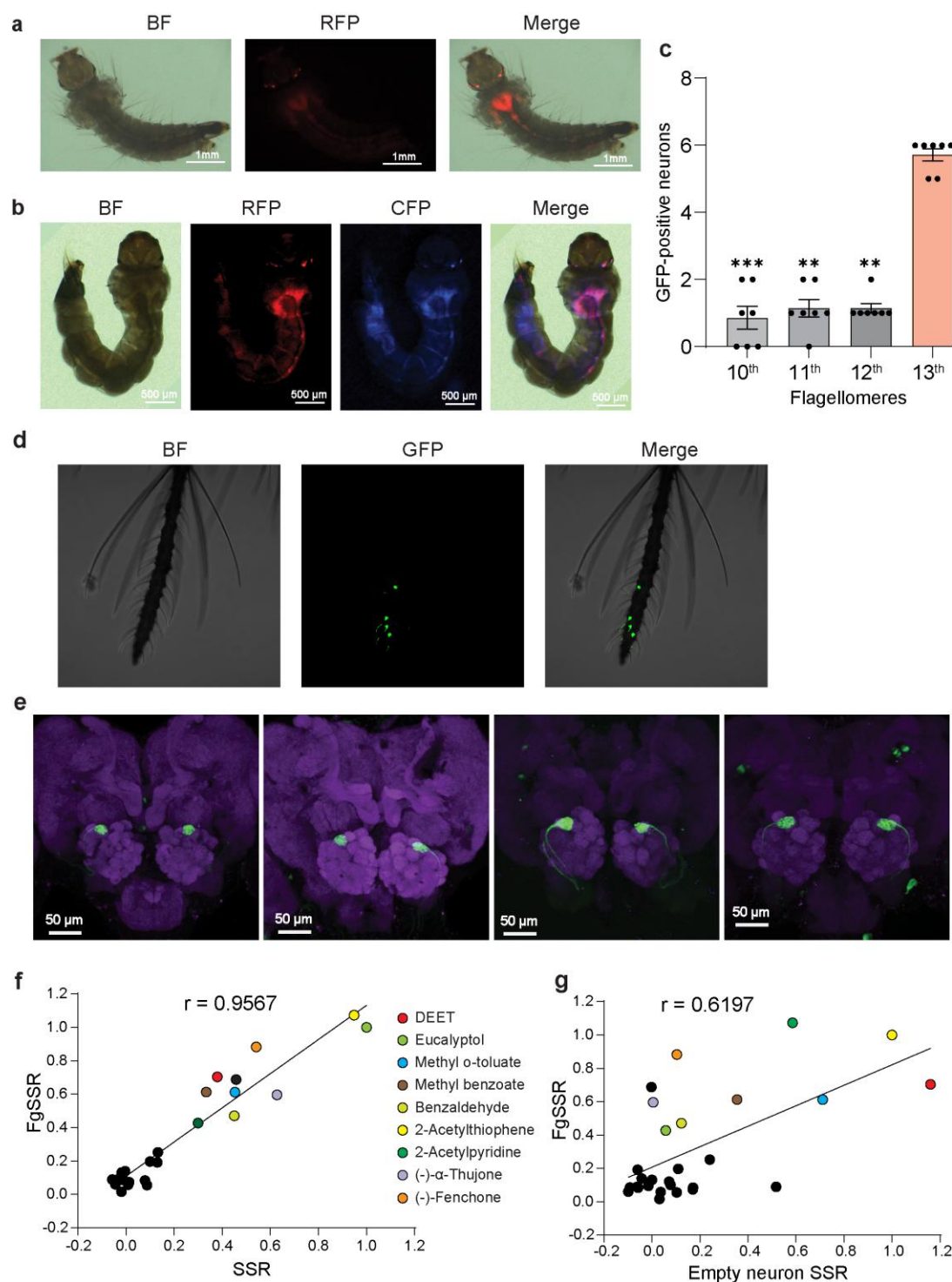


1117
 1118 **Fig. S3. Identification of *AegOr54* in *Ae. Aegypti*.** **a**, Comparison of odorant
 1119 receptor gene expression between the proximal antennal region (flagellomeres 1-9)
 1120 and the distal region (flagellomeres 10-13). Genes are ordered from left to right
 1121 according to decreasing fold-enrichment in the distal region. The y-axis shows fold-
 1122 change values on a log₂ scale. **b**, Representative two-electrode voltage-clamp (TEVC)
 1123 traces showing *AegOr54* responses to DEET (10⁻⁴ M). **c**, Representative TEVC
 1124 traces showing *AegOr55* responses to DEET (10⁻⁴ M). **d**, Representative SSR traces
 1125 from control fruit flies (*Or22ab*^{-/-}; *Dr/TM3*, top) and transgenic flies expressing
 1126 *AegOr54* (*Or22ab*^{-/-}; *UAS-AegOr54*, bottom) in the ab3A neuron in response to 2-
 1127 heptanone and paraffin oil (solvent). **e**, Dose-dependent SSR responses to DEET in
 1128 transgenic lines expressing *AegOr54* in *Drosophila* expression system (n = 5-8).
 1129



1130
 1131 **Fig. S4. Comparison of SSR recordings before and after OR54 knockout.**
 1132 Heatmaps depicting neuronal responses from SSR recordings to paraffin oil (P.O.) and
 1133 DEET (10⁻² v/v) in WT (70 records) and *AegOr54*^{-/-} (88 records) strains. Response

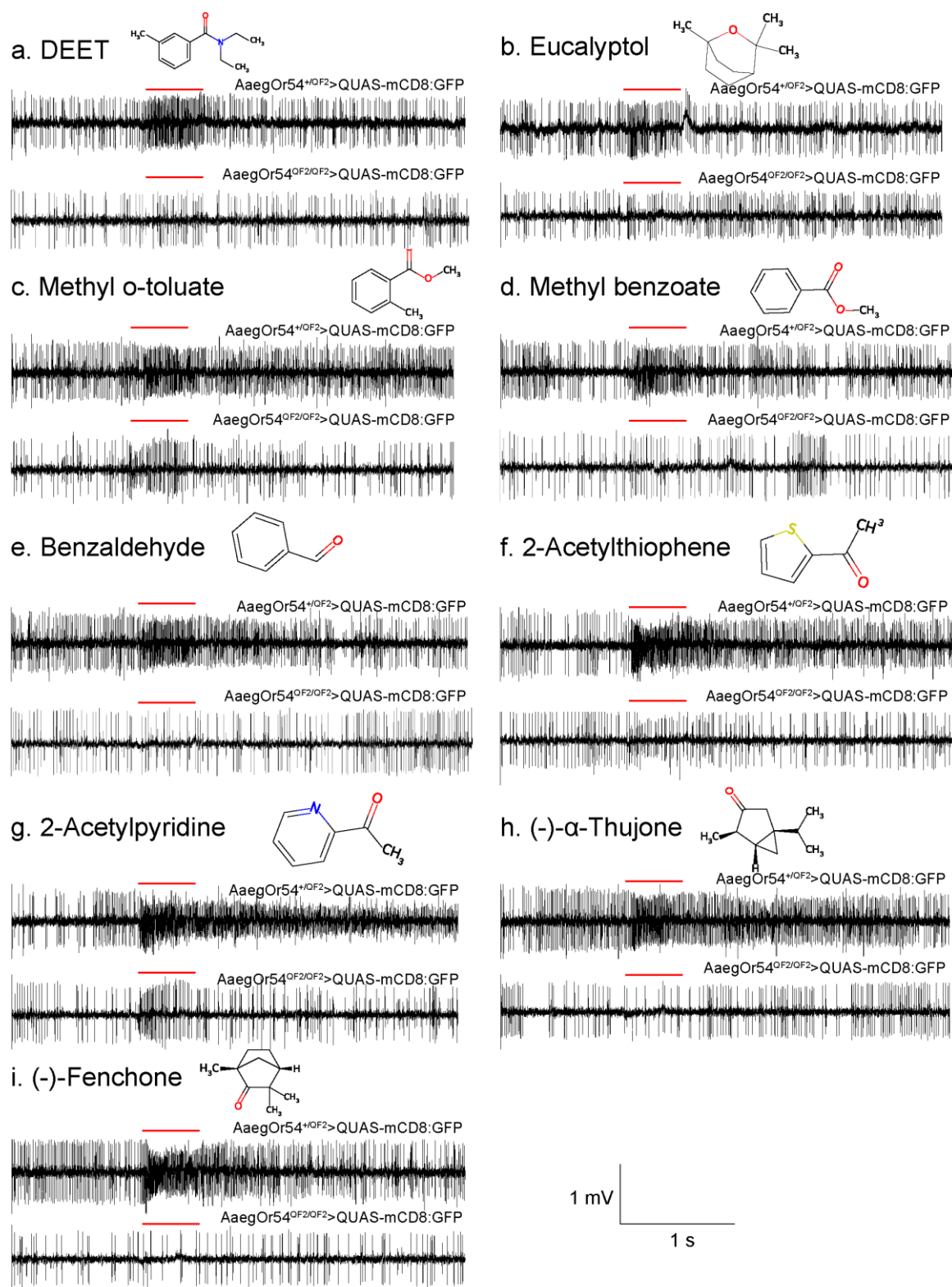
1134 amplitudes range from -20 spikes/s (dark blue) to 80 spikes/s (yellow). Each column
 1135 indicates the SSR recording from different individual sensillum, P.O served as a
 1136 negative control.
 1137



1138

1139 **Fig. S5. Fluorescent labeling of *AaegOr54-QF2* transgenic mosquitoes. a,**
 1140 **Representative images of the *AaegOr54-QF2* knock-in line showing DsRed**
 1141 **fluorescence driven by the $3 \times P3$ promoter in larval eyes. b, Representative images of**
 1142 ***AaegOr54^{+/QF2} > QUAS-mCD8:GFP* larvae showing both DsRed ($3 \times P3$ -DsRed) and**

1143 CFP ($3\times P3$ -CFP) fluorescence in the eyes. **c**, Bar chart summarizing the average
1144 number of *AaegOR54*-positive cell populations across the 10th-13th antenna
1145 flagellomeres (n=7). Statistical analysis was performed using a Kruskal–Wallis test
1146 followed by Dunn’s multiple-comparisons test (**0.001 < P < 0.01; ***0.0001 < P <
1147 0.001). **d**, Representative maximum-intensity projections of confocal z-stacks
1148 showing GFP-labeled SST sensilla on the 13th flagellomere of male antenna. **e**,
1149 Maximum-intensity projections of four additional adult female brains showing GFP-
1150 labeled axons of *AaegOr54*-expressing neurons innervating the antennal lobe. **f**,
1151 Correlation analysis of SST-1 response profiles obtained from wild-type SSR and
1152 fluorescence-guided SSR (FgSSR). Responses were normalized to 2-acetylthiophene
1153 (set to 1), which elicited the strongest response. Compounds eliciting strong response
1154 were labelled with different colors while other compounds eliciting weak response
1155 were labelled as black dots. **g**. Correlation analysis of response profiles obtained by
1156 FgSSR and the the *Drosophila* epmtv neuron expression system. Responses were
1157 normalized to 2-acetylthiophene (set to 1), which elicited the strongest response.
1158 Compounds eliciting strong response were labelled with different colors while other
1159 compounds eliciting weak response were labelled as black dots.
1160



1161

1162 **Fig. S6. Representative FgSSR traces from *AaegOr54*-expressing sensilla.**

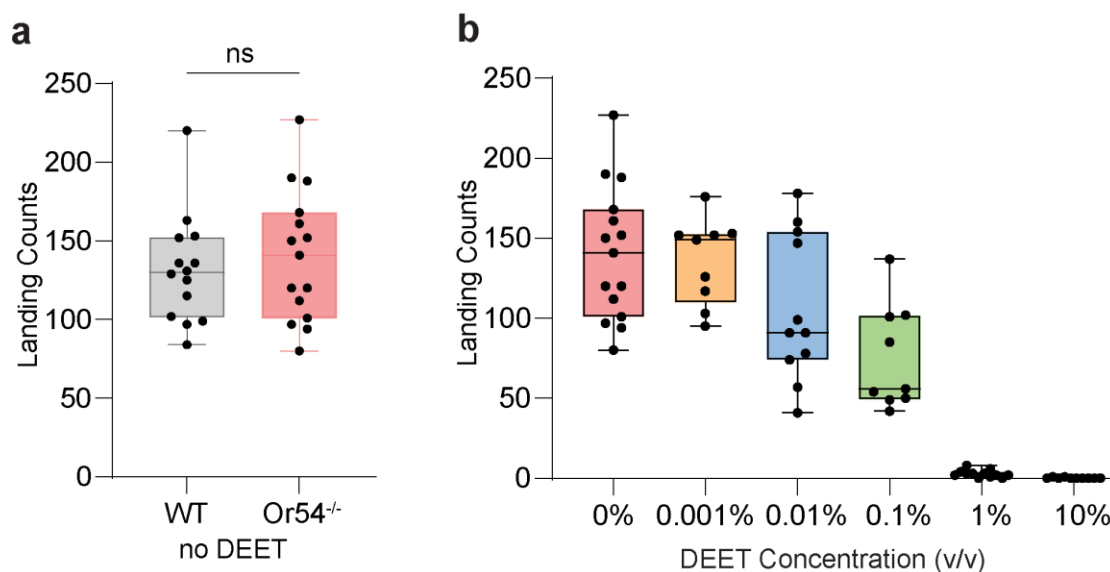
1163 Representative odorant-evoked responses recorded from *AaegOr54*-expressing SST

1164 sensilla in heterozygous (*AaegOr54*^{+/QF2} > *QUAS-mCD8:GFP*) and homozygous

1165 (*AaegOr54*^{QF2/QF2} > *QUAS-mCD8:GFP*) mosquitoes. Responses to (a) DEET, (b)

1166 eucalyptol, (c) methyl o-toluate, (d) methyl benzoate, (e) benzaldehyde, (f) 2-

1167 acetylthiophene, (g) 2-acetylpyridine, (h) (-)- α -thujone, and (i) (-)-fenchone are
 1168 shown.



1169

1170 **Fig. S7. Female *AegOr54* mutants are less sensitive to DEET.** **a**, Landing counts
 1171 of WT and *AegOr54*^{-/-} mosquitoes in the absence of DEET, assessed using the hand-
 1172 in-cage assay (n = 14-15, Mann-Whitney test; ns, not significant). **b**, Effect of DEET
 1173 concentration on landing counts in *AegOr54*^{-/-} mosquitoes (n = 9-15).

1174

1175 **Tables S1 to S5**

1176 **Table S1.** Transcription level of proximal part and distal part of antenna. Values are
 1177 presented as Transcripts Per Million (TPM).

Vectorbase Identifier	Gene name	Proximal flagellomeres (1 st -9 th)			Distal flagellomeres (10 th -13 th)		
		1#	2#	3#	1#	2#	3#
AAEL006003	Or10	42.519957	48.434036	43.835042	16.89739	16.079155	21.696584
AAEL011409	Or100	30.853886	31.183472	26.121842	2.762648	3.611861	9.656852
AAEL017050	Or101	1.601759	0.973521	0.558299	0	0	0
AAEL023017	Or102	0.111459	1.25283	0.284456	0	0	0
AAEL017505	Or103	74.207979	57.040915	74.693258	0	0	1.971402
AAEL016966	Or104	21.09108	31.177808	35.628358	9.3291	16.317778	23.997413
AAEL019984	Or105	17.529422	27.313024	19.569427	18.608793	22.74269	20.358128
AAEL022439	Or107	1.229419	2.316561	0.487761	0.041439	0	0
AAEL018070	Or108	24.550382	34.55031	26.328332	0	0	0
AAEL011583	Or11	57.954556	65.482079	53.317584	64.129978	53.773472	55.664036
AAEL016983	Or110	5.903525	6.917759	13.521553	0	0.313382	0
AAEL024663	Or111	64.671407	82.227519	35.897007	54.07746	39.627936	53.310376
AAEL026829	Or112	74.178339	119.93099 2	66.535789	33.952905	58.687378	56.893589
AAEL017123	Or113	58.543959	42.291562	44.209985	38.151295	22.028645	43.376522
AAEL017219	Or114	17.186495	27.91063	8.148854	0	0	0
AAEL017361	Or115	18.442348	2.518555	18.142895	0	0	0.1561

AAEL025139	Or116	13.024107	12.861399	15.089517	0	0	0
AAEL017377	Or117	8.268929	4.46984	5.435997	0	0	0.571057
AAEL018107	Or118	4.007587	3.063586	1.33536	0	0	0
AAEL017009	Or119	2.626067	1.489589	3.571017	0	0.070838	0
AAEL017104	Or121	21.595388	17.601057	26.417171	0.066994	0	0.148769
AAEL013563	Or122	0.278892	0.675004	9.840987	0	0	0
AAEL017537	Or123	21.152573	21.253609	13.929717	0	0.743462	1.049402
AAEL013893	Or125	1.768132	12.972459	8.519073	0	0	0
AAEL008368	Or13	7.885558	8.118209	7.552106	0	0	0
AAEL010669	Or132	84.069011	83.968643	85.493968	98.008312	72.547283	84.527769
AAEL022696	Or133	3.596177	0.885094	2.181615	10.620224	11.651715	6.084959
AAEL008442	Or14	10.776184	11.237592	17.027646	16.952874	17.332973	16.183721
AAEL008448	Or15	4.379668	4.595396	4.81098	4.398111	7.77585	8.420259
AAEL007110	Or16	5.1817	1.346018	3.361195	64.489554	56.019165	42.18492
AAEL005999	Or2	41.336906	26.177998	41.800004	0.060872	9.09508	15.731008
AAEL025135	Or20	0	0	0	4.912806	16.916373	26.052273
AAEL017398	Or21	3.84233	3.345013	3.73862	43.780125	11.626573	18.656099
AAEL021097	Or22	2.711714	2.308809	0.806281	7.9391	13.453561	7.042364
AAEL001510	Or23	20.374974	14.494314	18.384089	19.456625	7.67917	7.89595
AAEL017557	Or24	5.234801	0	0.464602	34.429322	10.581584	21.055492
AAEL003629	Or25	0.221991	1.458031	0	28.309935	4.332465	12.923096
AAEL010428	Or26	36.735797	14.350762	22.532251	20.471254	13.321798	30.480514
AAEL010418	Or27	19.036547	16.011751	17.797389	74.879633	64.428596	97.172763
AAEL027053	Or28	4.132741	7.29695	4.604707	18.706537	12.425736	25.138376
AAEL022663	Or29	35.203956	24.164046	38.742821	2.702786	0.85283	0.227099
AAEL010409	Or30	5.036191	0.549613	2.218202	36.787288	48.574173	23.896847
AAEL013217	Or31	0	0	0.129782	44.06654	44.107516	49.958059
AAEL023626	Or32	7.767832	2.264384	4.354597	3.540125	3.009558	12.146217
AAEL017362	Or33	0	0.144548	0	7.958718	10.936858	6.89353
AAEL003395	Or34	1.787674	1.975865	2.71854	1.898118	1.708444	3.639511
AAEL016981	Or36	8.715433	8.565562	6.731705	8.355754	4.017164	3.390896
AAEL020435	Or37	1.8018	1.708632	1.701659	0.944058	1.441398	4.466741
AAEL005489	Or38	51.43408	51.012967	70.789334	0.320671	0	0
AAEL015147	Or4	105.907549	103.71533	102.103917	137.480425	76.766313	111.67983 3
AAEL003045	Or41	8.607749	3.461432	8.921876	22.061982	38.231791	28.520972
AAEL000616	Or42	1.522016	0.071315	1.16577	1.16221	5.937422	2.077543
AAEL017294	Or43	2.327413	1.310046	0.198262	8.367358	9.213612	5.391255
AAEL006465	Or44	5.390471	2.058954	1.771207	42.779675	24.023272	36.156871
AAEL000613	Or45	2.053126	1.564385	0.546379	25.312923	20.816809	24.382658
AAEL017079	Or47	53.59655	69.419248	61.223757	99.624919	64.012508	127.63325 7
AAEL020825	Or48	0.867646	0.567268	0.31696	0.373297	0.259248	0

AAEL010426	Or50	3.32819	5.193696	2.404213	17.653887	13.011453	18.503409
AAEL018080	Or51	5.276703	3.695085	5.093447	14.832841	26.724128	22.777232
AAEL013507	Or52	21.497034	18.642898	15.213825	7.39507	12.974361	6.930257
AAEL015286	Or54	0.248278	0	0.422908	25.68359	36.680545	41.651783
AAEL010415	Or55	0	0.116063	0	48.768198	37.668346	49.309785
AAEL027258	Or57	0	0	0.117552	2.607585	3.155483	1.803345
AAEL001342	Or59	30.465925	24.049809	42.960108	0	2.805619	0
AAEL017548	Or6	5.985996	2.06708	6.104397	33.738909	39.439437	41.201224
AAEL000628	Or63	5.410647	3.607426	1.310384	64.942789	57.404427	52.582181
AAEL000614	Or64	4.595981	5.482498	1.636963	55.612639	65.394832	99.222281
AAEL017227	Or66	0	0	0	0.12582	0	0
AAEL001221	Or69	29.029789	19.661665	22.595371	54.441654	44.729253	62.1732
AAEL026167	Or70	7.373551	7.269333	9.21689	13.385967	10.487171	7.763477
AAEL017564	Or71	15.507825	21.547977	14.040062	35.545124	24.310442	43.670332
AAEL017129	Or72	13.741332	13.595023	6.914704	8.594551	6.425981	9.415534
AAEL013418	Or76	0	1.095218	1.591424	0.876916	1.736938	1.017316
AAEL013422	Or77	2.103481	0.193323	1.228608	0.220273	0.420972	3.099583
AAEL013423	Or78	11.074172	9.327964	8.868783	10.619847	8.544367	19.107013
AAEL013420	Or79	62.221278	26.880435	49.060503	26.037745	21.388288	78.7681
AAEL012254	Or8	0	0.143607	0	0	0	0
AAEL017221	Or80	2.698282	1.073987	1.950253	2.508401	1.423277	4.444964
AAEL017305	Or81	5.292919	5.166776	7.161095	6.63495	5.265073	7.50724
AAEL026266	Or82	4.425141	3.717901	2.039762	18.029121	12.778124	11.04622
AAEL017043	Or84	354.026256	337.30365	263.628769	0.649102	9.250079	43.786247
AAEL004218	Or85	32.764468	27.737753	21.418044	0.412431	0.071535	0.533371
AAEL017246	Or86	8.714051	4.04442	5.033863	0	0	1.265734
AAEL017347	Or87	257.069359	236.69734 4	335.914379	40.876451	75.912416	146.19848 4
AAEL014197	Or88	23.473893	18.019226	12.243807	21.491198	19.627504	7.659892
AAEL017149	Or91	12.173119	16.73356	19.850639	0	0	0
AAEL017296	Or93	0.80144	0.666558	2.793444	0	0	0
AAEL017201	Or94	76.346778	53.179572	48.465069	0.162977	0.075367	6.903438
AAEL017427	Or96	10.875248	10.167399	11.273167	0	0.254429	0
AAEL027077	Or96	10.875248	10.167399	11.273167	0	0.254429	0
AAEL017236	Or99	0.996164	1.398813	1.429303	0	0	0

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Table S2. Responses of *AeegOr54* against chemical compounds in TEVC system.

Compounds	<i>AeegOr54</i>		
	Mean	SEM	n
Acetic acid	13.33	6.67	3
Myristic acid	10.00	0.00	3
Hexanoic acid	3.33	3.33	3
Heptanoic acid	3.33	3.33	3

Propionic acid	3.33	3.33	3
Heptadecanoic acid	6.67	3.33	3
(L)-(+)-Lactic acid	0.00	3.65	6
n-Pentadecanoic acid	-1.67	3.07	6
Benzoic acid	-5.00	4.28	6
trans-23-Dimethylacrylic acid	-1.67	3.07	6
(d/l)-3-Methylvaleric acid	-1.67	9.10	6
Octanoic acid	-8.33	6.01	6
Decanoic acid	-13.33	5.58	6
Undecanoic acid	-12.00	5.83	5
Lauric acid	-5.71	2.97	7
n-Tridecanoic acid	-5.00	2.24	6
Adipic acid	1.67	4.01	6
Pimelic acid	-1.67	3.07	6
4-Hydroxybenzoic acid	1.67	1.67	6
Acrylic acid	-1.67	1.67	6
n-Nonanoic acid	-1.67	4.77	6
Oleic acid	3.33	6.67	3
Palmitic acid	-22.00	3.74	5
Hexanal	22.86	2.86	7
Propanal	-13.33	6.67	3
Decanal	10.00	3.78	7
Nonanal	11.43	4.04	7
Benzaldehyde	24.29	7.82	7
Heptanal	17.14	8.65	7
Pentanal	18.57	3.40	7
Octanal	30.00	7.56	7
Butanal	32.86	12.29	7
Isobutanal	22.86	2.86	7
2-Methylbutanal	15.71	5.28	7
Citronellal	15.71	2.02	7
(S)-(-)-Perillaldehyde	-12.86	2.86	7
Citral	2.50	2.50	4
2-Decanol	-16.67	3.33	3
Phenol	0.00	15.28	3
trans-2-Hexen-1-ol	14.29	2.97	7
1-Tetradecanol	0.00	5.77	3
2-Hexadecanol	10.00	5.77	3
cis-2-Hexen-1-ol	22.86	4.21	7
Glycerol	-6.67	3.33	3
1-Hexen-3-ol	3.33	6.67	3
1-Octen-3-ol	0.00	5.77	3
trans-2-Octen-1-ol	7.14	5.22	7

4-Ethyl phenol	0.00	10.00	3
p-Cresol	28.57	5.95	7
Phenelethyl alcohol	-6.67	3.33	3
Isoamyl alcohol	-23.33	8.82	3
Cinnamyl alcohol	13.33	17.64	3
(S)-(-)-Perillyl alcohol	-5.00	2.89	4
α -Terpineol	2.86	1.84	7
(+)-Terpinen-4-ol	5.00	2.89	4
d-Neomethol	5.00	2.89	4
Menthol	-12.50	6.29	4
Geraniol	0.00	7.07	4
Citronellol	-4.29	3.69	7
(S)-cis-Verbenol	4.29	2.97	7
Linalool	2.50	4.79	4
n-Heptadecane	-6.67	3.33	3
Toluene	5.71	4.81	7
1-Hexadecene	0.00	0.00	3
1-Tetradecene	3.33	3.33	3
n-Nonane	-6.67	3.33	3
Benzene	30.00	4.88	7
Squalene	-3.33	3.33	3
Propylbenzene	3.33	6.67	3
n-Pentadecane	3.33	3.33	3
Hexadecane	36.00	11.22	5
trans-2-Octene	10.00	0.00	3
n-Octadecane	3.33	3.33	3
Styrene	13.33	3.33	3
n-Decane	-6.67	3.33	3
Xylene	90.00	41.06	7
Ethylbenzene	8.57	5.08	7
2,4-Dimethylhexane	16.67	3.33	3
trans-4-Octene	13.33	6.67	3
trans-3-Octene	13.33	6.67	3
n-Octane	0.00	0.00	3
2-Pentene	3.33	3.33	3
Hexane	-3.33	3.33	3
Heptane anhydrous	0.00	5.77	3
Thymol	15.00	2.89	4
Carvacrol	10.00	0.00	3
Methyl nonanoate	6.67	8.82	3
Methyl tridecanoate	3.33	6.67	3
Dimethyl phthalate	308.33	38.59	6
Dibutyl phthalate	21.67	1.67	6

Menthyl acetate	111.43	17.52	7
Linalyl acetate	30.00	9.76	7
2-Hexanone	32.86	6.44	7
2-Pentanone	-6.67	3.33	3
3-Pentanone	3.33	3.33	3
2-Decanone	3.33	8.82	3
2-Butanone	17.14	4.74	7
Sulcatone	51.43	14.55	7
(-)-Menthone	55.71	11.31	7
(+)-Menthone	25.00	4.28	6
Geranyl acetone	-7.50	4.79	4
Camphor	15.71	7.19	7
(-)- α -Thujone	50.00	10.00	7
Propylamine	0.00	5.77	3
Butylamine	-3.33	3.33	6
Ammonia	2.00	2.00	5
Carbon disulfide	-2.00	2.00	5
Methyl disulfide	2.00	3.74	5
Urea	10.00	5.77	3
Methylurea	-3.33	6.67	3
Thiourea	135.00	21.41	6
1-Chloroheptane	1.67	4.77	6
Lauryl chloride	421.67	80.31	6
1-Chlorotetradecane	146.00	16.00	5
1-Chlorohexadecane	-11.43	3.40	7
1-Chlorohexane	15.00	22.02	6
Benzyl chloride	-4.29	4.81	7
Indole	8.00	10.68	5
3-Aminopyridine	-3.33	2.11	6
4-Aminopyridine	6.67	3.33	3
1-Methylpiperazine	10.00	0.00	3
2-Methylfuran	-20.00	10.00	2
Thiazolidine	23.33	3.33	3
2,6-Dimethylpyrazine	-4.29	4.81	7
Skatole	-10.00	17.32	3
Coumarin	33.33	79.65	3
n-Piperidineethanol	-30.00	20.00	2
4-Peridinemethanamine	5.00	2.24	6
Pyrazine	28.57	5.95	7
(<i>R</i>)-(+)-Limonene	6.67	3.33	3
(<i>S</i>)-(-)-Limonene	0.00	0.00	3
α -Terpinene	18.57	9.86	7
Myrcene	0.00	0.00	3

α -Pinene	10.00	0.00	4
(+)- α -Pinene	5.00	2.89	4
(-)- α -Pinene	5.00	2.89	4
(-)- β -Pinene	0.00	3.16	5
(+)- β -Pinene	10.00	0.00	4
(+)-3-Carene	5.00	2.89	4
Eugenol	126.67	21.55	6
Terpenolene	24.29	6.12	7
Eucalyptol	22.86	5.22	7
β -Caryophyllene	2.86	2.86	7
(-)-Caryophyllene oxide	5.00	2.89	4
Phytol	4.00	2.45	5
DEET	1462.00	155.09	5
Permethrin	7.50	2.50	4
Naphthalene	14.00	2.45	5
β -Cypermethrin	3.33	3.33	3
Deltamethrin	6.67	3.33	3
IR3535	-3.33	6.67	3
Picaridin	-13.33	6.67	3
Methyl salicylate	0.00	5.77	3
(+)-Fenchone	-13.33	3.33	3
Methyl dihydrojasmonate	3.33	6.67	3
Methyl jasmonate	0.00	5.77	3
PMD	-3.33	3.33	3

1180

1181 **Table S3.** Response profiles of *AeagOr54* in *Drosophila* empty neuron expression
1182 system.

Compound	Responses			Mean	SEM
	1#	2#	3#		
Amylacetate	-5	-8	-12	-8.33	2.03
trans-2-Octen-1-ol	-5	0	-12	-5.67	3.48
DMSO	3	-8	-12	-5.67	4.48
Geraniol	-11	-4	0	-5.00	3.21
Citronellic acid	-7	-2	-4	-4.33	1.45
2,3-Butanedione	-7	2	-6	-3.67	2.85
α -Terpineol	-5	-2	-2	-3.00	1.00
Phenol	-3	-6	0	-3.00	1.73
2-Decanol	-3	-6	0	-3.00	1.73
Furfural	-9	8	-4	-1.67	5.04
2-Propylphenol	-1	-2	-2	-1.67	0.33
2-Picoline	-3	2	-4	-1.67	1.86
Methanol	1	0	-6	-1.67	2.19
1-Pentanol	-5	-2	2	-1.67	2.03

Ethyl propanoate	5	2	-12	-1.67	5.24
Methyl hexanoate	1	0	-6	-1.67	2.19
Hexyl hexanoate	-7	6	-4	-1.67	3.93
Decanoic acid	3	2	-10	-1.67	4.18
Terpinolene	-5	0	2	-1.00	2.08
Methyl disulfide	1	0	-4	-1.00	1.53
2-Methylphenol	1	0	-4	-1.00	1.53
1-Hexanol	1	6	-10	-1.00	4.73
1-Penten-3-ol	-1	-2	0	-1.00	0.58
1-Hexen-3-ol	-7	8	-4	-1.00	4.58
Ethyl hexanoate	-3	2	0	-0.33	1.45
Ethyl benzene	-5	4	2	0.33	2.73
Styrene	-9	2	8	0.33	4.98
Xylene	-5	-2	8	0.33	3.93
Indole	1	8	-8	0.33	4.63
Skatole	-5	0	6	0.33	3.18
4-Methylthiazole	-1	2	0	0.33	0.88
3-Methyl-2-buten-1-ol	-1	-4	6	0.33	2.96
1-Octen-3-ol	-9	16	-6	0.33	7.88
Cinnamyl alcohol	5	0	-4	0.33	2.60
(<i>E</i>)-3-Hexenol	-3	2	2	0.33	1.67
Isopentyl acetate	-5	2	4	0.33	2.73
Ethyl propionate	7	-2	-4	0.33	3.38
Ethyl octanoate	3	0	-2	0.33	1.45
trans-3-Octene	-3	0	4	0.33	2.03
Hexanal	3	0	-2	0.33	1.45
Nonanal	1	2	-2	0.33	1.20
α -(-)-Thujone	-5	0	6	0.33	3.18
Butanoic acid	1	6	-6	0.33	3.48
(<i>Z</i>)-2-Hexenol	-3	2	4	1.00	2.08
Hexyl acetate	-5	4	4	1.00	3.00
ethyl-3-Hydroxybutyrate	-1	10	-6	1.00	4.73
Propional	-5	6	2	1.00	3.21
Acetaldehyde	1	8	-6	1.00	4.04
Undecanoic acid	-3	2	4	1.00	2.08
β -Myrcene	-3	12	-2	2.33	4.84
Limonene	-5	-2	14	2.33	5.90
Eugenol	-1	8	0	2.33	2.85
(-)-Linalool	-13	6	14	2.33	8.01
(-)-Menthone	1	4	2	2.33	0.88
3-methylthio-1-propanol	1	6	0	2.33	1.86
Benzyl alcohol	1	10	-4	2.33	4.10
2-Ethyl toluene	-3	2	8	2.33	3.18

2-Methyl furan	5	4	-2	2.33	2.19
Ethyl formate	-5	8	4	2.33	3.84
Methyl nonanoate	3	-2	6	2.33	2.33
trans-4-Octene	-3	4	6	2.33	2.73
trans-Cinnamaldehyde	1	4	2	2.33	0.88
Heptanal	5	6	-4	2.33	3.18
α -Terpinene	-1	8	2	3.00	2.65
Eucalyptol	-5	12	2	3.00	4.93
Benzyl methyl ether	3	12	-6	3.00	5.20
Benzyl acetate	3	12	-6	3.00	5.20
3-Methylphenol	5	0	4	3.00	1.53
4-Methylphenol	-3	10	2	3.00	3.79
2-Pentanol	3	4	2	3.00	0.58
(E)-2-Hexenyl acetate	-3	0	14	3.67	5.24
Ethyl butyrate	-5	6	10	3.67	4.48
Dodecanoic acid	-1	4	8	3.67	2.60
Pentanoic acid	-7	10	8	3.67	5.36
(S)-(-)-Perillaldehyde	5	4	4	4.33	0.33
6-Methyl-5-hepten-2-one	3	14	-4	4.33	5.24
Methyl acetate	-7	12	8	4.33	5.78
Menthol	-1	6	10	5.00	3.21
Citronellal	9	6	0	5.00	2.65
(+)-Terpinen-4-ol	-5	20	0	5.00	7.64
Dimethylsulfide	5	2	10	5.67	2.33
Thiazole	5	6	6	5.67	0.33
γ -Hexalactone	1	12	8	7.00	3.21
Benzene	3	12	6	7.00	2.65
3-Octanone	5	18	2	8.33	4.91
4,5-Dimethylthiazole	11	14	0	8.33	4.26
Methyl octanoate	-3	14	14	8.33	5.67
2-Acetylthiazole	11	14	8	11.00	1.73
2,4,5-Trimethylthiazole	11	22	10	14.33	3.84
1-Indanone	9	24	20	17.67	4.48
Methyl-2-methyl-benzoate	23	40	6	23.00	9.81
2-Acetylthiophene	25	40	28	31.00	4.58
DEET	21	40	46	35.67	7.54
Acetophenone	15	44	18	25.67	9.21
2-Acetylpyridine	5	28	24	19.00	7.09
Naphthalene	7	28	16	17.00	6.08
2-Isobutylthiazole	9	24	4	12.33	6.01
Methyl benzoate	13	16	4	11.00	3.61
Propylbenzene	7	10	14	10.33	2.03
β -Caryophyllene	5	8	12	8.33	2.03

Carbon disulfide	1	10	12	7.67	3.38
Neomenthol	5	8	10	7.67	1.45
2-Ethoxythiazole	1	12	6	6.33	3.18
Hexyl butyrate	-3	10	12	6.33	4.70
Geranyl acetate	-7	6	18	5.67	7.22
4-Methylcyclohexanol	-1	12	4	5.00	3.79
Pentyl acetate	3	6	6	5.00	1.00
Ethyl lactate	3	14	-2	5.00	4.73
(L)-(+)-lactic acid	3	6	6	5.00	1.00
Methyl acetate	-7	12	8	4.33	5.78
(-)-Fenchone	1	8	2	3.67	2.19
Linalyl acetate	-3	6	8	3.67	3.38
2-Hexanone	5	10	-4	3.67	4.10
Benzaldehyde	-1	2	10	3.67	3.28
2,6-Dimethylpyrazine	-9	6	14	3.67	6.74
1-Heptanol	1	4	6	3.67	1.45
1-Hepten-3-ol	3	8	0	3.67	2.33
(E)-2-hexenol	1	4	4	3.00	1.00
Dimethyl phthalate	7	4	-2	3.00	2.65
Pentanal	-1	6	4	3.00	2.08
Octanal	-5	8	6	3.00	4.04
Decanal	-1	12	-2	3.00	4.51
(S)-(-)-Limonene	-5	6	4	1.67	3.38
Citronellol	-1	0	6	1.67	2.19
(-)-Carvone	3	0	2	1.67	0.88
(+)-Fenchone	-7	2	10	1.67	4.91
Linalool oxide	3	-2	4	1.67	1.86
Linalool	5	-4	4	1.67	2.85
2-Nonanone	9	-4	0	1.67	3.84
Phenethyl acetate	-3	-2	10	1.67	4.18
Methyl salicylate	9	14	-18	1.67	9.94
Phenylacetaldehyde	-7	-2	14	1.67	6.33
Coumarin	-5	4	6	1.67	3.38
3-Methyl-1-butanol	3	6	-4	1.67	2.96
2,3-Butanediol	-3	10	-2	1.67	4.18
Ethyl methanoate	-3	8	0	1.67	3.28
Ammonia	-5	10	0	1.67	4.41
α -Pinene	-7	6	4	1.00	4.04
Nerol	-5	6	2	1.00	3.21
2-Heptanone	1	6	-4	1.00	2.89
Cyclohexanone	-5	10	-2	1.00	4.58
Ethyl cinnamate	-3	-4	10	1.00	4.51
4-Ethylphenol	-5	-2	10	1.00	4.58

1-Dodecanol	1	4	-2	1.00	1.73
(R)-(+)-Limonene	-11	6	4	-0.33	5.36
3-Carene	-1	8	-8	-0.33	4.63
(-)- α -Pinene	-3	10	-8	-0.33	5.36
(-)- β -Pinene	-5	4	0	-0.33	2.60
Citral	-7	8	-2	-0.33	4.41
2-Decanone	-1	4	-4	-0.33	2.33
Phenethyl alcohol	1	0	-2	-0.33	0.88
Ethyl benzoate	-5	8	-4	-0.33	4.18
1-Butanol	-1	-2	2	-0.33	1.20
Propyl acetate	-5	2	2	-0.33	2.33
Methyl propanoate	1	2	-4	-0.33	1.86
Ethyl hexanoate	-3	2	0	-0.33	1.45
trans-2-Octene	-5	4	0	-0.33	2.60
Tridecanoic acid	1	0	-2	-0.33	0.88
(+)-Pulegone	-3	8	-6	-0.33	4.26
(1S)-(+)-3-Carene	-7	4	4	0.33	3.67
(+)- α -Pinene	-5	6	0	0.33	3.18
Geranyl acetone	-1	0	2	0.33	0.88
o-Cresol	-5	4	2	0.33	2.73
Butyl acetate	-5	4	-2	-1.00	2.65
Isoamyl acetate	3	-2	-4	-1.00	2.08
Methyl butylate	3	2	-8	-1.00	3.51
Isobutanal	-1	0	-2	-1.00	0.58
2-Methylbutanal	-3	10	-10	-1.00	5.86
Pyruvic acid	-5	4	-2	-1.00	2.65
Paraffin oil	1	-4	0	-1.00	1.53
(S)-cis-Verbenol	-9	6	-2	-1.67	4.33
2-Pentanone	-5	6	-6	-1.67	3.84
3-Pentanone	1	0	-6	-1.67	2.19
Thymol	-7	0	0	-2.33	2.33
(+)-Carvone	-9	6	-4	-2.33	4.41
Menthyl acetate	-13	10	-4	-2.33	6.69
Estragole	-7	6	-6	-2.33	4.18
1-Octanol	-1	4	-10	-2.33	4.10
2-Ethyl-1-hexanol	-1	-2	-4	-2.33	0.88
Hexanoic acid	-1	2	-8	-2.33	2.96
Ethyl trans-2-butenoate	-1	-2	-6	-3.00	1.53
Butanal	-3	2	-8	-3.00	2.89
Linoleic acid	-7	8	-10	-3.00	5.57
2-Ethylphenol	-3	-6	-2	-3.67	1.20
1-Propanol	-1	0	-10	-3.67	3.18
Isobutyl acetate	-3	8	-16	-3.67	6.94

γ -Octalactone	-5	4	-18	-6.33	6.39
Camphor	-17	0	-6	-7.67	4.98

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Table S4. Sequencing of the mutants. The genomic sequence, inserted sequence, and stop codon are shown in black, red, and green, respectively.

Mutant	Sequence
AegOr54 ^{-/-}	<p>GATAATCCAATTACCATCCGCGTTATTTAAAGATAATTGCGTTTTTATTGTCAGGGA GTGAGTTTGCTTAAAACTCGTTTAGATCCAATTCGAGCTCGCCCGGGATCTAATT CAATTAGAGACTAATTCAATTAGAGCTAATTCAATTAGGATCCAAGCTTATCGATTTT GAACCCTCGACCGCCGGAGTATAAATAGAGGCGCTTCGTCTACGGAGCGACAATTC AATTCAAACAAGCAAAGTGAACACGTCGCTAAGCGAAAGCTAAGCAAATAAACAA GCGCAGCTGAACAAGCTAAACAATCGGGGTACCGCTAGAGTCGACGGTACCGCGG GCCCCGGATCCACCGTCCACCATGGCCTCCTCCGAGAACGTCATCACCGAGTT CATGCGCTTCAAGGTGCGCATGGAGGGCACCGTGAACGGCCACGAGTTCGAGATCG AGGGCGAGGGCGAGGGCCGCCCTACGAGGGCCACAACACCGTGAAGCTGAAGGT GACCAAGGGCGGCCCTTCCCTTCGCCTGGGACATCCTGTCCCCCAGTTCCAGT ACGGCTCCAAGGTGTACGTGAAGCACCCCGCCGACATCCCCGACTACAAGAAGCTG TCCTTCCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGT GGCGACCGTGACCCAGGACTCCTCCCTGCAGGACGGCTGCTTCATCTACAAGGTGA AGTTCATCGGCGTGAACCTCCCCTCCGACGGCCCCGTGATGCAGAAGAAGACCATG GGCTGGGAGGCCTCCACCGAGCGCCTGTACCCCCGCGACGGCGTGCTGAAGGGCG AGACCCACAAGGCCCTGAAGCTGAAGGACGGCGGCCACTACCTGGTGGAGTTCAA GTCCATCTACATGGCCAAGAAGCCCGTGCAGCTGCCCGGCTACTACTACGTGGACG CCAAGCTGGACATCACCTCCCACAACGAGGACTACACCATCGTGGAGCAGTACGAG CGCACCGAGGGCCGCCACCACCTGTTCCCTGTAGCGGCCGCGACTCTAGATCATAATC AGCCATAACCACATTTGTAGAGGTTTTACTTGCTTTAAAAAACCTCCACACCTCCCC CTGAACCTGAAACATAAAATGAATGCAATTGTTGTTGTTAACTTGTTTATTGCAGCTT ATAATGGTTACAAATAAAGCAATAGCATCACAATTTACAAATAAAGCATTTTTTTC ACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTATAATGGATGCAGA GTGAGTCACC</p>
AegOr54-QF	<p>GATAATCCAATTACCATCCGCGGAGGGCAGAGGAAGTCTTCTAACATGCGGTGAC GTGGAGGAGAATCCCGGCCCTATGCCACCCAAGCGCAAAACGCTTAACGCTGCGGC TGAGGCTAACGCTCATGCCGACGGACACGCCGACGAAACGCCGACGGACACGTG GCCAATACGGCCGCGTCTCGAATAATGCGAGGTTTCGTGATCTACTAACATCGAT ACTCCGGGTCTGGGACCCACAACACTACGACCCTGCTCGTGAACACGACGCTCAA GCGTCAACGAGTGTCCCGCGCATGCGACCAGTGCCGTGCAGCCGAGAGAAATGC GACGGAATACAGCTGCGTGTTCCTGCGTTCCTCCAGGGAAGGTCCTGCACTTAT CAGGCTTCGCCGAAAAGAGGGGAGTTCAAACCGTTATATTTCGTACGCTGGAGCT CGCCCTCGCCTGGATGTTGAAAATGTGCGCGGTTCCGAAGATGCCCTGCATAACCT CCTCGTCCGTGACGCCGACAAGGATCAGCTCTGCTCGTTGGTAAAGATTGCCCGG CTGCCGAGCGACTCCATGCCCGTTGGGCTACTAGCCGTGCAATAAGAGCATTACCC</p>

	<p>GCCTCTCCGTCAGTTGGAGCTCCCTCTACCGCCACGGCTACGGCCTCGATAATGC CGCACGTGATGGAGCAGCCTCTCAGTACCAGCATTAAACCCCGTCAACGACCGCTTC AACGGTATTCCCAACCCCACTCCGTATAACTCCGATGCAGCTCTCGATGCTATCACTC AGACCAACGATTATGGAAGCGTAAATACACATGGTATCCTCTCTACTTACCCGCCAC CGGCTACGCACCTTAATGAAGCTTCCGTCGCTCTCGCTCCCGGTGGCGCCCCCCCC GACCGCCTCTCCGTATGTTGACAGCACGACCAATCACCCGCGTACCACTCGAATC TGGTTCCAATGGCGAACTTTGGTTACTCGACCGTTGATTACGATGCCATGGTTGACG ATTTGGCTAGCATTGAATACACGGACGCTGTGGATGTGACCCACAGTTTATGACCA ATCTGGGATTCGTTCCCTGGATGTAACCTTCTCCGACATTAATACATACGAACAGTGATG ACGTCTATCGATACCGTCGACTAAAGCCAAATAGAAAATTATTAGTTCCCTGGCTTAA GTTTTTAAAAGTGATATTATTTATTTGGTTGTAACCAACCAAAAAGAATGAAATAACT AATACATAATTATGTTAGTTTTAAGTTAGCAACAAATTGATTTTAGCTATATTAGCTAC TTGGTTAATAAATAGAATATATTTATTTAAAGATAATTGCGTTTTTATTGTCAGGGAGT GAGTTTGCTTAAAACTCGTTTAGATCCAATTCGAGCTCGCCCGGGGATCTAATTCA ATTAGAGACTAATTCAATTAGAGCTAATTCAATTAGGATCCAAGCTTATCGATTTTCA ACCCTCGACCGCCGGAGTATAAATAGAGGCGCTTCGTCTACGGAGCGACAATCAAT TCAAACAAGCAAAGTGAACACGTCGCTAAGCGAAAGCTAAGCAAATAAACAAGCG CAGCTGAACAAGCTAAACAATCGGGGTACCGCTAGAGTCGACGGTACCGCGGGCCC GGGATCCACCGGTCCGACCATGGCCTCCTCCGAGAACGTCATCACCGAGTTCATG CGTTCAAGGTGCGCATGGAGGGCACCGTGAACGGCCACGAGTTCGAGATCGAGG GCGAGGGCGAGGGCCGCCCTACGAGGGCCACAACACCGTGAAGCTGAAGGTGAC CAAGGGCGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCCCCCAGTTCAGTACG GCTCCAAGGTGTACGTGAAGCACCCCGCCGACATCCCCGACTACAAGAAGCTGTCC TTCCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGG CGACCGTGACCCAGGACTCCTCCCTGCAGGACGGCTGCTTCATCTACAAGGTGAAG TTCATCGGCGTGAACCTCCCCCTCCGACGGCCCCGTGATGCAGAAGAAGACCATGGG CTGGGAGGCCTCCACCGAGCGCCTGTACCCCCGCGACGGCGTGCTGAAGGGCGAG ACCCACAAGGCCCTGAAGCTGAAGGACGGCGGCCACTACCTGGTGGAGTTCAAGT CCATCTACATGGCCAAGAAGCCCGTGCAGCTGCCCGGCTACTACTACGTGGACGCC AAGCTGGACATCACCTCCCAACGAGGACTACACCATCGTGGAGCAGTACGAGCG CACCGAGGGCCGCCACCACCTGTTCCCTGTAGCGGCCGCGACTCTAGATCATAATCAG CCATACCACATTTGTAGAGGTTTTACTTGCTTTAAAAAACCTCCACACCTCCCCCTG AACCTGAAACATAAAATGAATGCAATTGTTGTTGTTAACTTGTTTATGACGCTTATA ATGGTTACAAATAAAGCAATAGCATCACAAATTCACAAATAAAGCATTTTTTTCACT GCATTCTAGTTGTGGTTTGTCAAACTCATCAATGTATCTTATAATGGATGCAGAGTG AGTCACC</p>
<p>AagOr55^{-/-}</p>	<p>GGATTGGTTCCCGCTATTTTAAACCAACCCGGTGCTAGGCAGCCATGTTTTCGTGGTC AGCATCAAACCTCAAGAGCAATAACGTACATTGGCTTAATCATATAAAGGAATTTTTCC TCCTATACTAAATGTGTTTCTAGCTAATCATCAACCCAAATTGACTTGCTGTATT GCAATCCAATATTTGTAAACCGTTTATTTGTTTCAATAACTGTTGTTTTTACGGAAAA ATATTTTTTTCGGAGAAAAAGATTTCCGCATCATCGATTGTTATTCCGCAAACGGTTA TTTGTTTACAAACATTGAGCTGTCAGTGGGAACCACTTCCAGTGGGAACAGAGA</p>

	<p style="color: red;">TGTTTGCTCGTTATTTTTCAATGGGGTTGTATGGGCGGTGTCAGGAGGCTGATTTTAA</p> <p style="color: red;">CGACATACCGGTACTGTA ACTCC</p>
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1187 **Table S5.** Primers used in the project.

Primer name	Sequence
pT7Ts-AaegOr55F	GCAGATCTGATATCGCCACCATGCAGCAAAAACCTCCG
pT7Ts-AaegOr55R	GGGGTACCGGGCCCACTAGTCTATTCTCCGTAGA ACTGCT
pT7Ts-AaegOr31F	GCAGATCTGATATCGCCACCATGGCGCCCAACCAAAATG
pT7Ts-AaegOr31R	GGGGTACCGGGCCCACTAGTTTAATTATCAGTGAATGATAAAAGCAAAGC
pT7Ts-AaegOr54F	GCAGATCTGATATCGCCACCATGAATACTAATTCACAA
pT7Ts-AaegOr54R	GGGGTACCGGGCCCACTAGTTCACTTATCTCCATAGAATT
pT7Ts-AaegOr57F	GCAGATCTGATATCGCCACCATGGTCGTAATCGCATCGATG
pT7Ts-AaegOr57R	GGGGTACCGGGCCCACTAGTCTACTCTTCTCCGTAGA ACCG
pT7Ts-AaegOr20F	GCAGATCTGATATCGCCACCATGGATTTGCTGAACAAACTACAG
pT7Ts-AaegOr20R	GGGGTACCGGGCCCACTAGTTCAATCCTTTACACGCAAGAGC
pT7Ts-AaegOr25F	GCAGATCTGATATCGCCACCATGGAGGATGCAAAAATTCGG
pT7Ts-AaegOr25R	GGGGTACCGGGCCCACTAGTCTATGAATTTGTTTTTTGCTTAGTATGG
pT7Ts-AaegOr33F	GCAGATCTGATATCGCCACCATGGAACAGCACTTGAAGAAAATC
pT7Ts-AaegOr33R	GGGGTACCGGGCCCACTAGTTTATTTGCTAACCGTTCTAAACAAAG
pUASTattBBackboneF	GAATTCGTTAACAGATCTGCG
pUASTattBBackboneR	GAATTCCTCAATCCCTATTCA
pUASTattB-AaegOr54F	TGAATAGGGAATTGGGAATTCATGAATACTAATTCACAACCTCCG
pUASTattB-AaegOr54R	CGCAGATCTGTTAACGAATTCTCACTTATCTCCATAGAATTGCTGC
AaegOr54gRNA	TCACTCTGCATCCATTACCGCGG
AaegOr54ExamF	CCATCGTATTTGTTCAAGCTGG
AaegOr54ExamR	CACGGTATGGCCGAACAGTAC
AaegOr55gRNA	ATTACCTTCAGCGGTTATGGAGG
AaegOr55ExamF	GATCTACGTATCCATCGTATTC
AaegOr55ExamR	GCAACCATATCCAGTAATTGG
HDR-AaegOr54LAF	GTTGTAAAACGACGGCCAGTTTCGGCTAAGATCCTATCCCTG
HDR-AaegOr54LAR	GAAGACTTCCTCTGCCCTCCGCGGATGGTAATTGGATTATC
HDR-AaegOr54RAF	CTCATCAATGTATCTTATAATGGATGCAGAGTGAGTCACC
HDR-AaegOr54RAR	CACAGGAAACAGCTATGACCTTTCATTCATATCTCACAAAG
HDR-(T2A-QF-DsRed)F	GTCATAGCTGTTTCCTGTGTG
HDR-(T2A-QF-DsRed)R	ACTGGCCGTCGTTTTACAAC
HDR-(AmpR)F	GAGGGCAGAGGAAGTCTTC
HDR-(AmpR)R	TAAGATACATTGATGAGTTTGGACAAACC

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