

1 **Predator-secreted sulfolipids induce fear-like defense responses in *C. elegans***

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14

15 **Abstract**

16 Animals respond to predators by altering their behavior and physiological states, but the
17 underlying signaling mechanisms are poorly understood. Using the interactions between
18 *Caenorhabditis elegans* and its predator, *Pristionchus pacificus*, we show that neuronal
19 perception by *C. elegans* of a predator-specific molecular signature induces instantaneous escape
20 behavior and a prolonged reduction in oviposition. Chemical analysis revealed this predator-
21 specific signature to consist of a class of sulfolipids, produced by a biochemical pathway
22 required for developing predacious behavior and specifically induced by starvation. These
23 sulfolipids are detected by four pairs of *C. elegans* amphid sensory neurons that act redundantly
24 and recruit cyclic nucleotide-gated (CNG) or transient receptor potential (TRP) channels to drive
25 both escape and reduced oviposition. Specific abolishment of predator-evoked *C. elegans*
26 responses by the anti-anxiety drug sertraline as well as functional homology of the delineated
27 signaling pathways suggests a conserved or convergent strategy for managing predator threats.

28

29 **Introduction**

30 Animal survival depends on the ability to sense predators and generate specific behavioral
31 responses, such as flight or freezing. Examples from multiple vertebrate and invertebrate species
32 indicate that prey use multiple sensory modalities (including vision, audition, and most
33 frequently olfaction) to detect predators¹⁻³. In particular, the chemosensory neurons in the rodent
34 vomeronasal organ (VNO), Grueneberg ganglion, and main olfactory epithelium have been
35 shown to have the ability to detect chemical signals from cat urine and generate stereotyped
36 defensive behaviours^{4,5}. The nature of these sensory circuits and signaling pathways that drive
37 these invariant defensive behaviors, however, have remained elusive.

38 We approached this question by analyzing the behavioral responses of the nematode
39 *Caenorhabditis elegans*⁶ to a predatory nematode *Pristionchus pacificus*⁷. These two nematodes
40 likely shared a common ancestor around 350 million years ago⁸. Recent studies have shown that
41 *P. pacificus* is a facultative predator. *P. pacificus* can bite and kill *C. elegans*⁹, a process
42 facilitated by the extensive re-wiring of the *P. pacificus* nervous system under crowded and/or
43 starvation conditions¹⁰. The nematode *C. elegans*, with its fully mapped neural network
44 comprising of just 302 neurons connected by identified synapses¹¹ and powerful genetic tools, is
45 ideally suited for a molecular and circuit-level analysis of complex behaviors¹²⁻¹⁵. Combining
46 chemical and genetic methods, we dissected the signaling circuits underlying *C. elegans*'
47 responses to *P. pacificus*. We found that a novel class of sulfated small molecules excreted by *P.*
48 *pacificus* trigger defensive responses in *C. elegans*. These *P. pacificus*-derived chemical signals
49 are detected by *C. elegans* via multiple sensory neurons and processed via conserved
50 neurotransmitter signaling pathways. Our results suggest that signaling pathways that process
51 predator threats are likely conserved between *C. elegans* and more complex animals.

52

53 **Results**

54 ***C. elegans* generates rapid avoidance and reduced egg laying in response to a predator**

55 *C. elegans* was originally isolated from compost heaps in the developmentally arrested dauer
56 stage^{16,17}. However, recent studies have isolated proliferating and feeding populations of *C.*
57 *elegans* from rotting flowers and fruits¹⁷⁻¹⁹, where they are often found to cohabit with other
58 nematodes including the Diplogastrid *Pristionchus*¹⁶. Previous reports have shown that the
59 terrestrial nematode, *P. pacificus* can kill and consume the smaller nematode *C. elegans*⁹.

60 Further, when these two nematodes are placed together on an agar plate, *C. elegans* rapidly
61 avoids *P. pacificus* (data not shown). We hypothesized that the prey, *C. elegans*, detects the
62 predator, *P. pacificus*, through chemical cues and thus tested *C. elegans* responses to *P. pacificus*
63 excretions. We found that *C. elegans* showed immediate avoidance behavior upon perceiving
64 excretions of starved, but not well-fed predators (Fig. 1a and Supplementary Fig. S1a). Based on
65 this result, we collected excretions from *P. pacificus* after 21 hours of starvation (“predator cue”)
66 and found that it consistently repelled genetically diverse *C. elegans* isolates (Supplementary
67 Fig. S1b). Chemotaxis assays indicated that these *C. elegans* avoidance behaviors were not in
68 response to volatile components of predator cue (Supplementary Figs. S1c, S1d).

69 We further found that *C. elegans* exposed to predator cue did not lay eggs for many
70 minutes following exposure, even when placed on food (bacterial lawn), suggesting that predator
71 cue-induced stress affects egg laying behavior. Consistent with this idea, previous studies have
72 shown that *C. elegans* retain eggs in the gonad when exposed to environmental stressors²⁰. To
73 test our hypothesis, we designed a behavioral assay wherein the prey was exposed to predator
74 cue for 30 minutes, and egg laying was monitored for many hours following cue removal.
75 Animals exposed to predator cue laid significantly fewer eggs than controls during the initial 60
76 minutes following cue removal. During the next hour (i.e., the 60–120-minute post-cue time
77 period), these animals laid more eggs than controls, suggesting that predator cue transiently
78 modified egg laying behavior, but not egg production (Fig. 1b). Collectively, these results
79 indicate that starving *P. pacificus* release a potent, non-volatile factor (predator cue) that elicits
80 multiple prey responses, namely urgent escape behavior followed by up to one hour of reduced
81 egg laying.

82

83 **Predator releases a novel family of sulfolipids to drive *C. elegans* responses**

84 We aimed to identify chemical structure(s) of the small molecule(s) excreted by *P. pacificus* that
85 caused *C. elegans* avoidance behavior. Because the *P. pacificus* exo-metabolome is highly
86 complex, consisting of more than 20,000 distinct compounds detectable by UPLC-HRMS (Fig.
87 1c), we used a multi-stage activity-guided fractionation scheme (see Supplementary Methods).
88 After three rounds of fractionation, comparative analysis of 2D NMR spectra^{21,22} and high-
89 resolution tandem mass spectrometry data of active and adjacent inactive fractions (Figs. 1d, 1e,
90 Supplementary Tables S1-S3), revealed several novel (ω -1)-branched-chain sulfolipids (sufac#1,

91 sufac#2, sufal#1, and sufal#2) as major components of active, but not inactive fractions (Fig. 1f).
92 We then synthesized these compounds and tested their activity in the avoidance assay. The
93 terminal alcohols sufal#1 and sufal#2 accounted for most of the isolated activity (Fig. 1g,
94 Supplementary Fig. S3a, Supplementary methods). Following identification of these compounds,
95 we re-analyzed the *P. pacificus* exo-metabolome by UPLC-MS/MS, which revealed a large
96 number of structurally related sulfolipids (Fig. 1h). None of the *P. pacificus* sulfolipids could be
97 detected in the metabolomes of *E. coli* (used as food for nematodes) (Supplementary Fig. S2), *C.*
98 *elegans*, or several other nematode species (Supplementary Table S4), which were extracted and
99 analyzed under identical conditions. Notably, the identified sulfolipids are structurally similar to
100 sodium dodecyl sulfate (SDS, Fig. 1i), which is a potent *C. elegans* avoidance cue²³. Additional
101 assays showed that the sulfolipids identified from *P. pacificus* excretions also attenuated *C.*
102 *elegans* egg laying (Supplementary Fig. S3b), demonstrating that these predator-specific small
103 molecules instruct both rapid and longer-lasting prey responses.

104

105 **Multiple *C. elegans* sensory neurons act redundantly to generate predator avoidance**

106 To define the prey neural circuit that detects predator cue, we tested the role of all 12 pairs of
107 amphid sensory neurons, which project dendrites to the nose of the animal to sense
108 environmental changes (Fig. 2a)^{11,24}. Previous studies have shown that sensory neurons in the
109 amphid ganglia located in the head of the worm detect repellents and generate reversals in an
110 attempt to avoid the noxious cues^{24,25}. We found that animals lacking pairs of ASJ, ASH, ASI, or
111 ADL neurons (but not other amphid neurons) were defective in their responses to predator cue
112 (Figs. 2b, 2c), indicating that *C. elegans* uses multiple sensory neurons to detect predators.
113 Responses to sulfolipids purified from predator cue and SDS were similarly reduced in animals
114 lacking these neurons (Supplementary Figs. S3f-g).

115 To confirm the role for ADL, ASH, ASI, and ASJ neurons, we monitored their responses
116 to predator cue using calcium imaging¹⁴. Calcium responses are strongly correlated with
117 neuronal activity in *C. elegans* neurons^{26,27}. We found that adding predator cue to the nose of the
118 prey activated ADL and ASH (Figs. 2d, 2e, Supplementary Fig. S4 for all traces), whereas
119 predator cue removal activated ASI and ASJ neurons (Figs. 2f, 2g, Supplementary Fig. S4 for all
120 traces). Also, whereas ADL and ASJ responded to both tested dilutions of predator cue (Figs. 2d,
121 2g), ASH and ASI only detected the more concentrated cue (i.e., 1:10 dilution, but not 1:50)

122 (Figs. 2e, 2f), suggesting different response thresholds for these four neuronal pairs. Collectively,
123 these results show that predator cue activates ADL and ASH neurons, whereas its removal
124 increases ASI and ASJ activity.

125

126 **Sensory neurons use CNG and TRP channels to drive predator avoidance**

127 To gain insight into the signal transduction machinery underlying these responses, we examined
128 the behavior of mutants lacking specific signaling components. We found that mutants lacking
129 the alpha subunit (*tax-4*), but not the beta subunit (*tax-2*), of the cyclic nucleotide gated (CNG)
130 ion channel exhibited defective responses to predator cue (Fig. 3a). Moreover, expressing the
131 full-length *tax-4* cDNA via a *tax-4* promoter, an ASI-specific promoter, or an ASJ-specific
132 promoter (but not via an ASH-selective promoter) restored normal behavior to the null mutants
133 (Fig. 3a). The ability of these transgenes to rescue avoidance behavior was largely dose
134 dependent, as it varied depending on the amount of *tax-4* transgene expressed in ASI and ASJ
135 neurons (Supplementary Fig. S5a). These data indicate that increased CNG signaling from ASI
136 neurons could compensate for the lack of signaling from ASJ, and vice-versa. Similarly, mutants
137 lacking the transient receptor potential (TRP) channel OCR-2, but not OSM-9, were defective in
138 their responses to predator cue. Further, we observed that OCR-2 functions in ADL and ASH
139 neurons, but not in ASI or ASJ neurons (Fig. 3b), and that responses to ASH- and ADL-specific
140 *ocr-2* transgenes were also dose dependent (Supplementary Fig. S5b), indicating that signaling
141 from ASH could compensate for the lack of ASJ signaling, and vice-versa. Testing samples of
142 purified sulfolipids confirmed that *tax-4* (but not *tax-2*) and *ocr-2* (but not *osm-9*) mutants were
143 defective in avoidance to these molecules (Supplementary Fig. S5c). Also, since TAX-4 but not
144 TAX-2 can form a homomeric CNG channel²⁸, we hypothesize that the OCR-2 TRP channel
145 may either form a homomeric channel that interacts with other non-OSM-9 TRP channel
146 subunits to generate a functional channel and drive avoidance behavior, results consistent with
147 previous studies^{29,30}.

148 To investigate possible interactions between CNG and TRP channel signaling, we
149 analyzed *tax-4;ocr-2* double mutants. Restoring TAX-4 function to ASI neurons and OCR-2 to
150 ASH neurons (in combination) conferred normal predator cue avoidance to the double mutants
151 (Fig. 3C). Moreover, partial avoidance was seen for other rescue combinations (TAX-4 in ASJ
152 and OCR-2 in ASH; TAX-4 in ASI and OCR-2 in ADL, and TAX-4 in ASI alone) (Fig. 3C).

153 Together, these data indicate that there are at least four neuronal signaling pathways that can
154 drive robust avoidance to *Pristionchus* cue: 1) ASI sensory neurons using TAX-4 channels, 2)
155 ASI and ASH neurons using TAX-4 and OCR-2 channels, respectively, 3) ASJ and ASH using
156 TAX-4 and OCR-2 channels, respectively, and 4) ASI and ADL using TAX-4 and OCR-2
157 channels, respectively (Figs. 3d). Similarly, we found that *tax-4* mutants, but not *ocr-2* mutants,
158 did not curtail their egg laying behavior (a longer-lasting effect) in response to predator cue, and
159 that restoring TAX-4 function to ASI or ASJ significantly improved this *tax-4* defect (Figs. 3e,
160 3f).

161

162 **Sertraline acts on GABA signaling to block predator-evoked *C. elegans*' responses**

163 Next, we asked whether *C. elegans* avoidance behavior could be suppressed by small molecules
164 that alleviate mammalian anxiety (Supplementary Table S5)^{31,32}. We screened a small library of
165 anti-anxiety drugs, and found that pre-treating prey with a selective serotonin reuptake inhibitor,
166 sertraline (brand name “Zoloft”) attenuated avoidance to predator cue and purified sulfolipids,
167 but not to other repellents (Fig. 4a, Supplementary Table S5). Suppression of avoidance behavior
168 by sertraline was dose-dependent (Supplementary Fig. S6a) and lasted for at least 30 minutes
169 after the drug was removed (Supplementary Fig. S6b). To test whether sertraline modifies
170 signaling from specific sensory neurons, we analyzed mutants expressing different rescuing
171 transgenes. Sertraline had no detectable effect on the behavior of *tax-4* or *ocr-2* mutants, but it
172 attenuated avoidance to predator cue of: 1) *tax-4* mutants expressing *tax-4* in ASI or ASJ, and 2)
173 *ocr-2* mutants expressing *ocr-2* in ADL or ASH (Fig. 4b). Thus, sertraline affected signaling
174 from all four sensory neurons. Next, we found that sertraline required GABA, but not serotonin
175 signaling. Animals lacking glutamic acid decarboxylase (*unc-25*, enzyme required for GABA
176 synthesis³³), but no other neurotransmitter biosynthetic enzymes were defective in sertraline
177 attenuation (Fig. 4c, Supplementary Fig. S6c). Additionally, adding GABA exogenously to the
178 agar plate was sufficient to restore normal behavior to *unc-25* mutants confirming that GABA
179 signaling is required to modify predator avoidance (Fig. 4c). Further, we found that restoring
180 UNC-25 function to all 26 GABAergic neurons³⁴ or under a RIS interneuron-selective promoter
181 was sufficient to restore sertraline attenuation of predator avoidance (Fig. 4d). RIS interneuron
182 has been previously shown to play a role in inducing a sleep-like state in *C. elegans*^{35,36} and these
183 results suggest an additional for this neuron in modifying predator behavior. Moreover, we found

184 that mutants in a solute carrier 6 plasma membrane re-uptake transporters [*snf-10*³⁷], but no
185 other GABA transporters, were partially defective in their response to predator cue after
186 sertraline treatment (Fig. 4e). Consistently, rodent and human homologs of this protein have been
187 shown to bind multiple SSRIs using a non-competitive mechanism³⁸. Finally, we found that
188 sertraline treatment also reduced the longer-lasting egg laying response (Fig. 4f) showing that the
189 drug blocks *C. elegans*' responses on multiple timescales. Taken together, these results indicate
190 that the anti-anxiety drug sertraline specifically abolished predator-induced *C. elegans* responses
191 by acting on GABA signaling in RIS interneuron.

192

193 **Discussion**

194 We show that *P. pacificus* releases a mixture of sulfolipids that *C. elegans* perceives as a
195 predator-specific molecular signature, or kairomone³⁹. Perception of these sulfolipids via
196 multiple sensory neurons initiates a fear-like avoidance response and reduces egg laying
197 behavior (Fig. 4f). Among the nematode species whose metabolomes we have analysed, *P.*
198 *pacificus* is the only one that excretes copious amounts of sulfolipids. Sulfated fatty acids and
199 related lipids have previously been described primarily from marine sources, including
200 tunicates⁴⁰, sponges⁴¹, crustaceans⁴², and algae⁴³. In addition, a family of sulfated fatty acids, the
201 caeliferins, has been identified from grasshopper oral secretions^{44,45}. In a striking parallel to the
202 role of sulfated lipids in the nematode predator-prey system studied here, these herbivore-
203 associated sulfolipids have been shown to elicit specific defense responses in plants⁴⁵.
204 Furthermore, the sulfolipids we identified from *P. pacificus* resemble sodium dodecyl sulfate
205 (SDS), a known nematode repellent²³. We found that, similar to avoidance triggered by predator
206 cue, ASJ, ASH and ASI neurons are necessary for avoidance to SDS (Supplementary Fig. S3g).
207 Given the similarity of the neuronal circuitry required for the avoidance responses, it appears that
208 *C. elegans* avoid SDS because of its structural similarity to the *Pristionchus*-released sulfates,
209 which are interpreted as a molecular signature of this predator.

210 The sulfolipids we identified from *P. pacificus*, *sufac#1* and *sufal#2*, and several related
211 compounds, appear to be derived from the monomethyl branched-chain fatty acid (mmBCFA),
212 C15ISO, which is also produced by *C. elegans* and has been shown to be essential for *C. elegans*
213 growth and development⁴⁶. The biosynthesis of C15ISO in *C. elegans* requires the fatty acid
214 elongase ELO-5, and several homologous elongases in *P. pacificus* exist that may be involved in

215 the biosynthesis of the fatty acid precursors of sufac#1 and sufal#2. Additionally, the
216 biosynthesis of sufac#1 and sufal#2 requires oxygenation at the (ω -5) or (ω -6) position in the
217 fatty acid chain, respectively, followed by sulfation by sulfotransferase(s), a family of genes that
218 has undergone major expansion in *P. pacificus*⁴⁷. Notably, at least one sulfotransferase, EUD-1,
219 functions as a central switch determining whether *P. pacificus* larvae will develop into a
220 primarily bacterivorous, narrow-mouthed adult, or into a predacious, wide-mouthed adult that
221 can feed on other nematodes⁴⁸. It is intriguing that *C. elegans* has evolved the ability to detect a
222 *Pristionchus*-specific trait (the extensive sulfation of small molecules) that is directly connected
223 to the endocrine signaling pathway that controls development of the morphological features
224 required for predation.

225 Detection of predator cue relies on a sensory neural circuit consisting of at least four
226 different amphid neurons (ASI, ASH, ASJ and ADL, Fig. 4g). These neurons have well-
227 described roles in detecting chemicals from the environment: the ASI and ASJ sensory neurons
228 play a major role in the detection of ascaroside pheromones, while ASH neurons are nociceptive
229 and drive avoidance to glycerol and copper, and ASH and ADL act together to promote social
230 feeding^{23,49-53}. Therefore, whereas ASH and ADL have been shown to drive avoidance behavior
231^{23,52,54}, our finding that ASI and ASJ are involved in generating avoidance are novel.
232 Participation of these additional neurons facilitates redundancy, such that signaling from ASI,
233 ASI and ASH, ASI and ADL, ASJ and ASH, and ASJ and ADL is sufficient to drive avoidance
234 to predator cue. Similarly, signaling from either ASI or ASJ neurons alters egg-laying behavior.
235 Such redundant circuit(s) are likely to decrease the failure rate for signaling, thereby increasing
236 the robustness of the behavioral output. Similar redundant circuits have been described for
237 sensory neurons detecting temperature⁵⁵ or odors⁵⁶, and in neural circuits driving feeding in the
238 crab⁵⁷.

239 The neuronal signaling machinery in the ASI, ASJ, ASH and ADL sensory neurons relies
240 on CNG and TRP channels to mediate responses to predator cue. CNG ion channels typically
241 consist of alpha and beta subunits and have been shown to play a central role in regulating
242 chemosensory behaviors across multiple species^{58,59}. Because the alpha subunit homolog TAX-4
243 is required for detecting predator cue, whereas the beta subunit TAX-2 is not, we suggest that
244 homomeric TAX-4 channels act in the ASI and ASJ sensory neurons. *In vitro* experiments have
245 shown that *C. elegans* TAX-4 subunits can form a functional homomeric channel when

246 expressed in HEK293 cells⁶⁰. Similarly, alpha subunits of the CNG channels have also been
247 shown to function as homomeric channels both *in vitro* and *in vivo*^{61,62}. Our studies also indicate
248 a role for a subunit of the TRP channel OCR-2, but not its heteromeric partner OSM-9⁶³. We
249 suggest that OCR-2 can either form a homomeric channel or interact with other non-OSM-9 TRP
250 channel subunits to generate a functional channel and drive avoidance behavior. These results are
251 consistent with previous studies where OCR-2 has been shown to act independently of OSM-9 in
252 regulating *C. elegans* larval starvation²⁹ and egg-laying behaviors³⁰. Our results for the role of
253 TRP channels are reminiscent of rodent studies where TRP channels have been found to play a
254 crucial role in initiating responses to predator odors from cats⁴, suggesting broad conservation of
255 the molecular machinery that detects predators. Taken together, we hypothesize that signaling
256 from homomeric CNG and TRP channels acting in distinct, but redundant sensory circuits
257 enables reliable detection of predators by the prey.

258 We further show that the anti-anxiety drug, sertraline, acts downstream of CNG and TRP
259 channels and requires GABA signaling in RIS interneurons to suppress predator-evoked
260 responses. Sertraline has been shown to be particularly effective in alleviating human anxiety
261 disorders⁶⁴ and, classified as an SSRI, is thought to act in part by elevating serotonin levels at
262 synapses^{65,66}. Our studies show that sertraline requires GABA, but not serotonin signaling, to
263 exert its effects on *C. elegans* avoidance behavior. Other SSRIs have also been shown to require
264 GABA signaling in mammalian^{67,68} and *C. elegans* nervous systems⁶⁹, in addition to effects on
265 other neurotransmitter pathways including dopamine, glutamate, histamine, and acetylcholine⁷⁰.
266 We further show that sertraline action in *C. elegans* requires functional glutamic acid
267 decarboxylase, a GABA biosynthesis enzyme³³ specifically in RIS interneurons, defining the site
268 of action of the drug. RIS interneurons have been implicated in modulating a sleep-like state in
269 *C. elegans*^{35,36}. We also found that sertraline effects require a plasma membrane re-uptake
270 transporter that belongs to the solute carrier 6 family (*snf-10*). This protein has been previously
271 implicated in activating *C. elegans* sperm in response to male protease activation signals³⁷.
272 While our results showing a role for *snf-10* in animal behavior are novel, the closely related *snf-*
273 *11* has been shown to act to clear GABA from synaptic clefts⁷¹. We suggest that sertraline might
274 act on the SNF-10 protein at RIS synapses to modify *C. elegans* behavior. This idea is consistent
275 with results from *in vitro* studies where rodent and human homologs of this protein have been
276 shown to bind multiple SSRIs using a non-competitive mechanism³⁸. Collectively, our analysis

277 of the mechanisms by which sertraline attenuates *C. elegans* avoidance responses to an external
278 stressor (predator) suggest broad conservation of the involved signaling pathways from worms to
279 humans.

280 In summary, our results uncover the chemosensory and neuronal basis of a predator-prey
281 relationship between *P. pacificus* and *C. elegans*, in which predator detection is based on a
282 characteristic molecular signature of novel sulfate-containing molecules. The prey uses multiple
283 sensory neurons acting in parallel and conserved CNG and TRP channel signaling to detect these
284 sulfates and drive rapid avoidance and longer lasting reduced egg laying. Additionally, we show
285 that sertraline acts on GABA signaling in RIS interneurons likely targeting a plasma membrane
286 re-uptake transporter to attenuate *C. elegans* avoidance behavior. Based on these results, we
287 hypothesize that *C. elegans* evolved mechanisms to detect *Pristionchus*-released sulfolipids as a
288 kairomone, and that the identified neuronal signaling circuitry is representative of conserved or
289 convergent strategies for processing predator threats.

290

291 **Methods Summary**

292 Single animal avoidance assay and calcium imaging were performed as described^{14,23}. Avoidance
293 indices of all strains tested and controls are shown in Supplementary Table S6, while all the
294 neuronal responses are shown in Supplementary Fig. S4.

295 Egg laying assays were modified from assays previously described²⁰, where synchronized adults
296 were exposed to test compound for 30 minutes and the number of eggs laid monitored for the
297 times indicated.

298 Additional methods and sulfolipid purification and synthesis are shown in supplementary
299 information online.

300

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498 **Supplementary Information**

499 Supplemental information includes 6 figures and 7 tables, methods, and NMR spectra.

500

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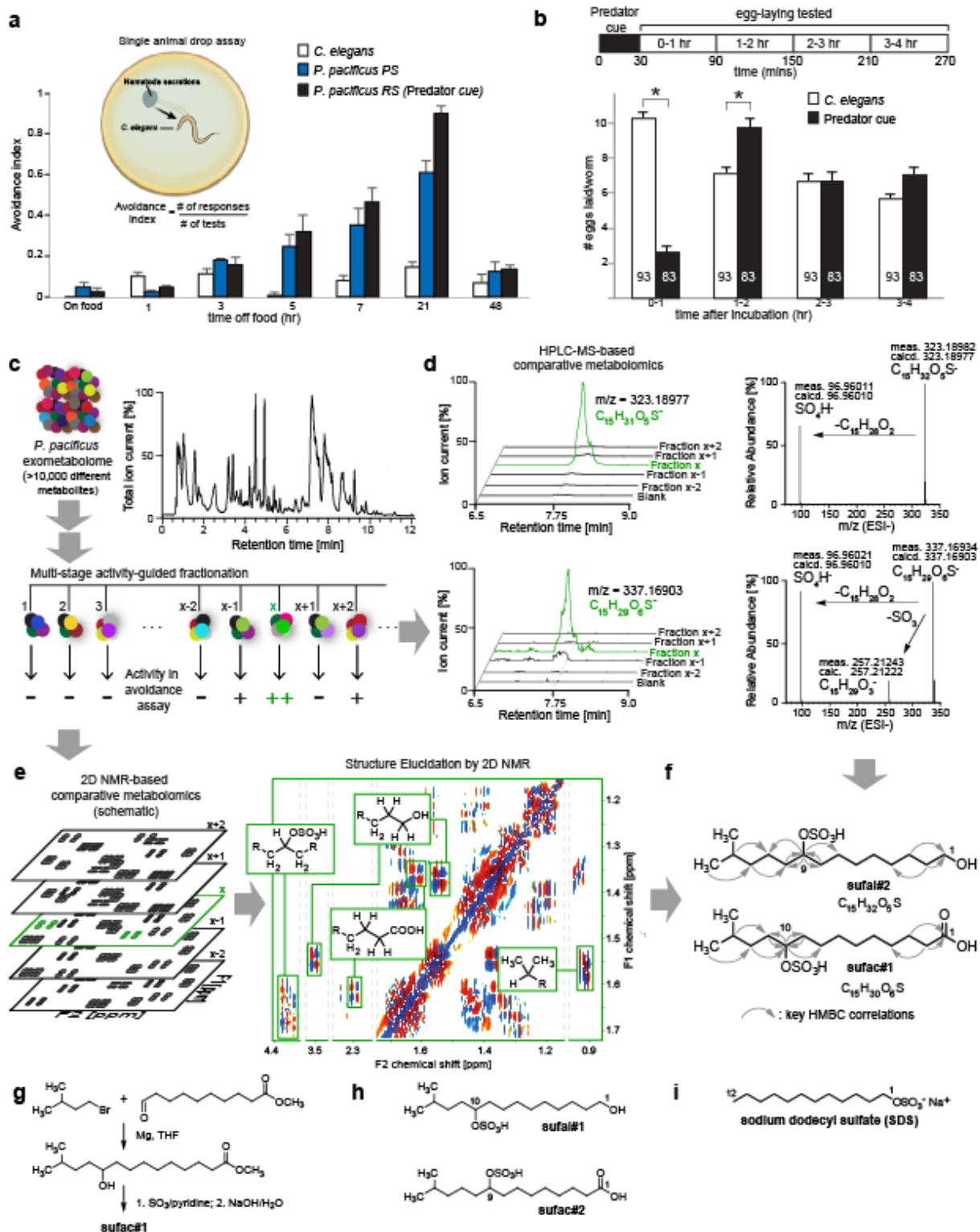
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510

511 **Author Contributions**

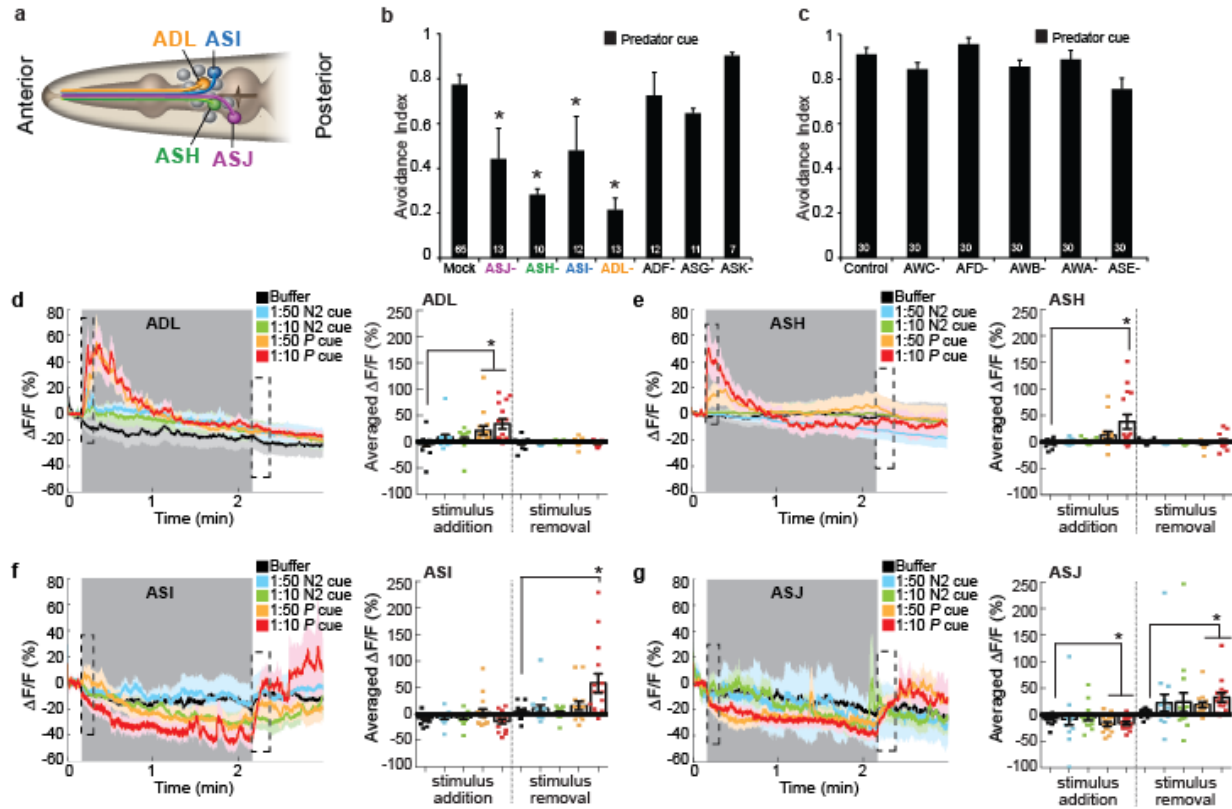
512 Z.L., M.J.K., C.D.C., A.K.P., S.G.L., A.T., K.P.C., and N.B. performed experiments, developed
513 experimental methods and reagents. F.C.S., J.S., and S.H.C. designed and interpreted the
514 experiments and wrote the paper.

515



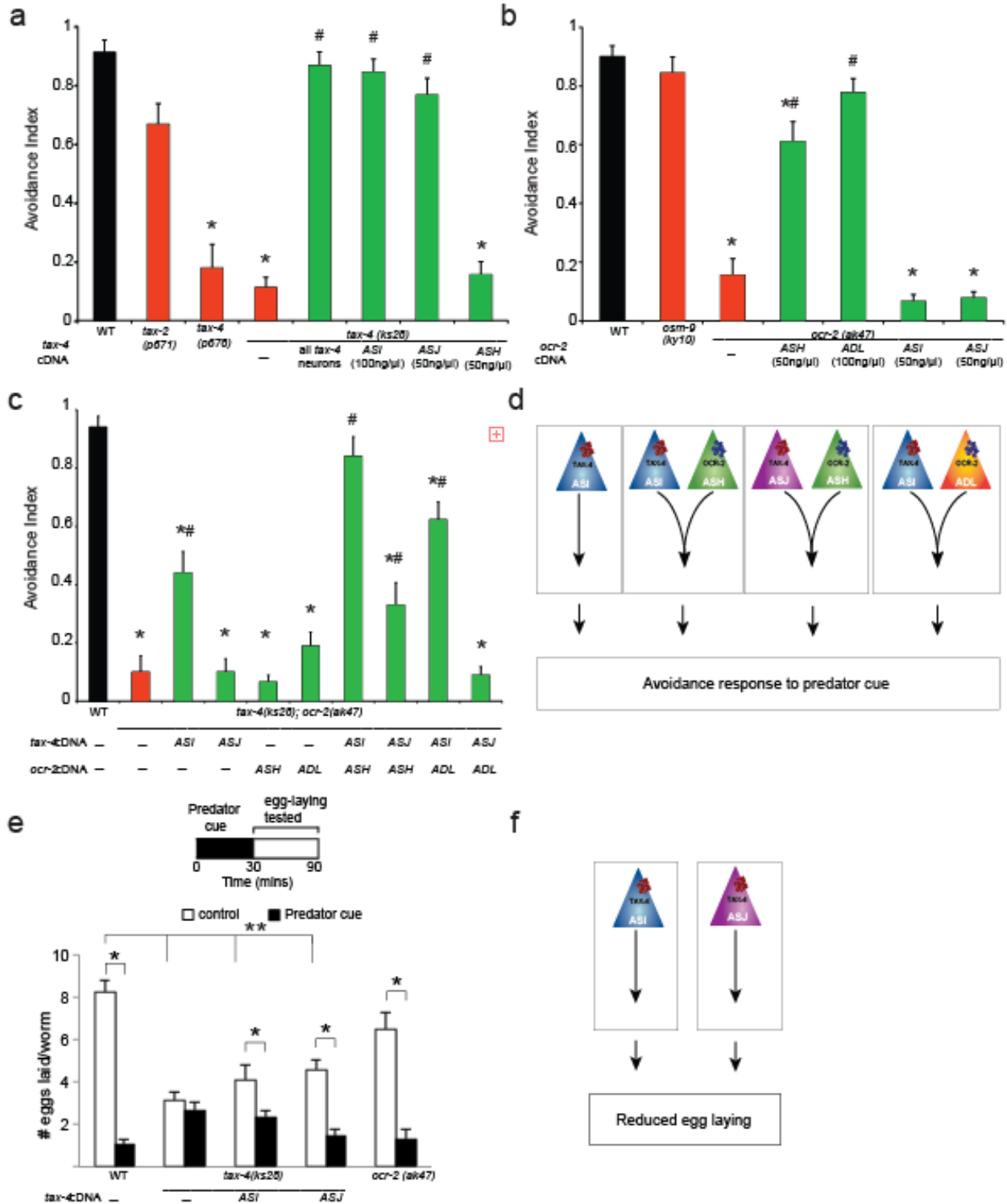
516
 517 **Figure 1| Predator-released sulfolipids drive *C. elegans* behaviors.** (a) *C. elegans* avoid
 518 excretions from starving *P. pacificus* PS (PS312, domesticated) and RS (RS5725B a wild isolate,
 519 predator cue) strains. Inset, shows a schematic of the avoidance assay. (b) **Top** shows schematic
 520 of the modified egg laying assay and **bottom**, *C. elegans* lay fewer eggs after a 30-minute
 521 exposure to concentrated predator cue, but recovers after 2 hours. (c) UHPLC-HRMS analysis

522 reveals a complex mixture of more than 10,000 metabolites, which was subjected to multistage
523 activity-guided fractionation using reverse-phase chromatography. After four fractionation steps,
524 most of the activity (++) was found in fraction x. Averages and s.e.m. are shown. $n > 90$ for each
525 condition. **(d)** UHPLC-HRMS ion chromatograms (m/z value ± 5 ppm) of active fraction x and
526 adjacent fractions for two sulfate containing metabolites that were strongly enriched in the active
527 fraction (left). MS-MS analysis (right) confirms presence of sulfate moieties in both compounds.
528 **(e)** Schematic representation of 2D NMR-based comparative metabolomics (left) of consecutive
529 fractions (x-2 to x+2) used to identify signals specific to fraction x. Cropped 2D NMR
530 (dqfCOSY) spectrum (right) of active fraction highlighting signals that represent specific
531 features of the identified metabolites (grey lines define edges of shown subsections). **(f)**
532 Chemical structures of metabolites identified via comparative metabolomics from active fraction
533 x, sufac#1 and sulfal#2. Grey arrows indicate important correlations observed in heteronuclear
534 2D NMR (HMBC) spectra. **(g)** Synthesis of sufac#1; THF: tetrahydrofuran. **(h)** Homologous
535 metabolites sufac#2 and sulfal#1 were also detected by UHPLC-HRMS. **(i)** Chemical structure of
536 sodium dodecyl sulfate (SDS). Averages and s.e.m. are shown and number of animals tested are
537 indicated on each bar or condition. * $p < 0.05$ obtained by comparison with controls using
538 Fisher's exact t-test with Bonferroni correction.
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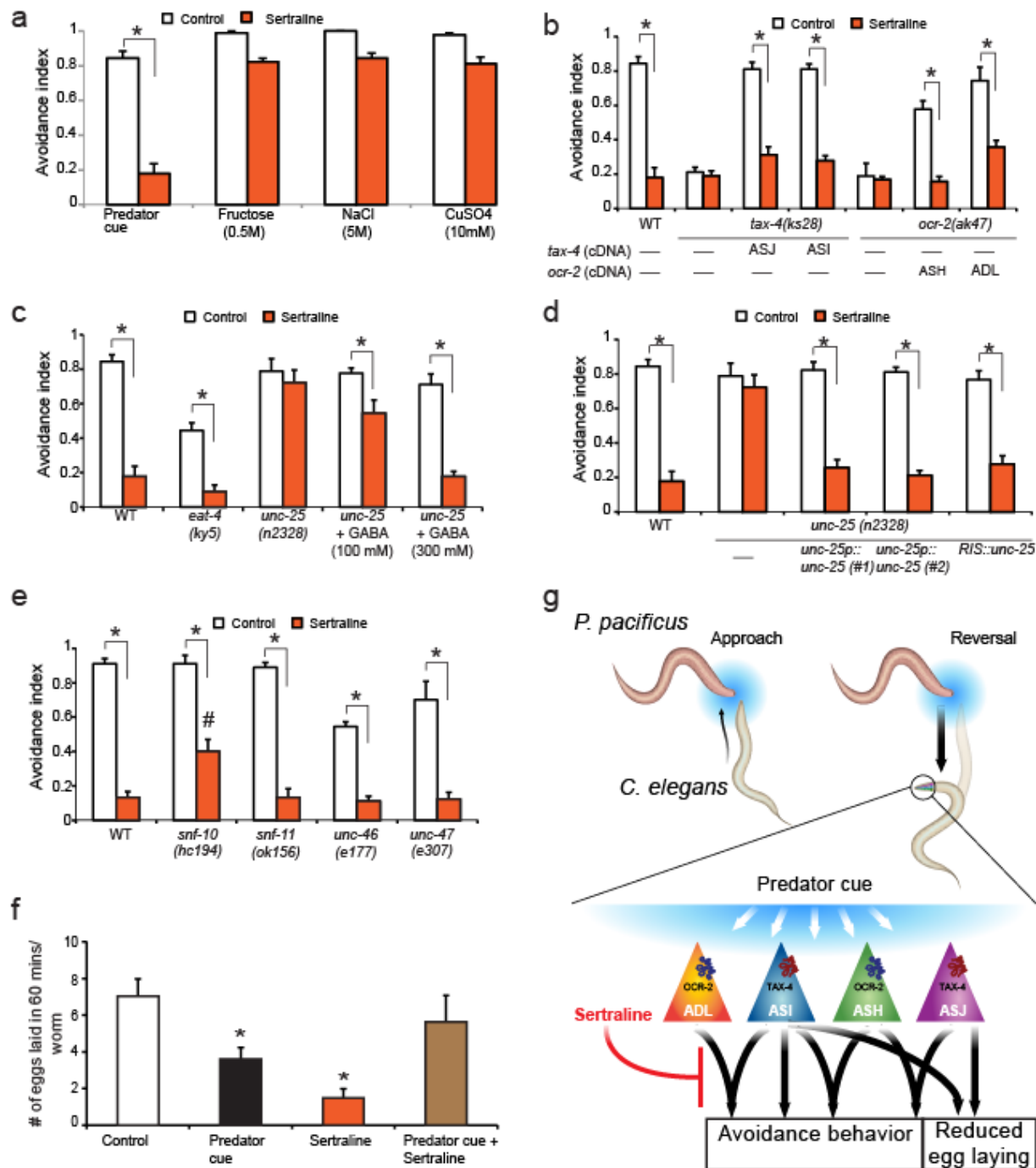
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Figure 2 | Multiple sensory neurons are required for avoidance of predator cue. (a) Schematic showing amphid sensory neurons with key neurons highlighted. **(b)** Cell and **(c)** genetic ablations showing that ASJ, ASH, ASI, and ADL sensory neurons, but not other amphid neurons, are required for avoidance of predator cue. Averages and s.e.m. and numbers of animals tested are shown on each bar. **(d-g)** Average calcium responses of transgenic animals ($n > 13$ for each condition) expressing the GCaMP family of indicators in **(d)** ADL, **(e)** ASH, **(f)** ASI, or **(g)** ASJ sensory neurons to predator cue (*P* cue) or *C. elegans* secretions (N2 cue). Each experiment was a 180 second recording where control (M9 buffer), *C. elegans* secretions (N2 cue), or predator cue (*P* cue) in different dilutions was added at 10 seconds and removed at 130 seconds (stimulus is indicated by a shaded grey box). Bar graphs, average percentage change during the 10 seconds after stimulus addition (dashed box), or 15 seconds after stimulus removal (dashed box) are shown. Error bars and shaded regions around the curves represent s.e.m. * $p < 0.05$ obtained by comparison with controls using Fisher's exact t-test with Bonferroni correction.



555
 556 **Figure 3 | A redundant *C. elegans* signaling network enables responses to predator cue. (a)**
 557 Mutants lacking the alpha subunit of the CNG channel (*tax-4*), but not the beta subunit (*tax-2*),
 558 are defective in their response to predator cue. Restoring wild-type *tax-4* cDNA using an ASI- or
 559 ASJ-specific promoter is sufficient to restore normal behavior to *tax-4* mutants. **(b)** Mutants
 560 lacking the TRPV channel subunit *ocr-2*, but not *osm-9*, are defective in their response to

561 predator cue. OCR-2 is specifically required in ASH sensory neurons. **(c)** A *tax-4;ocr-2* double
562 mutant is also defective in avoiding predator cue and wild-type behaviour is restored when
563 TAX-4 is restored to ASI at the same time OCR-2 is restored to ASH neurons. Restoring TAX-4
564 to ASJ and OCR-2 to ASH or ADL or restoring TAX-4 to ASI and OCR-2 to ADL is able to
565 partially restore wild-type behaviour to the double mutants. Moreover, expressing *tax-4* in ASI
566 but not ASJ is sufficient to rescue of the double mutant phenotype. **(d)** *C. elegans* egg-laying
567 reduction requires functional TAX-4 signaling in ASI and ASJ neurons. OCR-2 is not required
568 for this behaviour. Schematic showing **(e)** four redundant pathways (ASI acting independently,
569 ASI and ASH, or ASI and ADL, or ASJ and ASH acting together) driving avoidance to the *P.*
570 *pacificus* predator cue. **(f)** Schematic showing that TAX-4 acts in ASI and/or ASJ neurons to
571 reduce egg laying upon longer-term exposure to predator cue. Averages and s.e.m. are shown.
572 **(a-c)** n = 90 animals and in **(e)** n > 35 tested for each condition, *p < 0.05 compared with
573 controls, #p < 0.05 compared to mutants, **p < 0.05 comparing eggs laid by mutants and wild
574 type animals obtained using Fisher's exact t-test with Bonferroni correction.
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577

578 **Figure 4 | Sertraline attenuates *C. elegans* responses to the *P. pacificus* predator. (a)**

579 Sertraline specifically attenuates *C. elegans* responses to predator cue, but not to fructose, salt, or

580 copper. (b) The effect of sertraline is lost in *tax-4* and *ocr-2* mutants, and modulation is restored

581 when TAX-4 is restored to either ASI or ASJ, or when OCR-2 is restored to ASH or ADL. (c)

582 Sertraline requires GABA, but not glutamate signaling. Sertraline modulates avoidance

583 responses of *eat-4* (glutamate receptor), but not *unc-25* (glutamic acid decarboxylase, required

584 for GABA synthesis) mutants. Adding GABA exogenously to plates is able to restore sertraline

modulation to *unc-25* mutants. (d) Defective *unc-25* response is rescued by expressing wild-type

585 *unc-25* cDNA under the *unc-25* promoter or a RIS-selective promoter. (e) Sertraline partially
586 modulates mutants in a plasma membrane transporter (*snf-10*) and completely attenuates mutants
587 in other GABA transporters, *unc-47*, *snf-11*, and *unc-46* (a transmembrane protein that recruits
588 UNC-47). (f) Animals were treated with predator cue or 1 mM sertraline or predator cue and
589 sertraline for 30 minutes and egg-laying was monitored for 60 minutes after removal of these
590 compounds. (g) *C. elegans* detects secretions from a starving *P. pacificus* (predator cue) using
591 sensory circuits consisting of ASI, ASJ, ASH, and ADL neurons that use CNG and TRP
592 channels and act in a redundant manner to generate rapid avoidance. In contrast, CNG channels
593 act in ASI and ASJ neurons to reduce egg laying for many minutes. Sertraline attenuates both
594 predator-induced avoidance behaviour and egg laying behaviour downstream of these sensory
595 neurons. Averages of either $n > 90$ (a-e) or $n > 35$ (f) and s.e.m. are shown. * $p < 0.05$ compared
596 to controls obtained using Fisher's exact t-test with Bonferroni correction
597