



HAL
open science

Natural Variation in a Dendritic Scaffold Protein Remodels Experience-Dependent Plasticity by Altering Neuropeptide Expression

Isabel Beets, Gaotian Zhang, Lorenz Fenk, Changchun Chen, Geoffrey Nelson, Marie-Anne Félix, Mario de Bono

► **To cite this version:**

Isabel Beets, Gaotian Zhang, Lorenz Fenk, Changchun Chen, Geoffrey Nelson, et al.. Natural Variation in a Dendritic Scaffold Protein Remodels Experience-Dependent Plasticity by Altering Neuropeptide Expression. *Neuron*, 2019, 10.1016/J.neuron.2019.10.001 . hal-02405476

HAL Id: hal-02405476

<https://hal.science/hal-02405476>

Submitted on 21 Jul 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

1 **Natural variation in a dendritic scaffold protein remodels experience-**
2 **dependent plasticity by altering neuropeptide expression**

3 Isabel Beets¹, Gaotian Zhang², Lorenz A. Fenk¹, Changchun Chen¹, Geoffrey M. Nelson¹,
4 Marie-Anne Félix^{2*}, Mario de Bono^{1,3,4*}

5
6 ¹Cell Biology Division, MRC Laboratory of Molecular Biology, Cambridge, CB2 0QH, UK

7 ²Institut de Biologie de l'École Normale Supérieure, CNRS, Inserm, PSL Research
8 University, Paris, 75005, France^[1]_[SEP]

9 ³Present address: IST Austria, Klosterneuburg, 3400, Austria

10 ⁴Lead Contact

11 ***Correspondence:** debono@mrc-lmb.cam.ac.uk, mdebono@ist.ac.at, felix@biologie.ens.fr^[1]_[SEP]

12

13 **Summary**

14 The extent to which behavior is shaped by experience varies between individuals. Genetic
15 differences contribute to this variation but the neural mechanisms are not understood. Here,
16 we dissect natural variation in the behavioral flexibility of two *C. elegans* wild strains. In one
17 strain, a memory of exposure to 21% O₂ suppresses CO₂-evoked locomotory arousal; in the
18 other, CO₂ evokes arousal regardless of previous O₂ experience. We map that variation to a
19 polymorphic dendritic scaffold protein, ARCP-1, expressed in sensory neurons. ARCP-1
20 binds the Ca²⁺-dependent phosphodiesterase PDE-1, and co-localizes PDE-1 with molecular
21 sensors for CO₂ at dendritic ends. Reducing ARCP-1 or PDE-1 activity promotes CO₂ escape
22 by altering neuropeptide expression in the BAG CO₂ sensors. Variation in ARCP-1 alters
23 behavioral plasticity in multiple paradigms. Our findings are reminiscent of genetic
24 accommodation, an evolutionary process by which phenotypic flexibility in response to
25 environmental variation is reset by genetic change.

26 **Introduction**

27 Animals reconfigure their behavior and physiology in response to experience, and many
28 studies highlight mechanisms underlying such plasticity (Bargmann, 2012; Owen and
29 Brenner, 2012). While plasticity is presumed crucial for evolutionary success, it has costs, and
30 often varies across species and between individuals (Coppens et al., 2010; Dewitt et al., 1998;
31 Mery, 2013; Niemelä et al., 2012). Variation in behavioral flexibility is thought to underlie
32 inter-individual differences in cognitive ability and capacity to cope with environmental
33 challenges (Coppens et al., 2010; Niemelä et al., 2012). The genetic and cellular basis of
34 inter-individual variation in experience-dependent plasticity is, however, poorly understood.

35 Genetic accommodation and assimilation are concepts used to describe variation in plasticity
36 on an evolutionary timescale. Waddington and Schmalhausen suggested genetic assimilation
37 occurs when a phenotype initially responsive to the environment becomes fixed in a specific
38 state (Renn and Schumer, 2013; Schmalhausen, 1949; Waddington, 1942; 1953). This loss of
39 plasticity may reflect genetic drift or selection against the costs of expressing adaptive
40 behaviors (Niemelä et al., 2012). Studies of genetic assimilation led to the broader concept of
41 genetic accommodation, referring to evolutionary genetic variation leading to any change in
42 the environmental regulation of a phenotype (Crispo, 2007; West-Eberhard, 2005). Many
43 studies, in insects, fish, rodents and primates, highlight inter-individual variation in behavioral
44 plasticity; in some cases this has been shown to be heritable (Dingemanse and Wolf, 2013;
45 Izquierdo et al., 2007; Mery et al., 2007), but the mechanisms responsible for these
46 differences remain enigmatic.

47 Many animals use gradients of respiratory gases to help locate prey, mates, or predators, and
48 have evolved sophisticated behavioral responses to environmental changes in oxygen (O₂)
49 and carbon dioxide (CO₂) levels (Carrillo and Hallem, 2015; Cummins et al., 2013;

50 Guerenstein and Hildebrand, 2008; Prabhakar and Semenza, 2015). Where studied, behavioral
51 responses to CO₂ have been shown to depend on environmental context, past experience, and
52 life stage (Carrillo et al., 2013; Fenk and de Bono, 2017; Guillermin et al., 2017; Hallem and
53 Sternberg, 2008; Sachse et al., 2007; Vulesevic et al., 2006). This flexibility makes CO₂-
54 sensing an attractive paradigm to study natural variation in behavioral plasticity.

55 CO₂ responses in *Caenorhabditis elegans* are sculpted by previous O₂ experience (Carrillo et
56 al., 2013; Fenk and de Bono, 2017; Kodama-Namba et al., 2013). Acclimation to surface O₂
57 levels, i.e. 21%, generates a memory that suppresses aversion of high CO₂. The O₂ memory is
58 written over hours by O₂ sensors, called URX, AQR and PQR, whose activity is tonically
59 stimulated by 21% O₂ (Busch et al., 2012; Fenk and de Bono, 2017). 21% O₂ is itself aversive
60 to *C. elegans*, most likely because it signals surface exposure (Persson et al., 2009). By
61 suppressing CO₂ aversiveness, *C. elegans* acclimated to 21% O₂ may increase their chance of
62 escaping the surface into buried environments with elevated CO₂ (Fenk and de Bono, 2017).

63 Here, we show that the impact of O₂ experience on CO₂ aversion varies across
64 *Caenorhabditis* species and between wild *C. elegans* isolates. By characterizing differences
65 between *C. elegans* isolates, we identify a polymorphism in a dendritic ankyrin-repeat
66 scaffold protein, ARCP-1, that alters plasticity in one strain. ARCP-1 biochemically interacts
67 with the conserved cyclic nucleotide phosphodiesterase PDE-1 and localizes it with molecular
68 sensors for CO₂ to the dendritic ends of BAG sensory neurons. Disrupting ARCP-1 resets
69 CO₂ sensitivity and experience-dependent plasticity of CO₂ escape, in part by altering
70 neuropeptide expression, and conferring strong aversion to CO₂.

71 **Results**

72 **Natural Variation in Experience-Dependent Plasticity in *Caenorhabditis***

73 In *C. elegans*, a memory of recent O₂ levels reprograms aversive responses to CO₂ (Fenk and
74 de Bono, 2017). We hypothesized this experience-dependent plasticity is evolutionarily
75 variable. To investigate this, we compared the CO₂ responses of different *Caenorhabditis*
76 species grown at 21% or 7% O₂ (Figure S1A). Animals were transferred to a thin bacterial
77 lawn in a microfluidic chamber kept at 7% O₂, stimulated with 3% CO₂, and their behavioral
78 responses quantified. We used a background level of 7% O₂ in all assays because *C. elegans*
79 dwell at this O₂ concentration, making locomotory arousal by CO₂ prominent. By contrast,
80 21% O₂ evokes sustained rapid movement, making CO₂ responses above this high baseline
81 proportionally small. As a representative *C. elegans* strain we used LSJ1, a wild-type (N2-
82 like) laboratory strain bearing natural alleles of the neuropeptide receptor *npr-1(215F)* and the
83 neuroglobin *glb-5(Haw)*. We did not use the standard N2 strain, since it has acquired
84 mutations in these genes that confer gas-sensing defects (McGrath et al., 2009; Persson et al.,
85 2009). As expected, *C. elegans* was aroused more strongly by CO₂ when acclimated to 7% O₂
86 (Figure S1B). By contrast, O₂ experience did not alter the absolute speed of representative
87 strains of *C. latens* and *C. angaria* at 3% CO₂ (Figure S1B). Since *C. angaria* was not
88 aroused by 3% CO₂, we tested its response to 5% and 10% CO₂. These levels evoked
89 locomotory arousal that, as in *C. elegans*, was stronger in animals acclimated to 7% O₂
90 (Figure S1C). Thus, *C. angaria* is less sensitive to CO₂ than *C. elegans*, but its arousal by
91 CO₂ remains dependent on O₂ experience. By contrast, CO₂ responses of *C. latens* were
92 unaffected by previous O₂ experience at any concentration tested (Figure S1D).
93 Unexpectedly, acclimation to 7% O₂ suppressed rather than enhanced the locomotory

94 response of *C. nigoni* to CO₂ (Figure S1B). Thus the effect of O₂ memory on CO₂-evoked
95 behavioral responses is evolutionarily variable.

96 **Effect of O₂ Memory on CO₂ Responses Varies Between *C. elegans* Wild Isolates**

97 Our findings prompted us to seek intra-species variation in how O₂ experience influences CO₂
98 responses, by studying a genetically diverse collection of wild *C. elegans* isolates (Figure
99 S2A). Most strains responded like the reference strain (Figures 1A-1C and S2A). However,
100 two isolates, the French JU1249 and German MY16 strains, responded more strongly than
101 other isolates to a rise in CO₂ regardless of O₂ experience (Figures 1B and 1D). For MY16
102 CO₂ aversion was stronger when animals were acclimated to 7% O₂, recapitulating the cross-
103 modulation of CO₂ responses observed in most strains (Figures 1C and 1E). By contrast,
104 JU1249 animals acclimated to 21% O₂ further enhanced rather than suppressed CO₂ escape
105 (Figures 1C and 1F). To probe further if the O₂-dependent plasticity of CO₂ escape had
106 changed in JU1249, we quantified escape responses at different CO₂ concentrations. *npr-1*;
107 *glb-5* animals always responded more strongly to CO₂ when acclimated to 7% O₂, but this
108 was not the case for JU1249 at any CO₂ concentration tested (Figure 1G). CO₂ arousal was
109 enhanced more strongly in JU1249 animals acclimated to 21% O₂ than in those acclimated to
110 7% O₂ (Figure 1H), suggesting that JU1249 fails to suppress CO₂ escape at 21% O₂.

111 The increased locomotory arousal of JU1249 and MY16 in response to CO₂ could reflect
112 reduced inhibitory input from the neural circuit signaling 21% O₂. To probe this, we asked if
113 these isolates show altered behavioral responses to 21% O₂. All isolates we tested responded
114 similarly when we switched O₂ from 7% to 21% (Figure S2B), suggesting they retained a
115 functional O₂-sensing circuit.

116 In our assays, animals acclimated to 21% O₂ experienced a downshift to 7% O₂ 3 minutes
117 before the CO₂ stimulus. To ask if this drop in O₂, rather than O₂ experience, altered the CO₂

118 response in JU1249 we extended the time animals spent at 7% O₂ prior to receiving the CO₂
119 stimulus to 24 minutes. JU1249 was still more strongly aroused by CO₂ when acclimated to
120 21% rather than 7% O₂; as expected, O₂ experience had the opposite effect on plasticity in
121 *npr-1*; *glb-5* controls (Figure S2C). We also compared the behavioral responses of JU1249
122 and *npr-1*; *glb-5* animals to a 21% to 7% O₂ stimulus, and found no significant differences
123 (Figure S2D). Thus, the ability of an O₂ memory to modify CO₂ escape appears to be altered
124 in JU1249, recapitulating the phenotype observed in *C. nigoni*.

125 **Natural Variation in the Ankyrin Repeat Protein ARCP-1 Alters Plasticity of CO₂** 126 **Responses**

127 We sought the genetic changes conferring altered plasticity of CO₂ responses in JU1249.
128 Besides altering this phenotype, JU1249 exhibited reduced aggregation and bordering
129 behavior on an *E. coli* food lawn compared to other *C. elegans* wild isolates (Figures 2A and
130 2B). We speculated JU1249 aggregated poorly because increased avoidance of CO₂ shifted
131 the balance between attraction and repulsion as aerobic animals come together. In this model
132 the aggregation phenotype, which is easy to score, is linked to altered JU1249 CO₂ responses.

133 Before testing this hypothesis, we ruled out the possibility that JU1249 is genetically
134 contaminated by the non-aggregating N2 lab strain, by genotyping the *npr-1*, *glb-5* and *nath-*
135 *10* loci, which have acquired polymorphisms during N2 domestication (Duveau and Félix,
136 2012; McGrath et al., 2009; Persson et al., 2009; Weber et al., 2010). JU1249 exhibited the
137 natural alleles found in other wild isolates at all three loci (Figure S3).

138 To map the JU1249 aggregation defect, we used a selection-based QTL mapping approach in
139 which we crossed JU1249 to the aggregating *C. elegans* wild isolate JU2825 (Figure 2A). To
140 find conditions for selection-based QTL mapping, we first defined two treatments that
141 differentially selected for aggregating and solitary animals and performed competition tests

142 between JU1249 and JU2825 under these treatments. Starting with a 50:50% mix of each
143 strain, JU1249 (solitary) outcompeted JU2825 (aggregating) when the populations were
144 transferred by liquid harvest and aliquot (Figure S4A, Treatment A), indicating that JU1249
145 has higher fitness in these conditions than JU2825. When cultivated by transferring an agar
146 chunk from the border of the food lawn, where aggregating animals accumulate (Figure S4A,
147 Treatment B), JU2825 outcompeted JU1249, which indicates the aggregation trait in *C.*
148 *elegans* is selectable. We used treatments A and B as selection regimes on populations of
149 cross-progenies of JU1249 and JU2825 (Figure 2C), sequenced their genomes and compared
150 allele frequencies of paired replicate populations under the two treatments (Data Table S1)
151 (see STAR Methods). Populations selected for aggregation (Treatment B) were expected to
152 have higher frequencies of JU2825 alleles at the QTL that affect the variation in aggregation
153 behavior compared to the paired populations (Treatment A). Our analysis showed large
154 variation in allele frequencies among replicates, suggesting founder effects due to the
155 moderate population sizes in the first crosses (Figures 2D and S4B). We used two criteria to
156 identify candidate QTL regions associated with the aggregation phenotype. First, we
157 identified regions that show consistent differences in allele frequencies among all replicate
158 pairs for the two treatments (Figures S4B and S4C). Second, we narrowed down these regions
159 by examining replicates for the position of the closest recombination event that was selected
160 (Figure S4D). Based on these criteria, we identified a genomic interval on chromosome III
161 (3361869-4086899 bp) as a candidate region, showing a highly significant difference in allele
162 frequencies among the eight population pairs (Figures 2E and S4C).

163 The 725 kb QTL region in JU1249 contained 3 polymorphisms in protein-coding genes
164 compared to N2 and JU2825 (Data Table S1J). An 8 bp deletion (*mfp22*) in the open reading
165 frame of the gene F34D10.6, which we named *arcp-1* (for *ankyrin repeat containing protein*,
166 see below), stood out as a promising candidate for two reasons. First, *mfp22* is the only

167 polymorphism predicted to abolish protein function (Data Tables S1J and S1K), introducing a
168 frame-shift and premature stop codon in both transcripts of the *arcp-1* gene (Figures 2F and
169 S5A). Second, we independently found several alleles of *arcp-1* in a collection of sequenced
170 mutants that suppress aggregation behavior of *npr-1(null)* animals, including two that
171 introduced premature stop codons. The number and kind of these alleles made it likely that
172 disrupting *arcp-1* caused an aggregation defect. Consistent with this hypothesis, the
173 aggregation defect of one strain (*db1082* allele) mapped to a 1 Mb interval on chromosome
174 III, centered on *arcp-1* (Figures 2F and S5A). Two mutants from the million mutation project
175 (Thompson et al., 2013), harboring *arcp-1* alleles (*gk856856* and *gk863317*) that introduce
176 premature stop codons, were also defective in aggregation and bordering (Figures S5B and
177 S5C). To show conclusively that mutations in *arcp-1* disrupt aggregation, we performed
178 transgenic rescue experiments. Expressing wild-type *arcp-1* in JU1249 or in *arcp-1(db1082)*;
179 *npr-1(null)* mutants restored aggregation and bordering behavior (Figure 2G).

180 To gain insight into the distribution of the *arcp-1(mfP22)* polymorphism in *C. elegans*, we
181 examined other wild isolates. Our analysis suggests *mfP22* is a rare allele, because we did not
182 find it in a set of 151 worldwide *C. elegans* isolates, including MY16 (Data Table S2).

183 Does disrupting *arcp-1* alter responses to CO₂? *arcp-1(db1082); npr-1(null)* animals behaved
184 like JU1249: they showed no overt defect in their response to a 21-to-7% O₂ downshift
185 (Figure S5D) but failed to suppress escape from different CO₂ concentrations when
186 acclimated to 21% O₂ (Figures 2H, S5E and S5F). A wild-type *arcp-1* transgene rescued this
187 CO₂ plasticity defect (Figure 2I). *arcp-1* is thus required for animals acclimated to 21% O₂ to
188 suppress escape from high CO₂ environments.

189 Gene predictions and cDNA cloning revealed *arcp-1a* and *b* transcripts that overlap at their 3'
190 end (Figure 2F; Wormbase WS265). The *db1082* and *mfP22* alleles affect both *arcp-1*

191 transcripts (Figure 2F). Expressing *arcp-1b* fully rescued the heightened CO₂ response of
192 these animals, whereas a transgene for the longer *arcp-1a* transcript did not (Figures 2J and
193 2K). A mutation that only disrupted *arcp-1a* also failed to recapitulate the enhanced CO₂
194 response and aggregation phenotype of mutants defective in both *arcp-1* transcripts (Figures
195 S5C and S5G). Thus *arcp-1*, and more specifically the product of its *b* transcript, is required
196 for animals to suppress CO₂ escape following acclimation to 21% O₂.

197 **ARCP-1 Acts in BAG Sensory Neurons to Suppress CO₂ Escape Behavior**

198 *arcp-1* encodes an ankyrin repeat protein (Figure 3A) homologous to *C. elegans* ankyrin
199 UNC-44 and vertebrate ankyrins (Otsuka et al., 1995). These proteins are important for the
200 subcellular localization of neural signaling complexes, e.g. anchoring components of the axon
201 initial segment and allowing cyclic nucleotide-gated channels to accumulate in photoreceptor
202 cilia (Kizhatil et al., 2009; Leterrier et al., 2017; Maniar et al., 2011). Besides ankyrin repeats,
203 ARCP-1 contains a DPY-30 domain (Figure 3A). Both domains are common protein
204 interaction motifs that regulate the function and spatial organization of diverse signaling
205 complexes (Gopal et al., 2012; Jones and Svitkina, 2016; Monteiro and Feng, 2017; Sivadas
206 et al., 2012). ARCP-1's domain structure suggests it serves a similar role trafficking or
207 localizing signaling proteins in the nervous system.

208 A fosmid-based bicistronic transgene that co-expressed *arcp-1* and free GFP was expressed in
209 the main CO₂ and O₂ sensors: the URX, AQR, PQR and BAG neurons (Figures 3B and 3C).
210 We also observed expression in a subset of other sensory neurons, i.e. AFD, ASE, AWC and
211 AWB (Figure 3C). This raised the possibility that disrupting *arcp-1* modifies plasticity in
212 multiple paradigms. To test this we assayed *arcp-1* mutants in a salt-based associative
213 learning paradigm (Figure S6A) (Beets et al., 2012; Hukema et al., 2008). *arcp-1* mutants
214 were defective in gustatory plasticity: although mock-conditioned animals showed normal

215 attraction to NaCl, upon salt conditioning they failed to downregulate salt chemotaxis
216 behavior (Figure S6B).

217 To gain insight into *arcp-1* function, we focused on the failure of *arcp-1* mutants to suppress
218 CO₂ escape when acclimated to 21% O₂. Since *arcp-1* is expressed in the BAG CO₂ sensors,
219 we asked if it acts in these neurons to suppress CO₂ escape. Cell-specific expression of wild-
220 type *arcp-1* in BAG using the *flp-17* promoter (Kim and Li, 2004) rescued the increased
221 locomotory activity of *arcp-1* mutants at 3% CO₂ (Figure 3D). We also tested if *arcp-1* can
222 act in URX, AQR and PQR neurons, which sense 21% O₂, to suppress CO₂ escape.

223 Expressing *arcp-1* in these neurons, using the *gcy-32* promoter (Yu et al., 1997), did not
224 rescue the CO₂ phenotype of *arcp-1* mutants (Figure 3D). By contrast, the *arcp-1* aggregation
225 defect could be rescued by expressing *arcp-1* either in BAG or in URX, AQR and PQR
226 (Figures S5H and S5I). Together, these data show that *arcp-1* functions in gas-sensing
227 neurons and cell-autonomously suppresses CO₂ escape in the BAG CO₂ sensors.

228 **BAG Responses to CO₂ are Tuned by ARCP-1**

229 We investigated if the increased behavioral response of *arcp-1* animals to CO₂ was associated
230 with increased CO₂-evoked Ca²⁺ responses in BAG neurons. Using the ratiometric sensor
231 YC3.60, we quantified fluorescence changes at the cell body of BAG in response to CO₂.
232 Animals acclimated to 21% O₂ were transferred to a microfluidic chamber kept at 7% O₂, and
233 stimulated with different CO₂ concentrations. BAG Ca²⁺ responses evoked by 1% and 3%
234 CO₂ were significantly higher in *arcp-1* mutants compared to controls (Figure 4A). Unlike for
235 CO₂ escape, expressing *arcp-1* either in BAG or in URX, AQR and PQR rescued the CO₂
236 Ca²⁺ phenotype in BAG (Figure 4B). At 3% CO₂ animals with *arcp-1* rescued in URX, AQR
237 and PQR even showed a smaller increase in Ca²⁺ activity compared to *npr-1* controls, which
238 may be due to an overexpression effect of the *gcy-32p::arcp-1b* transgene (Figure 4B). Since

239 BAG neurons respond tonically to CO₂, we also measured Ca²⁺ responses during prolonged
240 CO₂ stimulation. BAG tonic responses to 3% CO₂ were reduced in *arcp-1* mutants, although
241 the effect was small (Figure S6C).

242 BAG neurons respond not only to a rise in CO₂, but also to a fall in O₂ (Zimmer et al., 2009).
243 We asked if the CO₂ phenotypes of *arcp-1* animals could be indirectly linked to changes in
244 BAG's ability to respond to O₂. BAG Ca²⁺ activity at 7% O₂, measured by YC2.60, was
245 similar for *arcp-1* mutants and *npr-1* controls, although *arcp-1* animals displayed higher Ca²⁺
246 at 21% O₂ (Figure S6D). Ca²⁺ responses in URX to a 7% to 21% O₂ stimulus were unaffected
247 in *arcp-1* animals (Figure S6E).

248 Disrupting *arcp-1* could potentiate escape from CO₂ by altering BAG responses to O₂. BAG
249 O₂ responses are mediated by the guanylate cyclases GCY-31/GCY-33 and are abolished in
250 mutants of these genes (Zimmer et al., 2009). Animals lacking *gcy-33* and *gcy-31*, like *arcp-1*
251 mutants, were aroused more strongly by CO₂, but the effects on CO₂ escape were additive in
252 an *arcp-1; gcy-33; gcy-31; npr-1* quadruple mutant (Figure S6F). Moreover, in *gcy-33; gcy-*
253 *31* mutants, CO₂ arousal was suppressed when animals were acclimated to 21% O₂ – unlike in
254 *arcp-1* animals (Figure S6G). These results indicate that *arcp-1* can act in a separate genetic
255 pathway from *gcy-33* and *gcy-31* to regulate CO₂ escape. Together with our rescue and Ca²⁺
256 imaging data, these findings are consistent with *arcp-1* suppressing CO₂ escape by inhibiting
257 BAG responses to CO₂.

258 **ARCP-1 Inhibits BAG-Mediated Turning Downstream of the CO₂ Receptor GCY-9**

259 *C. elegans* respond to a rise in CO₂ not only by becoming aroused and moving faster but also
260 by re-orienting their direction of travel and increasing the frequency of sharp (omega) turns.
261 This behavior is also mediated by BAG (Fenk and de Bono, 2015; Hallem and Sternberg,
262 2008). Since ARCP-1 acts in BAG to suppress CO₂-evoked Ca²⁺ responses and locomotory

263 arousal, we asked if it also inhibits CO₂-evoked turning. Both *arcp-1* mutants and JU1249
264 showed increased turning in response to a rise in CO₂ compared to controls (Figures 4C and
265 4D). This phenotype was rescued by expressing *arcp-1* in BAG, but not by expressing it in
266 URX, AQR and PQR (Figure 4D).

267 To gain insight into the molecular functions of *arcp-1*, we examined its effect on CO₂-evoked
268 turns further. This part of the locomotory response to CO₂ is driven by cGMP signaling from
269 the guanylyl cyclase receptor GCY-9 in BAG neurons (Fenk and de Bono, 2015; Hallem et
270 al., 2011). GCY-9 is a molecular receptor for CO₂ and appears to be specifically expressed in
271 BAG (Hallem et al., 2011; Smith et al., 2013). To examine if *arcp-1* regulates turning
272 downstream of GCY-9, we measured CO₂-evoked turns in a *gcy-9; arcp-1* mutant. Disrupting
273 *gcy-9* abolished turning evoked by 3% CO₂ in both *npr-1* and *arcp-1; npr-1* animals (Figure
274 4E), which implies that the mutant's turning phenotype depends on GCY-9, and that ARCP-1
275 antagonizes GCY-9 signaling in BAG.

276 **ARCP-1 Localizes Phosphodiesterase PDE-1 to BAG Cilia**

277 The ankyrin repeats and DPY-30 motif of ARCP-1 suggest it serves as an interaction partner
278 or scaffold for other proteins. To identify its molecular partners we took a biochemical
279 approach (Figure 5A). We first made a transgenic strain that expressed GFP-ARCP-1B and
280 showed that it rescued the enhanced CO₂ response of the *arcp-1* mutant (Figure S7A). We
281 then used anti-GFP nanobodies to pull down GFP-ARCP-1B fusion proteins from *C. elegans*
282 lysates, and identified putative interacting proteins by mass spectrometry (Figure 5A; Data
283 Table S3). As a negative control we immunoprecipitated other GFP-tagged cytoplasmic
284 proteins in parallel. Across two independent experiments, we identified phosphodiesterase 1
285 (PDE-1) as the top specific hit, i.e. the protein having the highest number of spectral counts in
286 ARCP-1B IPs while having none in control IPs (Figure 5B; Data Table S3).

287 PDE-1 is a Ca²⁺-activated cGMP/cAMP phosphodiesterase orthologous to mammalian
288 Ca²⁺/calmodulin-dependent PDE1, and is expressed in many neurons, including BAG (Couto
289 et al., 2013; Hallem et al., 2011). As expected from our biochemical data, PDE-1 and ARCP-
290 1 localize to similar compartments in BAG. GFP-tagged ARCP-1B was enriched at sensory
291 endings (Figure 5C), similar to what we observe and has been reported for PDE-1 (Martínez-
292 Velázquez and Ringstad, 2018) (Figure 5D).

293 The biochemical interaction of ARCP-1 and PDE-1, and their co-localization at dendritic
294 endings led us to hypothesize that ARCP-1 regulates PDE-1 localization. To test this, we
295 compared enrichment of PDE-1 at BAG cilia in *arcp-1* and control animals. Overall PDE-1
296 expression was slightly higher in *arcp-1* mutants, but enrichment of PDE-1 at the cilia was
297 reduced by more than half in these animals (Figure 5E). To extend this observation, we
298 investigated the subcellular localization of other signaling components of the gas-sensing
299 neurons in *arcp-1* mutants. We observed a reduction of GCY-9 levels in BAG cilia, as well as
300 reduced levels of the O₂-sensing guanylate cyclase GCY-35 at the sensory endings of URX
301 (Figures S7B and S7C). These phenotypes were not due to a general defect in dendritic
302 localization, because *arcp-1* mutants showed normal levels of the cGMP-gated channel
303 subunit TAX-4 and the O₂-sensing guanylate cyclase GCY-33 in BAG cilia (Figures S7D and
304 S7E). *arcp-1* mutants did not exhibit overt defects in dendritic morphology, based on
305 expression of a *flp-17p::gfp* transgene and DiI filling of amphid sensory neurons (Figure S7F
306 and S7G). Together, our data suggest that ARCP-1 acts as a scaffold that helps co-localize
307 signal transduction components at sensory endings of some neurons.

308 Our behavioral, Ca²⁺ imaging, and cell biological results led us to speculate that ARCP-1
309 promotes a Ca²⁺-dependent feedback mechanism mediated by PDE-1, which keeps BAG CO₂
310 responses in check by degrading cGMP following activation of the CO₂ receptor GCY-9. If
311 this is correct, disrupting *pde-1* should phenocopy *arcp-1* and increase the frequency of CO₂-

312 evoked turns. Moreover, the *arcp-1* and *pde-1* phenotypes should not be additive. As
313 predicted, *pde-1* mutants turned more in response to 3% CO₂ than controls and even *arcp-1*
314 mutants, likely because *pde-1* is more widely expressed and serves broader functions than
315 *arcp-1*. The turning phenotype was comparable for *pde-1*, *arcp-1* and *pde-1; arcp-1* mutants
316 (Figure 5F). These results are consistent with *pde-1* and *arcp-1* acting in the same genetic
317 pathway to keep CO₂ responses in check.

318 **PDE-1 and ARCP-1 Inhibit Expression of FLP-19 Neuropeptides**

319 To investigate further how disrupting *arcp-1* alters BAG function, we specifically labeled
320 these neurons with GFP, used FACS to isolate the fluorescent cells from acutely dissociated
321 *arcp-1;npr-1* and *npr-1* control animals, and profiled their gene expression using RNA-Seq
322 (see STAR Methods). Genes that are hallmarks of BAG, such as those involved in CO₂
323 signaling (*gcy-9*, *pde-1*, *flp-17*) and BAG cell fate determination (*ets-5*) (Guillermin et al.,
324 2011; Hallem et al., 2011) were among the top enriched genes in our dataset (Data Table S4).
325 *arcp-1* itself was among the 100 most highly expressed genes in BAG. The BAG profiles
326 highlighted significant gene expression differences between *arcp-1* mutants and controls,
327 notably changes in the abundance of mRNAs encoding neuropeptides, genes involved in
328 ciliary intraflagellar transport, ion channels and gap junction subunits (see Discussion) (Data
329 Table S4D). These data suggest that loss of ARCP-1 leads to altered gene expression.

330 One of the most abundant transcripts expressed in BAG whose expression was significantly
331 altered by defects in *arcp-1* was the neuropeptide *flp-19*. *flp-19* expression was upregulated
332 2.4 fold in *arcp-1* animals, which would be consistent with increased BAG signaling.

333 Previous work has shown that GCY-9, PDE-1 and the cGMP-gated Ca²⁺ channel TAX-4
334 control *flp-19* expression in BAG (Romanos et al., 2017), making it an interesting candidate
335 for altering CO₂ responses in the *arcp-1* mutant. To confirm that defects in *arcp-1* increased

336 expression of *flp-19*, we introduced a *flp-19p::gfp* reporter transgene (Kim and Li, 2004) into
337 *arcp-1* mutants and quantified fluorescence in BAG neurons. Disrupting *arcp-1* significantly
338 increased BAG expression of the neuropeptide reporter (Figure 6A). This phenotype was
339 rescued by expressing wild-type *arcp-1* in BAG, but not in the O₂ sensors URX, AQR and
340 PQR (Figure 6A). Thus, *arcp-1* controls *flp-19* expression cell-autonomously in BAG. We
341 observed a similar increase in expression of the *flp-19* reporter when *pde-1* was mutated
342 (Figure 6A). BAG expression of *flp-19* in mutants lacking both *arcp-1* and *pde-1* was similar
343 to that of the single mutants (Figure S7H). These data suggest that ARCP-1 and PDE-1
344 together reduce BAG signaling by lowering the expression of some neuropeptides. However,
345 disrupting *arcp-1* does not generally increase BAG neuropeptide expression as judged from
346 our BAG profiling experiments (Data Table S4) and analysis of a *flp-17* neuropeptide reporter
347 in BAG (Figure S7I).

348 To ask if *flp-19* expression was elevated in JU1249, we backcrossed the *flp-19p::gfp*
349 transgene ten times to this isolate. We did the same for JU2825 that, unlike JU1249,
350 suppressed CO₂ escape when acclimated to 21% O₂ (Figure S2A). BAG expression of *flp-19*
351 was low in JU2825 and high in JU1249 (Figure 6B). Restoring *arcp-1* in BAG significantly
352 reduced *flp-19* expression (Figure 6B). Thus, disrupting *arcp-1* also increases *flp-19*
353 expression in JU1249.

354 **FLP-19 Neuropeptide Signaling from BAG Potentiates Behavioral Responses to CO₂**

355 Does increased BAG expression of *flp-19* in *arcp-1* mutants enhance the behavioral responses
356 of these animals to CO₂? If increased *flp-19* expression heightened aversion to CO₂ in *arcp-1*
357 animals, then disrupting *flp-19* should reverse this phenotype. Consistent with this hypothesis,
358 deleting *flp-19* restored turning at 3% CO₂ in the *arcp-1* mutant, while it had no effect on this
359 behavior in *npr-1* animals (Figure 6C).

360 To confirm that FLP-19 release from BAG potentiates CO₂ responses, we knocked down *flp-*
361 *19* expression specifically in these neurons by expressing RNAi sense and antisense
362 sequences of *flp-19* from a BAG-specific *gcy-33* promoter (Hallem et al., 2011; Yu et al.,
363 1997). As a negative control, we expressed sense and antisense sequences for *gfp* under the
364 same promoter and found it had no effect on CO₂ responses (Figure 6D and 6E). By contrast,
365 BAG-specific knockdown of *flp-19* in *arcp-1* mutants restored the frequency of CO₂-evoked
366 turns (Figure 6D) and reduced CO₂-evoked locomotory arousal in animals acclimated to 21%
367 O₂ (Figure 6E). These data suggest increased *flp-19* expression in BAG mediates the
368 enhanced behavioral responses to CO₂ in *arcp-1* mutants.

369 The neuropeptide gene *flp-19* is also expressed in URX. However, knockdown of *flp-19* in
370 these neurons, using the *gcy-32* promoter, enhanced rather than reduced locomotory arousal at
371 3% CO₂ in *arcp-1* animals, and increased baseline locomotion in the absence of CO₂ (Figure
372 S7K). This result is consistent with previous reports (Carrillo et al., 2013) and suggests that
373 the RNAi effect in BAG is specific to these neurons. We wondered if altered expression of
374 *flp-19* from URX contributes to the enhanced CO₂ aversion in *arcp-1* animals as well. If this
375 is the case, *flp-19* expression in URX should be reduced in *arcp-1* mutants. Indeed, disrupting
376 *arcp-1* decreased expression of the *flp-19* reporter in URX. This phenotype was rescued by
377 expressing *arcp-1* either in URX or BAG neurons (Figure S7L), suggesting that BAG
378 signaling indirectly influences *flp-19* expression in URX.

379 Does FLP-19 release from BAG promote escape from CO₂ in animals that retain functional
380 *arcp-1*? In *npr-1* animals, BAG-specific knockdown of *flp-19* did not compromise CO₂
381 escape in animals acclimated to 21% O₂ or 7% O₂ (Figure 6F). Thus, *flp-19* is not required for
382 the O₂-dependent modulation of CO₂ responses. Consistent with this finding, we observed
383 similar expression of the *flp-19* reporter in *npr-1* animals acclimated to 21% or 7% O₂,
384 suggesting that *flp-19* expression is not regulated by O₂ experience (Figure S7J).

385 We next asked if increased *flp-19* expression in BAG is sufficient to boost *C. elegans*'
386 locomotory arousal by CO₂. To test this we overexpressed *flp-19* specifically in the BAG
387 neurons of *npr-1* animals, acclimated these transgenic animals to 21% O₂, and quantified their
388 speed at 3% CO₂. Animals overexpressing *flp-19* in BAG moved significantly faster at 3%
389 CO₂ than *npr-1* controls, although their locomotory arousal was weaker than that of *arcp-1*
390 animals or of *npr-1* animals grown at 7% O₂ (Figure 6G). Thus acclimation to 7% O₂ or
391 disrupting *arcp-1* alters other signals besides *flp-19* to heighten CO₂ responses. However, in
392 both scenarios - disruption of *arcp-1* or O₂ acclimation - increasing *flp-19* expression in BAG
393 can potentiate behavioral responses to CO₂, leading to increased CO₂ aversion.

394 **Discussion**

395 Individuals differ in how they respond to altered circumstances in their environment. This is
396 generally ascribed to a combination of genetic variation and different life experiences. How
397 neural circuits encoding behavioral plasticity vary across individuals is, however, poorly
398 understood. Here we show that *Caenorhabditis* species and wild isolates of *C. elegans* can
399 differ in how past O₂ experience influences CO₂ escape behavior. We uncover a genetic
400 variant and neuronal mechanism responsible for this variation in behavioral flexibility in one
401 natural *C. elegans* isolate.

402 The behavioral phenotypes that we observe are reminiscent of genetic accommodation, when
403 the reaction norm of a flexible phenotype responsive to the environment is altered by genetic
404 change (Figure 7A). Underlying this behavioral change, we find that disrupting ARCP-1 both
405 increases CO₂ sensitivity and alters the effect of previous O₂ experience on CO₂ escape.
406 Animals lacking this dendritic scaffold protein become strongly aroused by CO₂ regardless of
407 previous O₂ experience, and acclimation to 21% O₂ further enhances, rather than suppresses,

408 escape from this aversive cue. We show that loss of *arcp-1* mediates these phenotypes by
409 directly altering CO₂ responses, rather than by affecting the ability to respond to O₂.

410 We identify the BAG CO₂ sensors as the main site where ARCP-1 suppresses CO₂ escape in
411 animals acclimated to 21% O₂. Together with previous work (Couto et al., 2013; Romanos et
412 al., 2017), our results suggest a model (Figure 7B) in which ARCP-1 binds and co-localizes
413 the Ca²⁺-activated phosphodiesterase PDE-1 with guanylyl cyclase receptors for CO₂ at the
414 BAG cilia. ARCP-1 and PDE-1 keep signaling from these neurons in check by suppressing
415 CO₂-evoked Ca²⁺ responses and neuropeptide expression. Natural genetic variation has been
416 found to directly alter sensory systems in other animals (McGrath, 2013; Prieto-Godino et al.,
417 2017). We identify BAG as a major cellular focus for variation in CO₂ responses, but the
418 possibility remains that loss of *arcp-1* disrupts plasticity in other sensory circuits, which may
419 indirectly promote CO₂ aversion as well. Some evidence points to changes in URX, but these
420 are not sufficient to explain the heightened CO₂ escape behavior in *arcp-1* mutants.

421 Mounting evidence suggests that natural variation in behavioral flexibility is genetically
422 determined (Izquierdo et al., 2007; Mery, 2013; Mery et al., 2007). One well-established
423 example is the natural variation seen at the *foraging* gene in *Drosophila melanogaster*. This
424 polymorphism causes individual variation in learning and memory, amongst other
425 phenotypes, by altering the activity of cGMP-dependent protein kinase G (Mery et al., 2007).
426 It is notable that both in flies and worms genetic variation affecting cGMP signaling underlies
427 inter-individual variation in experience-dependent plasticity. Besides gas sensors, ARCP-1 is
428 expressed in olfactory, gustatory and thermosensory neurons that all signal using cGMP, and
429 *arcp-1* mutants show reduced plasticity in a gustatory paradigm. Correlated differences in the
430 plasticity of different sensory modalities have been described as coping styles or behavioral
431 syndromes in other animal models (Coppens et al., 2010), and may also reflect a common
432 genetic or molecular basis. Identifying how loss of *arcp-1* compromises plasticity in other

433 sensory circuits should provide a better understanding of such correlated changes in
434 behavioral flexibility.

435 We have shown that the absence of ARCP-1 alters expression of a range of genes in BAG.
436 One way this influences CO₂ aversion is by altering the expression of neuropeptide
437 messengers. Neuropeptides are a diverse group of neuromodulators that, both in vertebrates
438 and invertebrates, are involved in circuit plasticity (Jékely et al., 2018; Taghert and Nitabach,
439 2012). Natural genetic variation in neuropeptide pathways has been linked to individual
440 differences in aging and social behaviors (Donaldson and Young, 2008; Yin et al., 2017). Our
441 results suggest that they also contribute to heritable differences in behavioral plasticity
442 between individuals. In humans and other primates, natural polymorphisms in serotonergic
443 and dopaminergic systems have been associated with individual differences in memory and
444 cognitive ability (Izquierdo et al., 2007; Zhang et al., 2007). Changing the neuromodulatory
445 tone of circuits likely represents a general mechanism by which genetic variation sculpts
446 individual behavioral plasticity.

447 Disrupting ARCP-1 increases expression of FLP-19 neuropeptides in BAG. This potentiates
448 or disinhibits both CO₂-evoked turning and locomotory arousal in animals acclimated to 21%
449 O₂. A FLP-19 receptor is currently unknown; the *C. elegans* genome encodes about 150
450 predicted neuropeptide receptors but none have been reported to bind FLP-19 (Peymen et al.,
451 2014). FLP-19 neuropeptides belong to the ancient and conserved family of RFamide
452 neuropeptides (Peymen et al., 2014). Previous work suggested that CO₂-evoked cGMP and
453 Ca²⁺ signaling promote *flp-19* expression in BAG, and that this effect is counterbalanced by
454 PDE-1 (Romanos et al., 2017). In *arcp-1* mutants, the GCY-9 CO₂ receptor and PDE-1 are
455 less enriched at BAG cilia. Although *gcy-9* expression is slightly reduced, disrupting ARCP-1
456 increases BAG Ca²⁺ activity in response to CO₂. This is consistent with proper ciliary
457 localization of PDE-1 keeping BAG Ca²⁺ signaling in check, and could explain the increased

458 *flp-19* expression. ARCP-1 and PDE-1 may also orchestrate microdomains of cGMP that can
459 regulate gene expression (Arora et al., 2013; O'Halloran et al., 2012). In vertebrate neurons,
460 nanodomains of the ankyrin G protein, a homolog of ARCP-1, localize to the dendritic spines
461 and the axon initial segment, and contribute to neural plasticity (Grubb and Burrone, 2010;
462 Smith et al., 2014). Likewise, mammalian PDE1 has been implicated in the experience-
463 dependent adaptation of sensory responses. In mouse olfactory neurons, PDE1 is specifically
464 enriched at the cilia, although a molecular anchor that localizes the protein to this
465 compartment has not yet been identified (Cygnar and Zhao, 2009).

466 The molecular mechanism by which ARCP-1 controls *flp-19* expression and whether this
467 relates to its ciliary function, remains to be understood. Interestingly, our transcriptional
468 profiling of BAG neurons revealed a suite of genes involved in intraflagellar transport,
469 including the axonemal dynein *che-3*, that show about twofold increased expression in *arcp-1*
470 animals, although we did not observe obvious defects in cilia morphology. This suggests a
471 feedback mechanism exists by which signaling at the cilium regulates expression of genes
472 involved in ciliary transport. Identifying the molecular factors involved is the next step
473 forward toward understanding this transcriptional regulation.

474 The mechanisms through which natural genetic variation in *arcp-1*, acting on an evolutionary
475 timescale, and O₂ experience, acting over an animal's lifetime, sculpt CO₂ responsiveness
476 seem to be at least partly distinct. However, in both scenarios – disruption of *arcp-1*, or
477 acclimation to different O₂ environments – release of FLP-19 neuropeptides from BAG can
478 boost the animal's response to this aversive cue, and through alterations in neuropeptide
479 expression, a strong aversive response may become fixed.

480 CO₂ responses vary between *Caenorhabditis* species (Carrillo and Hallem, 2015; Pline and
481 Dusenbery, 1987; Viglielichio, 1990). Our results show this variation is at least in part due to

482 differences in O₂-dependent modulation, suggesting it is an adaptive trait. We speculate that
483 the influence of O₂ memory on other sensory responses enables animals to reconfigure their
484 behavioral priorities according to past experience (Fenk and de Bono, 2017). Animals at the
485 surface may prioritize escape from 21% O₂, and gradually suppress their CO₂ aversion to
486 facilitate migration to buried environments with less aeration and elevated CO₂ levels. Natural
487 variation in the O₂-dependent modulation of CO₂ escape may result in animals occupying
488 different ecological niches. Alternatively, there could be selection against the costs to
489 maintain sensory systems for behavioral plasticity (Dewitt et al., 1998), which may account
490 for the reduced plasticity of CO₂ responses in some nematode species. We do not know the
491 O₂ and CO₂ conditions in which the *arcp-1* deletion may have been selected, and can
492 therefore only speculate about its potential fitness benefits. The *arcp-1* mutation was not
493 found in any other wild isolate so is likely recent. However, our data indicate substantial
494 variation among both *Caenorhabditis* species and *C. elegans* isolates in the response to CO₂
495 (Figures 1 and S2). These findings are consistent with what has been found for other traits
496 (Frézal et al., 2018), where the phenotypic variation for the strain chosen for study is caused
497 by a rare allele found only in that strain, yet phenotypic variation itself is not restricted to that
498 strain. Understanding evolutionary mechanisms that might select for altered plasticity requires
499 more in-depth knowledge on the ecology of these species. The behavioral phenotypes that we
500 observe are consistent with genetic accommodation for a cross-modal gene-environment
501 interaction (Pigliucci et al., 2006). In summary, our study illustrates how natural genetic
502 variation, by altering the neuromodulatory control of aversive behavior, contributes to
503 individual differences in behavioral flexibility.

504

505 **Acknowledgements**

506 We thank Roger Pocock (Monash University, Australia), Niels Ringstad (New York

507 University School of Medicine, USA) and the *Caenorhabditis* Genetics Center, funded by
508 NIH Office of Research Infrastructure Programs (P40 OD010440), for strains. We gratefully
509 acknowledge Tim Stevens, Gurpreet Ghattaoraya, and members of the de Bono and Schafer
510 labs for advice and comments. This work was funded by the Medical Research Council
511 (United Kingdom). I.B. received an EMBO Long-term Fellowship (ALTF 387-2015) and EU
512 Marie-Curie Individual Fellowship (MSCA-IF 704383), and is a fellow of the Research
513 Foundation - Flanders (FWO). Work in the Félix lab was funded by a grant from the
514 Fondation pour la Recherche Médicale DEQ20150331704. G.Z. was funded in part by a
515 Fellowship from the China Scholarship Council.

516

517 **Author Contributions**

518 I.B., G.Z., M.A.F., and M.d.B. designed experiments. I.B., G.Z., L.A.F., and C.C. performed
519 experiments. I.B., G.Z., M.A.F., and M.d.B. analyzed the data. G.Z. and G.N. did the genome
520 sequence data analyses. I.B., M.A.F., and M.d.B. wrote the manuscript.

521

522 **Declaration of Interests**

523 The authors declare no competing interests.

524

525 **Figure Legends**

526 **Figure 1. Natural variation in the regulation of CO₂ escape by previous O₂ experience**

527 A) A *C. elegans* reference strain is more strongly aroused by CO₂ when acclimated to 7%
528 rather than 21% O₂. Two-way ANOVA with Šidák test; n = 6 assays. In this and all
529 subsequent Figures the background O₂ level in the assay is 7%.

530 B) Natural variation in the CO₂ response of *C. elegans* wild isolates acclimated to 21% O₂.
531 Bars represent average increase in speed ± SEM when CO₂ rises from 0% to 3%. The CO₂-
532 evoked speed increase is significantly different ($P < 0.05$) between isolates labeled with
533 different letters (a-d). One-way ANOVA with Tukey test; n = 6 assays.

534 C) The effect of O₂ memory on CO₂ responses in wild *C. elegans* isolates. Bars show mean ±
535 SEM for time intervals indicated in Figures 1A and S2A. Two-way ANOVA with Šidák test;
536 n = 6 assays.

537 D) JU1249 and MY16 are more strongly aroused by CO₂, regardless of previous O₂
538 experience. Bars plot mean ± SEM. Two-way ANOVA with Tukey test; n = 6 assays.

539 E-F) CO₂ responses of MY16 (E) and JU1249 (F) animals acclimated to 21% or 7% O₂. Two-
540 way ANOVA with Šidák test; n = 6 assays.

541 G) Acclimation to 21% O₂ in JU1249, unlike the reference strain LSJ1, enhances rather than
542 suppresses locomotory arousal at different CO₂ concentrations. n = 30 – 61 animals for *npr-1*;
543 *glb-5*, n = 59 – 66 animals for JU1249. Mann-Whitney *u* test.

544 H) CO₂ arousal is increased more strongly in JU1249 animals acclimated to 21% rather than
545 7% O₂. Bars plot mean ± SEM for time intervals indicated in panel G. Two-way ANOVA
546 with Šidák test. n = 4 assays.

547 For panels A and E-F, solid lines plot mean and shaded areas show SEM. Black bars indicate
548 time intervals used for statistical comparisons. For A-H, 20-30 animals were assayed in at
549 least 4 trials for each condition. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$; ns,
550 not significant. See also Figures S1 and S2.

551

552 **Figure 2. Natural variation in ARCP-1 alters CO₂ responses**

553 A-B) Individuals of JU2825, like most *C. elegans* wild isolates, aggregate at the border of an
554 *E. coli* lawn (A). By contrast, JU1249 animals disperse across the lawn (B).

555 C) Selection-based QTL mapping approach to establish the genetic basis of solitary behavior
556 in JU1249.

557 D) Line plots showing differences in JU1249 allele frequencies between Treatment A and B
558 for each replicate pair, using a sliding window 5 SNPs wide and a step size of one SNP.
559 Replicates are indicated by different colors. Chromosome I shows little consistent deviations
560 from equal frequencies in the two treatments, whereas chromosome III shows a strong
561 enrichment at the 3 – 4 Mb interval.

562 E) Read count frequency differences between Treatment A and B analyzed for consistency
563 across eight replicates using the Cochran-Mantel-Haenszel test. Only chromosome III is
564 shown. P values are shown as $-\log_{10}(P\text{-value})$ adjusted by the Bonferroni correction.

565 F) Gene structure of *arcp-1* (F34D10.6). Boxes represent exons and lines indicate introns.
566 The wild isolate JU1249 has an 8 bp deletion that introduces a frame-shift. The *db1082* allele,
567 isolated in a genetic screen for aggregation-defective mutants, replaces a Gln codon with a
568 premature stop codon.

569 G) Wild-type *arcp-1b* rescues bordering and aggregation phenotypes of JU1249 and *db1082*
570 animals. For each assay, 50-60 animals were transferred to a bacterial lawn and behaviors
571 were scored after 6 hours. One-way ANOVA with Tukey test. $n \geq 6$ assays.

572 H) *arcp-1(db1082)* animals, like JU1249, fail to suppress CO₂ responses when acclimated to
573 21% O₂. $n = 5 - 6$ assays. Two-way ANOVA with Šidák test.

574 I) Expressing wild-type *arcp-1* restores the O₂-dependent modulation of CO₂ responses in
575 *arcp-1; npr-1* mutants. $n = 67 - 105$ animals. Mann-Whitney u test.

576 J) An *arcp-1b* transgene, but not *arcp-1a*, restores locomotory arousal by CO₂ in *arcp-1; npr-*
577 *1* animals acclimated to 21% O₂. $n \geq 4$ assays for all genotypes. One-way ANOVA with
578 Tukey test.

579 K) An *arcp-1b* transgene rescues the enhanced CO₂ response of JU1249 animals acclimated
580 to 21% O₂. n = 6 assays. One-way ANOVA with Tukey test.
581 For H-K, each genotype was tested in at least 4 assays with 20-30 animals per trial. Solid
582 lines plot mean; shaded areas show SEM; horizontal black bars indicate time intervals for
583 statistical comparisons; vertical bars plot mean ± SEM. ** *P* < 0.01; *** *P* < 0.001; **** *P* <
584 0.0001; ns, not significant. See also Figures S3-S5 and Data Tables S1 and S2.

585

586 **Figure 3. ARCP-1B acts in BAG sensors to suppress CO₂ escape behavior**

587 A) Protein domain architecture of ARCP-1B.

588 B) Schematic model of the core neural circuits for O₂ and CO₂ responses in *C. elegans* (Fenk
589 and de Bono, 2017; Guillermin et al., 2017; Laurent et al., 2015). O₂-sensing neurons URX,
590 AQR and PQR tonically signal 21% O₂. CO₂ stimuli and O₂ downshifts are detected by BAG
591 and other neurons. The O₂ sensors cross-modulate the neural circuit underlying CO₂ escape.
592 The role of RIA, RIG, AIA and AIZ in the CO₂ circuit is hypothesized based on their function
593 in CO₂ aerotaxis (Guillermin et al., 2017).

594 C) A fosmid reporter transgene for *arcp-1* is expressed in all major O₂ and CO₂ sensors, and
595 other sensory neurons. Scale bar = 10 μm; A = anterior; V = ventral.

596 D) Cell-specific expression of *arcp-1b* in BAG, using the *flp-17* promoter (*BAGp*), rescues
597 locomotory arousal by CO₂, whereas expression in URX, AQR and PQR, using the *gcy-32*
598 promoter (*URX-AQR-PQRp*), does not. One-way ANOVA with Tukey test. n ≥ 5 assays with
599 20-30 animals per trial. * *P* < 0.05; *** *P* < 0.001; **** *P* < 0.0001; ns, not significant. See
600 also Figures S5 and S6.

601

602 **Figure 4. ARCP-1 suppresses BAG responses to CO₂**

603 A-B) Mean traces of BAG Ca²⁺ activity in *npr-1* and *arcp-1*; *npr-1* animals in response to
604 different CO₂ concentrations. Mutants for *arcp-1* show increased Ca²⁺ activity at 1% and 3%
605 CO₂ (A), which is rescued by expressing *arcp-1* either in BAG (*flp-17p*) or URX, AQR and
606 PQR (*gcy-32p*) (B). n = number of animals. Two-way ANOVA with Šidák test in A. One-
607 way ANOVA with Holm-Šidák test in B.

608 C-E) CO₂-evoked turning behavior: C) Rising CO₂ levels stimulate stronger turning behavior
609 in JU1249 (n = 85 animals) than in *npr-1(215F)* animals (n = 81). Mann-Whitney *u* test. (D)
610 CO₂-evoked turning is also increased in *arcp-1(db1082); npr-1(ad609)* animals. BAG-
611 specific expression of a *flp-17p::arcp-1b* transgene rescues this phenotype, whereas
612 expression of *arcp-1b* in URX, AQR and PQR (*gcy-32p*) does not. One-way ANOVA with
613 Tukey test. n ≥ 5 assays with 20-30 animals per trial. E) The increased turning of *arcp-1; npr-*
614 *1* animals in response to CO₂ requires the GCY-9 CO₂ receptor. One-way ANOVA with
615 Tukey test. n = 9 assays with 20-30 animals per trial.

616 For A-E, solid lines plot mean; shaded areas show SEM; black bars indicate time intervals for
617 statistical comparisons; bar graphs plot mean ± SEM for these intervals. * *P* < 0.05; ** *P* <
618 0.01; *** *P* < 0.001; **** *P* < 0.0001; ns, not significant. See also Figure S6.

619

620 **Figure 5. ARCP-1 is a scaffolding protein that localizes phosphodiesterase PDE-1 to**
621 **dendritic endings**

622 A) Schematic of co-IP approach to identify ARCP-1B interactors, by pull-down of an N-
623 terminal GFP tag.

624 B) Top ten specific putative interactors of GFP-ARCP-1B identified in two independent co-
625 IPs. IPs of other cytoplasmic GFP-tagged proteins provide negative controls.

626 C-D) GFP-tagged ARCP-1B and PDE-1B proteins are both enriched at the sensory endings of
627 BAG. Scale bar = 10 μm; A = anterior; V = ventral.

628 E) Disrupting *arcp-1* reduces enrichment of PDE-1, expressed from the *flp-17p*, at BAG cilia.
629 Bars plot mean \pm SEM. n (in bars) = number of animals. Mann-Whitney *u* test.
630 F) *pde-1* mutants phenocopy the increased turning frequency of *arcp-1* mutants in response to
631 CO₂. *pde-1*; *arcp-1* double mutants do not show an additive phenotype. Solid lines plot mean;
632 shaded areas show SEM; black bars indicate time intervals for statistical comparisons; bar
633 graphs plot mean \pm SEM for these intervals. One-way ANOVA with Tukey test. n \geq 8 assays
634 with 20-30 animals per trial. * *P* < 0.05; ** *P* < 0.01; **** *P* < 0.0001; ns, not significant.
635 See also Figure S7 and Data Table S3.

636

637 **Figure 6. PDE-1 and ARCP-1 inhibit BAG expression of FLP-19 neuropeptides that**
638 **potentiate behavioral responses to CO₂**

639 A) Mean fluorescence \pm SEM of a *flp-19* neuropeptide reporter (*flp-19p::gfp*) in BAG,
640 indicating that PDE-1 and ARCP-1 inhibit *flp-19* expression. BAG-specific expression of
641 *arcp-1b*, using the *flp-17* promoter (*BAGp*), rescues this phenotype, whereas expression in
642 URX, AQR and PQR, using the *gcy-32* promoter (*URX-AQR-PQRp*), does not. n (in bars) =
643 number of animals. One-way ANOVA with Tukey test.
644 B) Mean fluorescence \pm SEM of *flp-19* neuropeptide reporter in BAG neurons of JU1249 and
645 JU2825. Increased expression of *flp-19* in JU1249 is rescued by expressing *arcp-1b* from the
646 BAG-specific *flp-17* promoter (*BAGp*). n (in bars) = number of animals. Kruskal-Wallis with
647 Dunn test.
648 C) Disrupting *flp-19* suppresses the potentiated turning phenotype of *arcp-1*; *npr-1* animals in
649 response to 3% CO₂. One-way ANOVA with Holm Šidák test. n = 9 assays.
650 D) CO₂-evoked turning of *arcp-1*; *npr-1* mutants following cell-specific knockdown of *flp-19*
651 expression in BAG. Knockdown of *flp-19* in the mutant background suppresses turning at 3%
652 CO₂, whereas knockdown of *gfp* does not. One-way ANOVA with Dunnett test. n \geq 7 assays.

653 E) Knockdown of *flp-19* expression in BAG partially rescues the increased arousal phenotype
654 of *arcp-1*; *npr-1* animals at 3% CO₂. One-way ANOVA with Dunnett test. $n \geq 7$ assays with
655 20-30 animals per trial.

656 F) BAG-specific knockdown of *flp-19* in *npr-1* animals does not affect the plasticity of CO₂
657 escape in response to previous O₂ experience. Two-way ANOVA with Šidák test. $n = 7 - 8$
658 assays.

659 G) Animals overexpressing *flp-19* in BAG move significantly faster at 3% CO₂ compared to
660 *npr-1* controls, although their response is still lower than *npr-1* animals grown at 7% O₂ and
661 *arcp-1* mutants. $n \geq 3$ assays. One-way ANOVA with Tukey test.

662 For C-G, 20-30 animals were tested per assay. Solid lines plot mean; shaded areas show
663 SEM; black bars indicate time intervals for statistical comparisons; bars plot mean \pm SEM for
664 these intervals. * $P < 0.05$; *** $P < 0.001$; **** $P < 0.0001$; ns, not significant. See also
665 Figure S7 and Data Table S4.

666

667 **Figure 7. A model for how genetic variation in *arcp-1* affects CO₂ escape behavior**

668 A) Effect of the natural *arcp-1* allele on experience-dependent plasticity, shown as behavioral
669 reaction norms. *C. elegans* wild isolates acclimated to a high (21%) O₂ environment suppress
670 their aversion to CO₂ (left panel). A shift to a low (7%) O₂ environment results in a
671 heightened CO₂ response. A mutation in *arcp-1* alters experience-dependent plasticity and
672 genetically fixes a strong aversive response to CO₂ in part by increasing *flp-19* neuropeptide
673 expression in BAG CO₂ sensors (right panel).

674 B) CO₂ is detected by the receptor guanylate cyclase GCY-9, expressed in BAG cilia. The
675 ankyrin-repeat scaffold protein ARCP-1 is also enriched at dendritic sensory endings,
676 interacts with PDE-1, and localizes this phosphodiesterase to the cilia of BAG CO₂-sensory
677 neurons. PDE-1 and ARCP-1 inhibit CO₂-evoked Ca²⁺ activity and expression of FLP-19

678 neuropeptide messengers in BAG. In the absence of ARCP-1, less GCY-9 and PDE-1 localize
679 to BAG cilia, and *flp-19* is more strongly expressed. Increased FLP-19 expression in BAG
680 contributes to resetting a strong aversive response to CO₂ in *arcp-1; npr-1* animals regardless
681 of previous O₂ experience.

682 **STAR Methods**

683 **LEAD CONTACT AND MATERIALS AVAILABILITY**

684 Further information and requests for resources and reagents should be directed to and will be
685 fulfilled by the Lead Contact, Mario de Bono (debono@mrc-lmb.cam.ac.uk,
686 mdebono@ist.ac.at).

687 **EXPERIMENTAL MODEL AND SUBJECT DETAILS**

688 **Animals**

689 *C. elegans* and other *Caenorhabditis* species were maintained under standard conditions
690 (Stiernagle, 2006) on nematode growth medium (NGM) plates seeded with *E. coli* OP50.
691 Young adult hermaphrodites were used in all experiments. For gonochoristic *Caenorhabditis*
692 species, young adult females were used. For a list of strains and transgene details, see Table
693 S1 and the Key Resources Table.

694 The mutations in *arcp-1* alleles obtained by forward genetics, and in the JU1249 wild isolate,
695 are shown in Figure S5A. The *C. elegans* strain JU1249 was isolated from a rotten apple
696 collected in 2007 in Santeuil, France (Zhang et al., 2016). A detailed description of the
697 forward genetic screen that isolated the *db1082* allele will be described elsewhere. Causal
698 variants in aggregation-defective mutants from this screen were identified by SNP-based
699 mapping in combination with WGS (Minevich et al., 2012).

700 **Microbe strains**

701 The *Escherichia coli* OP50 strain was used as a food source for *C. elegans* and other
702 *Caenorhabditis* species.

703 **METHOD DETAILS**

704 **Molecular biology**

705 Transgenes were cloned using the Multisite Gateway Three-Fragment cloning system (12537-
706 023, Invitrogen) into pDEST4R3 II. For transgenic lines, the promoter lengths were: *arcp-*
707 *1p* (1.2 kb for *arcp-1a* and 2 kb for *arcp-1b*), *flp-17p* (3.3 kb), *gcy-32p* (0.6 kb), and *gcy-33p*
708 (1.0 kb). For rescue experiments, cDNA of *arcp-1* isoforms was amplified and cloned into
709 pDONR221, using primers listed in Table S2.

710 For immunoprecipitation and subcellular localization of ARCP-1, a functional *arcp-*
711 *1p::gfp::arcp-1b* transgene was made by fusing GFP coding sequences upstream of the *arcp-*
712 *1b* cDNA sequence. To investigate the subcellular localization of PDE-1 in BAG neurons, the
713 *pde-1b* cDNA sequence was cloned into pDONR221 using primers listed in Table S2. This
714 plasmid was used to generate a *flp-17p::pde-1b::gfp* transgene, by cloning the GFP reporter
715 sequence in frame and downstream of the *pde-1b* cDNA sequence. Details of strains and
716 transgenes used to study the subcellular localization of *gcy-9*, *tax-4*, *gcy-33* and *gcy-35* are
717 provided in Table S1. The *gcy-9p::gcy-9::mCherry* and *gcy-9p::tax-4::gfp* strains were a kind
718 gift from Dr. Niels Ringstad (New York University School of Medicine, USA).

719 For *flp-19* RNAi, 469 bp of *flp-19* cDNA starting from the sequence GCTTTTCCTGTAA
720 was cloned in both the sense and antisense orientations. For cell-specific RNAi experiments,
721 we expressed these fragments in BAG using the *gcy-33p* (1.0 kb) and in URX neurons using
722 *gcy-32p* (0.6 kb). To overexpress *flp-19* in BAG, we amplified *flp-19* cDNA using primers
723 listed in Table S2, and fused this sequence to the *flp-17* (3.3 kb) promoter.

724 To characterize the expression pattern of *arcp-1*, we made a fluorescent reporter transgene by
725 fosmid recombineering. pBALU9 was used to amplify a reporter cassette, containing the *gpd-*
726 *2* intergenic SL2 sequence and a GFP coding sequence, which was inserted downstream of
727 the *arcp-1* stop codon in the WRM0633bA06 fosmid as described (Tursun et al., 2009). The

728 reporter strain for *flp-19* neuropeptide expression (*flp-19p::gfp*) was a kind gift from Dr.
729 Roger Pocock (Monash University, Australia).

730 **Genotyping of natural polymorphisms**

731 Polymorphisms of *npr-1*, *glb-5*, *nath-10* and *arcp-1* genes in *C. elegans* wild isolates were
732 genotyped by PCR. Primers used are listed in Table S2.

733 **Behavioral assays**

734 All experiments used young adult hermaphrodite animals, therefore sample stratification was
735 not required within each genotype/condition. For most experiments, measurements were
736 scored using an automated algorithm so blind scoring was not undertaken: see each
737 subsection for details. For details of statistical tests, see the relevant Figure legend for each
738 experiment and also the subsection “Quantification and Statistical Analysis.” All recordings
739 that passed the automated analysis pipeline were included in the final dataset. For rescue and
740 RNAi experiments, behavioral responses and phenotypes were confirmed by testing at least
741 two independent transgenic strains.

742 *Locomotory responses to CO₂ and O₂*: Behavioral responses to gas stimuli were assayed as
743 described (Fenk and de Bono, 2017; Laurent et al., 2015). Animals were acclimated to
744 different O₂ levels by growing them for one generation at 21% O₂ (room air) or in a gas-
745 controlled incubator kept at 7% O₂. For each assay, 20-30 young adult hermaphrodites were
746 transferred onto NGM plates seeded 16–20 h earlier with 20 µl of *E. coli* OP50. To control
747 gas levels experienced by *C. elegans*, animals were placed under a 200 µm deep square
748 polydimethylsiloxane (PDMS) chamber with inlets connected to a PHD 2000 Infusion syringe
749 pump (Harvard apparatus). Humidified gas mixtures were delivered at a flow rate of 3.0
750 ml/min. Behavioral responses to changes in O₂ levels were measured by exposing animals to

751 a stimulus train of 7% O₂ - 21% O₂ - 7% O₂ (upshift) or 21% O₂ - 7% O₂ - 21% O₂
752 (downshift), in which each stimulus comprised a 3 min time interval. Locomotory responses
753 to CO₂ were measured by exposing animals to a series of 0% CO₂ (3 min) - X% CO₂ (3 min)
754 - 0% CO₂ (3 min), with X corresponding to 1%, 3%, 5% or 10% CO₂ depending on the
755 experiment. In all CO₂ assays, a background level of 7% O₂ was used. Movies were recorded
756 during the stimulus train using FlyCapture (Point Grey Research) on a Leica MZ6 dissecting
757 microscope with a Point Grey Grasshopper camera running at 2 frames/s. Video recording
758 was started 2 min after animals were placed under the PDMS chamber to ensure that the
759 initial environment was in a steady state. In assays where we prolonged the exposure to 7%
760 O₂ before CO₂ stimulation, video recording was started 21 min after animals were placed
761 under the PDMS chamber kept at 7% O₂, and animals were stimulated with 3% CO₂ at t = 24
762 min. Videos were analyzed in Zentracker, a custom-written MATLAB software
763 (<https://github.com/wormtracker/zentracker>). All worms in the field of view were analyzed
764 except those in contact with other animals. Speed was calculated as instantaneous centroid
765 displacement between successive frames. Omega turns were identified as described (Laurent
766 et al., 2015). In total 2-4 assay plates with 20-30 animals per plate were tested per day, and
767 each genotype or condition was assayed in at least two independent experiments. As
768 locomotion measurements were conducted using an automated algorithm, genotypes were not
769 blinded prior to analysis.

770 *Aggregation and bordering behavior:* L4 animals were picked to a fresh plate 24 h before the
771 assay. Sixty animals were then re-picked to the assay plate (an NGM plate seeded 2 days
772 earlier with 100 µl of *E. coli* OP50), and bordering and aggregation was scored 2 and 6 h
773 later. The scorer was blind to genotype. Behavior was always scored on 2-4 assay plates (each
774 containing 60 animals) per day and tested in at least two independent experiments.

775 *Salt-based associative learning:* Gustatory plasticity was tested as described (Beets et al.,

776 2012; Hukema et al., 2008), in a climate-controlled room set at 20°C and 40% relative
777 humidity. Synchronized young adult hermaphrodites were grown at 25°C on culture plates
778 seeded with *E. coli* OP50. Animals were collected and washed three times over a period of 15
779 minutes with chemotaxis buffer (CTX, 5 mM KH₂PO₄/K₂HPO₄ pH 6.6, 1 mM MgSO₄, and 1
780 mM CaCl₂). Mock-conditioned animals were washed in CTX buffer without NaCl, whereas
781 NaCl-conditioned animals were washed in CTX containing 100 mM NaCl for salt
782 conditioning. Salt chemotaxis behavior of mock- and NaCl-conditioned animals was then
783 tested on four-quadrant plates (Falcon X plate, Becton Dickinson Labware) filled with
784 buffered agar (2% agar, 5 mM KH₂PO₄/K₂HPO₄ pH 6.6, 1 mM MgSO₄, and 1 mM CaCl₂) of
785 which two opposing pairs have been supplemented with 25 mM NaCl. Assay plates were
786 always prepared fresh and left open to solidify and dry for 60 min. Plates were then closed
787 and used on the same day. After the washes, 50 - 150 animals were pipetted on the
788 intersection of the four quadrants and allowed to crawl for 10 minutes on the quadrant plate.
789 A chemotaxis index was calculated as $(n(A) - n(C)) / (n(A) + n(C))$ where $n(A)$ is the number
790 of worms within the quadrants containing NaCl and $n(C)$ is the number of worms within the
791 control quadrants without NaCl. The scorer was blind to genotype.

792 **Selection-based QTL mapping**

793 *Competition assays:* The *C. elegans* strains JU1249 and JU2825 were competed for several
794 generations using different transfer methods. At the start of the assay, ten JU1249 and JU2825
795 L4 larvae were put together on a 10 cm NGM plate seeded with *E. coli* OP50. Five biological
796 replicates were maintained at 23°C. Before the cultures starved, a small fraction of the
797 population (200 to 400 animals) was used to seed a fresh culture plate. In Treatment A, the
798 worms were harvested with M9 buffer and 2 µL of worm pellet was transferred to the next
799 plate. In Treatment B, an agar cube (chunk) was cut at the edge of the bacterial lawn and
800 deposited onto the next plate. After each transfer, the remaining animals were stored in M9

801 buffer at -80 °C to quantify the relative proportions of JU1249 and JU2825 alleles.

802 The genomes of JU1249 and JU2825 were sequenced on an Illumina Hiseq4000 at 20x
803 coverage with paired-end 150 bp reads. For each genome, the raw data were aligned to the
804 reference genome (*C. elegans* WS243 masked from Wormbase.org) and analyzed using
805 BWA, SAMtools, Picard and Genome Analysis Toolkit (GATK) (Li and Durbin, 2009; Li et
806 al., 2009; Van der Auwera et al., 2013). The accession number for the genomic sequence data
807 of JU1249 and JU2825 is NCBI: PRJNA514933
808 (<https://www.ncbi.nlm.nih.gov/genome/?term=PRJNA514933>).

809 From the output BAM files, homozygous SNPs between the two strains were called and
810 filtered with a raw read depth threshold of 10-300. Allele quantification for the III_663310
811 polymorphism was performed using pyrosequencing as previously described (Duveau and
812 Félix, 2012). Primers for pyrosequencing are listed in Table S2. In brief, *C. elegans* samples
813 harvested after each transfer were centrifuged at 3,000 rpm for 2 minutes. Lysates of 2 µL of
814 the worm pellets were used as PCR templates and allele frequencies were quantified with a
815 pyrosequencer (PyroMark Q96 ID; Biotage). The accuracy of this quantification method was
816 estimated by measuring the allele frequencies of PCR products that were amplified using *C.*
817 *elegans* lysates of known proportions of JU1249 and JU2825 individual L4 larvae. On
818 average, a 2% difference was measured between expected and observed allele frequencies.

819 *Selection-based QTL mapping experiment:* Segregating populations were generated by
820 crossing the parental JU1249 and JU2825 *C. elegans* wild isolates in both directions, using
821 ten males and two self-sperm exhausted hermaphrodites in each cross. From the F1 progeny,
822 eight biological replicates were set up to generate F2 by crossing again ten males and two
823 self-sperm exhausted hermaphrodites. From each F2 replicate, six males and two L4 stage
824 hermaphrodites were crossed to have plenty of F3 progeny. In the F3 generation, two paired

825 founding populations of 200 L4 larvae (100 from each initial cross direction) were set up per
826 replicate and submitted to contrasted selection regimes. Treatment A transferred worms
827 through liquid harvest and Treatment B by chunking, as described for the competition assay
828 above. In both Treatments, 200-400 animals were transferred before starvation. Males were
829 maintained in the population during each of the first five transfers by picking 50 males. In
830 total 19-20 transfers were done for populations under Treatment A and 17-19 transfers for
831 populations with Treatment B. Genomic DNA of each population (about 10^5 individuals) was
832 extracted as a pool and sequenced as described above.

833 The reads of each pool were aligned to the N2 reference genome as described above. The
834 BAM files were filtered for allele information on the positions of homozygous SNPs between
835 the two parents. Allele frequencies were analyzed in each pool. A Cochran-Mantel-Haenszel
836 (CMH) test was used to analyze the consistency of the allele frequency difference between
837 populations with different treatments among the eight replicates, except in the genomic
838 positions 4396879-16406352 on Chromosome IV, where replicate 3 was excluded because
839 one parental genome was fixed in this region in both treatments (McDonald, 2009). The null
840 hypothesis for this CMH test is an equal distribution of sequence reads between the two
841 treatments, and does not consider noise due to allelic drift in the populations, thus inflating
842 the $-\log(p\text{-value})$. Drift could not be simulated because, for experimental simplicity,
843 population sizes and generations were not controlled during the transfers. We note that,
844 although population size in the experiment was low, the mapping had a relatively good
845 resolution due to the number of populations (16 in total), which yielded independent
846 recombination events.

847 RStudio (v 0.99.903) and packages (ggplot2, plyr, evobiR) were used for statistical analysis,
848 plots of allele frequencies and CMH tests. Pindel (Ye et al., 2009) was used to detect
849 homozygous indels in the candidate region between JU1249 and JU2825, but no additional

850 polymorphism was found. High quality homozygous variants of the parental strains in the
851 candidate region were annotated using VEP
852 (www.ensembl.org/info/docs/tools/vep/index.html) (McLaren et al., 2016). The *mfP22*
853 deletion was verified by PCR and Sanger sequencing, using primers listed in Table S2. The
854 accession number for the genomic sequence data of the replicate populations is NCBI:
855 PRJNA515248 (<https://www.ncbi.nlm.nih.gov/genome/?term=PRJNA515248>).

856 **Distribution of *mfP22* allele in wild isolates**

857 To examine the distribution of the *mfP22* deletion in *C. elegans* wild isolates, we monitored
858 the presence of the deletion visually, using Tablet 1.16.09.06 (Milne et al., 2013), for 151
859 isotypes with whole genome sequences in the CeNDR database (Cook et al., 2016). The
860 *mfP22* allele was only found in JU1249 (Data Table S2).

861 **Confocal microscopy and image analysis**

862 Confocal images were acquired using a Zeiss LSM 710 microscope or a Nikon Eclipse Ti
863 inverted setup coupled to an Andor Ixon EMCCD camera and a spinning disk confocal unit.
864 Projections of z-stacks were generated using Fiji (Image J).

865 Expression of *arcp-1* in URX, AQR and PQR was confirmed by co-expression with a *gcy-*
866 *32p::mCherry* transgene. Expression in BAG and AWB neurons was verified by crossing
867 *arcp-1* reporter strains with *flp-17p::mCherry* and *str-1p::mCherry* marker strains,
868 respectively. Expression in AWC and ASE was confirmed by co-expression with *ceh-*
869 *36p::RFP* and *odr-1p::RFP* transgenes. For DiI staining, animals were incubated in DiI
870 solution (0.01 mg/ml) for 3 hours and washed with M9 buffer before mounting for confocal
871 microscopy.

872 To quantify the fluorescence of reporter-tagged proteins in cilia and neuron cell bodies, z-
873 stack images were taken on a spinning disk confocal microscope using a 60x lens and 100 ms
874 exposure time. Z-projections of image stacks were generated with Fiji (Image J). Regions of
875 interest (ROIs) were selected by centering a 50-pixel by 50-pixel square region over the distal
876 dendrite or soma of the BAG neurons, respectively. All measurements were background-
877 corrected by subtracting the mean values of a 50-pixel by 50-pixel square region drawn
878 outside of the neuron.

879 To quantify the expression of neuropeptide reporters in BAG soma, z-stack images were
880 taken on a spinning disk confocal microscope using a 60x lens and 100 ms exposure time. 3D
881 images were reconstituted using the IMARIS software package (Bitplane). GFP pixel
882 intensities brighter than a threshold value (1000 for *flp-19* and 3000 for *flp-17* reporters) were
883 cropped by creating a surface with 0.25 μ m details. The mean pixel intensities inside the
884 surface were calculated after background subtraction.

885 **Calcium imaging**

886 L4 animals expressing a ratiometric yellow cameleon sensor were picked 24 h before
887 imaging. Animals were glued to agarose pads (2% agarose in M9 buffer, 1 mM CaCl₂) using
888 Dermabond tissue adhesive (Ethicon) with the nose immersed in a mix of bacterial food (*E.*
889 *coli* OP50) and M9 buffer. To deliver gas stimuli, glued animals were placed under a
890 microfluidic chamber with inlets connected to a PHD 2000 Infusion syringe pump (Harvard
891 Apparatus) running at a flow rate of 2.5 ml/min. An electronic valve system placed between
892 the syringes and the microfluidic chamber allowed switching between two different gas
893 mixtures in a controlled manner at pre-specified time intervals. Imaging data were analysed
894 using Neuron Analyzer, a custom-written Matlab program (code available at

895 <https://github.com/neuronanalyser/neuronanalyser>). As measurements were conducted using
896 an automated algorithm, genotypes were not blinded prior to analysis.

897 *CO₂-evoked Ca²⁺ activity*: Animals expressing a *flp-17p::YC3.60* (yellowameleon 3.60)
898 transgene were used for ratiometric imaging of relative calcium concentration in BAG cell
899 bodies (Bretscher et al., 2011). After immobilization, animals were placed under a
900 microfluidic PDMS chamber and exposed to a 0% CO₂ (3 min) - X% CO₂ (3 min) - 0% CO₂
901 (3 min) stimulus train, with X corresponding to 1%, 3% or 5% CO₂ depending on the
902 experiment. To measure CO₂-evoked tonic Ca²⁺ activity in BAG, the time interval for CO₂
903 stimulation was prolonged from 3 min to 18 min. In all experiments, the background O₂ level
904 was 7% O₂. Calcium imaging was done at 2 frames/s on an AZ100 microscope (Nikon)
905 bearing a TwinCam adaptor (Cairn Research) mounted with two ORCA-Flash4.0 V2 digital
906 cameras (Hamamatsu) using an AZ Plan Fluor 2x lens with 2x zoom and an exposure time of
907 500 ms.

908 *O₂-evoked Ca²⁺ activity*: We used animals expressing a *gcy-37p::YC2.60* transgene to
909 measure Ca²⁺ activity of URX neurons in response to O₂ stimuli (Fenk and de Bono, 2017).
910 To measure O₂ responses in BAG, we used animals expressing a *flp-17p::YC2.60* transgene
911 (Gross et al., 2014). After immobilization, animals were placed under a Y-shaped
912 microfluidic chamber and exposed to an O₂ upshift (7% - 21% - 7% O₂) in case of URX
913 imaging, or an O₂ downshift (21% - 7% - 21% O₂) for BAG. Each stimulus comprised a 2
914 min time window. Images were recorded at 2 frames/s with an exposure time of 100 ms for a
915 total of 6 min, on a Zeiss Axiovert inverted microscope with an EMCCD Evolve 512
916 Deltacamera (Photometrics) and a 40x C-Apochromat lens, using MetaMorph acquisition
917 software (Molecular Devices). To reduce photobleaching an optical density filter 2.0 or 1.5
918 was used. Excitation light was passed through an excitation filter for CFP (438/24-25,
919 Semrock) and a dichroic filter for YFP (DiO2-25x36, Semrock). A beam splitter (Optical

920 Insights) was used to separate the cyan and yellow emission light using a dichroic filter for
921 483/32-25 nm (CFP) and 542/27-25 nm (YFP) (Semrock).

922 **Immunoprecipitation from *C. elegans***

923 Two independent co-IP experiments were performed to identify putative interactors of ARCP-
924 1B. Samples for GFP-ARCP-1B were always processed in parallel with control samples of
925 other cytoplasmic GFP-tagged proteins (MALT-1-GFP and EIF-3.L-GFP), providing
926 negative controls. For co-IP experiments, lysis buffer was prepared with 50 mM HEPES (pH
927 7.4), 1 mM EGTA, 1 mM MgCl₂, 100 mM KCl, 10% glycerol, 0.05% Tergitol type-NP40
928 (Sigma-Aldrich), 1mM DTT, 0.1M PMSF with 1 complete EDTA-free proteinase inhibitor
929 cocktail tablet (Roche Applied Science) per 12ml. Worms were washed twice in ice-cold M9
930 and once in ice-cold lysis buffer, and then snap-frozen in liquid nitrogen. Frozen worm pellets
931 (~10 g) were pulverized using a Freezer/Mill (SPEX SamplePrep). Crude extract was clarified
932 at 4°C for 10 min at 20,000 g, and again for 20 min at 100,000 g with a TLA-100 rotor
933 (Beckman Coulter). For immunoprecipitation, samples were incubated with GFP-Trap
934 (ChromoTek) for 4 h at 4°C, then washed 3 times with 50 mM HEPES, 100 mM KCl.
935 Purified complexes were eluted in SDS-sample buffer at 95°C and further fractionated by
936 SDS-PAGE prior to mass spectrometry analysis.

937 Proteins were identified by Orbitrap-mass spectrometry and MASCOT database searching.
938 Gel samples were destained with 50% v/v acetonitrile and 50 mM ammonium bicarbonate,
939 reduced with 10 mM DTT, and alkylated with 55 mM iodoacetamide. Digestion was with 6
940 ng/μl trypsin (Promega) overnight at 37°C, and peptides extracted in 2% v/v formic acid 2%
941 v/v acetonitrile, and analysed by nano-scale capillary LC-MS/MS (Ultimate U3000 HPLC,
942 Thermo Scientific Dionex) at a flow of ~ 300 nL/min. A C18 Acclaim PepMap100 5 μm, 100
943 μm x 20 mm nanoViper (Thermo Scientific Dionex), trapped the peptides prior to separation

944 on a C18 Acclaim PepMap100 3 μm , 75 μm x 250 mm nanoViper. Peptides were eluted with
945 an acetonitrile gradient. The analytical column outlet was interfaced via a nano-flow
946 electrospray ionisation source with a linear ion trap mass spectrometer (Orbitrap Velos,
947 Thermo Scientific). Data dependent analysis was performed using a resolution of 30,000 for
948 the full MS spectrum, followed by ten MS/MS spectra in the linear ion trap. MS spectra were
949 collected over a m/z range of 300–2000. MS/MS scans were collected using a threshold
950 energy of 35 for collision-induced dissociation. LC-MS/MS data were searched against the
951 UniProt KB database using Mascot (Matrix Science), with a precursor tolerance of 10 ppm
952 and a fragment ion mass tolerance of 0.8 Da. Two missed enzyme cleavages and variable
953 modifications for oxidised methionine, carbamidomethyl cysteine, pyroglutamic acid,
954 phosphorylated serine, threonine and tyrosine were included. MS/MS data were validated
955 using the Scaffold programme (Proteome Software Inc).

956 **RNA-Seq of sorted BAG neurons**

957 *Adult cell isolation:* Synchronized young adult hermaphrodites with GFP-labelled BAG
958 neurons (expressing a *flp-17p::gfp* transgene) were acutely dissociated as described (Kaletsky
959 et al., 2015). Synchronized adult worms were washed with M9 buffer to remove excess
960 bacteria. The pellet (~250 μl) was washed with 500 μl lysis buffer (200 mM DTT, 0.25%
961 SDS, 20 mM Hepes pH 8.0, 3% sucrose) and resuspended in 750 μl lysis buffer. Worms were
962 incubated in lysis buffer for 6.5 minutes at room temperature. The pellet was washed 5 times
963 with M9 and resuspended in 20 mg/ml pronase from *Streptomyces griseus* (Roche). Worms
964 were pipetted up and down for 12 min at room temperature; then ice-cold PBS buffer
965 containing 2% fetal bovine serum (Gibco) was added. Cell suspensions were passed over a 5
966 μm syringe filter (Millipore). The filtered cells were diluted in PBS and sorted using a Sony
967 Biotechnology Synergy High Speed Cell Sorter. Gates for detection were set by comparison
968 to *npr-1* cell suspensions prepared on the same day alongside the experimental samples.

969 Positive fluorescent events were sorted directly into Eppendorf tubes containing 10 μ l of
970 0.2% (vol/vol) Triton X-100 and 2 U μ l⁻¹ RNase inhibitor. Six biological replicates were
971 prepared for each genotype, i.e. *npr-1(ad609)* and *arcp-1(db1082); npr-1(ad609)* animals.
972 For each replicate sample, approximately 4,000 GFP positive events were collected.

973 *RNA amplification and library preparation:* RNA-Seq was done using a Smart-seq2 protocol
974 as described (Picelli et al., 2014). After neuron isolation by FACS, cDNA was prepared from
975 each sample by reverse transcription using SuperScript II reverse transcriptase (18064-014,
976 Invitrogen), Oligo-dT₃₀ and Template-Switching Oligonucleotide (TSO) primers listed in
977 Table S2. After the first strand reaction, the cDNA was amplified with the KAPA Hifi
978 HotStart kit (KK2601, KAPA Biosystems) and IS PCR primers listed in Table S2. cDNA was
979 then purified using Ampure XP beads (A 63881, Beckman Coulter), tagmented and 1 μ g was
980 used for preparing libraries with the Illumina Nextera XT DNA sample preparation kit (FC-
981 131-1096, Illumina), as per manufacturer suggested practices. Sequencing libraries were then
982 submitted for sequencing on the Illumina HiSeq 4000 platform.

983 *RNA-Seq data analysis:* Prior to analysis the raw files were pre-processed using Bowtie2
984 version 0.11.0 to remove ribosomal RNA that mapped to a ribosomal RNA sequence library
985 (Wormbase, WS255). Additionally, FASTQ files relating to the same sample but sequenced
986 over multiple flow cell lanes were concatenated to give a single file. Custom rRNA_remover
987 and rna_seq_lane_merger scripts were used (available on GitHub: [https://github.com/lmb-
988 seq/RNA-Seq_utilities](https://github.com/lmb-seq/RNA-Seq_utilities)). The files were then processed by PRAGUI - a Python3 pipeline for
989 RNA-Seq data analysis. PRAGUI automates analysis by incorporating widely used RNA-Seq
990 processing packages including: Trim Galore, FastQC, STAR, DESeq2, HTSeq, Cufflinks and
991 MultiQC. PRAGUI can be found at: <https://github.com/lmb-seq/PRAGUI>. The following
992 parameters were used with PRAGUI: DESeq2 analysis (labeled as “DESeq”), unstranded

993 paired-end library, worm organism with *C. elegans* genome fasta file and canonical gene set
994 gtf file (Wormbase, WS255), STAR arguments set to “--outSAMstrandFieldDESeq
995 intronMotif --readFilesCommand zcat -c --outSAMtype BAM SortedByCoordinate”, mapq
996 set to 20. All other PRAGUI parameters were kept default. 5.5 – 17 million reads were
997 obtained per sample and mapped to the *C. elegans* genome. Sequences are deposited at GEO
998 (GSE135687).

999 **QUANTIFICATION AND STATISTICAL ANALYSIS**

1000 The number of animals and replicates used per experiment is described in detail in the
1001 “Methods Details” subsection for each assay and in the relevant Figure legends. Specifically,
1002 for the main behavioral assays: locomotory responses to CO₂ and O₂ were measured in > 4
1003 trials per condition with 20-30 animals each; aggregation and bordering assays were
1004 conducted with > 4 trials per genotype of 50 - 60 animals each.

1005 Statistical analyses used GraphPad Prism 7.0 and Mathworks MATLAB R2014b (8.4). Exact
1006 tests used are indicated in figure legends. In general, where more than two groups tested with
1007 a single condition were compared, a one-way ANOVA with Tukey's or Šidák's multiple
1008 comparisons test was used. Where multiple groups tested with multiple conditions were
1009 compared, a two-way ANOVA with Tukey's or Šidák's post-hoc test was used. Where
1010 appropriate, a D'Agostino & Pearson or Shapiro-Wilk normality test was conducted to assess
1011 if the data fit a normal distribution. For locomotory assays where two groups were compared
1012 over one time interval, we chose time intervals where we expected the locomotory changes to
1013 have plateaued and used a Mann-Whitney *u* test for statistical comparisons as described
1014 (Laurent et al., 2015). For the intervals of interest, we determined independent per-subject
1015 means derived from individuals flagged as continuously valid for at least 10 s during the
1016 interval. We considered all individuals in the field of view as valid except those in contact

1017 with other animals and those that were off the food lawn or less than half a body-length from
1018 the border. Following these criteria, each individual was sampled at most once per interval.

1019 **DATA AND SOFTWARE AVAILABILITY**

1020 **Datasets**

1021 The genome sequencing data of JU1249 and JU2825 is available on NCBI: PRJNA514933
1022 (<https://www.ncbi.nlm.nih.gov/genome/?term=PRJNA514933>). The genomic sequence data
1023 of the replicate populations for QTL mapping is available on NCBI: PRJNA515248
1024 (<https://www.ncbi.nlm.nih.gov/genome/?term=PRJNA515248>). Sequence data from the
1025 RNA-Seq analysis of sorted BAG neurons is deposited on GEO: GSE135687
1026 (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE135687>).

1027 **Codes**

1028 *Locomotory assays:* Videos of locomotory assays were analyzed in Zentracker, a custom-
1029 written MATLAB software available on <https://github.com/wormtracker/zentracker>.

1030 *Calcium imaging:* Recordings were analysed using Neuron Analyzer, a custom-written
1031 MATLAB program available at <https://github.com/neuronanalyser/neuronanalyser>.

1032 *RNA-Seq analysis:* Codes for removing rRNA sequences from datasets and for concatenating
1033 FASTQ files relating to the same sample but sequenced over multiple flow cell lanes, are
1034 available on GitHub: https://github.com/lmb-seq/RNA-Seq_utilities. The git repository for
1035 PRAGUI can be found at: <https://github.com/lmb-seq/PRAGUI>.

1036 **Supplementary Information**

1037 **Data Table S1, related to Figure 2: Data used for selection-based QTL mapping**

- 1038 A) List of single-nucleotide polymorphisms (SNPs) between JU1249 and JU2825.
- 1039 B) Number of reads in the eight replicates of treatment A, by SNP.
- 1040 C) Number of reads in the eight replicates of treatment B, by SNP.
- 1041 D) Allele frequencies in the eight replicates of treatment A, by SNP. Data in red were used to
1042 generate Figure S4C.
- 1043 E) Allele frequencies in the eight replicates of treatment B, by SNP. Data in red were used to
1044 generate Figure S4C.
- 1045 F) Allele frequencies in the eight replicates of treatment A, displayed using a sliding window
1046 10 SNPs wide and a step size of one SNP for all chromosomes except chromosome IV, where
1047 we used a sliding window 100 SNPs wide and a step size of one SNP.
- 1048 G) Allele frequencies in the eight replicates of treatment B, displayed using a sliding window
1049 10 SNPs wide and a step size of one SNP for all chromosomes except chromosome IV, where
1050 we used a sliding window 100 SNPs wide and a step size of one SNP.
- 1051 H) Differences in allele frequencies between treatment A and treatment B in each of the eight
1052 replicates, displayed using a sliding window 5 SNPs wide and a step size of one SNP for all
1053 chromosomes, except a sliding window 100 SNPs wide and a step size of one SNP for
1054 chromosome IV. In each pair of replicates, the same genomic positions were first selected
1055 between treatment A and treatment B, before subtracting allele frequencies between
1056 treatments.
- 1057 I) Read statistics used for the CMH analysis. The same genomic positions were first selected
1058 for both treatments among all the replicates, before the CMH analysis. The part in gray on
1059 chromosome IV for replicate 3 was not used in the test as one parental allele was fixed in both
1060 treatments.

1061 J) Annotation of variants detected in JU1249 compared to the reference N2, using the VEP
1062 algorithm. The F34D10.6 deletion (in red) appears as the only high impact variation.

1063 K) Annotation of variants detected in JU2825 compared to the reference N2.

1064

1065 **Data Table S2, related to Figure 2: Distribution of the *mfp22* deletion**

1066 List of wild isolates where the *mfp22* deletion is absent, based on mapped sequence reads
1067 available at the *Caenorhabditis elegans* Natural Diversity Resource, elegansvariation.org
1068 (Cook et al., 2016).

1069

1070 **Data Table S3, related to Figure 5: Protein identifications from co-IP of GFP-ARCP-1B**

1071 Proteins identified by mass spectrometry in two independent co-IP experiments for interactors
1072 of GFP-ARCP-1B. IP of GFP-tagged cytoplasmic proteins (MALT-1-GFP and EIF-3.L-GFP)
1073 provided a negative control. Total spectrum counts in GFP-ARCP-1B and control samples are
1074 listed for proteins that were at least 3-fold enriched in the GFP-ARCP-1B sample in both
1075 experiments.

1076

1077 **Data Table S4, related to Figure 6: Expression profiling of BAG neurons in *npr-1* and
1078 *arcp-1; npr-1* animals using RNA-Seq**

1079 A-D) Genes expressed in BAG neurons, which were isolated by FACS from adult *npr-1* and
1080 *arcp-1; npr-1* animals, with six biological replicates per genotype. A-B) Values indicate
1081 transcripts per kilobase million (TPM). C-D) Values show fragments per kilobase million
1082 (FPKM). Genes are listed based upon an expression detection threshold of 1 count per million
1083 reads per gene in at least 6 samples.

1084 E) Genes differentially expressed in BAG neurons of *npr-1* and *arcp-1; npr-1* animals.

1085

1086 **References**

- 1087 Arora, K., Sinha, C., Zhang, W., Ren, A., Moon, C.S., Yarlagadda, S., and Naren, A.P.
1088 (2013). Compartmentalization of cyclic nucleotide signaling: a question of when, where, and
1089 why? *Pflugers Arch - Eur J Physiol* *465*, 1397–1407.
- 1090 Bargmann, C.I. (2012). Beyond the connectome: How neuromodulators shape neural circuits.
1091 *Bioessays* *34*, 458–465.
- 1092 Beets, I., Janssen, T., Meelkop, E., Temmerman, L., Suetens, N., Rademakers, S., Jansen, G.,
1093 and Schoofs, L. (2012). Vasopressin/oxytocin-related signaling regulates gustatory
1094 associative learning in *C. elegans*. *Science* *338*, 543–545.
- 1095 Bretscher, A.J., Kodama-Namba, E., Busch, K.E., Murphy, R.J., Soltesz, Z., Laurent, P., and
1096 de Bono, M. (2011). Temperature, oxygen, and salt-sensing neurons in *C. elegans* are carbon
1097 dioxide sensors that control avoidance behavior. *Neuron* *69*, 1099–1113.
- 1098 Busch, K.E., Laurent, P., Soltesz, Z., Murphy, R.J., Faivre, O., Hedwig, B., Thomas, M.,
1099 Smith, H.L., and de Bono, M. (2012). Tonic signaling from O₂ sensors sets neural circuit
1100 activity and behavioral state. *Nat. Neurosci.* *15*, 581–591.
- 1101 Carrillo, M.A., and Hallem, E.A. (2015). Gas sensing in nematodes. *Mol. Neurobiol.* *51*, 919–
1102 931.
- 1103 Carrillo, M.A., Guillermin, M.L., Rengarajan, S., Okubo, R.P., and Hallem, E.A. (2013). O₂-
1104 sensing neurons control CO₂ response in *C. elegans*. *J. Neurosci.* *33*, 9675–9683.
- 1105 Cook, D.E., Zdraljevic, S., Roberts, J.P., and Andersen, E.C. (2016). CeNDR, the
1106 *Caenorhabditis elegans* natural diversity resource. *Nucleic Acids Res.* gkw893–gkw898.
- 1107 Coppens, C.M., de Boer, S.F., and Koolhaas, J.M. (2010). Coping styles and behavioural
1108 flexibility: towards underlying mechanisms. *Phil. Trans. R. Soc. B* *365*, 4021–4028.
- 1109 Couto, A., Oda, S., Nikolaev, V.O., Soltesz, Z., and de Bono, M. (2013). In vivo genetic
1110 dissection of O₂-evoked cGMP dynamics in a *Caenorhabditis elegans* gas sensor. *Proc. Natl.*
1111 *Acad. Sci. U.S.A.* *110*, E3301–E3310.
- 1112 Crispo, E. (2007). The Baldwin effect and genetic assimilation: revisiting two mechanisms of
1113 evolutionary change mediated by phenotypic plasticity. *Evolution* *61*, 2469–2479.
- 1114 Cummins, E.P., Selfridge, A.C., Sporn, P.H., Sznajder, J.I., and Taylor, C.T. (2013). Carbon
1115 dioxide-sensing in organisms and its implications for human disease. *Cell. Mol. Life Sci.* *71*,
1116 831–845.
- 1117 Cygnar, K.D., and Zhao, H. (2009). Phosphodiesterase 1C is dispensable for rapid response
1118 termination of olfactory sensory neurons. *Nat. Neurosci.* *12*, 454–462.
- 1119 de Bono, M., and Bargmann, C.I. (1998). Natural variation in a neuropeptide Y receptor
1120 homolog modifies social behavior and food response in *C. elegans*. *Cell* *94*, 679–689.
- 1121 Dewitt, T.J., Sih, A., and Wilson, D.S. (1998). Costs and limits of phenotypic plasticity.

- 1122 Trends in Ecology & Evolution *13*, 77–81.
- 1123 Dingemanse, N.J., and Wolf, M. (2013). Between-individual differences in behavioural
1124 plasticity within populations: causes and consequences. *Animal Behaviour* *85*, 1031–1039.
- 1125 Donaldson, Z.R., and Young, L.J. (2008). Oxytocin, vasopressin, and the neurogenetics of
1126 sociality. *Science* *322*, 900–904.
- 1127 Duveau, F., and Félix, M.-A. (2012). Role of pleiotropy in the evolution of a cryptic
1128 developmental variation in *Caenorhabditis elegans*. *PLoS Biol* *10*, e1001230.
- 1129 Fenk, L.A., and de Bono, M. (2015). Environmental CO₂ inhibits *Caenorhabditis elegans*
1130 egg-laying by modulating olfactory neurons and evokes widespread changes in neural
1131 activity. *Proc. Natl. Acad. Sci. U.S.a.* *112*, E3525–E3534.
- 1132 Fenk, L.A., and de Bono, M. (2017). Memory of recent oxygen experience switches
1133 pheromone valence in *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. U.S.a.* *204*, 201618934.
- 1134 Félix, M.-A., and Duveau, F. (2012). Population dynamics and habitat sharing of natural
1135 populations of *Caenorhabditis elegans* and *C. briggsae*. *BMC Biol.* *10*, 59.
- 1136 Frézal, L., Demoinet, E., Braendle, C., Miska, E., and Félix, M.-A. (2018). Natural genetic
1137 variation in a multigenerational phenotype in *C. elegans*. *Curr Biol* *28*, 2588–2596.e2588.
- 1138 Gopal, R., Foster, K.W., and Yang, P. (2012). The DPY-30 domain and its flanking sequence
1139 mediate the assembly and modulation of flagellar radial spoke complexes. *Mol Cell Biol* *32*,
1140 4012–4024.
- 1141 Gross, E., Soltesz, Z., Oda, S., Zelmanovich, V., Abergel, Z., and de Bono, M. (2014).
1142 GLOBIN-5-dependent O₂ responses are regulated by PDL-1/PrBP that targets prenylated
1143 soluble guanylate cyclases to dendritic endings. *J. Neurosci.* *34*, 16726–16738.
- 1144 Grubb, M.S., and Burrone, J. (2010). Activity-dependent relocation of the axon initial
1145 segment fine-tunes neuronal excitability. *Nature* *465*, 1070–1074.
- 1146 Guerenstein, P.G., and Hildebrand, J.G. (2008). Roles and effects of environmental carbon
1147 dioxide in insect life. *Annu. Rev. Entomol.* *53*, 161–178.
- 1148 Guillermin, M.L., Carrillo, M.A., and Hallem, E.A. (2017). A single set of interneurons drives
1149 opposite behaviors in *C. elegans*. *Curr Biol* 1–23.
- 1150 Guillermin, M.L., Castelletto, M.L., and Hallem, E.A. (2011). Differentiation of carbon
1151 dioxide-sensing neurons in *Caenorhabditis elegans* requires the ETS-5 transcription factor.
1152 *Genetics* *189*, 1327–1339.
- 1153 Hallem, E.A., and Sternberg, P.W. (2008). Acute carbon dioxide avoidance in *Caenorhabditis*
1154 *elegans*. *Proc. Natl. Acad. Sci. U.S.a.* *105*, 8038–8043.
- 1155 Hallem, E.A., Spencer, W.C., McWhirter, R.D., Zeller, G., Henz, S.R., Rättsch, G., Miller,
1156 D.M., Horvitz, H.R., Sternberg, P.W., and Ringstad, N. (2011). Receptor-type guanylate
1157 cyclase is required for carbon dioxide sensation by *Caenorhabditis elegans*. *Proc. Natl. Acad.*
1158 *Sci. U.S.a.* *108*, 254–259.

- 1159 Hukema, R.K., Rademakers, S., and Jansen, G. (2008). Gustatory plasticity in *C. elegans*
1160 involves integration of negative cues and NaCl taste mediated by serotonin, dopamine, and
1161 glutamate. *Learn Mem* *15*, 829–836.
- 1162 Izquierdo, A., Newman, T.K., Higley, J.D., and Murray, E.A. (2007). Genetic modulation of
1163 cognitive flexibility and socioemotional behavior in rhesus monkeys. *Proc Nat Acad Sci* *104*,
1164 14128–14133.
- 1165 Jékely, G., Melzer, S., Beets, I., Kadow, I.C.G., Koene, J., Haddad, S., and Holden-Dye, L.
1166 (2018). The long and the short of it – a perspective on peptidergic regulation of circuits and
1167 behaviour. *J Exp Biol* *221*, jeb166710–jeb166714.
- 1168 Jones, S.L., and Svitkina, T.M. (2016). Axon initial segment cytoskeleton: architecture,
1169 development, and role in neuron polarity. *Neural Plast* *2016*, 1–19.
- 1170 Kaletsky, R., Lakhina, V., Arey, R., Williams, A., Landis, J., Ashraf, J., and Murphy, C.T.
1171 (2015). The *C. elegans* adult neuronal IIS/FOXO transcriptome reveals adult phenotype
1172 regulators. *Nature* *529*, 92–96.
- 1173 Kim, K., and Li, C. (2004). Expression and regulation of an FMRamide-related
1174 neuropeptide gene family in *Caenorhabditis elegans*. *J. Comp. Neurol.* *475*, 540–550.
- 1175 Kiontke, K.C., Félix, M.-A., Ailion, M., Rockman, M.V., Braendle, C., Pénigault, J.-B., and
1176 Fitch, D.H.A. (2011). A phylogeny and molecular barcodes for *Caenorhabditis*, with
1177 numerous new species from rotting fruits. *BMC Evol. Biol.* *11*, 1–18.
- 1178 Kizhatil, K., Baker, S.A., Arshavsky, V.Y., and Bennett, V. (2009). Ankyrin-G promotes
1179 cyclic nucleotide-gated channel transport to rod photoreceptor sensory cilia. *Science* *323*,
1180 1614–1617.
- 1181 Kodama-Namba, E., Fenk, L.A., Bretscher, A.J., Gross, E., Busch, K.E., and de Bono, M.
1182 (2013). Cross-modulation of homeostatic responses to temperature, oxygen and carbon
1183 dioxide in *C. elegans*. *PLoS Genet.* *9*, e1004011.
- 1184 Langmead, B., and Salzberg, S.L. (2012). Fast gapped-read alignment with Bowtie 2. *Nat*
1185 *Meth* *9*, 357–359.
- 1186 Laurent, P., Soltesz, Z., Nelson, G.M., Chen, C., Arellano-Carbajal, F., Levy, E., and de
1187 Bono, M. (2015). Decoding a neural circuit controlling global animal state in *C. elegans*. *Elife*
1188 *4*.
- 1189 Letierrier, C., Clerc, N., Rueda-Boroni, F., Montersino, A., Dargent, B., and Castets, F.
1190 (2017). Ankyrin G membrane partners drive the establishment and maintenance of the axon
1191 initial segment. *Front Cell Neurosci* *11*, 6.
- 1192 Li, H., and Durbin, R. (2009). Fast and accurate short read alignment with Burrows-Wheeler
1193 transform. *Bioinformatics* *25*, 1754–1760.
- 1194 Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G.,
1195 Durbin, R., 1000 Genome Project Data Processing Subgroup (2009). The sequence
1196 alignment/map format and SAMtools. *Bioinformatics* *25*, 2078–2079.

- 1197 Maniar, T.A., Kaplan, M., Wang, G.J., Shen, K., Wei, L., Shaw, J.E., Koushika, S.P., and
1198 Bargmann, C.I. (2011). UNC-33 (CRMP) and ankyrin organize microtubules and localize
1199 kinesin to polarize axon-dendrite sorting. *Nat. Neurosci.* *15*, 48–56.
- 1200 Martínez-Velázquez, L.A., and Ringstad, N. (2018). Antagonistic regulation of trafficking to
1201 *Caenorhabditis elegans* sensory cilia by a *Retinal Degeneration 3* homolog and retromer.
1202 *Proc. Natl. Acad. Sci. U.S.a.* *115*, E438–E447.
- 1203 McDonald, J.H. (2009). *Handbook of biological statistics* (Baltimore: Sparky House
1204 Publishing).
- 1205 McGrath, P.T. (2013). Varieties of behavioral natural variation. *Curr Opin Neurobiol* *23*, 24–
1206 28.
- 1207 McGrath, P.T., Rockman, M.V., Zimmer, M., Jang, H., Macosko, E.Z., Kruglyak, L., and
1208 Bargmann, C.I. (2009). Quantitative mapping of a digenic behavioral trait implicates globin
1209 variation in *C. elegans* sensory behaviors. *Neuron* *61*, 692–699.
- 1210 McLaren, W., Gil, L., Hunt, S.E., Riat, H.S., Ritchie, G.R.S., Thormann, A., Flicek, P., and
1211 Cunningham, F. (2016). The Ensembl variant effect predictor. *Genome Biol.* *17*, 122.
- 1212 Mery, F. (2013). Natural variation in learning and memory. *Curr Opin Neurobiol* *23*, 52–56.
- 1213 Mery, F., Belay, A.T., So, A.K.-C., Sokolowski, M.B., and Kawecki, T.J. (2007). Natural
1214 polymorphism affecting learning and memory in *Drosophila*. *Proc Nat Acad Sci* *104*, 13051–
1215 13055.
- 1216 Milne, I., Stephen, G., Bayer, M., Cock, P.J.A., Pritchard, L., Cardle, L., Shaw, P.D., and
1217 Marshall, D. (2013). Using Tablet for visual exploration of second-generation sequencing
1218 data. *Brief. Bioinformatics* *14*, 193–202.
- 1219 Minevich, G., Park, D.S., Blankenberg, D., Poole, R.J., and Hobert, O. (2012). CloudMap: a
1220 cloud-based pipeline for analysis of mutant genome sequences. *Genetics* *192*, 1249–1269.
- 1221 Monteiro, P., and Feng, G. (2017). SHANK proteins: roles at the synapse and in autism
1222 spectrum disorder. *Nat Rev Neurosci* *18*, 147–157.
- 1223 Niemelä, P.T., Vainikka, A., Forsman, J.T., Loukola, O.J., and Kortet, R. (2012). How does
1224 variation in the environment and individual cognition explain the existence of consistent
1225 behavioral differences? *Ecol Evol* *3*, 457–464.
- 1226 O'Halloran, D.M., Hamilton, O.S., Lee, J.I., Gallegos, M., and L'Etoile, N.D. (2012). Changes
1227 in cGMP levels affect the localization of EGL-4 in AWC in *Caenorhabditis elegans*. *PLoS*
1228 *ONE* *7*, e31614–12.
- 1229 Otsuka, A.J., Franco, R., Yang, B., Shim, K.H., Tang, L.Z., Zhang, Y.Y.,
1230 Boontrakulpoontawee, P., Jeyaprakash, A., Hedgecock, E., and Wheaton, V.I. (1995). An
1231 ankyrin-related gene (*unc-44*) is necessary for proper axonal guidance in *Caenorhabditis*
1232 *elegans*. *J. Cell Biol.* *129*, 1081–1092.
- 1233 Owen, G.R., and Brenner, E.A. (2012). Mapping molecular memory: navigating the cellular
1234 pathways of learning. *Cell Mol Neurobiol* *32*, 919–941.

- 1235 Persson, A., Gross, E., Laurent, P., Busch, K.E., Bretes, H., and de Bono, M. (2009). Natural
1236 variation in a neural globin tunes oxygen sensing in wild *Caenorhabditis elegans*. *Nature* 458,
1237 1030–1033.
- 1238 Peymen, K., Watteyne, J., Frooninckx, L., Schoofs, L., and Beets, I. (2014). The
1239 FMRFamide-like peptide family in nematodes. *Front Endocrinol (Lausanne)* 5, 90.
- 1240 Picelli, S., Faridani, O.R., rklund, A.S.K.B.O., Winberg, G.O.S., Sagasser, S., and Sandberg,
1241 R. (2014). Full-length RNA-seq from single cells using Smart-seq2. *Nat Protoc* 9, 171–181.
- 1242 Pigliucci, M., Murren, C.J., and Schlichting, C.D. (2006). Phenotypic plasticity and evolution
1243 by genetic assimilation. *J Exp Biol* 209, 2362–2367.
- 1244 Pline, M., and Dusenbery, D.B. (1987). Responses of plant-parasitic nematode *Meloidogyne*
1245 *incognita* to carbon dioxide determined by video camera-computer tracking. *Journal of*
1246 *Chemical Ecology* 13, 873–888.
- 1247 Prabhakar, N.R., and Semenza, G.L. (2015). Oxygen sensing and homeostasis. *Physiology* 30,
1248 340–348.
- 1249 Prieto-Godino, L.L., Rytz, R., Cruchet, S., Bargeton, B., Abuin, L., Silbering, A.F., Ruta, V.,
1250 Peraro, M.D., and Benton, R. (2017). Evolution of acid-sensing olfactory circuits in
1251 *Drosophilids*. *Neuron* 93, 661–675.e667.
- 1252 R Development Core Team (2015). R: a language and environment for Statistical Computing.
1253 R Foundation for Statistical Computing www.r-project.org.
- 1254 Renn, S.C.P., and Schumer, M.E. (2013). Genetic accommodation and behavioural evolution:
1255 insights from genomic studies. *Animal Behaviour* 85, 1012–1022.
- 1256 Richaud, A., Zhang, G., Lee, D., Lee, J., and Félix, M.-A. (2018). The local coexistence
1257 pattern of selfing genotypes in *Caenorhabditis elegans* natural metapopulations. *Genetics*
1258 208, 807–821.
- 1259 Romanos, T.R., Petersen, J.G., and Pocock, R. (2017). Control of neuropeptide expression by
1260 parallel activity-dependent pathways in *Caenorhabditis elegans*. *Sci Rep* 1–11.
- 1261 Sachse, S., Rueckert, E., Keller, A., Okada, R., Tanaka, N.K., Ito, K., and Vosshall, L.B.
1262 (2007). Activity-dependent plasticity in an olfactory circuit. *Neuron* 56, 838–850.
- 1263 Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T.,
1264 Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., et al. (2012). Fiji: an open-source platform
1265 for biological-image analysis. *Nat Meth* 9, 676–682.
- 1266 Schmalhausen, I.I. (1949). *Factors of Evolution* (Philadelphia: Blakiston).
- 1267 Sivadas, P., Dienes, J.M., St Maurice, M., Meek, W.D., and Yang, P. (2012). A flagellar A-
1268 kinase anchoring protein with two amphipathic helices forms a structural scaffold in the radial
1269 spoke complex. *J. Cell Biol.* 199, 639–651.
- 1270 Smith, K.R., Kopeikina, K.J., Fawcett-Patel, J.M., Leaderbrand, K., Gao, R., Schürmann, B.,
1271 Myczek, K., Radulovic, J., Swanson, G.T., and Penzes, P. (2014). Psychiatric risk factor

- 1272 ANK3/Ankyrin-G nanodomains regulate the structure and function of glutamatergic
1273 synapses. *Neuron* 84, 399–415.
- 1274 Smith, Martinez-Velazquez, L., and Ringstad, N. (2013). A chemoreceptor that detects
1275 molecular carbon dioxide. *J Biol Chem* 288, 37071–37081.
- 1276 Stiernagle, T. (2006). Maintenance of *C. elegans* (WormBook).
- 1277 Taghert, P.H., and Nitabach, M.N. (2012). Peptide neuromodulation in invertebrate model
1278 systems. *Neuron* 76, 82–97.
- 1279 Thompson, O., Edgley, M., Strasbourger, P., Flibotte, S., Ewing, B., Adair, R., Au, V.,
1280 Chaudhry, I., Fernando, L., Hutter, H., et al. (2013). The million mutation project: A new
1281 approach to genetics in *Caenorhabditis elegans*. *Genome Res.* 23, 1749–1762.
- 1282 Troemel, E.R., Félix, M.-A., Whiteman, N.K., Barrière, A., and Ausubel, F.M. (2008).
1283 Microsporidia are natural intracellular parasites of the nematode *Caenorhabditis elegans*.
1284 *PLoS Biol* 6, 2736–2752.
- 1285 Tursun, B., Cochella, L., Carrera, I., and Hobert, O. (2009). A toolkit and robust pipeline for
1286 the generation of fosmid-based reporter genes in *C. elegans*. *PLoS ONE* 4, e4625.
- 1287 Van der Auwera, G.A., Carneiro, M.O., Hartl, C., Poplin, R., Del Angel, G., Levy-
1288 Moonshine, A., Jordan, T., Shakir, K., Roazen, D., Thibault, J., et al. (2013). From FastQ data
1289 to high confidence variant calls: the Genome Analysis Toolkit best practices pipeline. *Curr*
1290 *Protoc Bioinformatics* 43, 11.10.1–10.33.
- 1291 Viglierchio, D.R. (1990). Carbon dioxide sensing by *Panagrellus silusiae* and *Ditylenchus*
1292 *dipsaci*. *Revue De Nematologie* 13, 425–432.
- 1293 Vulesevic, B., McNeill, B., and Perry, S.F. (2006). Chemoreceptor plasticity and respiratory
1294 acclimation in the zebrafish *Danio rerio*. *J Exp Biol* 209, 1261–1273.
- 1295 Waddington, C.H. (1942). Canalization of development and the inheritance of acquired
1296 characters. *Nature* 150, 563–565.
- 1297 Waddington, C.H. (1953). Genetic assimilation of an acquired character. *Evolution* 7, 118–
1298 126.
- 1299 Weber, K.P., De, S., Kozarewa, I., Turner, D.J., Babu, M.M., and de Bono, M. (2010). Whole
1300 genome sequencing highlights genetic changes associated with laboratory domestication of *C.*
1301 *elegans*. *PLoS ONE* 5, e13922.
- 1302 West-Eberhard, M.J. (2005). Developmental plasticity and the origin of species differences.
1303 *Proc Nat Acad Sci* 102, 6543–6549.
- 1304 Ye, K., Schulz, M.H., Long, Q., Apweiler, R., and Ning, Z. (2009). Pindel: a pattern growth
1305 approach to detect break points of large deletions and medium sized insertions from paired-
1306 end short reads. *Bioinformatics* 25, 2865–2871.
- 1307 Yin, J.-A., Gao, G., Liu, X.-J., Hao, Z.-Q., Li, K., Kang, X.-L., Li, H., Shan, Y.-H., Hu, W.-
1308 L., Li, H.-P., et al. (2017). Genetic variation in glia-neuron signalling modulates ageing rate.

- 1309 Nature 551, 198–203.
- 1310 Yu, S., Avery, L., Baude, E., and Garbers, D.L. (1997). Guanylyl cyclase expression in
1311 specific sensory neurons: a new family of chemosensory receptors. Proc Nat Acad Sci 94,
1312 3384–3387.
- 1313 Zhang, G., Sachse, M., Prevost, M.-C., Luallen, R.J., Troemel, E.R., and Félix, M.-A. (2016).
1314 A Large Collection of Novel Nematode-Infecting Microsporidia and Their Diverse
1315 Interactions with *Caenorhabditis elegans* and Other Related Nematodes. PLoS Pathog 12,
1316 e1006093.
- 1317 Zhang, Y., Bertolino, A., Fazio, L., Blasi, G., Rampino, A., Romano, R., Lee, M.-L.T., Xiao,
1318 T., Papp, A., Wang, D., et al. (2007). Polymorphisms in human dopamine D2 receptor gene
1319 affect gene expression, splicing, and neuronal activity during working memory. Proc. Natl.
1320 Acad. Sci. U.S.a. 104, 20552–20557.
- 1321 Zimmer, M., Gray, J.M., Pokala, N., Chang, A.J., Karow, D.S., Marletta, M.A., Hudson,
1322 M.L., Morton, D.B., Chronis, N., and Bargmann, C.I. (2009). Neurons detect increases and
1323 decreases in oxygen levels using distinct guanylate cyclases. Neuron 61, 865–879.
- 1324

Figure 1

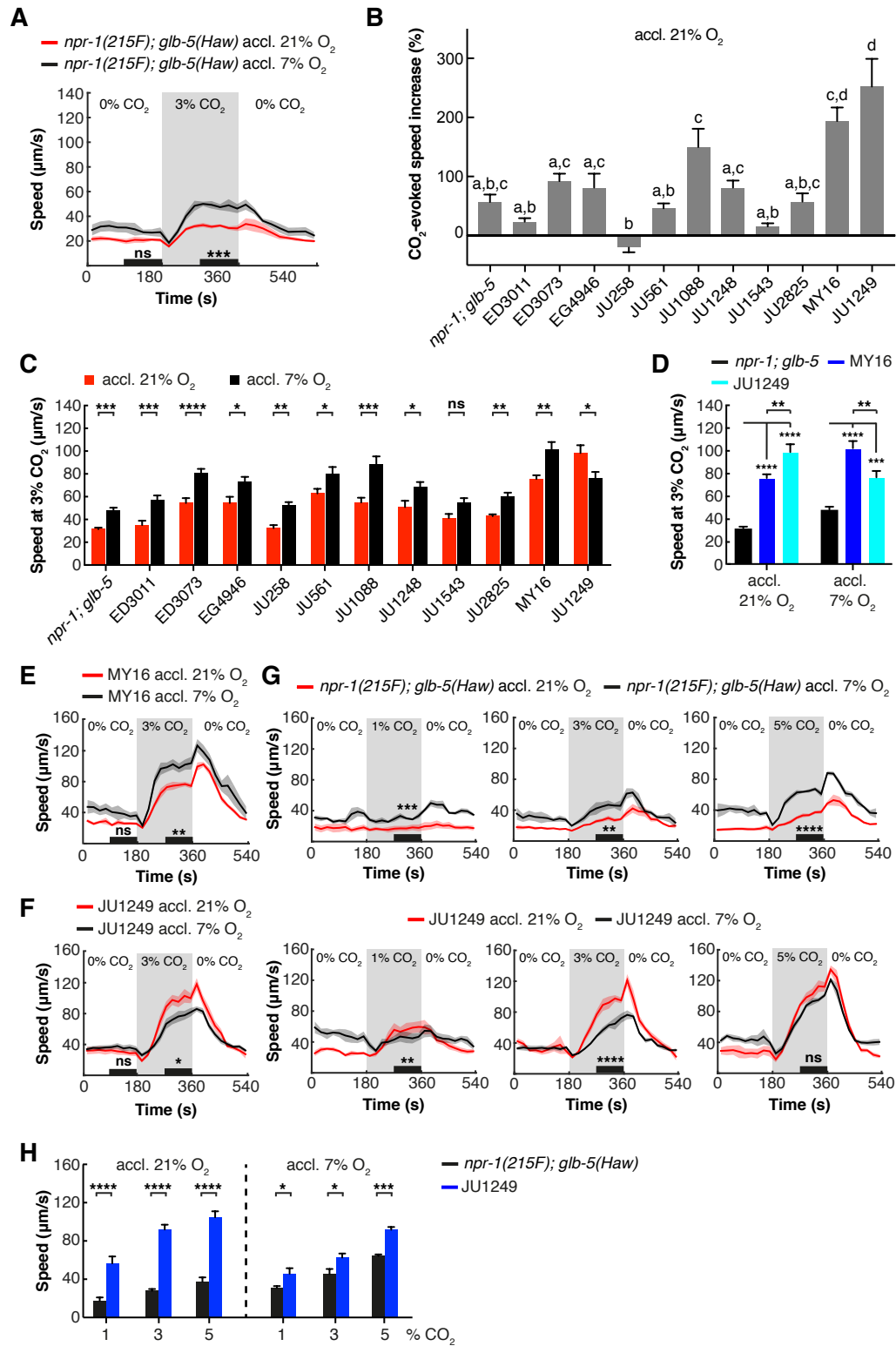


Figure 2

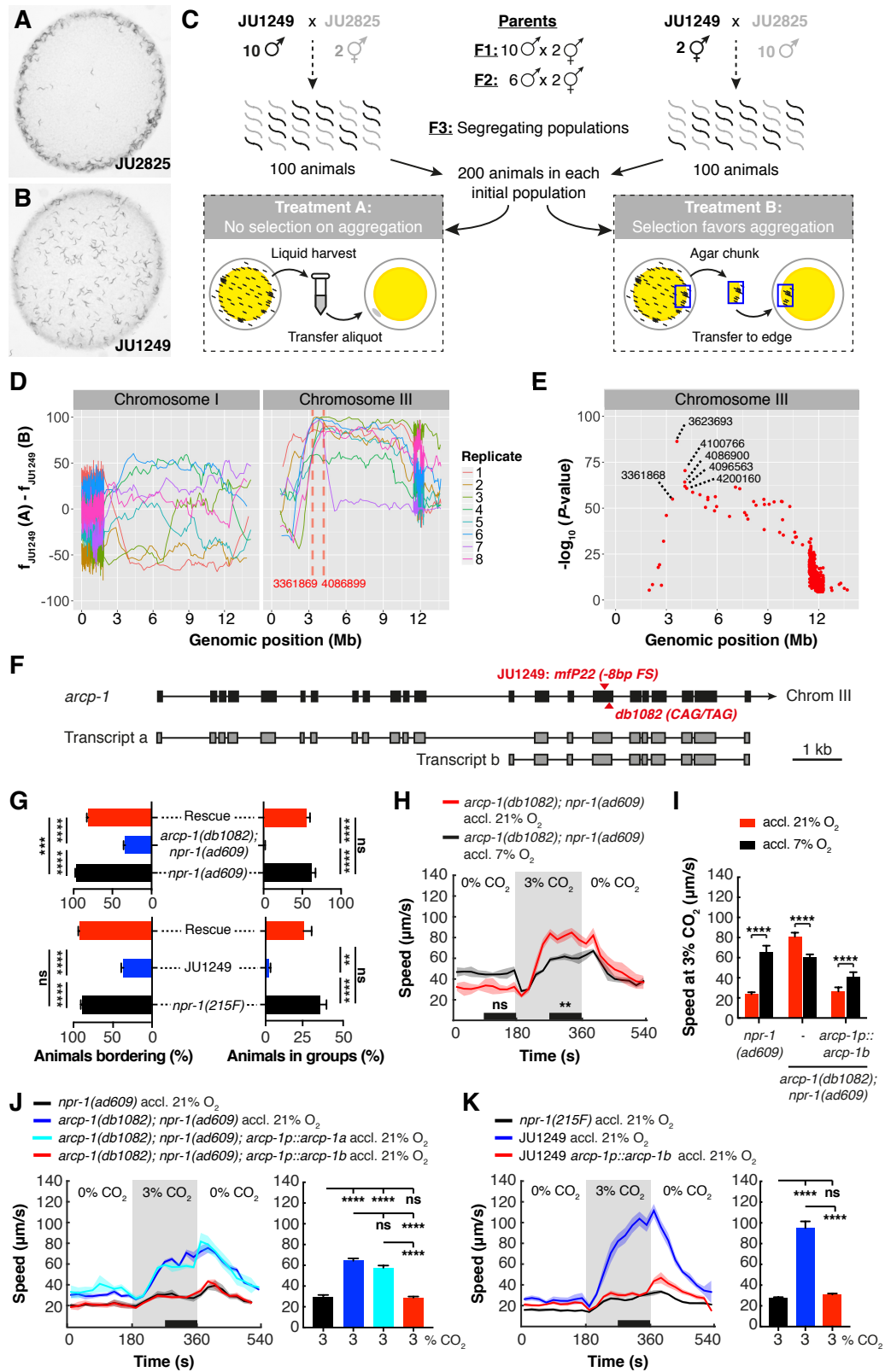


Figure 3

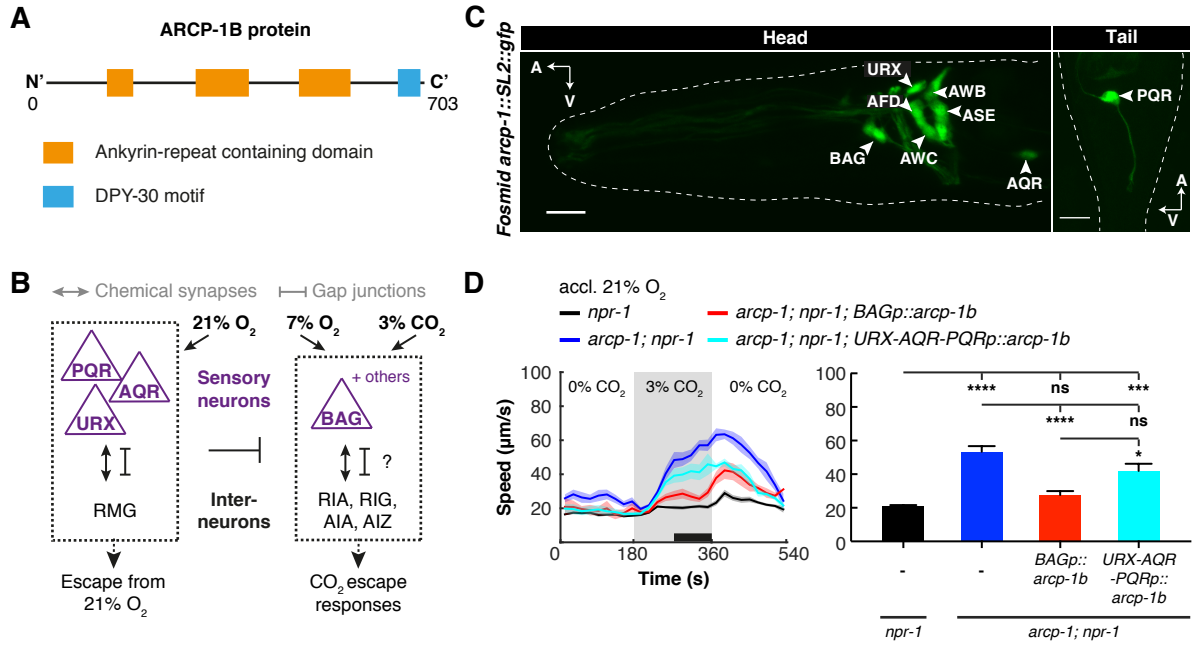


Figure 4

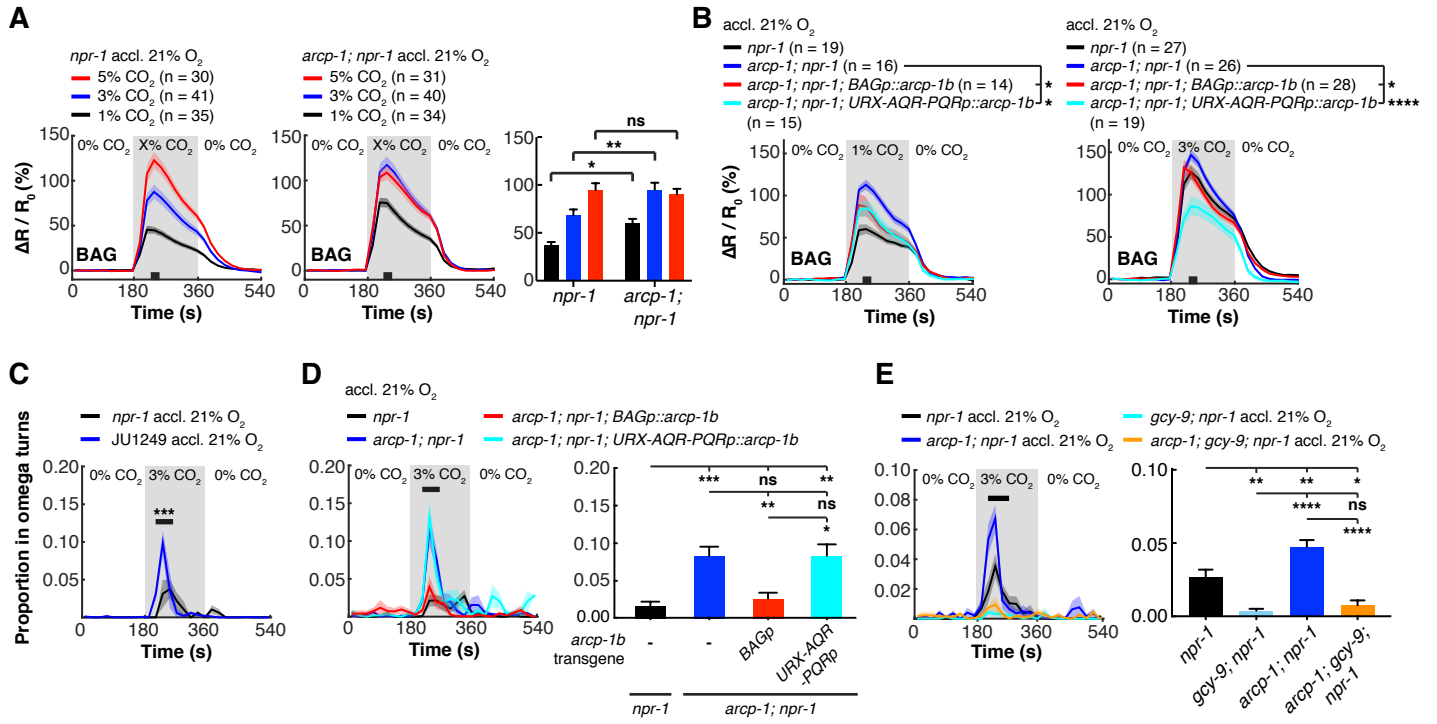


Figure 5

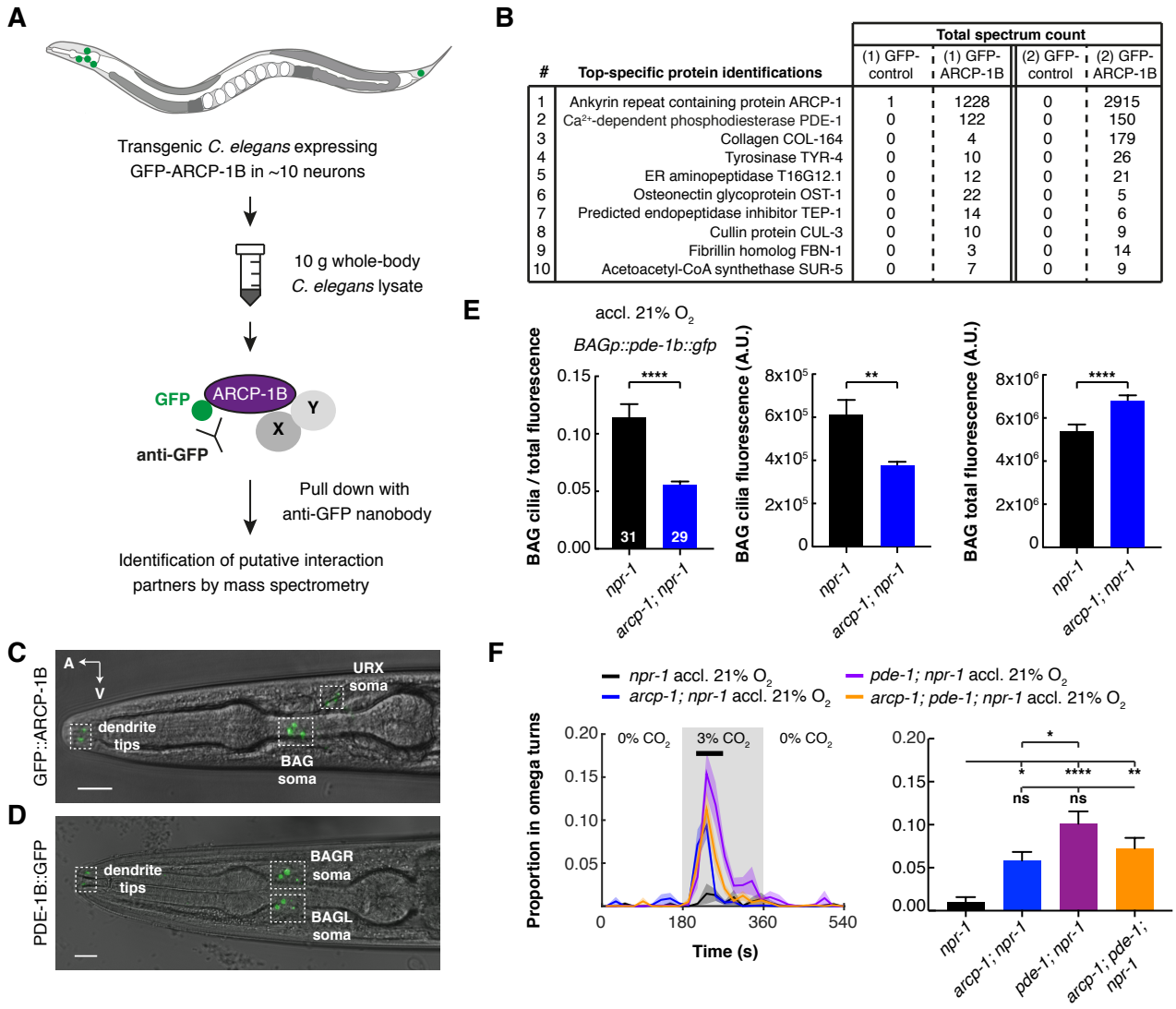


Figure 6

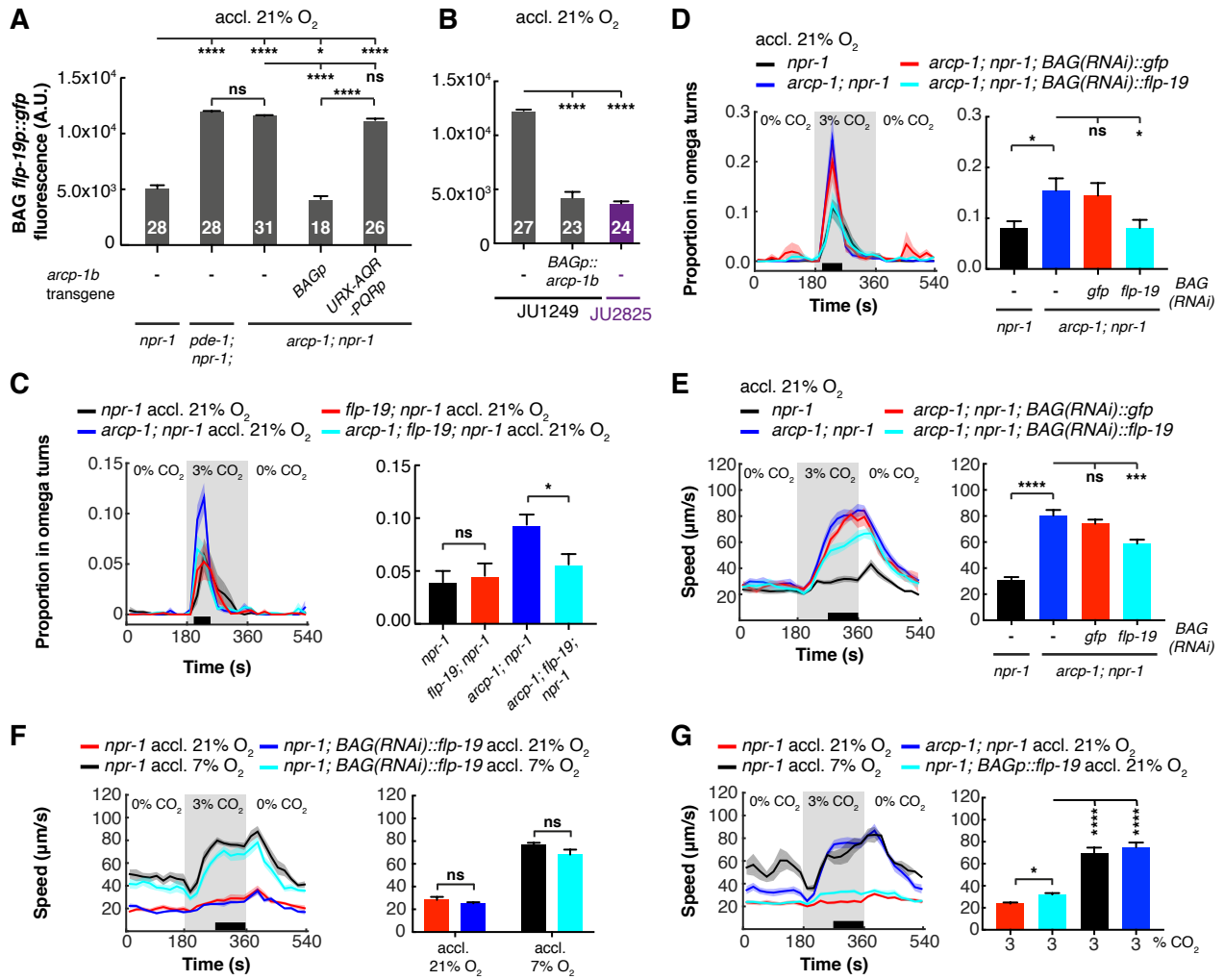


Figure 7

