Sensory experience controls dendritic structure and behavior by distinct pathways involving degenerins

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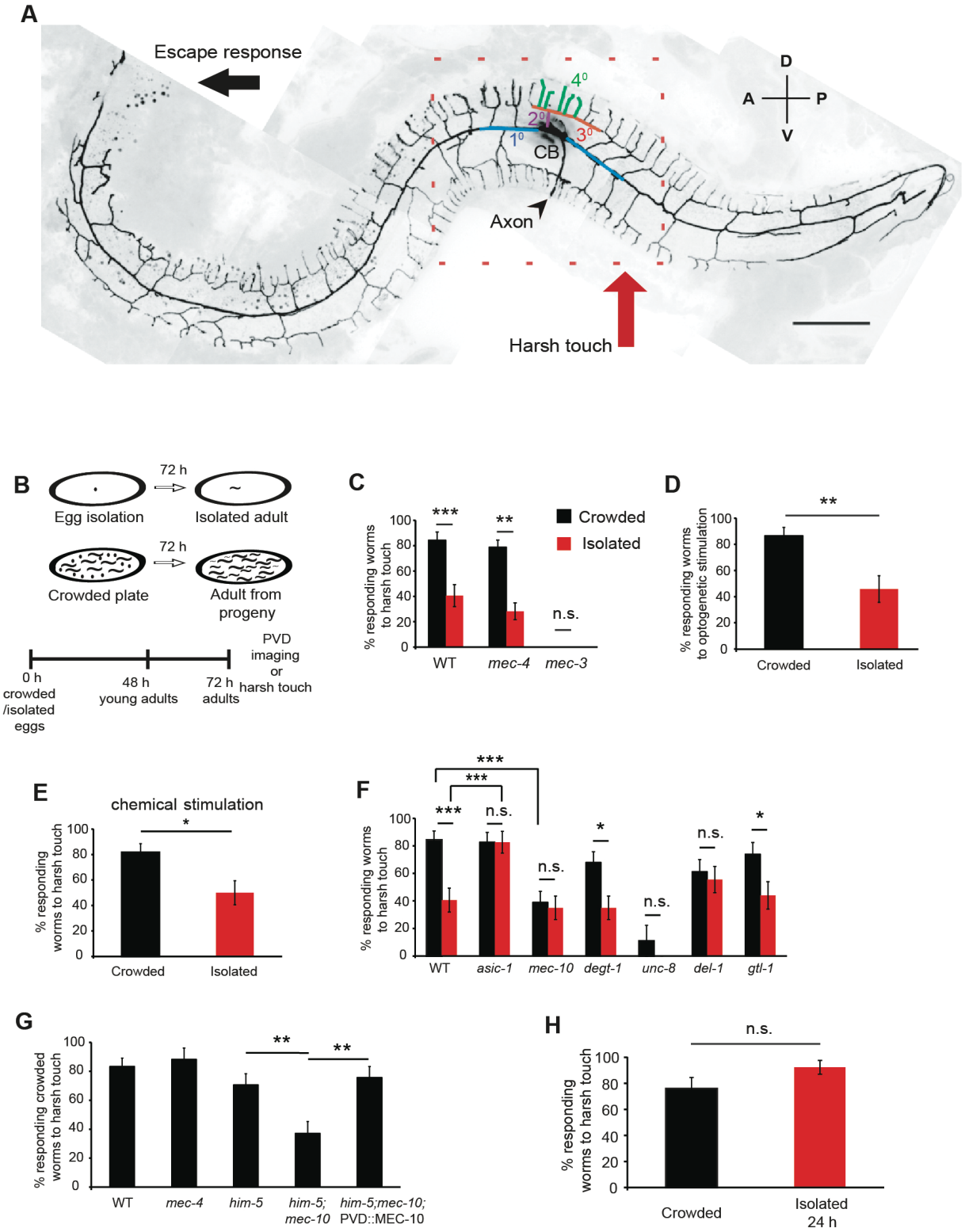
Abstract

Tree-like neurites are crucial for receiving information into neurons. It is assumed that nurturing affects the structure and function of dendrites, yet the evidence is scarce, and the mechanisms are unknown. To study whether mechanosensory experience affects dendritic morphology, we use natural mechanical stimulation of the *Caenorhabditis elegans'* polymodal PVD neurons, induced by physical contacts between individuals. We found that animal isolation affects the dendritic tree structure of the PVD. Moreover, developmentally isolated animals show a decrease in their ability to respond to harsh touch. The structural and behavioral plasticity following mechanosensory deprivation are functionally independent of each other and are mediated by an array of evolutionary conserved amiloride-sensitive epithelial sodium channels (degenerins). Our results suggest an activity-dependent homeostatic mechanism for dendritic structural plasticity, acting downstream to mechanosensory activation of degenerins.

Main Text:

The general structure of the nervous system has been known for over a century. Groundbreaking studies on synaptic plasticity and its underlying mechanisms have shown that before birth and in adult animals, brain waves of neural activity are needed for synaptic remodeling [1-4]. In contrast, the molecular mechanisms responsible for structural plasticity of dendritic trees, as an output of different sensory signals, especially during adulthood, are poorly understood [5-7].

Figure 1


A
Escape response
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A
P
V
4°
2°
1°
3°
CB
Axon
Harsh touch
B
72 h
Egg isolation
Isolated adult
72 h
Crowded plate
Adult from progeny
0 h crowded /isolated eggs
48 h young adults
72 h adults
PVD imaging or harsh touch
C
% responding worms to harsh touch
Crowded
Isolated
***
**
n.s.
WT
mec-4
mec-3
D
% responding worms to optogenetic stimulation
**
Crowded
Isolated
E
chemical stimulation
% responding worms to harsh touch
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Crowded
Isolated
F
% responding worms to harsh touch
***
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n.s.
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n.s.
n.s.
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WT
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mec-10
degt-1
unc-8
del-1
gtl-1
G
% responding crowded worms to harsh touch
**
**
WT
mec-4
him-5
him-5; mec-10
him-5;mec-10; PVD::MEC-10
H
% responding worms to harsh touch
n.s.
Crowded
Isolated 24 h

Figure 1. Mechanosensory deprivation during development and cell autonomous activity of MEC-10 reduces the behavioral response of the PVD neuron to activation. (A) Entire branching pattern of the PVD neuron is shown. The red arrow corresponds to the harsh touch contact point with platinum wire, while the left black arrow represents the response of the worm following application of harsh touch. The red dashed square represents the region of interest around the cell body (CB). One representative candelabrum is shown with color coded branch orders. A- anterior; P- posterior; V- ventral; D-dorsal. **(B)** Schematic for the isolation protocol, followed by posterior harsh touch assay or PVD imaging. **(C)** Egg isolation reduced the percentage of responding worms to harsh touch at adulthood. Harsh touch assay was performed for crowded (black bars) and isolated (red bars) worms: Wildtype (WT, N2) worms (Crowded, n=32; Isolated, n=32), *mec-4* (Crowded, n=52; Isolated, n=46. Here the experiment was performed at 96 h since *mec-4* animals develop slower and the worms were L4- young adults at 72 h), *mec-3* (Crowded, n=12; Isolated, n=12). **(D)** Reduced response to photostimulation after isolation. Channelrhodopsin expressing worms on All Trans Retinal were used for analysis of forward escape following photostimulation of the PVD (Crowded, n=30; Isolated, n=24, no response was observed for worms grown without All Trans Retinal). **(E)** Isolation of *mec-4* worms with pheromonal signals resulted in reduced percentage of responding worms (Here the experiment was performed at 96 h since at 72 h the worms were L4s- very young adults; Crowded, n=33; Isolated, n=28). **(F)** DEG-ENaCs and one TRP (*gtl-1*) mutants were tested for the effect of isolation on harsh touch response. Isolated worms were compared to crowded worms from the same strain. WT worms (The same set of worms as in Fig. 1c. Crowded, n=32; Isolated, n=32), *asic-1* (Crowded, n=46; Isolated, n=30), *mec-10* (Crowded, n=38; Isolated, n=31), *degt-1* (Crowded, n=37; Isolated, n=31), *unc-8* (Crowded, n=18; Isolated, n=15), *del-1* (Crowded, n=31; Isolated, n=27), *gtl-1* (Crowded, n=27; Isolated, n=25). **(G)** Strains containing the *ser-2Prom3*::Kaede were tested for their response to harsh touch in the crowded conditions. MEC-10 expression in the PVD rescues the reduction in the response to harsh touch induced by *mec-10* mutants (Crowded: WT, n=42 ;*mec-4,* n=17 ; *him-5,* n=34 (*him-5* was used as WT background for several strains after cross); *him-5;mec-10,* n=35; *him-5;mec-10*;PVD::MEC-10, n=33). **(H)** Isolation of young adult worms for 24 h did not affect the response to harsh touch. Worms were isolated for 24 h as young adults and were tested for harsh touch response, compared to worms that were grown in crowded conditions (Crowded, n=25; Isolated for 24 h, n=26). The proportion of responding worms (percentage) ± standard error of proportion is shown. Fisher exact test, *$p<0.05$ ** $p<0.01$, *** $p<0.001$, n.s. not significant.

81 Mechanistic understanding of experience-dependent dendritic structural plasticity
82 is focused on activity sensation by calcium channels and N-methyl-D-aspartate (NMDA)
83 receptors, that induce downstream signaling cascades including Rho family of small
84 GTPases, calcium metabolism and microtubule stability [8-11]. Since neurological
85 disorders like autism, Down syndrome, fragile X syndrome, and schizophrenia are
86 characterized by abnormal dendritic spine structures, uncovering the molecular basis of
87 dendritic tree instability during development and adulthood, is of great importance [12].

88 The morphology of the *C. elegans'* PVD bilateral neurons is composed of
89 repetitive and spatially organised structural units that resemble candelabra (Fig. 1A) [13].
90 These structural properties provide a useful platform to study dendritic morphogenesis.
91 While some of the genetically programmed molecular mechanisms responsible for the
92 development, morphogenesis and regeneration of PVD's dendritic trees are known [14-20],
93 the influence of nurture, e.g. sensory experience, on its structure and function during
94 development and in adulthood remain unexplored. The PVD mediates three sensory
95 modalities: response to harsh touch [21], response to low temperatures [21] and
96 proprioception [22]. Rose et al [23] found that deprivation of mechanosensory stimulation,
97 generated by colliding conspecifics in the growing plate, resulted in reduced response to
98 gentle tap stimulation and modified glutamatergic signalling in gentle touch circuits.
99 Here, we adapted this natural mechanosensory stimulation paradigm to identify the
100 mechanism that couples mechanosensory activity to structural and functional plasticity,
101 focusing on degenerins/epithelial Na^+ channels (DEG/ENaCs) expressed in the PVD [21,
102 24]. These channels form homo- and hetero-trimers involved in mechanosensation and
103 cognitive functions like learning and memory [25-28]. We investigate how mechanosensory

experience, mediated by activity through DEG/ENaCs, affects structural plasticity of the PVD dendritic trees in adult *C. elegans*. Sensory and social isolation affect the behavior and fitness of diverse animals [29], including primates [30]. Our findings on the plasticity of stereotypic dendritic trees of polymodal somatosensory neurons in adult nematodes, reveal mechanisms of dendritic plasticity induced by mechanosensation that may be conserved.

Experience induces behavioral plasticity

To evaluate PVD activity we tested one of its modal functions using a behavioral assay that measures escape from the noxious mechanical stimulus by prodding with a platinum wire (harsh touch assay; Fig. 1A) (*24*). To study whether mechanosensory deprivation affects the nociceptive functions of the PVD [13, 31], we isolated embryos into single plates, from hatching through adulthood and compared their behavioral response to harsh touch against adults that were grown for 72 h on crowded plates (Fig. 1B). We found that ~40% of isolated wildtype (WT) animals responded to harsh touch, compared to ~80% of animals grown in crowded plates (Fig. 1C). To exclude the possibility that gentle touch neurons are involved in this behavioral response, we isolated *mec-4* mutants, which are insensitive to gentle touch [32], and obtained similar results. As a negative control, we used *mec-3* mutants, that are touch insensitive (*31*) and found a similarly low response for both groups (Fig. 1C). Thus, isolation reduces the response to noxious stimuli during adulthood in a process that is independent of gentle touch.

To investigate whether the isolation-induced decreased response is a PVD-dependent function, we used optogenetic stimulation [24]. We found that isolation reduced the percentage of worms responding to optogenetic stimulation of the PVD (Fig. 1D),

127 indicating that the plasticity in the response is acting downstream to PVD activation and
128 mechanosensory channels.

129 Since mechanosensory experience can be induced not only by conspecifics
130 present on the plate, but also by solid inert objects [33], we used the *mec-4* strain to
131 compare responses of isolated worms to responses of isolated worms grown in the
132 presence of glass beads. Worms grown in isolation with beads had a similar response
133 compared to animals grown in crowded plates (Fig. S1a). We then used plates pre-
134 stimulated with pheromones (Fig. 1E) and *osm-6* mutants (defective sensory cilia,
135 including the PDE neuron [34] that reduces also harsh touch responses [35] (Fig. S1b)). For
136 both experiments we found that isolation induced a reduction in the response to harsh
137 touch. Thus, behavioral plasticity for harsh touch stimulation of the PVD is dependent
138 on mechanosensory stimulation during development and is chemosensory-independent
139 [35].

140 **MEC-10 affects behavioral plasticity**

141 To study the genetic mechanisms of plasticity during nociceptive response, we
142 performed a candidate gene screen for degenerins (DEG/ENaC) and transient receptor
143 potential (TRP) channels that are expressed in the PVD and affect its behavioral
144 responses [9, 20, 36]. The harsh touch response following isolation was reduced in WT,
145 *degt-1* and *gtl-1* mutants, suggesting that these channels are not directly involved in
146 behavioral plasticity following isolation (Fig. 1F). In contrast, for *del-1*, *asic-1* and *mec-*
147 *10* mutants the difference between isolated and crowded conditions was undetectable,
148 indicating that they are required for behavioral plasticity. Interestingly, while the harsh
149 touch response of *asic-1* mutants was high and similar to crowded WT worms, the

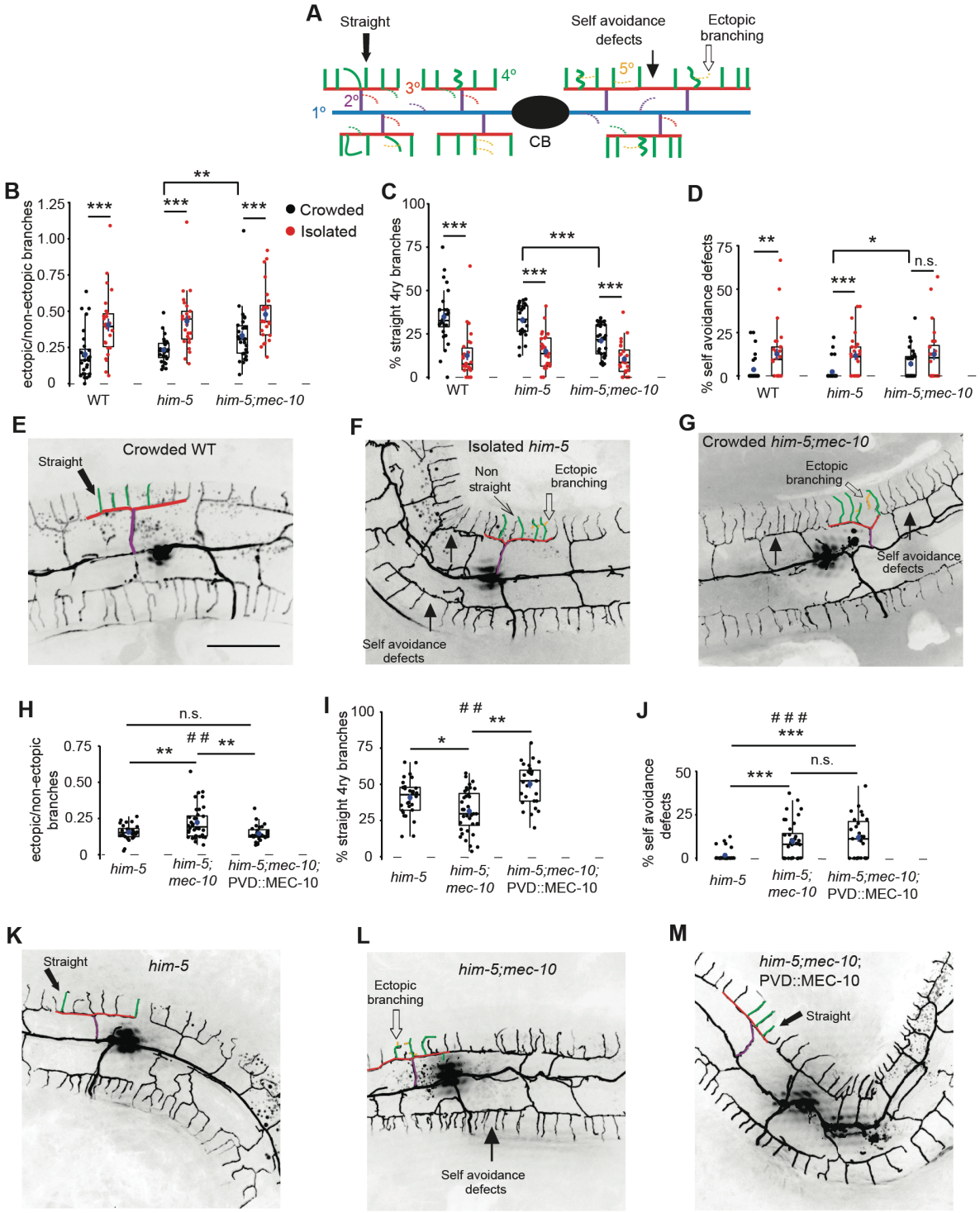
150 response for *mec-10* mutants was low, similar to isolated WT animals. To test whether
151 the response to harsh touch is dependent on DEG/ENaC activity, we used DEG/ENaC
152 blocker- amiloride for worms that were grown in crowded plates. We found that the
153 response to harsh touch was not affected by continuous growth in the presence of
154 amiloride (Fig. S1C). This result supports the idea that the combinatorial activities of
155 multiple amiloride-sensitive epithelial sodium channels have positive and negative
156 effects on the response to harsh touch (Fig. 1F).

157 Since MEC-10 is expressed in the PVD and is responsible for its response to
158 harsh touch [21], we asked how it mediates the behavioral plasticity following
159 mechanosensory experience in the crowded, but not isolated conditions. To test
160 whether the activity of MEC-10 is required autonomously in the PVD, we performed a
161 cell-specific rescue experiment, with a plasmid encoding for MEC-10, under a PVD-
162 specific promoter [13, 37]. We found that expression of MEC-10 in the PVD rescues the
163 reduction in response to harsh touch in crowded *mec-10* mutants, indicating that it acts
164 cell autonomously to modulate behavioral plasticity of mechanosensory signals (Fig.
165 1G) [21].

166 To determine whether isolation affects the response to harsh touch during
167 development or in adults, we isolated young adults for 24 h and found that isolation of
168 young adults did not affect the response to harsh touch (Fig. 1H). Thus, the isolation-
169 induced reduction in response to harsh touch is MEC-10-dependent and is determined
170 during development.

171

Figure 2


A
Straight
Self avoidance defects
Ectopic branching
1°
2°
3°
4°
5°
CB
B
ectopic/non-ectopic branches
Crowded
Isolated
WT
him-5
him-5;mec-10
C
% straight 4ry branches
D
% self avoidance defects
n.s.
E
Crowded WT
Straight
F
Isolated him-5
Non straight
Ectopic branching
Self avoidance defects
G
Crowded him-5;mec-10
Ectopic branching
Self avoidance defects
H
ectopic/non-ectopic branches
him-5;mec-10; PVD::MEC-10
I
% straight 4ry branches
J
% self avoidance defects
K
Straight
him-5
L
him-5;mec-10
Ectopic branching
Self avoidance defects
M
him-5;mec-10; PVD::MEC-10
Straight

172

Figure 2. Mechanosensory deprivation and cell autonomous activity of MEC-10 affects the architecture of the PVD. (A) Schematic representation for PVD candelabrum structure, with color coded branch orders, ectopic branches, quaternary branching geometry and self-avoidance defects marked and labeled. Dashed lines represent ectopic branching at each order. Isolation and *mec-10* affect the structure of the PVD: **(B)** Increase the fraction of ectopic branching, **(C)** decrease the percentage of straight quaternary branches and **(D)** increase the percentage of self-avoidance defects between two adjacent candelabra were quantified. Crowded (black dots) and isolated (red dots) animals were tested for each strain. WT (Crowded, n=28; Isolated n=26), *him-5* (Crowded, n=27; Isolated, n=25; *him-5* was used as WT background for several strains after cross), *him-5;mec-10* (Crowded, n=30; Isolated, n=24). **(E-G)** Representative pictures from the PVD. Scale bar on panel 'E' represents 50 µm. **(H)** In crowded conditions over- expression of MEC-10 in the PVD, on the background of *him-5;mec-10,* resulted in reduction of ectopic branching and **(I)** increased percentage of straight quaternary branches. **(J)** Over expression of MEC-10 in the PVD does not affect the amount of self-avoidance defects induced by *mec-10.* Crowded *him-5* (n=28), crowded *him-5;mec-10* (n=36), crowded *him-5;mec-10;*PVD::MEC-10 (n=27). **(K-M)** Representative pictures from the PVD. Each dot represents a single worm. The mean ± s.e.m. are shown in blue. Box plot with hinge for the first and third quartile. The median is represented by the thick horizontal black line and the whiskers are estimated calculation of the confidence interval 95%. Kruskal-Wallis test, # # $p<0.01$, # # # $p<0.001$, Mann Whitney test with Bonferroni Correction $\alpha=0.0167$. *$p<0.05$, ** $p<0.01$, *** $p<0.001$, n.s. not significant.

196 **Experience affects morphology via MEC-10**

197 Sensory experience drives synaptic plasticity in the nervous system [3, 4, 38]. To test
198 whether experience regulates the morphology of the dendritic tree of the PVD and its
199 function as a nociceptor [39], we used the mechanical deprivation paradigm (Fig. 1B) and
200 examined morphological features of the PVD (Fig. 2A): **(1)** The fraction of ectopic
201 (excess) out of non-ectopic branches (those that form the classical candelabrum), **(2)**
202 the percentage of straight quaternary branches and **(3)** the percentage of self-
203 avoidance defects between adjacent candelabra.

204 We found that isolation of both WT and *him-5* (the WT background for several
205 strains after cross) animals for 72 h, increased the fraction of ectopic branches
206 compared with age matched crowded worms (Fig 2B), and reduced the percentage of
207 straight quaternary branches (Fig 2C). Moreover, isolation increased self-avoidance
208 defects (Fig. 2D-G, Fig. S2). These results show that the PVD dendritic tree is sensitive
209 to the sensory signals generated by other worms on the plate. Using our quantitative
210 assays to determine changes in the structure of the PVD neuron following
211 mechanosensory isolation, we decided to investigate whether these morphological
212 phenotypes are induced via MEC-10 mechanoreceptor; we compared the PVD
213 arborisation patterns between crowded and isolated *mec-10* mutants. We found that
214 crowded *mec-10* animals were characterized by isolated-like morphological features
215 (more ectopic branches, fewer straight quaternary branches and more self-avoidance
216 defects, compared to crowded *him-5* animals; Fig. 2B-G). These results suggest that
217 PVD morphology is affected by sensory experience in a *mec-10*-dependent pathway. To
218 study whether MEC-10 acts cell autonomously to mediate morphological plasticity, we

219 used the PVDp::MEC-10 line in a *mec-10* background and found a reduced fraction in
220 the ectopic branches and an increased percentage in the number of straight quaternary
221 branches, compared to age-matched *mec-10* animals (Fig. 2H-I). Interestingly,
222 expression of MEC-10 in the PVD had no effect on the amount of self-avoidance
223 defects (Fig. 2J-M), suggesting that it acts cell non-autonomously to mediate loss of
224 self-avoidance. Activity and sodium influx via the DEG/ENaC UNC-8 promotes synapse
225 elimination in *C. elegans* [40]. To test how global inhibition of DEG/ENaCs affects the
226 morphology of the PVD neuron, we compared crowded worms that were grown on
227 plates with amiloride to control worms. We found that blocking DEG/ENaCs by amiloride
228 increased the fraction of ectopic branching and decreased the percentage of straight
229 quaternary branches, without affecting self-avoidance (Fig. S3). Thus, the structure of
230 the PVD is sensitive to activity and cation influx via DEG/ENaCs. To determine whether
231 the morphological effect on PVD structure is also mediated by mechanical (like friction
232 [41] and collisions [21]), rather than chemical cues, we used glass beads under isolated
233 conditions, to test the effect on the morphology. We found that mechano-stimulation
234 induced by glass beads did not restore the fraction of ectopic branching, but it did
235 significantly increase the number of straight quaternary branches and decreased the
236 percentage of self-avoidance defects (compared with isolated animals without beads,
237 Fig. 3A-E). Thus, mechanosensory stimuli induce morphological plasticity in the PVD
238 via DEG/ENaCs.

239 Similarly to Fig. 2B-D, animal isolation onto pheromone-conditioned plates [42],
240 increased the fraction of ectopic branching, decreased the percentage of straight
241 quaternary branches and increased the percentage of self-avoidance defects (Fig. S4).

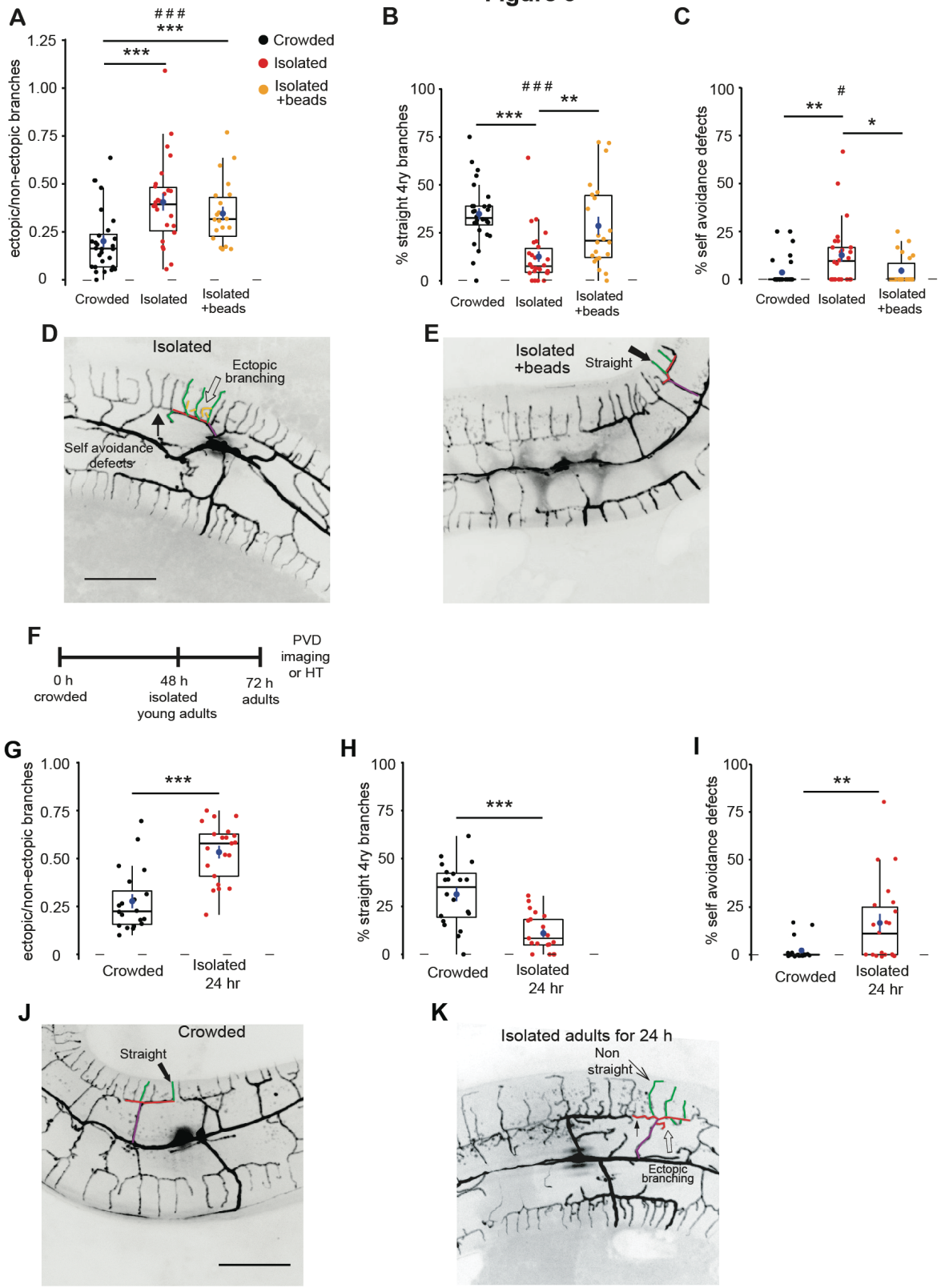
242 These results indicate that the effect of isolation on the structure of the PVD is not
243 mediated by chemical signals. Additionally, we looked at *mec-4* gentle-touch-insensitive
244 mutants [23, 32] and confirmed that isolation caused a similar change to PVD structure as
245 WT (Fig.S5, compare with Fig. 2B-D). In summary, mechanosensory experience
246 controls morphological plasticity of PVD dendritic trees via MEC-10 activity and
247 independently of MEC-4.

248 **Adult isolation induces tree complexity**

249 Isolation of eggs for 72 h comprises ~48 h of development followed by ~24 h of
250 adulthood. To determine whether the isolation-induced morphological phenotypes of the
251 PVD (Fig. 2A) occur during development or alternatively during adult maintenance, we
252 compared crowded and isolated WT worms after 48 h, as young adults. We found a
253 small but significant difference only for the percentage of straight terminal 4ry branches
254 (Fig. S6), suggesting that isolation affects maintenance of PVD morphology during
255 adulthood. To determine directly the period when mechanical isolation acts to cause
256 structural phenotypes, we studied the morphology of the PVD in worms that were grown
257 in crowded plates and then isolated as young adults for 2, 5 or 24 h. Isolation of adults
258 for 2 h had no significant consequence on the structure of the PVD (Fig. S7A-C), while
259 isolation of adults for 5 h reduced the percentage of quaternary straight branches
260 compared to crowded worms (Fig. S7D-F). In contrast, worms isolated as adults for 24
261 h (Fig. 3F) showed increased PVD complexity, similar to worms grown under
262 continuous isolation of eggs for 72 h (Fig. 2B-G, Fig. 3G-K). Thus, the architecture of
263 the adult PVD is sensitive to the duration of mechanosensory signals during adulthood.

264

Figure 3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% straight 4ry branches
% self avoidance defects
Isolated
Ectopic branching
Self avoidance defects
Isolated +beads
Straight
0 h crowded
48 h isolated young adults
72 h adults
PVD imaging or HT
Isolated 24 hr
Crowded
Isolated adults for 24 h
Non straight

265

Figure 3. The structure of the PVD is plastic during the adult stage and is mechanosensory- and not chemosensory-dependent. (A) Egg isolation with glass beads did not affect ectopic branching, but it **(B)** increased the percentage of straight quaternary branches and **(C)** reduced the percentage of self-avoidance defects, compared to isolated worms without beads. **(D, E)** Representative pictures from the PVD for isolated eggs with and without glass beads. Crowded (the same worms as used for Fig. 2B-D), n=28; Isolated (the same worms as used for Fig. 2B-D, n=26); Isolated eggs with beads, n=22. **(F)** Schematic description for the isolation of crowded young adult worms for 24 h, followed by PVD imaging. **(G)** Isolation of young adult worms for 24 h increased the fraction of ectopic branching, **(H)** reduced the percentage of straight quaternary branches and **(i)** increased the percentage of self-avoidance defects (Crowded, n=20; Isolated adults for 24 h, n=21). **(J, K)** Representative pictures from the PVD for crowded and isolated adult for 24 h. Scale bars in panels 'D' and 'J' represent 50 µm. Each dot represents one worm. The mean ± s.e.m. are shown in blue. Box plot with hinge for the first and third quartile. The median is represented by the thick horizontal black line and the whiskers are rough calculation of the confidence interval 95%. Kruskal-Wallis test, # $p<0.05$, # # # $p<0.001$, Mann Whitney test, * $p<0.05$, ** $p<0.01$, *** $p<0.001$, n.s. not significant.

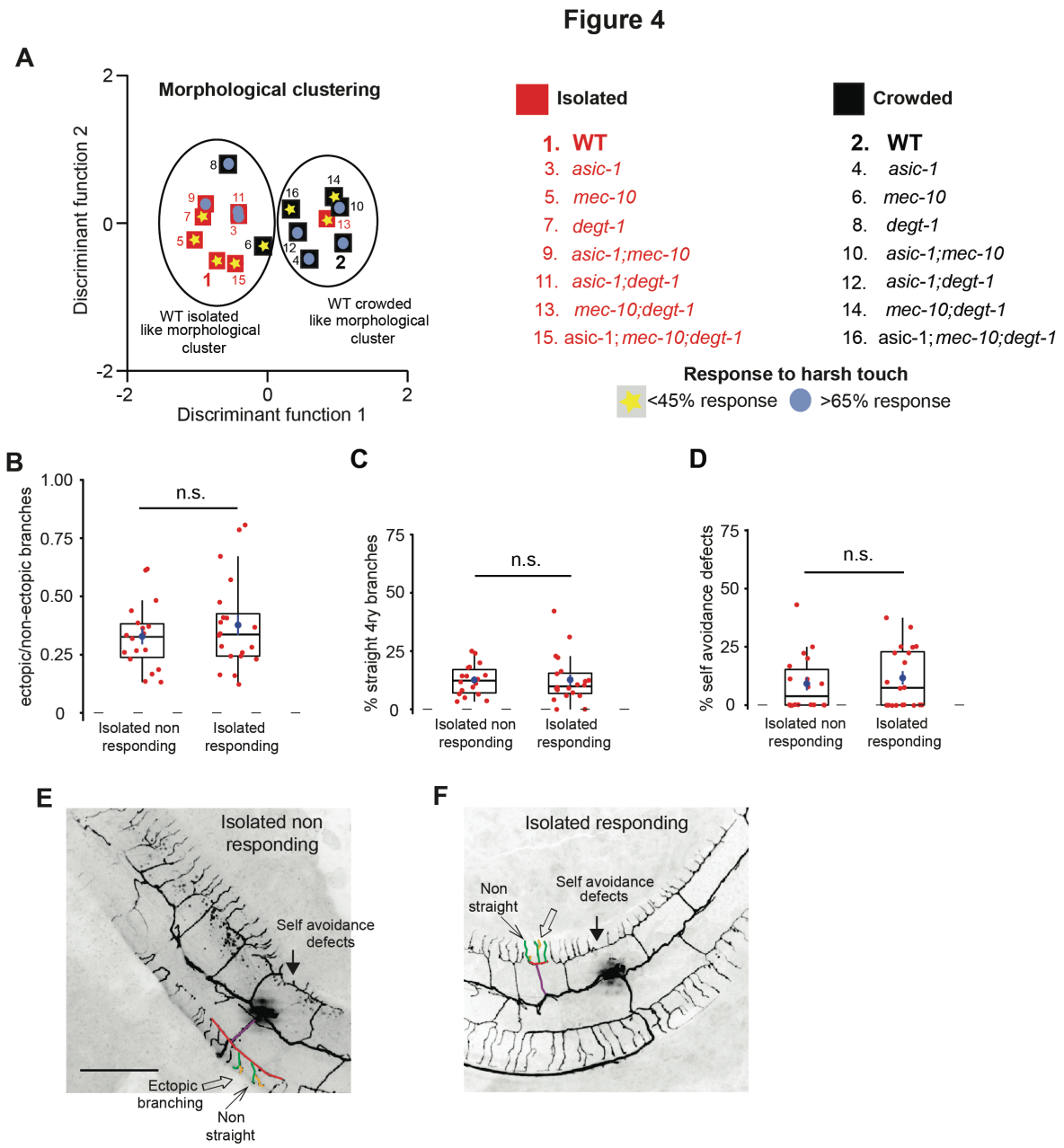


Figure 4. Response to harsh touch and morphology of the PVD are independent

(A) Discriminant analysis revealed the independence of harsh touch on PVD's morphological classification. Worms from a particular treatment (crowded or isolated) and genotype were classified based on their morphological characteristics of the PVD using discriminant analysis and by their response to harsh touch. The squares (black for crowded and red for isolated) are the centroids for the morphological characteristics analyzed in Fig. S9. The response to harsh touch (Fig. S8) is illustrated by its

magnitude (low, <45% in yellow asterisk; high, >65% in light blue circles). The different genotypes (numbers 1-16) are labeled in the list located on the right. Isolated worms were defined based on their response to harsh touch. Isolated harsh touch responding animals were compared to isolated non-responding. No significant difference was measured for **(B**) the fraction of ectopic branching, **(C)** the geometry of quaternary branches or **(D**) the percentage of self-avoidance defects (Isolated non-responding, n=18; Isolated responding, n=20). **(E, F)** Representative pictures from the PVD for responding and non-responding worms. Scale bar for panel 'E' represents 50 µm. The mean ± s.e.m. are shown in blue. Box plot with hinge for the first and third quartile. The median is represented by the thick horizontal black line and the whiskers are rough calculation of the confidence interval 95%. Each dot represents one worm. The mean ± s.e.m. are shown in blue. Mann Whitney test. n.s. not significant.

305 **Structure and function are independent**

306 To test the model proposing that there is a causal link between the morphology of the
307 dendritic tree of the PVD and its function as a nociceptor [39] we followed the isolation
308 protocol described in Fig. 1B for seven combinations of DEG/ENaC mutants and
309 analyzed their response to harsh touch (Fig. S8) and their PVD structure (Fig. S9). To
310 compare the morphological features of different DEG-ENaC genotypes and treatments
311 (crowded, isolated; Fig. S9), we used discriminant analysis as a supervised
312 classification method to combine all the morphological phenotypes analyzed (Fig. 4A).
313 In addition to the data based on morphological similarities between groups, we
314 superimposed the information obtained from behavioral harsh touch experiments for
315 DEG/ENaC mutants (from Fig. S8) on the morphological clustering. We utilized a binary
316 like distribution of the worms in terms of the response to harsh touch (<45% for isolated
317 versus >65% for crowded, as shown in Fig. S8*)*. We found no correlation between the
318 morphology of the PVD and the response to harsh touch when testing the different
319 combinations of genotypes and treatments. In particular, isolated *mec-10;degt-1*
320 mutants show crowded-like morphology with isolated-like behavioral response (Fig. 4A
321 and Fig. S8, S9). These findings suggest that these two proteins are required for the
322 isolation-induced morphological modification of the PVD and represent an example in
323 which the response to harsh touch is independent of the structural alteration of the PVD.
324 Since MEC-10 is important for laminar shear stress [41] and touch [21] we suggest that this
325 channel and its possible partners from the DEG/ENaC family, such as DEGT-1, ASIC-1
326 and DEL-1 [25, 43, 44], affect the structure and the function of the PVD independently, in an

327 experience-dependent manner and contrary to the original hypothesis by Hall and
328 Treinin [39].

329 Additional line of evidence supporting the independence was demonstrated for
330 isolation of young adult worms for 24 h (Fig. 3F). This isolation affects the structure of
331 the PVD (Fig. 3G-K) but has no effect on the response to harsh touch (Fig. 1H). Finally,
332 to directly demonstrate that these two features are independent, we analyzed harsh
333 touch responses of individual animals and then assayed each individual animal for its
334 PVD morphology. We compared the dendritic morphology of individually isolated worms
335 that either responded to harsh touch or didn't. We found that the three morphological
336 parameters were unchanged (Fig. 4B-F). Thus, analysis at the level of individual worms
337 failed to demonstrate a correlation between the morphology and the response to harsh
338 touch. In summary, the morphological and behavioral phenotypes were independently
339 affected by experience via degenerins. We cannot exclude the possibility that other
340 functions of the PVD, like the response to low temperatures [21] and proprioception [22] are
341 more tightly associated to the structure of the PVD.

342 **MEC-10 localization is experience-dependent**

343 Since MEC-10 and DEGT-1 tend to co-localize within the PVD [21] we analyzed the
344 genetic interaction between these two proteins, during mechanosensory experience.
345 Differential localization of degenerins can affect both the behavioral response to harsh
346 touch and also the structural properties of the neuron. We hypothesized that changes in
347 the localization patterns of DEG/ENaC can account for plasticity at both the behavioral
348 (Fig. 1) and the structural level (Figs. 2, 3). MEC-10 localization in the plasma
349 membrane and in intracellular vesicular compartments of the axon and the quaternary

350 branches was reduced after isolation (Fig. 5A-D, Movies S1 and S2). We also tested the
351 localization pattern of the DEGT-1 [21] in the PVD. In contrast to MEC-10 (Figs. 5A-D),
352 DEGT-1 localization is reduced only in the cell body following isolation (Fig. 5E-H).
353 Furthermore, *degt-1* mutants reduced the amount of MEC-10, and more importantly,
354 abrogated the isolation-induced reduction in MEC-10 localization at the quaternary
355 branches and the axon (Fig. 5A-D). In the reciprocal experiment, DEGT-1 localization at
356 the cell body was affected by *mec-10* mutation, as isolated worms exhibit increased
357 localization compared to WT isolated worms (Fig. 5E-H). Thus, mechanosensory
358 experience induced plasticity in the localization pattern of MEC-10 and DEGT-1. Such
359 experience-dependent plasticity is proposed to be part of the mechanism that locally
360 modulates dendritic and axonal properties, to affect both the structure and the function
361 of the PVD, respectively (Fig. 6).

Figure 5

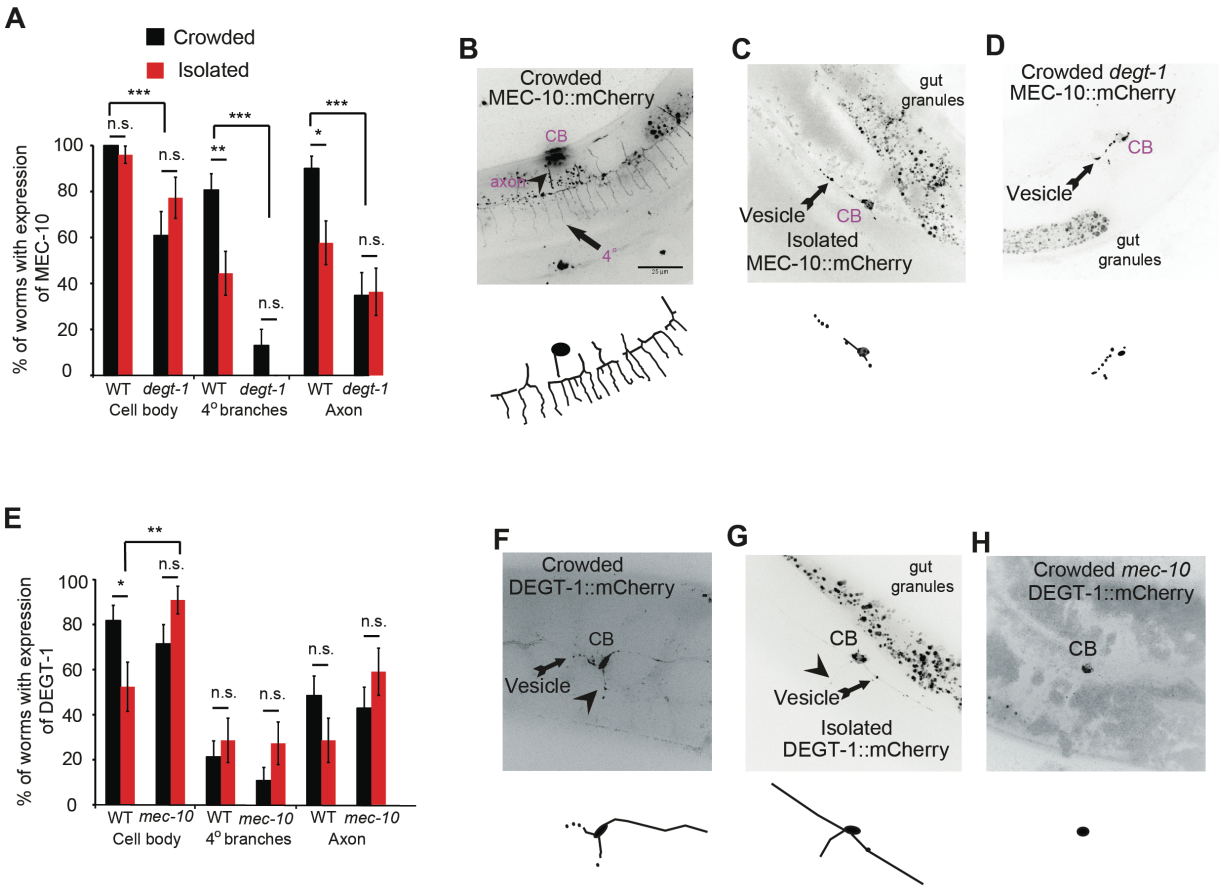
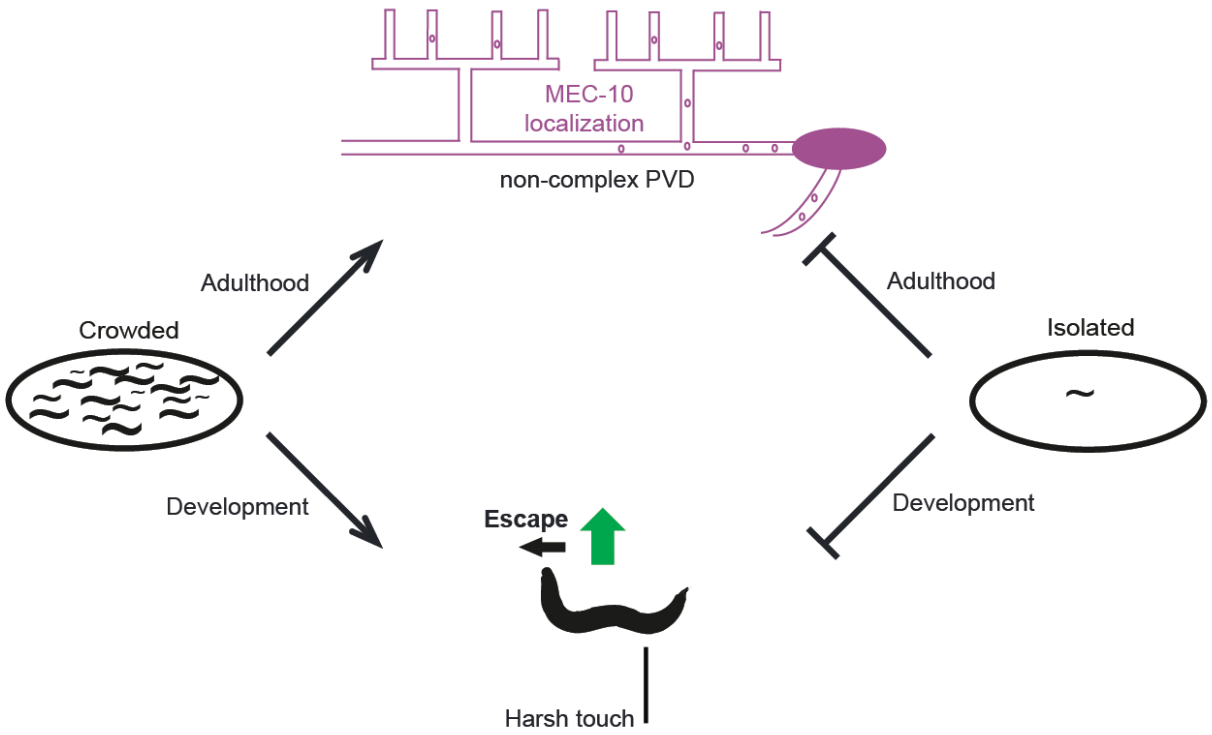


Figure 5. Localization of MEC-10 is mechanosensory- and DEGT-1-dependent. PVD promoter (*ser-2p*) drives the localization of MEC-10::mCherry and DEGT-1::mCherry. Crowded worms were compared to isolated in terms of the localization of MEC-10 and DEGT-1, at three different compartments- the cell body, quaternary branches and axon. **(A)** MEC-10::mCherry localization (shown and labeled in purple) is lower in the quaternary branches and the axon of isolated worms, in a mechanism that is DEGT-1 dependent (WT: Crowded, n=31; Isolated, n=27; *degt-1* mutants: Crowded, n=23; Isolated, n=22). For WT, the same worms as in Fig. 2C were used). **(B, C)** Representative and reconstructions for MEC-10::mCherry localization for crowded and isolated WT worms. Scale bar for panel 'B' represents 50 µm. **(D)** Representative for MEC-10:: mCherry localization for crowded *degt-1* mutants. **(E)** DEGT-1::mCherry localization level is reduced, in MEC-10 dependent-manner, at the cell body, but not in the quaternary branches or the axon of the PVD after isolation, (WT: Crowded, n=33; Isolated, n=21; *mec-10* mutants: Crowded, n=28; Isolated, n=22). **(F, G)** Representative and skeletonized images for DEGT-1::mCherry localization for crowded and isolated worms. **(H)** Representative skeletonized images for DEGT-1::mCherry localization for crowded *mec-10* mutants. The percentage expressing worms ± standard error of proportion is shown. Fisher exact test, *$p<0.05$, ** $p<0.01$, *** $p<0.01$ n.s. not significant.

Figure 6



380

381 **Figure 6. Model of experience-induced plasticity via MEC-10.** Mechanosensory
382 experience is a driving force for changes in the localization of MEC-10 in the PVD.
383 Changes in MEC-10 localization (and possibly its partners from the DEG/ENaC family)
384 can induce functional changes in the axon, during development, which in turn may
385 affect the behavioral response to harsh touch. In contrast, changes in localization of
386 MEC-10 (purple) in PVD's dendrites affect the structure of the PVD during adulthood.
387 We propose that the differential spatial and temporal effects of MEC-10 localization are
388 independent. MEC-10 localization in the cell body, axon, dendrites and vesicles is
389 represented in purple and the morphological and behavioral consequences of sensory
390 experience and MEC-10 are illustrated.

391 **Discussion**

392 From the evolutionary point of view, dendritic trees and their structural complexity
393 remain mysterious entities, despite many efforts to understand the contribution of
394 arborization complexity to dendritic physiology [45].

395 Previous research has demonstrated both cell autonomous [13, 14, 46] and cell non-
396 autonomous [15, 16] mechanisms that regulate PVD's dendritic morphogenesis during
397 development. Few studies have also focused on regeneration and aging effects on the
398 tree structure of the PVD revealing plastic mechanisms during the adult stage [17, 18],
399 similar to what has been shown for *Drosophila* sensory neurons [47]. We have uncovered
400 here that nurture, manifested as mechanosensory experience, activates
401 mechanotransduction signaling, via DEG/ENaCs amiloride-sensitive activity, to maintain
402 the structure of the dendritic tree in adults. Figure 6 shows our working model, where
403 the amount of mechanosensory stimulation, in crowded or isolated conditions, affects
404 the localization of MEC-10 in different compartments of the PVD. Localization of MEC-
405 10, probably by forming high order complexes with other DEG/ENaC, can affect the
406 structure of the PVD at the level of dendritic tree. Thus, experience induced structural
407 plasticity of the dendrites is homeostatic at the dendritic branch level. In contrast, we
408 demonstrate that the response to harsh touch is modulated by the duration and timing
409 of mechanosensory experience and by the presence of degenerins during development.
410 Behavioral plasticity is probably a synaptic property, mediated by DEG/ENaC [48] and
411 related to neurotransmission modulation, independently of the structure of the PVD
412 dendritic tree. These structural and behavioral plasticity are separated in time
413 (adulthood vs. development) and space (dendrite vs. axon).

414 Somatosensory activation in vertebrates plays a prominent role in shaping the
415 structural and functional properties of dendritic spines, mainly studied in the central
416 nervous system [49-52]. Here we suggest that degenerins mediate mechanosensation-
417 induced dendritic growth in sensory dendrites. In contrast to mammalian cortical
418 neurons, much less is known about their degree of plasticity and the molecular
419 mechanisms utilized during adulthood. We suggest that the dendritic plasticity we
420 described bears resemblance to the activity-dependent effect of glutamatergic signaling
421 and NMDA receptors. Activity via NMDA affects dendritic spines as an upstream
422 mechanism of cell signaling, resulting in structural modifications [53-55]. We propose that
423 degenerins mediate mechanosensory signaling sensation, by activating cationic
424 gradients [56], leading to activation of downstream intracellular signaling pathways [8-11],
425 which in turn enable local, actin-mediated [3, 57, 58] structural plasticity in the PVD dendritic
426 branches. In parallel, it is possible that DEG/ENaCs are also modulating
427 pre/postsynaptic homeostatic signaling in the harsh touch circuit, as has been shown in
428 neuromuscular junctions [59].
429 Thus, we demonstrated mechanosignaling functions for the combinatorial actions
430 of DEG/ENaCs that act as mediators of experience-induced structural plasticity of
431 sensory dendritic trees.
432

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585 **Author contributions** SI and BP conceived and designed the experiments. SI
586 performed the experiments. SI and BP analyzed the data and wrote the paper.

587 **Author information:**
588 **Competing interests** None
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590

591 **Materials and Methods**

592 **Strains**
593 Nematode strains were maintained according to standard protocols [60]. The list of the
594 strains is presented in Supplementary Table 1. Strains of the DEG/ENaCs family
595 obtained from the CGC (JPS282: *asic-1*(*ok415*) I; ZB2551: *mec-10*(*tm1552*) X; VC2633:
596 *degt-1*(*ok3307*) V) were crossed with BP709: IS[hmnEx133](*ser-2Prom3::kaede*). The
597 validation of F2 homozygotes for the DEG/ENaCs (including single, double and triple
598 mutants) was performed by PCR amplification of the deleted region in the genome.

599 **Primers for Multiplex PCR:**
600 ***asic-1(ok415)*** I: Forward-1: 5' aactggtgtggccacttcaactttc 3';
601 Forward-2: 5' aaggtttcagatgatcgcgtagtcaag 3'; Reverse: 5' catttctcttcttccgtcagcgc 3'.
602 ***mec-10(tm1552)* X:** Forward-1: 5' acacggctccttcttgagttccga 3'; Forward-2: 5'
603 attcggtttcctcctcttcttccaatgc 3' ; Reverse: 5' cgtttttttcagcgccctttcctgca 3'
604 ***degt-1(ok3307)* V:** Forward-1: **5'** cgagtagctgattatcaaaaagtcctcga 3';
605 Forward-2: 5' cggatattccagcattggcgaa 3'; Reverse: 5' ttccccgttgatcttctatgtattaca 3'.

606 **Spinning disk confocal microscopy**
607 Prior to imaging, the worms were placed on an agar pad (10%), mounted on top of a
608 microscope glass slide, with 1µl of polystyrene beads (100 nm diameter, from
609 *Polysciences*, Inc.) for their mechanical immobilization, followed by sealing with a
610 coverslip for complete physical immobilization [61] and taken to imaging:
611 The PVD neuron was visualized by spinning disk confocal microscope, CSU-X, with
612 Nikon eclipse Ti system and iXon3 camera (ANDOR).
613 Sequential z-series stacks (0.35 µm) from each worm were taken, with Plan Fluor 40X
614 (NA 1.3). The cell body of the PVD was positioned in the middle of the field and the
615 anterior and posterior branches were revealed. Images were captured with MetaMorph,
616 version 7.8.1.0. Figures were prepared with Adobe Illustrator CS version 11.0.0.

617 **Data analysis**
618 The analysis of the PVD structure was performed for the ~200 µm surrounding the cell
619 body. The pictures in TIFF format were analysed with ImageJ, version 1.48 (NIH). The
620 images were converted to their negative form using the "convert lookup table (LUT)"
621 function in ImageJ.

622 Maximal intensity projection was used for each PVD image.
623 Ectopic/excess branching defined as described previously [45]. Briefly, ectopic branches
624 defined as those branches that are not part of a wildtype "ideal" candelabrum (menorah)
625 of a late L4 or young adult and those that sprouted at an incorrect position (non-
626 quaternary terminal ends), as illustrated with dashed lines for ectopic branching in Fig.
627 2A. The total number of ectopic branching was calculated and presented as a fraction,
628 out of non-ectopic branches.
629 The geometry of each quaternary branch ("candle") was defined in the following
630 manner: Straight geometry- all the pixels that constitute the branch are positioned on a
631 straight line generated with ImageJ. The width of the line (1 pixel) was constant for the
632 entire sets of experiments. The number of straight quaternary branches were divided by
633 the total quaternary branches for each worm and presented as percentage. The
634 analysis was done only for worms which did not move through the Z stack. Moving
635 worms were excluded from the experiment.

636 Self-avoidance defects- the number of events where two adjacent candelabra
637 overlapped (no gap formation), was divided by the total number of gaps between the
638 candelabra within the frame (Fig. 2A). The self-avoidance values are presented as
639 percentage.

640 **Behavioral procedures**

641 **Harsh touch assay**
642 After 72 h, adult worms (both isolated and those from the crowded plate as described
643 below) were transferred using an eyelash separately to a new agar plate (each worm
644 was transferred to a different plate) with 150 µl of fresh OP50 (about 16 h after
645 seeding), in order to avoid accumulation of OP50 on the edge of the bacterial lawn,
646 which might interfere with harsh touch response measurement. After ~45 minutes in the
647 plate, the worms were prodded with a platinum wire posterior to the vulva, above the
648 interface between worm's body and the agar plate [31]. The number of responses to harsh
649 touch was counted every 10 sec. The non-responding worms were defined after two
650 harsh touch events that were observed sequentially without response. More than one
651 response was considered as responsive animal. The percentage of responding worms
652 was calculated for each genotype and treatment. The experimenter was blind to both
653 the genotype and the treatment- crowded or isolated.

654 **Isolation of embryos**
655 The worm isolation procedure was based on previous work [23] with few modifications, as
656 indicated. Isolated animals were grown on 5 cm agar plate with 150 µl of OP50 *E. coli*,
657 while crowded plate worms were grown on 600-700 µl. Adding sufficient amount of
658 OP50, to avoid starvation of the worms in the crowded plate is important. The embryos

659 and adult worms were isolated with platinum wire. The plates were sealed with one
660 layer of Parafilm M and placed into a plastic box, at 20 ºC, for the entire experiment.
661 Three experimental groups were used for the 72 h (96 h of experiment was performed
662 only for *mec-4* worms, since they were L4- very young adults at 72 h) isolation
663 experiment: **(1)** Single isolated embryos. **(2)** Crowded worms- the progeny of 30
664 young, non-starved, adults (approximately, 7,000-9,000 worms in different
665 developmental stages, without approaching starvation). **(3)** Crowded adult worms that
666 were isolated for certain amount of time as adults.
667 After 48/72/96 h (according to the experiment), age matched worms from each group
668 were transferred to 10% agar pad slides for imaging, as described [23].

669 **Isolation of adults**

670 **Isolation for 2 h:** Crowded plate - the progeny of 30 adults per plate was used. After
671 ~70 h in the crowded plate the worms were isolated using an eyelash into a new plate,
672 with 150 µl of OP50, for 2 h, followed by imaging of the PVD.

673 **Isolation for 5 h:** Crowded plate - the progeny of 30 adults per plate was used. After
674 ~67 h in the crowded plate the worms were isolated using an eyelash into a new plate,
675 with 150 µl of OP50, for 5 h, followed by imaging of the PVD.

676 **Isolation for 24 h:** Crowded plate- the progeny of 30 adults per plate was used. After
677 ~48 h in the crowded plate the worms (young adults) were isolated using an eyelash
678 into new plates, with 150 µl of OP50, for 24 h, followed by imaging of the PVD or
679 measurement of harsh touch response. The age of the worms was similar to the worms
680 in experiments of embryo isolation for 72 h experiments (Fig. 1B). The isolated worms
681 were compared to age matched worms from the crowded plate (their original plate).

682 **Optogenetic stimulation**

683 Crowded and isolated worms (ZX819: *lite-1(ce314) X;*
684 *xIs12[pF49H12.4::ChR2::mCherry; pF49H12.4::GFP*]) were grown on OP50 with 100
685 µM of All Trans Retinal, in order to have functional channel Rhodopsin [24]. After 72 h the
686 worms were singly mounted with eyelash on a chunk (1cm X 1cm) of agar that was
687 mounted on a microscope glass slide. The agar contained fresh but dry OP50, with 100
688 µM All Trans Retinal. About 30 minutes following the transfer the worms were tested for
689 the response to light. Worms were stimulated with the spinning disk confocal
690 microscope, CSU-X, with Nikon eclipse Ti system and iXon3 camera (ANDOR), at 488
691 nm wavelength, with laser intensity of 40% and exposure time of 100 ms, with 10X Plan
692 Fluor (NA 0.3) for ~1 second and the forward acceleration response was measured. The
693 experimenter determined the presence or the absence of forward acceleration response
694 to light activation.

695 **Isolation with chemical stimulation**

696 The *glp-4(bn2)* mutants which are sterile at 25 °C were used (~40 worms for each
697 plate), in order to prepare conditioned/chemically stimulated plates [42] prior to isolation of
698 the ser-2Prom3::Kaede (BP709) strain. The *glp-4* mutants were transferred at early
699 larval stage (L1, L2) to a new agar plate for 96 h at 25 °C. After the removal of the *glp-4*
700 mutants, the embryo isolation procedure was used, as described before at 20 °C.

701 **Isolation with glass beads**

702 Single embryos were isolated to agar plates with 150 µl OP50 and 2.5 g of glass beads
703 (1mm diameter) placed on the 150 µl OP50 lawn in the middle of the plate. The worms
704 were isolated for 72 h and tested for response to harsh touch as described above.

705 **Pharmacology**

706 Amiloride hydrochloride hydrate (Sigma, #A7410) 1M stock solution in DMSO was
707 stored at -20°C. A final concentration of 3 mM amiloride in 0.03 % DMSO was prepared
708 in OP50 bacteria and seeded on NGM plates. 650 µl OP50 were seeded on each plate.
709 As a control, 0.03 % DMSO was added to OP50 bacteria. For each plate (control 0.03%
710 DMSO or 3 mM amiloride) 30 non-starved adult worms were added. After 72 h at 20 °C
711 the progeny of the 30 adults were tested as young adults for their PVD morphology and
712 their response to posterior harsh touch, as described in the previous sections.

713 **Analysis of DEG/ENaCs localization in the PVD**

714 Two DEG/ENaCs proteins, PF49H12.4::MEC-10::mCherry and PF49H12.4::DEGT-
715 1::mCherry (the plasmids were provided by W. Schafer's lab, [21] were analysed for their
716 localization in the PVD, by comparing crowded to isolated worms, in a similar behavioral
717 assay as described in Fig. 1b. The presence of the co-injection marker Punc-122::gfp
718 was a prerequisite for analysis. For each individual worm a stack of images was
719 examined and maximal intensity projection was performed. The images were encoded
720 so the analysis was performed in a blind manner. The cell body of the PVD localized at
721 the center of the region of interest with 60X Apochromat (NA 1.40) and the presence or
722 absence of fluorescent signal was examined in three compartments: The cell body, the
723 quaternary branches and the axon of the PVD. The results are shown as percentage of
724 worms that localize the protein at any compartment.

725 **Rescue strain**

726 Worms from BP1022 (*mec-10(tm1552)* X; Is[*hmn*Ex133](*ser-2Prom3::kaede*); *him-*
727 *5(e1490)* V) were injected into the gonad with a rescuing plasmid for MEC-10, with a
728 PVD specific promoter (pWRS825: *ser-2Prom3::mec-10* genomic) kindly provided by W.
729 Schafer's lab [21]. The injection mix contained *myo-2::gfp* (20 ng/µl) as a co-
730 transformation marker and pWRS825 (80 ng/µl). For both behavioral and structural
731 characterization the *him-5;mec-10* strains, with and without rescuing plasmid, shared
732 the same crowded plate and were differentiated by the presence or absence of the co-
733 injection marker *myo-2::gfp*.

734 **Statistics**

735 For the morphological characterization of the PVD the results are expressed as means
736 (blue circle) ± standard error of the mean (s.e.m.). In the boxplot (first, third quartiles)
737 the upper whisker extends from the hinge to the highest value that is within 1.5 * IQR
738 (inter-quartile range), the distance between the first and third quartiles. The lower
739 whisker extends from the hinge to the lowest value within 1.5 * IQR of the hinge.
740 The statistical analyses were performed with SPSS software (IBM, version 20) and "R
741 package". Two-tailed tests were performed for the entire data sets.
742 Since for many experiments the distribution of the data was not normal, a-parametric
743 tests were used: Mann Whitney test for comparison between independent groups.
744 Kruskal-Wallis test was used for multiple comparisons for more than two groups.
745 For proportions (percentage worms) ± standard error of proportion was calculated.
746 Fisher's exact test was used for analysis of differences in proportions. To estimate the
747 variability in proportion we calculated the Standard Error of Proportion: $\sqrt{\frac{(1-p)\cdot p}{n}}$
748 The dot plot figures were prepared with "R package", the bar charts with Excel software.

749 **Discriminant analysis**

750 Eight different strains (WT and seven DEG/ENaCs), with two treatments (crowded,
751 isolated worms) for each strain were analyzed for Linear discriminant analysis for
752 morphological characteristics, to evaluate similarity between different strains and
753 treatments. Each worm in the data set was characterized by the three morphological
754 characteristics (the fraction of ectopic branching, the percentage straight quaternary
755 branches and the percentage self-avoidance defects). The centroid for morphological
756 characterization was calculated for each condition and represented in square. Data from
757 independent harsh touch experiments are shown for each group. The analysis was
758 performed using SPSS 20.

759

760 **Supplementary information**

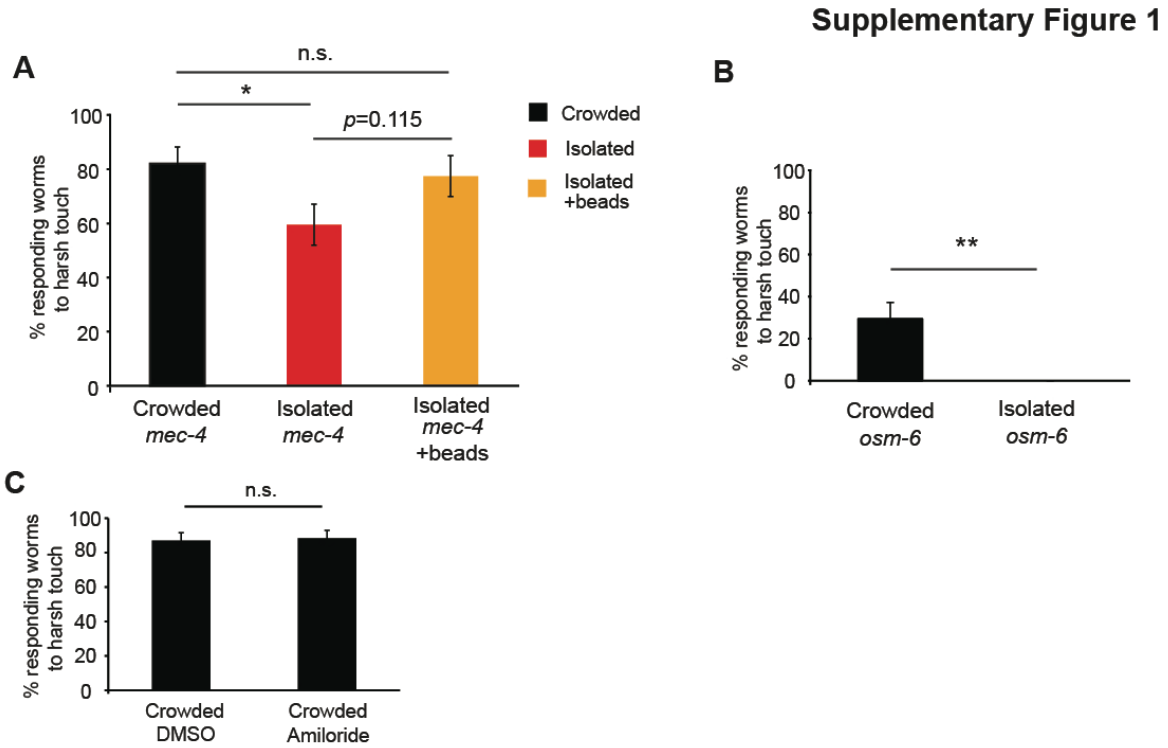
761 Table S1
762 Figures S1-S9
763 Movies S1 and S2

764 **Table S1: List of strains and transgenes used in this work**

Strain	Genotype	Details
N2	Wild-type	[60]
JPS282	*asic-1(ok415)* I; *vxEx282*	From Caenorhabditis Genetics Center (CGC)
SS104	*glp-4*(*bn2*) *I*	CGC [62]
CB1338	*mec-3(e1338) IV*	CGC [31]
MT1085	*unc-8(n491) IV*	CGC [36]
VC2633	*degt-1(ok3307) V*	CGC [21]
DR466	*him-5(e1490) V*	CGC, *him-5* was used as WT background for several strains after cross
NC279	*del-1(ok150) X*	CGC [21]
CB1611	*mec-4(e1611) X*	CGC [63]
ZB2551	*mec-10(tm1552) X*	CGC [21]
JPS478	*asic-1(ok415) I*; *mec-10(tm1552) X*; *vxEx478*	CGC
AQ3272	*ljEx637[PF49H12.4::DEGT-1::mCherry; Punc-122::gfp]*	Kindly provided by W. Schafer [21]
AQ3273	*ljEx638[PF49H12.4::MEC-10::mCherry; Punc-122::gfp]*	Provided by W. Schafer [21]
ZX819	*lite-1(ce314) X*; *zxIs12*[*pF49H12.4::ChR2::mCherry; pF49H12.4::GFP*]	Kindly provided by A. Gottschalk [24]
BP709	*Is*[*hmnEx133*](*ser-2Prom3::kaede*)	Kindly provided by C. Yip and M. Heiman [64]
BP925	*mec-4(e1611) X; Is*[*hmnEx133](ser-2Prom3::kaede*)	Cross [17]
BP1021	*him-5(e1490) V*; *Is*[*hmnEx133*](*ser-2Prom3::kaede*)	Cross: BP709 X DR466
BP1022	*mec-10(tm1552) X*; Is[*hmnEx133*](*ser2Prom3::kaede)*; *him-5(e1490) V*	Cross: BP1021 X JPS478
BP1023	*asic-1(ok415) I*; *him-5(e1490) V; Is[hmnEx133](ser-2Prom3::kaede)*	Cross: BP1021 X JPS478
BP1024	*asic-1(ok415) I; mec-10(tm1552) X*; *him-5(e1490) V Is*[*hmnEx133*](*ser-2Prom3::kaede)*	Cross: BP1021 X JPS478
BP1025	*asic-1(ok415) I; degt-1 (ok3307) V*; *mec-10(tm1552) X*	Cross: BP1024 X VC2633
BP1026	*degt-1(ok3307) V; mec-10(tm1552) X*	Cross: BP1024 X VC2633
BP1027	*degt-1(ok3307)* V; *Is*[*hmnEx133*](*ser-2Prom3::kaede)*	Cross: BP1021 X VC2633
BP1028	*asic-1(ok415) I; degt-1(ok3307) V; Is*[*hmnEx133*](*ser-2Prom3::kaede)*	Cross: BP1025 X BP1027
BP1029	*mec-10(tm1552); degt-1(ok3307) V; Is*[*hmnEx133*](*ser-2Prom3::kaede)*	Cross: BP1025 X BP1027
BP1030	*asic-1(ok415) I; mec-10(tm1552) X; degt-1* (*ok3307) V*; *Is*[*hmnEx133*](*ser-2Prom3::kaede)*	Cross: BP1025 X BP1027
BP1031	*degt-1(ok3307) V*; *ljEx638[PF49H12.4::MEC-10::mCherry; Punc-122::gfp*]	Cross: VC2633 X AQ3273
BP1033	*mec-10(tm1552) X*; ljEx637[*PF49H12.4::DEGT-1::mCherry; Punc-122::gfp*]	Cross: BP1022 X AQ3272
BP1034	*mec-10(tm1552) X*; *IS[hmnEx133](ser-2Prom3::kaede); him-5(e1490) V; pWRS825 Ex[ser-2Prom3::MEC-10genomic*]	pWRS825 plasmid provided by W. Schafer [21] was injected into BP1022

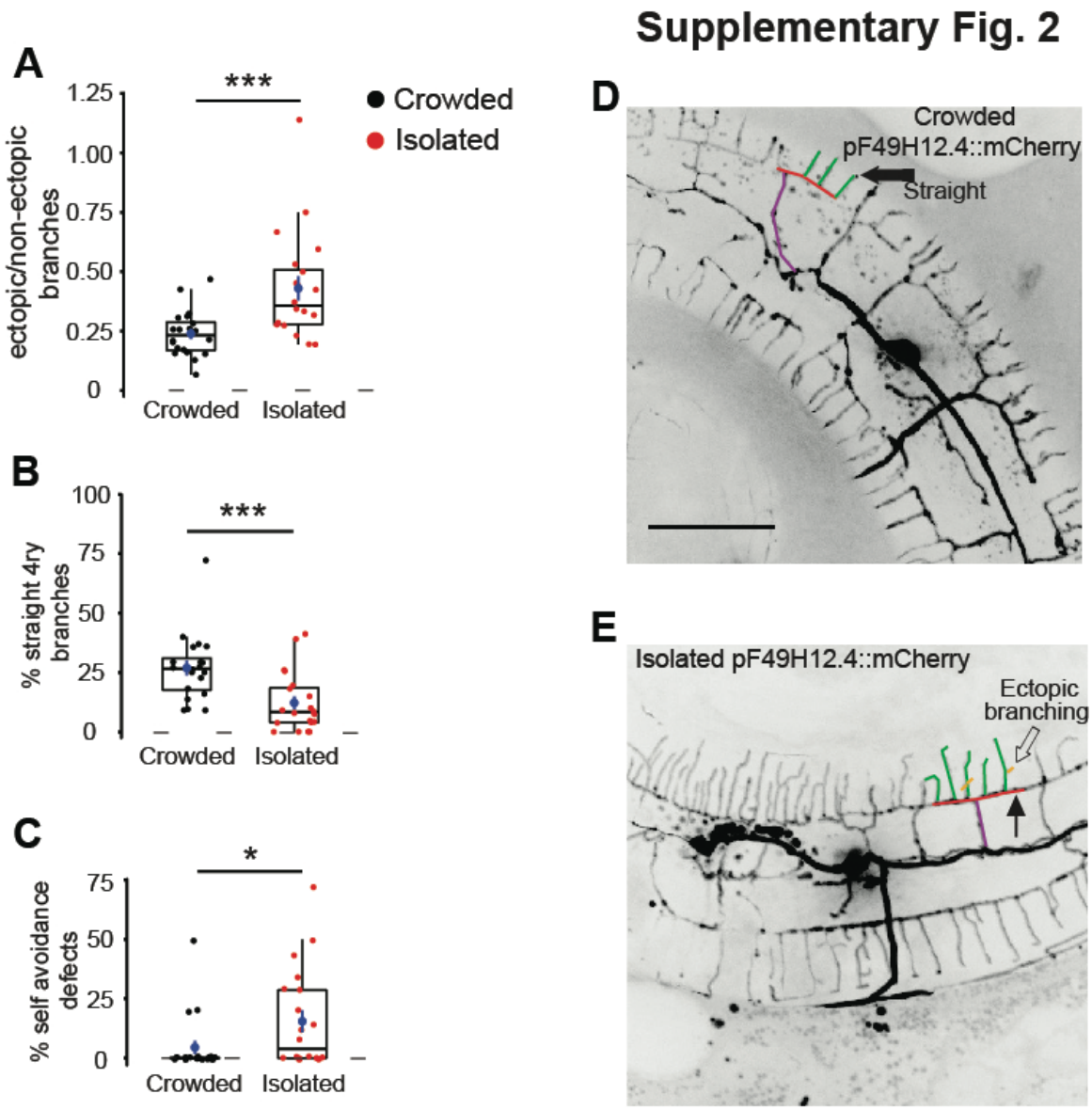
765

766 **Supplementary figures**



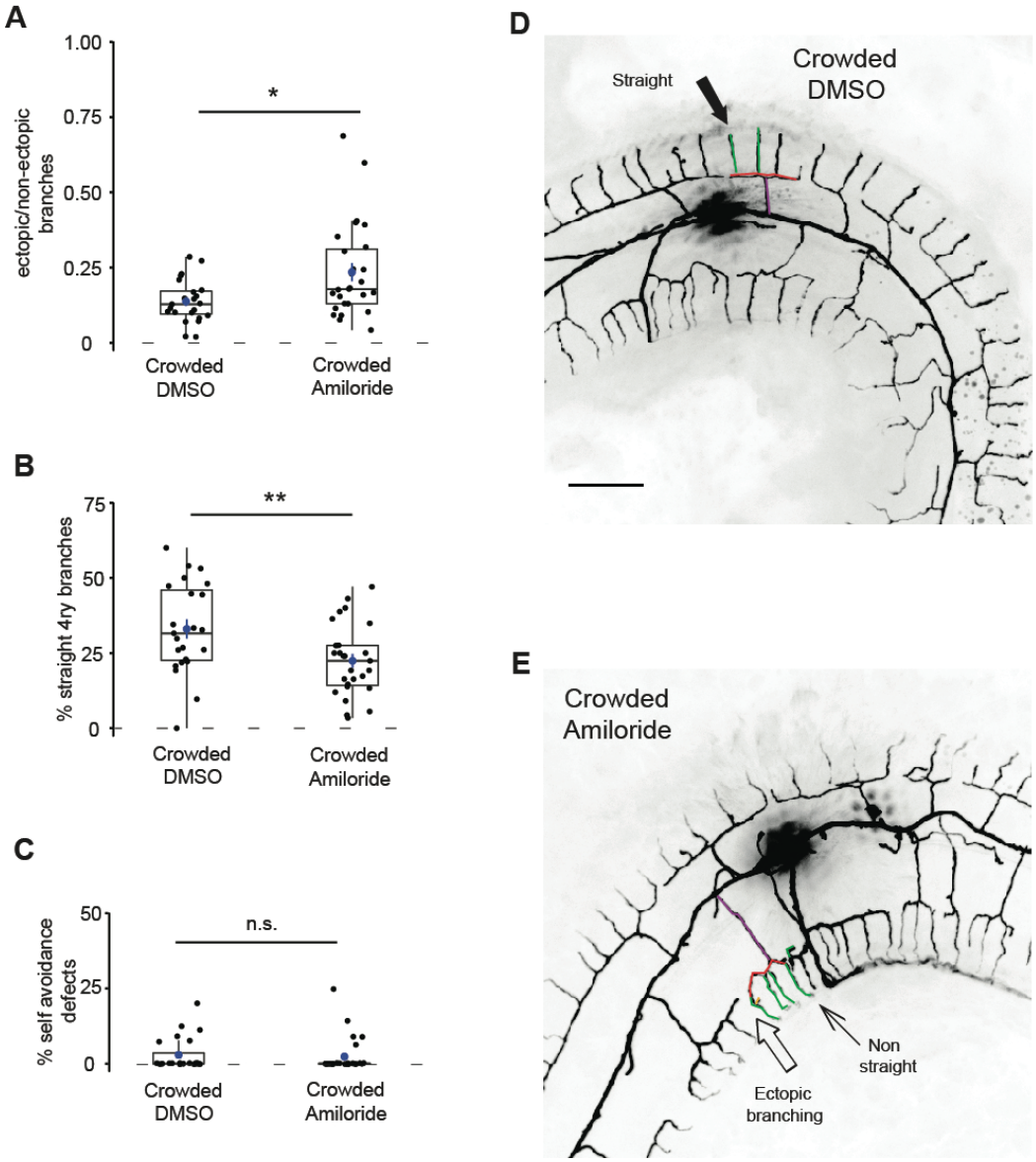
767

768 **Supplementary Figure 1: The reduction in response to harsh touch following**
769 **isolation is PVD-dependent and chemosensory-independent. (A)** Isolation of gentle
770 touch insensitive *mec-4* worms with glass beads resulted in non-significant increase in
771 the percentage of responding worms, compared to isolated without beads and non-
772 significant difference compared to crowded worms (This experiment was performed at
773 96 h since *mec-4* animals develop slower and the worms were L4- young adults at 72 h.
774 Crowded, n=39; Isolated, n=42; Isolated egg with beads, n=31). **(B)** Isolation of *osm-6*
775 mutants (impaired in their ciliated neurons, including the PDE which mediates response
776 to harsh touch) resulted in reduced response to harsh touch, when compared to worms
777 from a crowded plate. (Crowded, n=34; Isolated, n=27). **(C)** Continuous exposure to
778 amiloride does not affect the response to harsh touch in the crowded conditions
779 (Crowded DMSO, n=54; Crowded 3 mM amiloride, n=52). The proportion of responding
780 worms (percentage) ± the standard error of proportion is shown. Fisher exact test, *
781 $p<0.05$, ** $p<0.01$, n.s. not significant.

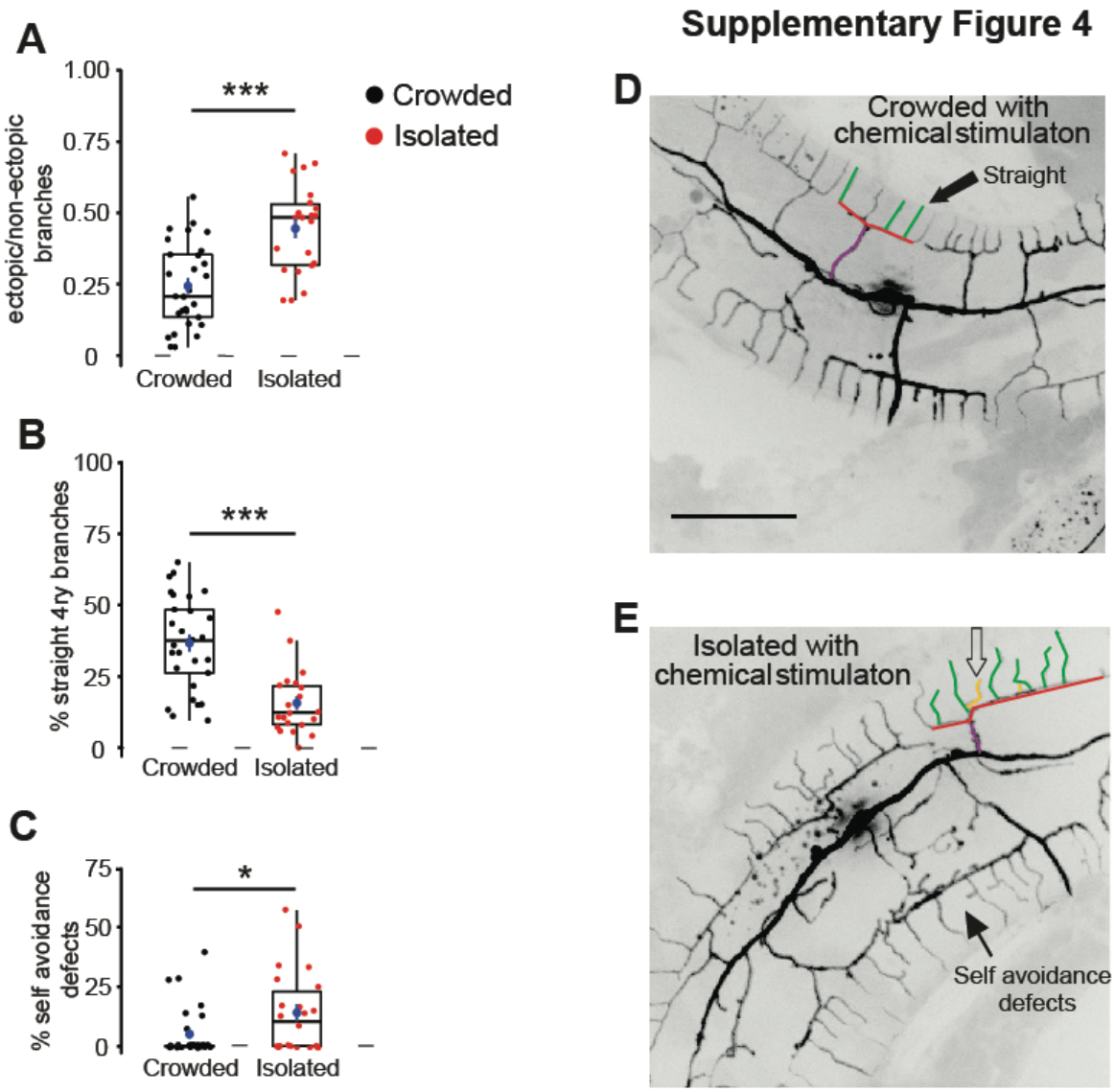


Supplementary Figure 2: The effect of isolation on the structure of the PVD is independent from the identity of the promoter driving expression of the reporter in the PVD. Worms from a different strain and fluorescent marker, F49H12.4::mCherry, were isolated as eggs for 72 h and compared to crowded worms from the same strain. **(A)** Egg isolation for 72 h increased total ectopic branching, **(B)** reduced the percentage of straight quaternary branches and **(C)** increased the percentage of self-avoidance defects between two adjacent candelabra (Crowded, n=20; Isolated, n=20). **(D, E)** Representative pictures from the PVD for crowded worms and isolated eggs. Scale bar for panel 'D' represents 50 µm. The mean ± s.e.m. are shown in blue. Box plot with hinge for the first and third quartile. The median is represented by the thick horizontal black line and the whiskers are estimated calculation of the confidence interval 95%. Mann Whitney test, * $p<0.05$, *** $p<0.001$.

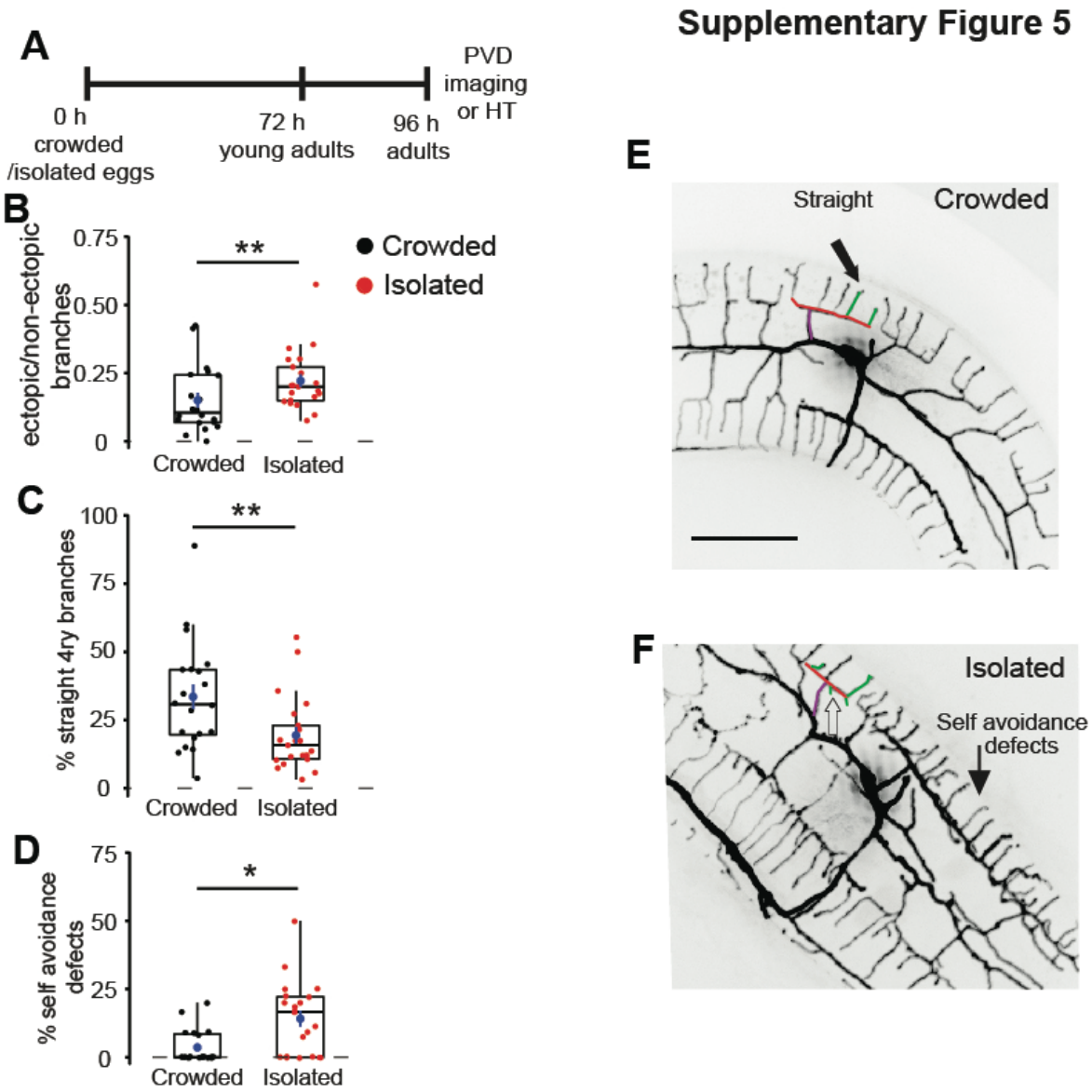
Supplementary Figure 3



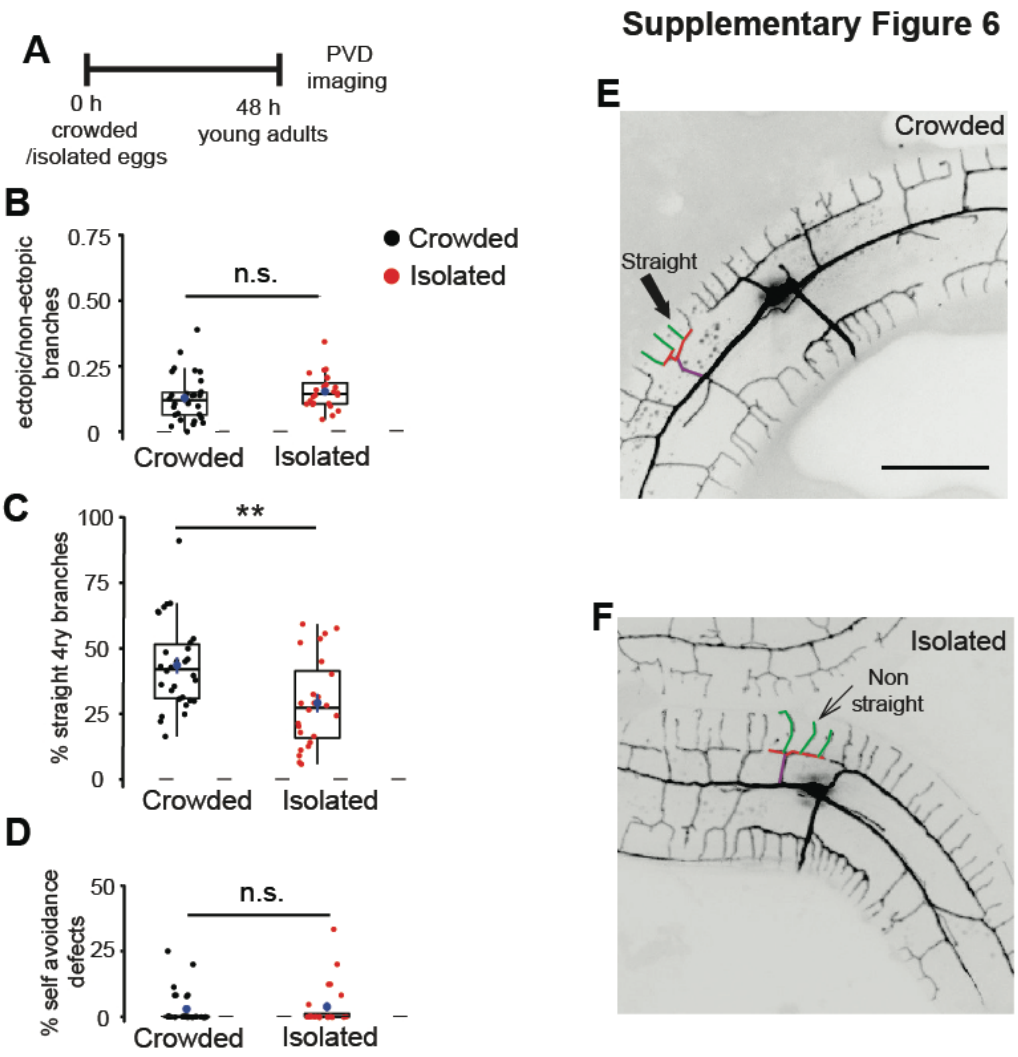
Supplementary Figure 3: Blocking DEG/ENaC with amiloride affects the structure of the PVD. Crowded worms that were exposed to 3 mM amiloride **(A)** increased the fraction of ectopic branches, **(B)** decreased the percentage of straight quaternary branches, **(C)** without affecting the percentage of self-avoidance defects (Crowded DMSO, n=23; Crowded 3 mM amiloride, n=27). **(D, E)** Representative images from the PVD for DMSO and amiloride treated worms. Scale bar for panel 'D' represents 50 µm. The mean ± s.e.m. are shown in blue. Box plot with hinge for the first and third quartile. The median is represented by the thick horizontal black line and the whiskers are estimated calculation of the confidence interval 95%. Mann Whitney test *$p<0.05$, ** $p<0.01$, n.s. not significant.



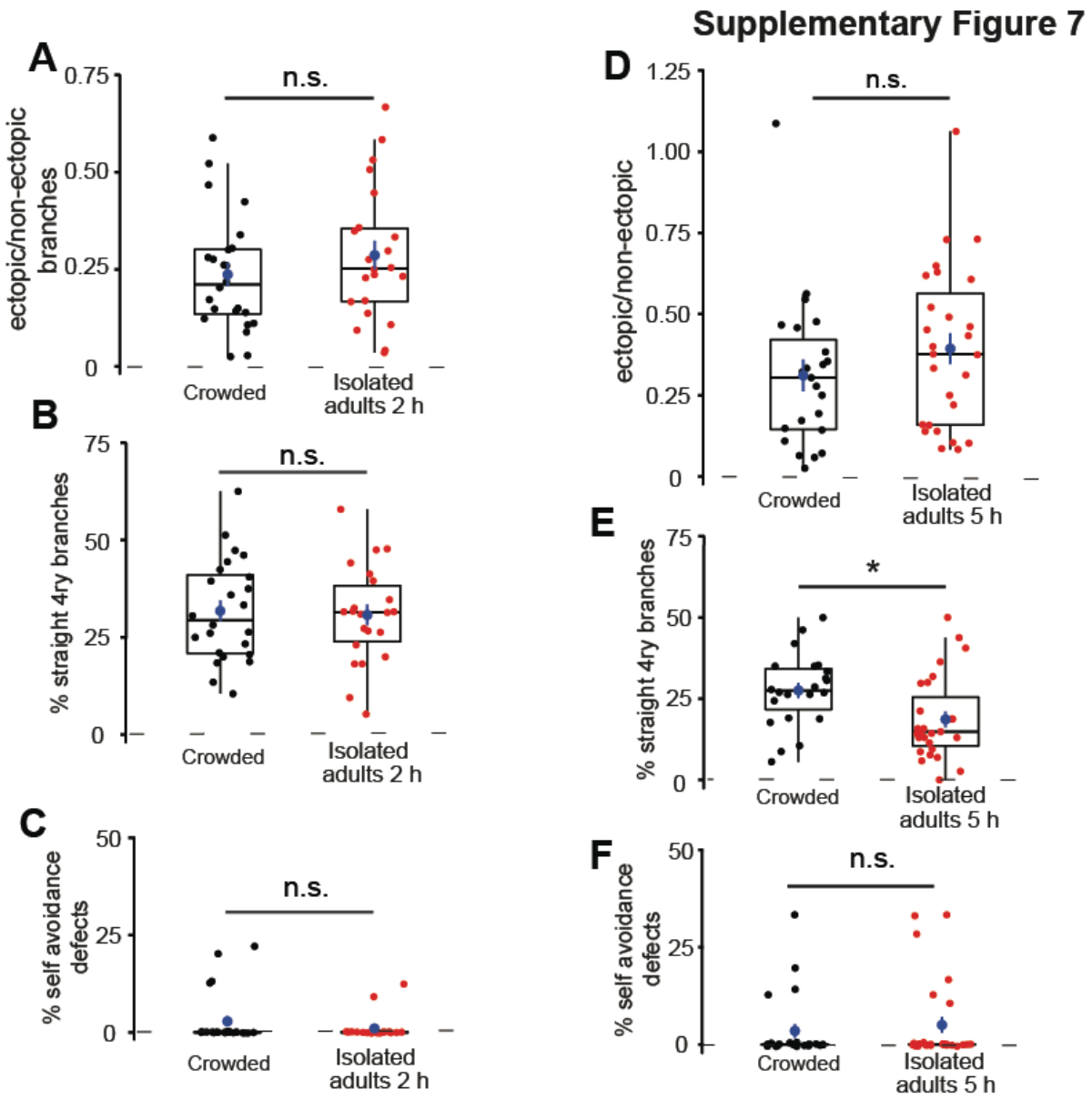
Supplementary Figure 4: The PVD undergoes isolation-dependent structural plasticity in the presence of pheromonal signals in the plate. Both crowded and isolated worms were pre-exposed to pheromonal signals, released by *glp-4* worms (~40 worms for 96 h. The *glp-4* worms were taken out of the plate and the isolation protocol described in Fig. 1b was used). **(A)** Chemical stimulation of plates with *glp-4*, followed by egg isolation, increased the fraction of ectopic branching, **(B)** decreased straight quaternary branches and **(C)** increased the percentage of self-avoidance defects (Crowded, n=24; Isolated, n=20). **(D, E)** Representative pictures from the PVD for crowded and isolated worms with chemical stimulation of the plates. Scale bar for panel 'D' represents 50 µm. The mean ± s.e.m. are shown in blue. Box plot with hinge for the first and third quartile. The median is represented by the thick horizontal black line and the whiskers are estimated calculation of the confidence interval 95%. Mann Whitney test, * $p<0.05$, *** $p<0.001$.



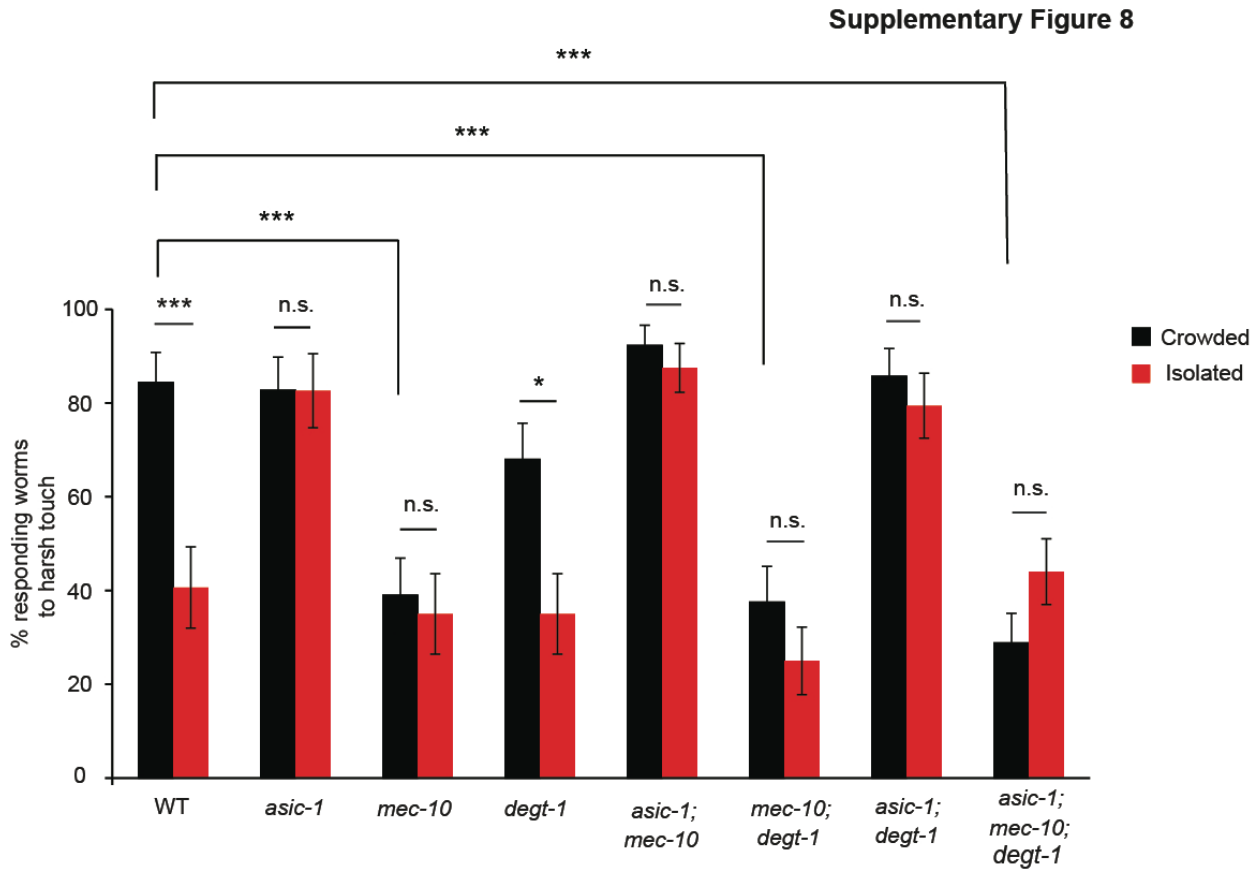
Supplementary Figure 5: The effect of isolation on the structure of PVD is not mediated by the gentle touch mechano-sensory neurons. (A) Description of the protocol for the isolation of *mec-4* eggs for 96 h (the experiment was performed for 96 h since the worms were L4s or very young adults at 72 h), followed by PVD imaging. **(B)** Isolation of the gentle touch impaired strain, *ser-2Prom3*::Kaede;*mec-4*, increased total ectopic branching, **(C)** reduced the percentage of straight quaternary branching and **(d)** increased the percentage of self-avoidance defects after isolation of eggs. Crowded, n=20; Isolated, n=21. **(E, F)** Representative pictures from the PVD for crowded worms and isolated eggs. Scale bar panel on 'E' represents 50 µm. The mean ± s.e.m. are shown in blue. Box plot with hinge for the first and third quartile. The median is represented by the thick horizontal black line and the whiskers are estimated calculation of the confidence interval 95%. Mann Whitney test with Bonferroni correction α=0.0167, * $p<0.05$, ** $p<0.01$.



Supplementary Figure 6: Isolation of eggs for 48 h is sufficient to induce some changes in the structure of the PVD's quaternary branches. (A) Schematic protocol for the isolation of WT eggs for 48 h, followed by PVD imaging. **(B)** Isolation of eggs for 48 h (resulted in young adult worms) did not affect the number of ectopic branches, it **(C)** decreased the percentage of straight quaternary branching and **(D)** it did not affect the percentage of self-avoidance defects (Crowded, n=30; Isolated, n=24). **(E, F)** Representative pictures from the PVD for crowded worms and isolated eggs. Scale bar for panel 'E' represents 50 µm. The mean ± s.e.m. are shown in blue. Box plot with hinge for the first and third quartile. The median is represented by the thick horizontal black line and the whiskers are estimated calculation of the confidence interval 95%. Mann Whitney test, ** $p<0.01$, n.s. not significant.

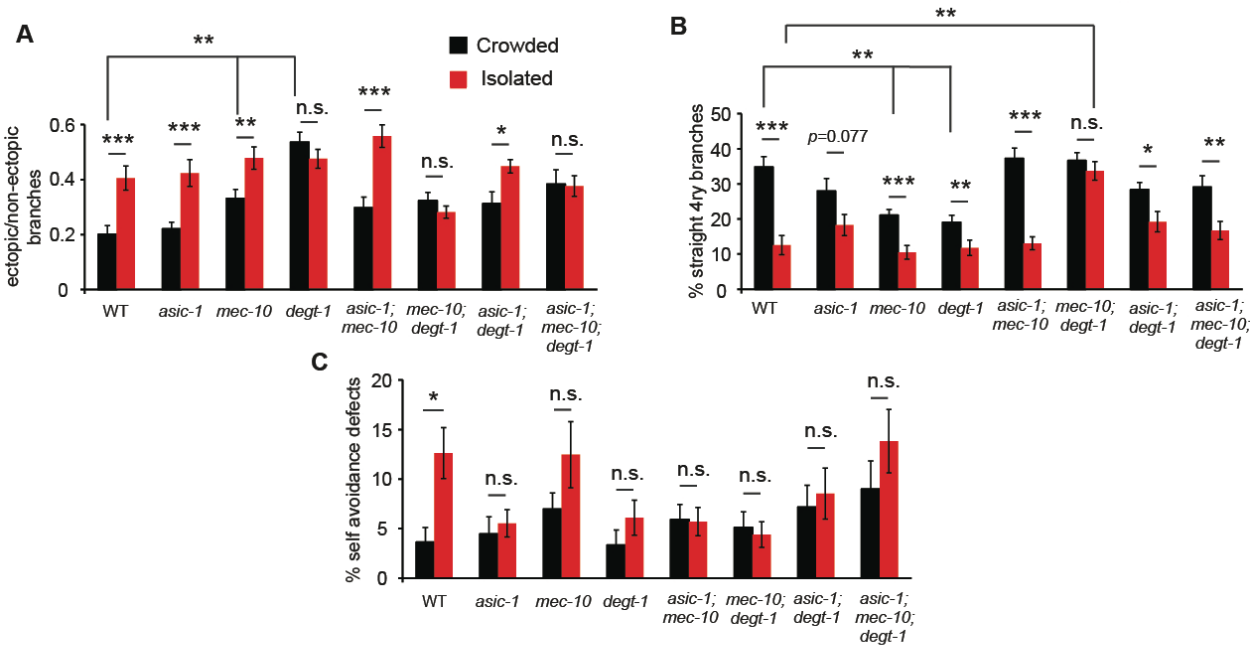


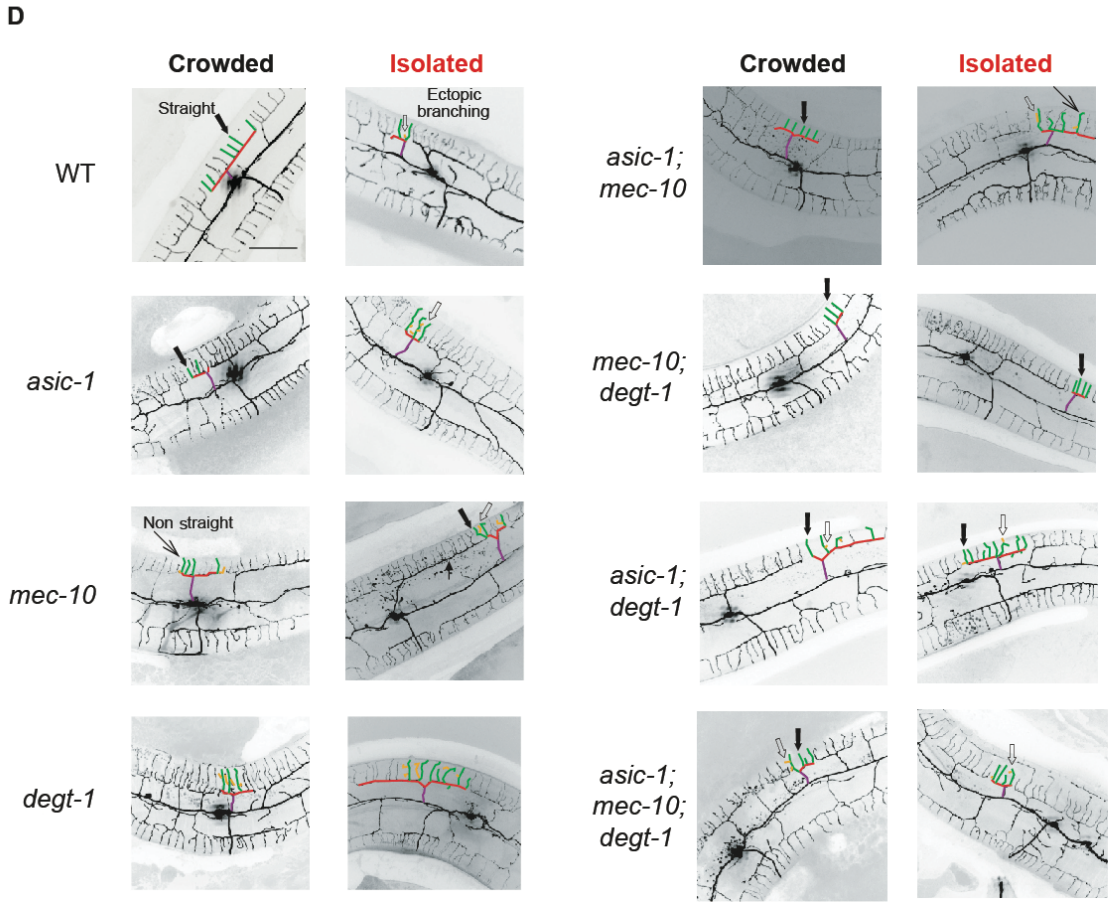
Supplementary Figure 7: Isolation of young adult worms for 5 h, but not 2 h, is sufficient to induce changes in the arborization pattern of the PVD. (A-C) Isolation of adult worms for 2 h was not sufficient to induce morphological changes on the PVD (Crowded, n=24; Isolated adults for 2 h, n=22)**. (D)** Isolation of adult worms for 5 h did not affect total ectopic branching, **(E)** it reduced the percentage of straight quaternary branching and **(F)** it did not affect the percentage of self-avoidance defects. (Crowded, n=24; Isolated adults for 5 h, n=27). The mean ± s.e.m. are shown in blue. Box plot with hinge for the first and third quartile. The median is represented by the thick horizontal black line and the whiskers are estimated calculation of the confidence interval 95%. Mann Whitney test, * $p<0.05$, n.s. not significant.



Supplementary Figure 8: The DEG/ENaC *asic-1* and *mec-1*0 mediate experience dependent behavioral plasticity following isolation. Posterior assay was performed for crowded and isolated WT and DEG-ENaC mutants, similar to the procedure described in Fig. 1b. Seven combinations of DEG-ENaC mutants were tested for the isolation effect on response. Isolated worms were compared to crowded worms from the same strain. N2 worms (The same set of worms as in Fig. 1C. Crowded, n=32; Isolated, n=32), *asic-1* (Crowded, n=46; Isolated, n=30), *mec-10* (Crowded, n=38; Isolated, n=31), *degt-1* (Crowded, n=37; Isolated, n=31), *asic-1;mec-10* (Crowded, n=20; Isolated, n=22), *mec-10*;*degt-1* (Crowded, n=40; Isolated, n=36), *asic-1*;*degt-1* (Crowded, n=35; Isolated, n=34), *asic-1*;*mec-10;degt-1* (Crowded, n=52; Isolated, n=50). The proportion (percentage) of responding worms ± the standard error of proportion. Fisher exact test, *p<0.05, *** p<0.001, n.s. not significant.

Supplementary Figure 9





869

870

871 **Supplementary Figure 9: The DEG-ENaCs, *mec-10* and *degt-1*, mediate**
872 **mechanosensory dependent structural changes in the PVD.**

873 Mutants of DEG-ENaC were isolated and were compared to crowded animals from the
874 same strain, similar to the procedure described in Fig. 1b. **(A)** Three DEG-ENaC
875 mutants: *degt-1, mec-10;degt-1*, and *asic-1*;*mec-10;degt-1* show no difference between
876 crowded and isolated worms in terms of ectopic branching. In addition, crowded *mec-10*
877 *and degt-1* animals increased the number of ectopic branches compared to crowded
878 WT worms while isolated *mec-10;degt-1* decreased it. **(B)** *mec-10;degt-1* mutants failed
879 to decrease the percentage of straight quaternary branches, while *mec-10* and *degt-1*
880 crowded decreased it, compared to the crowded WT animals. **(C)** The increase in self-
881 avoidance defects following isolation is DEG-ENaC dependent. WT (The same set of
882 worms as shown in Fig. 2B-D. Crowded, n=28; Isolated, n=26), *asic-1* (Crowded, n=22;
883 Isolated, n=25), *mec-10* (Crowded, n=27; Isolated, n=21. The same set of worms as
884 shown in Fig. 2B-D), *degt-1* (Crowded, n=28; Isolated, n=24), *asic-1;mec-10* (Crowded,
885 n=30; Isolated, n=31), *mec-10*;*degt-1* (Crowded, n=26; Isolated, n=28), *asic-1*;*degt-1*
886 (Crowded, n=23; Isolated, n=22), *asic-1*;*mec-10;degt-1* (Crowded, n=23; Isolated,
887 n=21). **(D)** Representative pictures from the PVD for crowded and isolated worms of WT
888 and seven different DEG-ENaC mutants. Scale bar for panel 'D' represents 50 µm. The
889 mean ± s.e.m. are shown. Mann Whitney test, *$p<0.05$, ** $p<0.01$, *** $p<0.001$, n.s. not
890 significant.

891 **Supplementary movie captions**

892 **Movie S1:** Localization pattern of MEC-10::mCherry in the PVD for crowded worms.
893 MEC-10 is localized in moving vesicles, indicated by red arrow. Six z-stacks series (~60
894 optical slices for each) were taken around the CB every 3 minutes.

895 **Movie S2:** Localization pattern of MEC-10::mCherry in the PVD for crowded worms.
896 MEC-10 is localized in moving vesicles, indicated by red arrow. Six z-stacks series (~60
897 optical slices for each) were taken around the CB every 2 minutes.