

1 **Extracellular matrix regulates morphogenesis and function of ciliated sensory**
2 **organs in *Caenorhabditis elegans***

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17 Keywords: Extracellular matrix; MEC-9; cilia; ciliopathy; extracellular vesicle; ADPKD,
18 polycystin; *C. elegans*

19
20 Running title: ECM and cilia

21 **ECM regulates morphogenesis and function of ciliated sensory organs in**

22 ***Caenorhabditis elegans***

23 **ABSTRACT**

24 Cilia and extracellular vesicles (EVs) are signaling organelles that play important
25 roles in human health and disease. In *C. elegans* and mammals, the Autosomal
26 Dominant Polycystic Kidney Disease (ADPKD) gene products polycystin-1 and
27 polycystin-2 localize to both cilia and EVs, act in the same genetic pathway, and
28 function in a sensory capacity, suggesting ancient conservation. Hence, the nematode
29 offers an excellent system in which to address central questions regarding the biology of
30 cilia, EVs, and the polycystins. We discovered an unexpected role of the *mec-1*, *mec-5*,
31 and *mec-9* genes encoding extracellular matrix (ECM) components. We determined that
32 these ECM encoding genes regulate polycystin localization and function, ciliary EV
33 release, cilia length, dendritic morphology, and neuron-glia interactions. Abnormal ECM
34 and fibrosis are observed in ciliopathies such as ADPKD, nephronophthisis, and Bardet-
35 Biedl Syndrome. Our studies reveal multifaceted roles for ECM proteins in the ciliated
36 nervous system of the worm and provide a powerful new *in vivo* model to study the
37 relationship between ECM, the polycystins, and ciliopathies.

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42 INTRODUCTION

43 Cilia are antenna-like structures that project from many eukaryotic cells (WOOD
44 AND ROSENBAUM 2015). Cilia play essential roles in human development and health, with
45 ciliary defects resulting in syndromic ciliopathies (REITER AND LEROUX 2017). Cilia are
46 endowed with receptors, channels, and signaling components, enabling cilia to act as
47 cellular sensors. In addition to their sensory abilities, cilia may transmit signals via
48 submicroscopic extracellular vesicles (WANG AND BARR 2018; WOOD AND ROSENBAUM
49 2015). The mechanisms that enable a cilium to simultaneously send and receive
50 information remain mysterious.

51 Cilia and the extracellular matrix (ECM) share an intimate association, with cilia
52 projecting into and being surrounded by ECM (SEEGER-NUKPEZAH AND GOLEMIS 2012).
53 ECM is made up of a network of interacting proteins that surround and support cells for
54 adhesive, structural and signaling functions (HYNES 2009). ECM is necessary for tissue
55 morphogenesis and homeostasis throughout the lifespan of an organism and
56 dynamically interacts with and regulates body systems, organs, and tissues. In the
57 brain and nervous system, ECM is important for neuronal development, anatomy, and
58 synaptic transmission. Dysregulation of ECM contributes to pathological conditions
59 such as invasive cancer and fibrosis (BONNANS *et al.* 2014). Fibrosis and abnormal ECM
60 are observed in ciliopathies such as autosomal dominant polycystic kidney disease
61 (ADPKD), nephronophthisis, and Bardet-Biedl Syndrome (SONG *et al.* 2017).

62 ADPKD is a genetic disorder characterized by the presence of fluid filled cysts
63 which form on and in the kidney epithelia lining renal tubules that replace normally
64 functioning renal tissue and result in enlarged, polycystic kidneys and end stage renal

65 failure (GHATA AND COWLEY 2017; ONG AND HARRIS 2015). ADPKD affects 1 in ~500
66 persons regardless of race or gender and can be caused by a mutation in either of the
67 polycystin encoding genes PKD1 or PKD2. The large extracellular domain of polycystin-
68 1 extends into the ECM and contains domains that may mediate protein-protein or
69 protein-ECM interactions. PKD2 has an often-mutated extracellular domain that is
70 necessary for polycystin channel assembly, stimulation, and gating (SHEN *et al.* 2016).
71 These two polycystins can be cleaved and have been found to act individually and
72 synergistically, performing myriad functions including acting as ion channels and
73 regulation of ECM components (HANAOKA *et al.* 2000; MANGOS *et al.* 2010). Polycystins
74 and ECM seem to mutually regulate each other, suggesting dynamic feedback.

75 The polycystins localize to cilia and extracellular vesicles, and this subcellular
76 localization is evolutionarily conserved and observed in *Chlamydomonas*, *C. elegans*,
77 and mammals (HOGAN *et al.* 2009; O'HAGAN *et al.* 2014; SEMMO *et al.* 2014; WANG *et al.*
78 2014; WOOD *et al.* 2013; WOOD AND ROSENBAUM 2015). EVs carry many ECM proteins
79 such as fibronectin and laminin, which provide communication necessary for altering
80 ECM composition, signaling between ECM and the cells it surrounds, tumor
81 proliferation, and inflammation (RILLA *et al.* 2017). In *Chlamydomonas*, ciliary EVs carry
82 a proteolytic enzyme that degrades ECM required for hatching (WOOD *et al.* 2013). In *C.*
83 *elegans*, ciliary proteins such as polycystins are EV cargo that function in animal-to-
84 animal communication (WANG *et al.* 2014).

85 The *C. elegans* polycystins LOV-1 and PKD-2 localize to cilia of male-specific
86 sensory neurons (BARR *et al.* 2001; BARR AND STERNBERG 1999). We previously
87 performed a forward genetic screen for regulators of PKD-2::GFP ciliary localization

88 (BAE *et al.* 2008). Here we identify a mutation in the collagen gene *mec-5* that produced
89 a PKD-2::GFP ciliary localization defect and discovered new functions for the *mec-1*,
90 *mec-5*, and *mec-9* ECM genes previously implicated in the function of non-ciliated touch
91 receptor neurons (KATTA *et al.* 2015). MEC-1 and MEC-9 contain EGF/Kunitz domains
92 and MEC-5 is a Type IV collagen (EMTAGE *et al.* 2004). MEC-1, MEC-5, and MEC-9
93 ECM proteins form the mantle surrounding non-ciliated touch receptor neurons, mediate
94 touch neuron attachment to the hypodermal skin of the worm, and regulate the
95 localization of the mechanosensitive DEG/ENaC (degenerin/sodium epithelial channel)
96 complex MEC-4 and MEC-10 (Du *et al.* 1996; Gu *et al.* 1996).

97 Here we demonstrate that *mec-1*, *mec-5*, and *mec-9* are required for polycystin
98 protein localization to the sensory cilia and discovered that proteins in the extracellular
99 matrix regulate the movement and activity of proteins inside the cell. We find that these
100 ECM components also regulate polycystin-mediated male mating behaviors, control
101 neuron-glia interactions important for ciliary and dendritic integrity, and modulate the
102 shedding and release of ciliary extracellular vesicles. While the polycystins have been
103 implicated in sensing and regulating collagen in zebrafish models, roles for ECM
104 proteins in regulating ciliary integrity, ciliary polycystin localization, and ciliary function
105 have not been previously appreciated.

106

107 **MATERIALS AND METHODS**

108 **Culture of *C. elegans* nematodes** Nematodes were maintained using standard
109 conditions (BRENNER 1974). Males and hermaphrodites were isolated at L4 stage
110 ≥ 24 hrs prior to experiments and kept at 20-22°C overnight. In *C. elegans*, the

111 predominant sex is hermaphrodite and males spontaneously arise only rarely (less than
112 1%). Therefore, in all experiments in which males were tested, we used animals in
113 either the *him-5(e1490)* or *him-8(e1494)* background. These backgrounds were
114 considered wild type. *him-5(e1490)* and *him-8(e1494)* males exhibit normal mating
115 behaviors and are used as wild-type controls for mating assays.

116

117 **General molecular Biology** PCR amplification was used for genotyping and building
118 transgenic constructs using the following templates: *C. elegans* genomic DNA, cDNA, or
119 prebuilt constructs. High fidelity LA Taq (TaKaRa Bio Inc., Otsu, Shiga, Japan) or
120 Phusion High Fidelity DNA Polymerase (Thermo Fisher Scientific, Vantaa, Finland)
121 were used for amplification of DNA for constructs. Sequencing reactions were
122 performed by Genewiz, (South Plainfield, NJ, USA). DNA and protein sequence
123 analysis BLAST was used for identification of gene orthologs in *C. elegans*. Human and
124 nematode protein sequence information was provided by NCBI smartBLAST, and *C.*
125 *elegans* gene and protein sequence information was also provided by WormBase.
126 Serial Analysis of Gene Expression (SAGE) data provided by WormBase (Release
127 WS221). Whole genome sequencing was performed and analyzed by Richard Poole
128 using CloudMap. ApE 1.17 was used for sequence manipulation.

129

130 **Imaging** Nematodes were anaesthetized with 10 mM levamisole and mounted on agar
131 pads for imaging at room temperature. Epifluorescence images were acquired using a
132 Zeiss Axioplan2 microscope with 10x, 63x (NA 1.4), and 100x (NA 1.4) oil-immersion
133 objectives with a Photometrics Cascade 512B CCD camera using Metamorph software

134 (www.moleculardevices.com) or Zeiss Axio Imager.D1m microscope using a 63x and
135 100X objective with a Q imaging Retiga-SRV camera. Optical Z-stack projections were
136 stored as TIFF files and manipulated using ImageJ and Adobe Illustrator. Scale bars are
137 10 microns for head and tail images. EM scale bars are 200 or 500 nm as marked in
138 image.

139

140 **Transmission Electron Microscopy** *mec-9(ok2853)* and wild-type young adult males
141 were fixed using high-pressure freeze fixation and freeze substitution in 2% OsO₄ + 2%
142 water in acetone as the primary fixative (WEIMER 2006). Samples were slowly freeze
143 substituted in an RMC freeze substitution device, before infiltration with Embed-812
144 plastic resin. For TEM, serial sections (70-75 nm thickness) of fixed animals were
145 collected on copper slot grids coated with formvar and evaporated carbon and stained
146 with 4% uranyl acetate in 70% methanol, followed by washing and incubating with
147 aqueous lead citrate. Images were captured on a Philips CM10 transmission electron
148 microscope at 80kV with a Morada 11-megapixel TEM CCD camera driven by iTEM
149 software (Olympus Soft Imaging Solutions). Images were analyzed using ImageJ (FIJI)
150 and manipulated with Adobe Illustrator.

151

152 **Extracellular vesicle release** EV release was analyzed by counting all EVs from one-
153 day old young adult males as described by Silva et al 2017. Individual animals were
154 mounted on agar into four quadrants of agar slide. Free and newly released EVs that
155 float to the cover slip were counted and reported as “number of EVs released.”

156

157 **Transmission Electron Microscopy EV measurements** Using ImageJ (FIJI) software,
158 WT and mutant electron micrographs were compared at and around the CEM transition
159 zone. Qualitative examination was performed. EV diameter measurements were
160 performed by drawing a line across the widest diameter of an EV and taking a
161 measurement via FIJI measurement tool reporting diameter in nm.

162

163 **Transmission Electron Microscopy cilia length measurements** Electron
164 micrographs were stacked using the TrakEM2 component of ImageJ(FIJI) software. The
165 transition zone was identified by Y-links and used as the bottom-most measurement.
166 The ciliary tip was used as the top measurement. The Z stack position was multiplied
167 by the thickness of the cut (~70-75 nm) and length was reported in μm .

168

169 **PKD-2 antibody generation and staining** Animals were staged young adults and
170 washed off plates with M9. Antibodies were prepared using Abmart protocols and
171 staining against PKD-2 was prepared and performed using a Finney Ruvkun protocol
172 (BETTINGER *et al.* 1996) [Wormatlas.org]. The monoclonal PKD-2 primary antibody was
173 created by Abmart against the intracellular N-terminal domain (amino acids:
174 DERWANPPQPVA) and an intracellular C-terminal domain (amino acids:
175 KRGKRPDAPGED). The secondary antibody was α mouse Alexa Fluor $\text{\textcircled{R}}$ 568 donkey
176 anti-mouse IgG (H + L) (2 mg/ml) by Invitrogen TM. The following dilutions and
177 incubation times were used: primary antibodies 1:200 overnight (18-24 hours);
178 secondary antibody 1:1000 for two hours.

179

180 **Response behavior assay** Control Strains used: CB1490: *him-5(e1490)*, CB1489: *him-*
181 *8(e1489)*, PT9: *pkd-2(sy606) him-5(e1490)*, and CB169: *unc-31(e169)IV*. L4 larval
182 males were moved to a fresh plate approximately 24 hours before mating. *unc-31*
183 mutant hermaphrodites were also picked as L4 larvae ~24 hours before experiments.
184 Male mating assays were conducted on a fresh NGM agar plate with a small lawn of *E.*
185 *coli* (OP50) containing 25 young-adult *unc-31* hermaphrodites. One, two, or three males
186 were placed in the center of the lawn and observed for four minutes. When a male
187 began scanning a hermaphrodite and the male tail maintained contact with a mate for at
188 least ten seconds, a response was scored and that male was removed from the test
189 plate.

190

191 **Location of vulva assay** was performed as described (BARR AND STERNBERG 1999).
192 Location of vulva efficiency is calculated by successful vulva location divided by the total
193 number of vulva encounters for each male. Total time measured was four minutes.

194

195 **Male leaving assay** was performed as described (BARRIOS *et al.* 2008). L4 males were
196 picked and isolated from hermaphrodites on plates, then assayed 24 hours later in 20 μ l
197 food on a 9 cm diameter plate. Animals are positive for leaving when males exhibit
198 tracks that approach within 1 cm of the edge of the plate. Time points scored were 2, 5,
199 8, and 24 hours after the males were placed on the spot of food. A minimum of 20
200 animals per strain and three replicates were scored for each genotype assayed.
201 Statistical significance was determined by R software.

202

203 **Dye filling assays** Standard dye-filling assays (PERKINS *et al.* 1986) were performed
204 using Dil (Invitrogen). The number of amphid and phasmid cell bodies were counted,
205 and the results reported as number of neurons out of 12 (amphid) or 4 (phasmid) that fill
206 with Dil.

207
208 **Dendritic trafficking velocity measurements** To directly measure in vivo velocity of
209 PKD-2::GFP in CEM dendrites, we acquired time lapse image stacks, which were later
210 converted to kymographs using the KymographClear V2.0 plugin in FIJI. Motile particles
211 were automatically and indiscriminately detected and traced, and velocities analyzed
212 with KymographDirect (MANGEOL *et al.* 2016). We observed a reduction of overall
213 observed velocities compared to our previous publication (BAE *et al.* 2006), likely due to
214 differences between automatic versus manual particle tracing. This reduction does not
215 affect our analysis or overall conclusions, as our model is not based on absolute
216 velocities, but rather on their relative changes.

217
218 **Ciliary localization and fluorescence intensity** Ciliary localization was performed by
219 blind assay of a stack of images collected into a maximum intensity image using the “Z
220 project” function in ImageJ software. Animals were scored as ciliary localization
221 defective (Cil) if excess or misplaced PKD-2::GFP or endogenous PKD-2 (detected by
222 α -PKD-2 antibodies) was detected (excess in cilium, ciliary base, or dendrite).
223 Fluorescence intensity was measured using ImageJ by drawing a range of interest and
224 using measurement tool. All measurements have background subtracted to create final
225 value.

226 **Strains used: (transgenic lines created by Knudra)**

CB1066 *mec-1(e1066)V*
CB1292 *mec-1(e1292)V*
CB1490 *him-5(e1490)*
CB1494 *mec-9(e1494)V*
CB1503 *mec-5(e1503)X*
CB169 *unc-31(e169)IV*
COP1472 *knuEx206[pNU1416-mec-9Sp::GFP::tbb-2utr,unc-119(+)];unc-119(ed3)III*
COP1473 *knuEx207[pNU1415-mec-9Lp::GFP::tbb-2utr,unc-119(+)];unc-119(ed3)III*
KU25 *pmk-1(km25)IV*
PT3296 *mec-9(ok2853)pmk-1(km25)IV;myls4 him-5(e1490) V*
PT3168 *him-8(e1489) myls1[PKD-2::GFP + cc::GFP] IV*
PT277 *unc-119(ed3)III; him-5(e1490) V*
PT1213 *myls4 him-5(e1490) V; mec-5(my2) X*
PT1852 *pha-1(e2123) III; him-5(e1490) V; Ex [LOV-1::GFP1]*
PT2434 *dyf-1(m335); myls1; him-5*
PT2679 *him-5(e1490)V;myls23[Pcil-7::gCIL-7::GFP_3'UTR+ccRFP]*
PT2962 *him-8(e1489) myls1; mec-1(e1066) V*
PT2963 *him-8(e1489) myls1; mec-1(e1336) V*
PT2964 *him-8(e1489) myls1; mec-1(e1292) V*
PT2965 *him-8(e1489) myls1; mec-9(e1494) V*
PT2966 *him-8(e1489) myls1; mec-9(ok2853) V*
PT2967 *him-5(e1490) myls4[PKD-2::GFP + cc::GFP] V; mec-5(e1790) X*
PT2968 *him-5(e1490) myls4; mec-5(e1503) X*
PT2969 *uls31[MEC-17::GFP] III; him-5(e1490) myls4[PKD-2::GFP + cc::GFP] V; mec-5(u444) X*
PT3038 *unc-31(e169)IV; him-5(e1490), myls4 V*
PT3203 *mec-9(ok2853); pha-1; him-5(e1490); syEx301[pBx+LOV-1::GFP1]*
PT3213 *mec-9(ok2853)V,him-5(e1490)V;myls23[pcil-7::gCIL-7::GFP::3'UTR+ccRFP]*
PT443 *myls1 pkd-2(sy606) IV; him-5(e1490) V*
PT621 *myls4 him-5(e1490) V*
RB2140 *mec-9(ok2853) V*

227

228 Strains and plasmids are available upon request. The authors affirm that all data
229 necessary for confirming the conclusions of the article are present within the article,
230 figures, and tables.

231 **RESULTS:**

232 ***mec-1*, *mec-5*, and *mec-9* regulate PKD-2::GFP localization.** The *C. elegans*
233 polycystins LOV-1 and PKD-2 localize to cilia and cell bodies of cephalic male-specific
234 (CEM), left and right B-type ray neurons in the tail (RnB; n=neuron 1-9, but not 6), and
235 Hook B (HOB) neurons. (Figure 1 A-B, Supplemental Figure 1 A-B) The polycystins
236 and these male specific neurons are necessary for male mating behaviors (BARR *et al.*
237 2001; BARR AND STERNBERG 1999; LIU AND STERNBERG 1995; SRINIVASAN *et al.* 2008).
238 We previously performed a forward genetic screen for genes necessary in PKD-2::GFP
239 localization and identified mutants defective in PKD-2 ciliary receptor localization (the
240 Cil phenotype) (BAE *et al.* 2008). The *cil-2(my2)* mutant displays abnormally high PKD-
241 2::GFP levels in the CEM cilia and ciliary base and also has abnormal extracellular,
242 extradendritic PKD-2::GFP accumulation along CEM dendrites (BAE *et al.* 2008). *cil-*
243 *2(my2)* hermaphrodites also displayed temperature sensitive sterility phenotype, that is
244 linked to the Cil phenotype. The extradendritic PKD-2::GFP accumulation and sterility
245 phenotypes are unique to the *cil-2(my2)* mutant and not the other ten Cil mutants. We
246 used genetic mapping, whole genome sequencing, complementation testing, and
247 rescue experiments of the Cil phenotype to map the *my2* lesion to *mec-5*.

248 Expression of the wild-type (WT) *mec-5* genomic region using either the *mec-5*
249 promoter or muscle-specific *myo-3* promoter rescued *cil-2(my2)* defects (data not
250 shown), confirming that *mec-5* was mutated in the *cil-2(my2)* mutant and that *mec-5*
251 acted non-cell autonomously to regulate PKD-2::GFP ciliary localization in male-specific
252 neurons. *mec-5* encodes a collagen protein that is produced and secreted by
253 hypodermal cells to anchor the degenerin complex in touch receptor neurons to the

254 extracellular matrix (ECM) (Du *et al.* 1996). In these non-ciliated neurons, *mec-5* and
255 the ECM encoding genes *mec-1* and *mec-9* act in concert (Du *et al.* 1996). We
256 therefore determined whether MEC-1, MEC-5, and MEC-9 also regulated PKD-2::GFP
257 ciliary localization.

258 We characterized PKD-2::GFP ciliary localization in two mutant alleles each of
259 *mec-1* (*e1066* and *e1292*), *mec-5* (*e1503* and *my2*), and *mec-9* (*e1494* and *ok2853*)
260 (Figure 1C-H). First, we visualized ciliary localization in CEM neurons of WT and mutant
261 males via blind examination. A male was scored as Cil if CEM cilia, ciliary base, and
262 dendrites in male worms had abnormally increased or mislocalized PKD-2::GFP.
263 Values were reported as percent of animals that are Cil (Figure 1I). In WT, we saw
264 occasional Cil animals (~16% of males). In contrast, *mec-5* males exhibited the most
265 penetrant Cil phenotype: over 60% of *mec-5(my2)* animals and over 70% of *mec-*
266 *5(e1503)* were Cil (p values < 0.0001). Also, *mec-9(ok2853)* animals had a significantly
267 increased number of Cil males (p=0.008), but *mec-9(e1494)* males were statistically
268 indistinguishable from WT. *mec-1(e1066)* males were also Cil (p= 0.0003), but *mec-*
269 *1(e1292)* males were similar to WT.

270 In *mec-1(e1066)*, *mec-5(e1503)*, *mec-5(my2)*, and *mec-9(ok2853)* males, we
271 observed abnormally increased PKD-2 localization in CEM cilia, ciliary bases, and
272 occasionally dendrites. (Figures 1C, E, F, and H) The extradendritic accumulation in
273 *mec-1(e1066)*, *mec-5(e1503)* and *mec-9(ok2853)* was reminiscent of that observed in
274 *mec-5(my2)*. The Cil defect was not observed in *mec-1(e1292)* and was only
275 occasionally seen in *mec-9(e1494)*. (Figures 1D and G)

276 *mec-1* encodes multiple ECM protein isoforms with multiple disulfide-linked EGF
277 and Kunitz-type protease inhibitor domains (EMTAGE *et al.* 2004). The *mec-1* alleles
278 cause gene truncations: *e1292* results in truncation after Kunitz Domain 3 whereas
279 *e1066* truncates after Kunitz Domain 6 (EMTAGE *et al.* 2004), both of which remove
280 Kunitz domains but might leave EGF domains intact. *mec-9* encodes two protein
281 isoforms of an ECM protein containing multiple EGF domains, multiple Kunitz domains,
282 and a glutamic acid-rich region (Du *et al.* 1996). *mec-9* mutations perturb EGF domains:
283 *e1494* is a point mutation in the first set of EGF domains and affects only the *mec-9*
284 long (*mec-9L*) isoform. *ok2853* perturbs the second set of EGF domains via deletions
285 and affects both short and long isoforms (Du *et al.* 1996). Using whole genome
286 sequencing, we found that both *mec-5* mutants had lesions in the third intron (data not
287 shown). We conclude that some but not all alleles of *mec-1*, *mec-5* and *mec-9*
288 perturbed PKD-2::GFP localization.

289 We observed that cilia and ciliary bases appeared brighter in some but not all
290 ECM mutants. We measured highest/maximum fluorescence intensity (FI) of PKD-
291 2::GFP in CEM neuron cell bodies and cilia (including both cilium and ciliary base) to
292 quantify PKD-2::GFP abundance. FI is a computed measurement of the pixels
293 illuminated in a selected region of interest. *mec-9* mutants had a brighter maximum FI
294 (~1.75x) than WT (Figure 2B): *mec-9(e1494)* p=0.011 and *mec-9(ok2853)* p<0.0001
295 (Figure 1J). *mec-1(e1066)* mutants were dimmer than WT: p=0.0088. We observed
296 variability in CEM FI of ECM gene mutant cilia, dendrites, and cell bodies (Supplemental
297 Table 1), with *mec-9(ok2853)* exhibiting the brightest overall FI. Similar to CEM
298 neurons, we also observed variable ray neuron FI in ECM mutant tails (Supplemental

299 Figure 1 and Supplemental Table 1), again with *mec-9(ok2853)* exhibiting the brightest
300 overall FI. Our data shows that although *mec-1*, *mec-5*, and *mec-9* were all necessary
301 for PKD-2 ciliary localization, *mec-9* also regulated PKD-2::GFP ciliary abundance.

302 To test for specificity of ECM components, we examined PKD-2::GFP localization
303 in a hemicentin mutant. Hemicentin is an ECM component required for adhesion
304 between tissues including touch neuron attachment to the epidermis and gonad (VOGEL
305 AND HEDGECOCK 2001). Analysis of the hemicentin mutant *him-4(e1267)* revealed no
306 differences in PKD-2::GFP ciliary localization or FI. We conclude that the *mec-1*, *mec-5*,
307 and *mec-9* ECM genes; but not the *him-4* ECM gene, were necessary for polycystin
308 localization and abundance.

309 We generated a monoclonal anti-PKD-2 antibody to visualize and measure
310 endogenous PKD-2 localization in WT and ECM gene mutant males and observed
311 similar Cil defects (Supplemental Figures 2 and 3). In WT, endogenous PKD-2 was
312 limited to the cell bodies and cilia of CEM head neurons and ray RnB and hook HOB tail
313 neurons (Supplemental Figure 3A and 3B). In *mec-9(ok2853)* males, endogenous PKD-
314 2 mislocalized to dendrites and had increased abundance in cilia and cell bodies as
315 shown in FI measurements (Supplemental Figure 3C and 3D). Endogenous PKD-2
316 localization was also abnormal in the ray cilia in *mec-1(e1066)* and *mec-5(e1503)* (data
317 not shown) male tail but not as severely as *mec-9(ok2853)*. We conclude that all three
318 ECM genes regulate PKD-2 localization with *mec-9* mutants displaying the most severe
319 Cil defects.

320 The partner of Polycystin-2 (PKD-2) is Polycystin-1 (LOV-1). We therefore,
321 examined LOV-1 localization in ECM gene mutants. In WT, LOV-1::GFP localizes to the

322 CEM, RnB, and HOB cell bodies and cilia. In *mec-9(ok853)* mutants, we observed distal
323 dendritic LOV-1::GFP mislocalization and increased ciliary fluorescence (Supp. Figure
324 4). We also observed significantly increased LOV-1::GFP FI in CEMs and RnBs of *mec-*
325 *5(e1503)* males (data not shown). We conclude that ECM encoding genes *mec-1*, *mec-*
326 *5*, and *mec-9* regulate polycystin localization in male-specific ciliated sensory neurons.

327

328 ***mec-1*, *mec-5*, and *mec-9* regulate male mating behaviors.** *pkd-2*, *lov-1*, and the
329 male-specific polycystin expressing neurons are required for the male mating behaviors
330 of mate searching, response to hermaphrodite contact, and location of hermaphrodite
331 vulva (O'HAGAN *et al.* 2014). We therefore determined whether the three ECM genes
332 were required for these male sensory behaviors.

333 *C. elegans* males leave a food source in search of a mate if no hermaphrodite is
334 present (LIPTON *et al.* 2004). *lov-1* and *pkd-2* mutant males do not leave food to search
335 for a mate (BARRIOS *et al.* 2008). Similarly, *mec-5(e1503)* males were leaving defective
336 (Figure 2A). In contrast, *mec-9(ok2853)* mutants left food more readily than WT
337 animals (Figure 2A). Hyper-leaving behavior is associated with defects in male-specific
338 and the shared inner labial type 2 IL2 ciliated neurons (MAGUIRE *et al.* 2015), suggesting
339 that the *mec-9* mutation may affect other neurons in addition to the polycystin-
340 expressing cells.

341 When male ray neurons detect contact with a hermaphrodite, males initiate the
342 response behavior by stopping forward locomotion, and initiating backing (BARR AND
343 GARCIA 2006; BARR *et al.* 2018). *lov-1* and *pkd-2* mutant males are response defective.
344 *mec-9(ok2853)* and *mec-1(e1066)* mutants were also defective in response to

345 hermaphrodite contact, while *mec-5(my2)* mutant males displayed normal response
346 behavior (Figure 2B) (BAE *et al.* 2006). After response, the male scans the
347 hermaphrodite's body for her vulva. *lov-1* and *pkd-2* mutants are location of vulva
348 defective (Lov). Only *mec-9(ok2853)* males displayed the Lov phenotype (Figure 2C).

349 We previously showed that mate searching, response, and location of vulva do
350 not require MEC-4 and MEC-10, the touch neuron specific degenerin epithelial sodium
351 channel (DEG/ENaC) receptors (BARR AND STERNBERG 1999; BARRIOS *et al.* 2008).
352 However, *mec-1*, *mec-5*, and *mec-9* ECM genes are required for these male-specific
353 sensory behaviors. We conclude that *mec-1*, *mec-5* and *mec-9* ECMs genes act beyond
354 DEG/ENaC localization and function in touch receptor neurons and are required more
355 broadly for the function of other sensory neurons. Moreover, only *mec-9* was required
356 for all examined male-specific behaviors suggesting a distinct role for *mec-9* in
357 polycystin-expressing male specific neurons.

358

359 ***mec-9* long and short isoforms have distinct expression patterns: MEC-9S is**
360 **expressed in ciliated sensory neurons.** *mec-9* encodes two predicated proteins (Du
361 *et al.* 1996). The *mec-9* long isoform encodes an 839-amino acid protein with five Kunitz
362 protease inhibitor domains, seven EGF domains, and a glutamic acid rich/coiled-coiled
363 region (Figure 3A) (Du *et al.* 1996). The *mec-9* short isoform encodes a 502-amino acid
364 protein with two Kunitz domains, three EGF domains, and a glutamic acid rich/coiled-
365 coiled region (Du *et al.* 1996) (Figure 3B). Both long and short isoforms encode an N-
366 terminal signal sequence consistent with secreted ECM proteins. *mec-9(ok2853)* is a

367 408-base pair in-frame deletion that is predicted to remove two of the three EGF like
368 domains in both isoforms (CONSORTIUM 2012) (Figure 3A).

369 Chalfie and colleagues showed *mec-9* long isoform expression in touch receptor
370 neurons and *mec-9* short isoform expression in other neurons in the nerve ring and
371 ventral cord in hermaphrodites (DU *et al.* 1996). We examined long and short isoform
372 expression patterns in males and hermaphrodites using transcriptional reporters (Figure
373 3A). We find that *mec-9Lp::GFP* (long isoform transcriptional reporter) was expressed
374 only in the shared touch receptor neurons (found in both males and hermaphrodites)
375 and not male-specific neurons. However, *mec-9Sp::GFP* (short isoform transcriptional
376 reporter) was expressed in polycystin-expressing CEM, RnB, and HOB male-specific as
377 well as shared ciliated sensory neurons found in both males and hermaphrodites
378 (Figures 3D and 3E).

379 *mec-9Sp::GFP* was coexpressed with the kinesin-3 gene *klp-6* transcriptional
380 reporter in the shared IL2 neurons and male-specific CEM neurons in the head and RnB
381 and HOB neurons in the tail (Figure 3D). These 27 *klp-6* expressing neurons are called
382 extracellular vesicle releasing neurons (EVNs) based on their ability to shed and release
383 ciliary EVs (Wang *et al.*, 2015). *mec-9Sp::GFP* was also expressed in the amphid
384 neurons in the head and phasmid in the tail that take up the lipophilic fluorescent dye Dil
385 (Figure 3E).

386

387 **MEC-9 regulates extracellular vesicle biogenesis and release.** EVs are shed and
388 released from ciliated IL2, CEM, and RnB neurons (WANG *et al.* 2014), all of which
389 expressed *mec-9Sp::GFP*. To determine if *mec-9* regulates EV biogenesis, we counted

390 PKD-2::GFP containing EVs that were shed and released into the local environment
391 from the male head and tail. WT adult males released an average of 26 PKD-2::GFP
392 labeled EVs from the CEM neurons compared to 78 EVs in *mec-9* mutants ($p=0.0008$):
393 a threefold increase (Figure 4C). The EV hypersecretion phenotype of *mec-9* mutants
394 contrasts other previously described EV hyposecretion mutants that are deficient in EV
395 release (MAGUIRE *et al.* 2015; O'HAGAN *et al.* 2017; SILVA *et al.* 2017; WANG *et al.* 2014).

396 Next, we used transmission electron microscopy (TEM) to more closely examine
397 the ultrastructure of the male cephalic organs containing EVs in ECM and luminal
398 spaces (WANG *et al.* 2014). In WT males, EVs are shed from the ciliary base of the CEM
399 neuron and occupy a luminal space formed by the glial support cells (Figure 4E). In
400 *mec-9* mutant males, we observed three striking defects. First, in the *mec-9* cephalic
401 lumen we observed a dramatic accumulation of EVs that ranged in diameter from 45nm
402 to 226nm (Figure 4F). Second, we saw an increase in the volume of the glial lumen
403 occupied by the EVs. The 2D area of the *mec-9* mutant lumen cross section was
404 massively distended, perhaps due to EV hypersecretion and increased EV storage
405 (Figure 4F). Eight WT and 12 *mec-9* cephalic sensilla were compared and revealed
406 increased occurrences and larger diameters of luminal spaces surrounding cilia and
407 distal dendrites. The enlarged EV containing lumen is a phenotype observed in mutants
408 that shed but do not environmentally release EV. Third, *mec-9* cephalic sensilla were
409 filled with remarkable light and dark matrix filled vesicles, themselves also containing
410 EVs. For example, the lightly shaded vesicle shown in Figure 4E was 506 nm in
411 diameter and contained smaller vesicles ranging in diameter from 40-90 nm. Dark
412 matrix vesicles found at the level of the distal CEM dendrite were 898 and 1056 nm in

413 diameter and contained vesicles that ranged from 51-171 nm (Figure 4G). For both
414 abnormal EV and matrix-filled vesicle phenotypes in *mec-9*, we observed larger
415 complex vesicles in the glial cytoplasm as well as in the luminal spaces surrounding the
416 cilia, ciliary transition zone (TZ), and distal dendrite (data not shown). The presence of
417 these larger vesicles with complex contents is a phenomenon not previously described
418 in any of our other EV biogenesis mutants (MAGUIRE *et al.* 2015; O'HAGAN *et al.* 2017;
419 SILVA *et al.* 2017; WANG *et al.* 2014).. We propose that the *mec-9* and likely the *mec-5*
420 extradendritic PKD-2::GFP ciliary localization (Cil) phenotype may correlate to extra-
421 dendritic accumulation of the abnormal EVs in the ECM surrounding the CEM dendrite.

422

423 ***mec-9* and *pmk-1* act antagonistically in EV biogenesis and release.** We wondered
424 if the EV hypersecretion and excessive EV storage phenotypes seen in *mec-9* mutants
425 were due to abnormal EV biogenesis or to defects in neuronal integrity. To test the
426 former possibility, we examined genetic interactions with a positive regulator of EV
427 shedding and release – the p38 MAPK *pmk-1*. We previously showed that *pmk-1*
428 mutants do not accumulate EVs in the cephalic lumen and release fewer PKD-2::GFP
429 labeled EV from ray neurons into the environment (WANG *et al.* 2015). In contrast, *mec-*
430 *9* mutants exhibit the opposite phenotypes: hypersecretion and excessive release of
431 cephalic EVs. WT and the *mec-9(ok2853); pmk-1(km25)* double mutant release
432 comparable numbers of PKD-2::GFP labeled EVs (Figure 5A). In a *mec-9(ok2853);*
433 *pmk-1(km25)* double mutant we see that EV release from ray neurons is restored to WT
434 levels (Figure 5B), suggesting that *mec-9* and *pmk-1* may act antagonistically, with *mec-*
435 *9* as a negative regulator, *pmk-1* as a positive regulator. Further, PKD-2::GFP

436 fluorescence intensity (FI) and blind study of ciliary localization was analyzed and
437 although the *mec-9* and *pmk-1* single mutants exhibit an increase of PKD-2::GFP at the
438 ciliary base, the ciliary localization phenotype and increased ciliary FI is ameliorated in
439 the double mutant (Figure 5C). We conclude that MEC-9 is a negative regulator of EV
440 biogenesis, with the mutant shedding and releasing excessive amounts of EVs.

441

442 ***mec-9* maintains CEM and IL2 neuronal morphology.** Given that *mec-9S* was
443 expressed in many ciliated sensory neurons, we used transmission electron microscopy
444 (TEM) to examine ultrastructure of sensory organs in the head of fixed age-matched
445 young adult WT and *mec-9* males (Figure 6A). The head contains four cephalic sensilla,
446 six inner labial sensilla, and two amphid sensilla, each of which performs distinct
447 sensory functions in the worm (INGLIS 2007).

448 In the male, the cephalic sensillum includes the cephalic male CEM neuron and
449 the sex-shared CEP neuron. We viewed the CEM in cross section and measured from
450 the ciliary transition zone to the ciliary tip and found WT CEM cilia to be ~2.1 μm in
451 length (Figure 6A). In *mec-9* mutants, the CEM neurons were 1.2-1.5 times longer than
452 WT. CEP cilia length was not significantly different between WT and *mec-9* mutant
453 animals.

454 The inner labial sensilla house the IL1 and IL2 neurons. WT dorsal and ventral
455 IL2 cilia averaged 1.155 μm and the lateral IL2s averaged 1.085 μm in length (Figure
456 6A). In the *mec-9(ok2853)* mutant, dorsal and ventral IL2 cilia were about twice as long
457 as WT and the *mec-9(ok2853)* mutant lateral IL2s were ~1.3 times longer than WT

458 (Figure 6A). IL1 ciliary lengths were similar in WT and *mec-9*, consistent with *mec-9S*
459 expression in IL2 but not IL1 neurons.

460 The ECM encoding genes *mec-1*, *mec-5*, and *mec-9* regulate touch receptor
461 neuron attachment and integrity (PAN *et al.* 2011). In *mec-5* and *mec-9* CEM neurons,
462 PKD-2::GFP accumulated in extradendritic spaces (Figure 1 E,F, H). We therefore
463 examined CEM neuronal morphology using a soluble GFP reporter. In WT and *mec-9*
464 CEMs, *pkd-2p::GFP* was expressed and localized throughout the neuron in the cell
465 body, dendrite, and cilium (Figure 6B). We did not observe extradendritic accumulation
466 of soluble GFP in *mec-9* mutant, therefore the neurons are not fragile or leaky.
467 However, *mec-9* mutant dendrites appeared brighter and more irregular than WT
468 (Figure 6C).

469 To determine if there were differences in the dendritic morphology of WT and
470 *mec-9* mutants, we compared FI peaks along the dendrite from the CEM cilium to cell
471 body. These peaks represent dendritic varicosities and the example shown is indicative
472 of the increased number and size of the varicosities in the *mec-9* mutant compared to
473 WT (Figure 6D). WT animals averaged 30.6 varicosities per animal, whereas *mec-9*
474 mutant animals averaged 36 varicosities ($p= 0.0211$) (Figure 6E).

475 We defined gross varicosities as those that are noticeably larger than normally
476 observed and measure this phenomenon by blind investigation and FI. WT averaged
477 3.7 gross varicosities per animal and *mec-9* mutants averaged almost double (7 gross
478 varicosities per animal) ($p=0.0007$; Figure 6F) The FI of the peaks/varicosities in WT
479 were measured and normalized to 100. In comparison, *mec-9* mutant animals'

480 varicosities averaged 159% of WT intensity ($p < 0.0001$) (Figure 6G). We conclude that
481 the ECM gene *mec-9* was important for CEM dendritic morphology.

482 To determine if defects in dendritic morphology impacted protein transport in
483 CEMs, we used time-lapse microscopy to measure the number and velocity of
484 PKD::GFP particles moving along the dendrite. *mec-9(ok2853)* mutants had significantly
485 fewer moving puncta (Table 1). In *mec-9(ok2853)*, PKD-2::GFP anterograde and
486 retrograde velocities were significantly slower than WT (Table 1). Combined, these
487 results indicate that the MEC-9 ECM component also regulated the CEM neuronal
488 cytoskeleton and transport.

489

490 ***mec-9* regulates amphid ciliary length, positioning, and fasciculation.** *mec-*
491 *9S* was also expressed in the dye-filling amphid sensory neurons that are housed in
492 bilateral amphid sensilla (Figure 2H-I). Dye filling assays assess ciliary integrity: WT
493 animals dye fill whereas ciliary mutants are dye filling defective (Dyf). *mec-1(e1066)*
494 animals have a slight Dyf phenotype (PERKINS *et al.* 1986). We performed dye filling
495 assays to determine whether *mec-5* and *mec-9* have similar ciliary defects as seen in
496 *mec-1*. *mec-1(e1066)*, *mec-5(e1503)*, and *mec-9(ok2853)* had subtle amphid Dyf
497 defects with 1-4 cells of 12 not filling (Figure 7A). *mec-1(e1066)* and *mec-5(e1503)*
498 phasmids are also Dyf, with 1-2 out of four cells not filling (Supplemental Figure 5).
499 Therefore, the amphid and phasmid ciliated sensory neurons also require proteins
500 encoded by *mec-1*, *mec-5*, and *mec-9* ECM genes.

501 We proceeded to examine amphid sensilla ultrastructure using TEM. In WT, 10
502 amphid cilia are enclosed in a pore of the bilateral papilla at the worm nose and
503 surrounded by the cuticle (Figures 7B and 7D). *mec-9* worms exhibited a misshapen
504 cuticular pore encapsulating only the ASE cilium (Figure 7C). In the WT amphid
505 channel, ten cilia are present in an invariant and precise arrangement (Figures 7D and
506 7G). In *mec-9* mutants, we observed only cilia from the ASE, ASG, ASH, ASI, ASJ, and
507 ASK (the single ciliated amphids) in the more proximal amphid channel; ADF and ADL
508 (the double ciliated neurons) were displaced in an adjacent, aberrant electron dense
509 space (Figure 7I). Consistent with a function in these neurons, *mec-9shp::GFP* was
510 expressed in ADL and ADF. We conclude that *mec-9* was necessary for cilia alignment
511 and placement in amphid sensilla.

512 We measured ciliary lengths in these double ciliated amphid neurons from the
513 bifurcation at the transition zone to ciliary tip. In contrast to the longer CEM and IL2, EV
514 releasing cilia in *mec-9* mutants, the cilia of the ADL and ADF amphid neurons were
515 significantly shorter than WT by ~1.5x (Figure 6A). We also measured single ciliated
516 amphid axonemes and found the ASI cilia significantly shorter than WT (Figure 6A).
517 These amphid cilia length defects may contribute to dye filling defects observed in *mec-*
518 *9* mutants (Figure 7A). Not only were *mec-9* amphid cilia shorter and misplaced, *mec-9*
519 mutant amphid transition zones were disorganized and abnormally dispersed along the
520 anterior-posterior axis (compare WT in Figure 7F to *mec-9* in Figure 7I), suggesting that
521 the *mec-9* ECM component is necessary for regulating dendritic fasciculation and/or
522 transition zone placement.

523 Perkins et al. describe the presence of dark matrix vesicles in the space between
524 the amphid cilia and sheath in WT animals and highlighted several mutants (*osm-1* and
525 *osm-3*) that abnormally accumulate large matrix vesicles (PERKINS *et al.* 1986). In *mec-*
526 *9(ok2853)* mutants, we observed a substantial increase in electron dense extracellular
527 spaces that we identified as large matrix filled vesicles (Figure 7H). *mec-9* mutants also
528 had excessive matrix filled spaces in the amphid neuron sheath surrounding the amphid
529 neurons that decouples cilia from glia (Figures 7H). Because of these spaces, there is a
530 dysmorphic amphid socket and sheath in *mec-9* mutant animals, which may contribute
531 to the disorganization of the amphid channel cilia (Figures 7G-I). We conclude that *mec-*
532 *9* regulates ECM deposition in amphid and cephalic sensilla (Figure 4H-I).

533

534 **DISCUSSION**

535 ECM is important for neuronal anatomy and organization of the brain and
536 nervous system (GARDINER 2011). For example, the ECM glycoprotein Reelin is
537 necessary for migration of neocortical radial cells in the mammalian brain (FRANCO *et al.*
538 2011). In *C. elegans*, ECM components *dex-1* and *dyf-7* regulate amphid dendritic
539 extension, which then affects cilia placement (HEIMAN AND SHAHAM 2009). Mutations in
540 the RNA splicing gene *mec-8* or ECM component *mec-1* mutants cause ciliary
541 fasciculation defects (PERKINS *et al.* 1986). The ECM gene *mec-9* is expressed in the *C.*
542 *elegans* nervous system where it provides mechanical support to multiple cell types
543 including non-ciliated touch receptor neurons (DU *et al.* 1996) and, as shown here,
544 ciliated sensory neurons.

545 *mec-9* encodes two isoforms (Du *et al.* 1996). The *mec-9* long isoform is
546 expressed in touch receptor neurons (Du *et al.* 1996). Here, we show that the *mec-9*
547 short isoform is expressed in the shared and male-specific ciliated nervous system. In
548 amphid and phasmid sensory organs, *mec-9* mutants are dye filling defective, which
549 reflects abnormalities in stereotypical ciliary positioning and fasciculation. In cephalic
550 sensory organs of males, *mec-9* mutants display distended extracellular spaces that
551 contain excessive amounts of ciliary EVs and longer cilia. In CEM neurons, *mec-9* is
552 also required for dendritic integrity, with *mec-9* mutant dendrites showing varicosities
553 and waves as opposed to more linear trajectories. This abnormal dendritic morphology
554 is typically observed in the nervous system of aged animals, and these age-dependent
555 defects are accelerated by mutations that disrupt neuronal excitability or
556 mechanosensation, including the *mec-1*, *mec-5*, and *mec-9* ECM genes (PAN *et al.*
557 2011). Further studies are needed to ascertain if MEC-9 protein physically restrains the
558 dendrite or attaches to the ciliary or dendrite membrane to properly position the neuron.
559 However, it is clear that MEC-9 and the ECM support the development and function of
560 the *C. elegans* sensory nervous system (Figure 8).

561 ECM and cilia share an intimate association. In aortic valves, primary cilia are
562 restricted to ECM zones (TOOMER *et al.* 2017). Chondrocytes have cilia embedded in
563 ECM (RUHLEN AND MARBERRY 2014). In umbilical cord mesenchyme, ECM regulates
564 ciliary orientation (NANDADASA *et al.* 2015). ECM regulates ciliogenesis and
565 organogenesis of Kupffer's vesicle, the zebrafish equivalent of the mammalian
566 embryonic node (COMPAGNON *et al.* 2014; HOCHGREB-HAGELE *et al.* 2013). In

567 *Drosophila*, the ECM protein artichoke is required for morphogenesis of ciliated organs
568 (ANDRES *et al.* 2014). The ECM gene spacemaker/Eyes shut/RP25 is necessary for
569 ciliary pocket morphology and photoreceptor survival (YU *et al.* 2016), with mutation
570 causing photoreceptor degeneration in retinitis pigmentosa patients (ABD EL-AZIZ *et al.*
571 2008).

572 ECM influences ciliary length in multicellular animals. Here we show that ECM
573 regulates ciliary length in a cell-type specific manner in *C. elegans*. In amphid channel
574 neurons, *mec-9* is a positive regulator, with *mec-9* mutants having shorter cilia.
575 Conversely, in EV-releasing IL2 and CEM neurons, *mec-9* is a negative regulator of
576 ciliary length, with *mec-9* mutants having significantly longer cilia. In mammalian skin,
577 ECM component laminin-511 and its receptor integrin-b1 are required for primary cilia
578 formation (GAO *et al.* 2008). In mouse embryonic fibroblast 3T3-L1 cells, type 1
579 collagen promotes primary ciliary growth by repressing the HDAC6-autophagy pathway
580 (XU *et al.* 2018). In zebrafish Kupffers Vesicle, laminin-1 is a positive regulator of ciliary
581 length (COMPAGNON *et al.* 2014; HOCHGREB-HAGELE *et al.* 2013). The mechanisms by
582 which ECM controls ciliary length are largely unknown. A positive or negative feedback
583 loop may act cell autonomously (between the ciliated cell and ECM secreted by the cell
584 itself) or non-autonomously (between the ciliated cell and ECM secreted by neighboring
585 cells). Our data are consistent with both possibilities. Rescue experiments indicate that
586 *mec-5* acts non-autonomously while *mec-9S* expression in ciliated neurons suggest cell
587 autonomous function.

588 EVs are components of the ECM and EVs themselves may carry ECM proteins
589 as cargo (RACKOV *et al.* 2018; RILLA *et al.* 2017). Chlamydomonas ciliary EVs carry
590 ECM proteins and ECM-degrading proteases, including a proteolytic enzyme that
591 degrades ECM necessary for hatching (LONG *et al.* 2016; WOOD *et al.* 2013). Here we
592 show that *mec-9* mutants display dramatic increases in EV shedding and release
593 (Figure 4C) and abnormal dark/light matrix vesicles containing EVs (Figure 4G),
594 suggesting that the MEC-9 ECM component negatively regulates both EV shedding and
595 release. We do not understand how ECM regulates EV biogenesis. However, genetic
596 analysis revealed that *mec-9* and the p38 MAPK *pmk-1* acted antagonistically in EV
597 biogenesis and release. *pmk-1* mutants were defective in EV shedding and EV release
598 (WANG *et al.* 2015). Interestingly, *pmk-1* suppressed the *mec-9* EV hypersecretion
599 phenotype and *mec-9* suppressed *pmk-1* EV hyposecretion (Figure 5), suggesting that
600 these genes act in opposing pathways that control EV biogenesis. An intriguing
601 possibility is that MEC-9/ECM and *pmk-1* kinase regulate the same target(s) such as a
602 cell surface ECM receptor. In mice, CELSR3 (cadherin EGF LAG seven-pass G-type
603 receptor 3) has MEC-9-like EGF domains in its N-terminal ectodomain and CELSR3
604 interacts with a kinase that regulates extension and guidance of sensory neurons
605 (GOFFINET AND TISSIR 2017). Mice deficient in CELSR2 and CELSR3 are defective in
606 ependymal cilia development and develop hydrocephalus, a ciliopathy phenotype
607 (GOFFINET AND TISSIR 2017). The ligand(s) that activates CELSR2 and CELSR3 are not
608 known.

609 The CELSR family is categorized as adhesion GPCRs (LIEBSCHER AND
610 SCHONEBERG 2016). Adhesion GPCRs (aGPCRs) contain a large N-terminal

611 ectodomain that contains a tetherized agonist *Stachel* sequence (LIEBSCHER AND
612 SCHONEBERG 2016). Removal or structural changes to the N-terminal ectodomain
613 exposes the *Stachel* sequence, which in turn activates the GPCR. Proposed
614 mechanisms of Stachel release and activation of aGPCR signaling include mechanical
615 stress and binding of ECM proteins to the N-terminus (LIEBSCHER *et al.* 2014; LUO *et al.*
616 2014; SCHOLZ *et al.* 2016).

617 The polycystin-1 family, while an 11-transmembrane spanning receptor class,
618 has some features similar to an aGPCR (CAZORLA-VAZQUEZ AND ENGEL 2018;
619 LANGENHAN *et al.* 2015; TRUDEL *et al.* 2016). The function of the polycystins remains an
620 enigma, even thirty years after the cloning of PKD1 and PKD2 (MA *et al.* 2017). Based
621 on their ciliary localization, the polycystins were thought to be ciliary mechanosensors,
622 but this model was disproven by the Clapham lab {Delling, 2013 #14868}(DECAEN *et al.*
623 2013) . In mice, an ECM receptor integrin signaling pathway is essential for the
624 development of ADPKD (LEE *et al.* 2015). An intriguing possibility is that the ECM itself
625 acts in permissive fashion to allow cell-type specific polycystin activation and signaling,
626 a mechanism used by adhesion GPCRs. Several lines of evidence support this idea. –
627 Polycystin-1 binds to many ECM proteins including collagen I, II, and IV, fibronectin, and
628 laminin (MALHAS *et al.* 2002). Inactivation of integrin- β 1 or integrin-linked kinase inhibits
629 cystogenesis in *Pkd1* mutant mice (LEE *et al.* 2015; RAMAN *et al.* 2017). In zebrafish,
630 *pkd2* deficiency causes increased collagen synthesis via upregulated protein secretion
631 and downregulation of this secretory pathway rescues cystogenesis (LE CORRE *et al.*
632 2014; MANGOS *et al.* 2010). Combined, these studies reflect the close but poorly
633 understood association between ECM, cilia, and the polycystins.

634 Our data reveal the profound importance of ECM components in nervous system
635 of the worm. Model Figure 8 depicts the activity of ECM genes on ciliated sensory
636 neurons. *mec-1*, *mec-5*, and *mec-9* regulated PKD-2::GFP localization and male mating
637 behaviors. *mec-9* also influenced PKD-2::GFP dendritic transport and negatively
638 regulated EV biogenesis, storage, and release. *mec-9* and ECM was also important for
639 neuronal anatomy, dendritic integrity, ciliary length and organization, and matrix
640 deposition. Notably, abnormal ECM is implicated in the pathogenesis of ADPKD
641 (CALVET 1993; SONG *et al.* 2017) and the polycystins interact with ECM and focal
642 adhesion proteins (DRUMMOND 2011; RETAILLEAU AND DUPRAT 2014). Renal fibrosis
643 observed in PKD is characterized by excessive deposition of ECM proteins (DRUMMOND
644 2011; SONG *et al.* 2017). Here we show that ECM is necessary for the health and well-
645 being of ciliated neurons and neural organs in the nematode. Our findings highlights the
646 promiscuity of ECM components, reveal ECM activity in ciliated neurons of the worm,
647 and broadens the scope of activity of the ECM proteins originally named for their roles
648 in mechanosensory touch receptor neurons. That ECM proteins contribute to ciliary
649 localization and function of the polycystins in *C. elegans* advances the understanding of
650 ciliopathies like ADPKD.

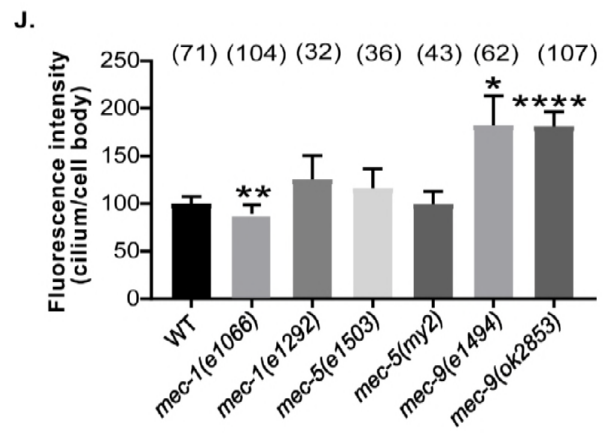
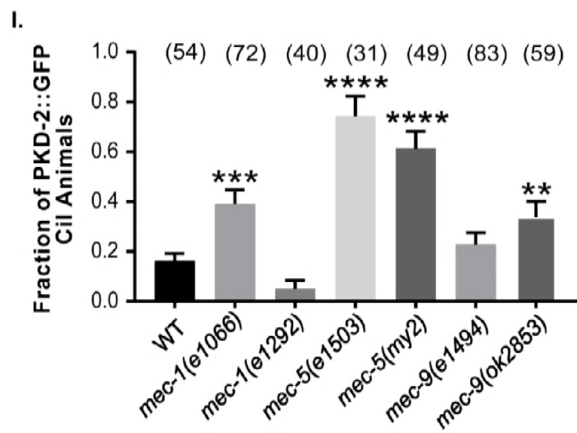
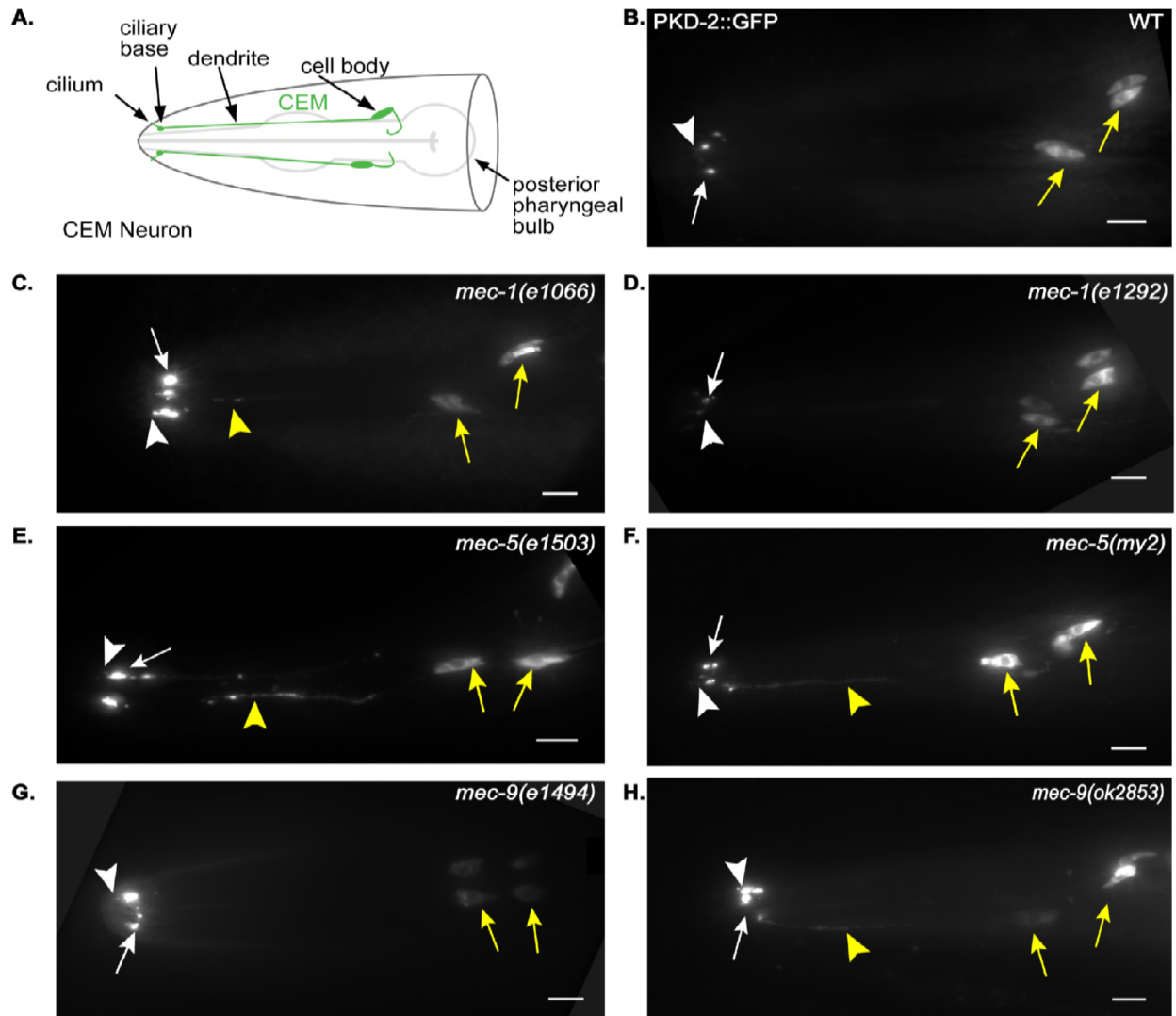
651

652 **Acknowledgements:** We thank Brian Coblitz and Martin Chalfie (Columbia University)
653 for *mec-5(+)* rescue constructs; Richard Poole (Hobert lab, Columbia University) for
654 assistance with whole genome sequencing of *cil-7/mec-5(my2)*; Aranzta Barrios for
655 advice on leaving assays and statistical analysis; Geoff Perumal and Leslie Gunther-
656 Cummins for their help with HPF/FS processing; Monica Driscoll, Marion Gordon,

657 Sunita Kramer, Barth Grant, Martha Soto, Christopher Rongo, and the Rutgers Super
658 Worm Group for essential feedback during D.D.'s graduate career; the Barr lab for
659 ongoing discussion and constructive criticisms, and especially Robert O'Hagan for his
660 expertise in mechanotransduction; Gloria Androwski for outstanding laboratory support;
661 WormBase and WormAtlas for online resources. This work was funded by National
662 Institutes of Health grants DK059418 and DK111214 (to M. M. B.), OD 010943 (to D. H.
663 H), and F31DK103550 (to D.D.). Some strains were provided by the National
664 BioResource Project and the *Caenorhabditis* Genetics Center (CGC), which is funded
665 by NIH Office of Research Infrastructure Programs [P40 OD010440]. Authors declare
666 no competing financial interests or any funding that can compromise the integrity of this
667 work.

668

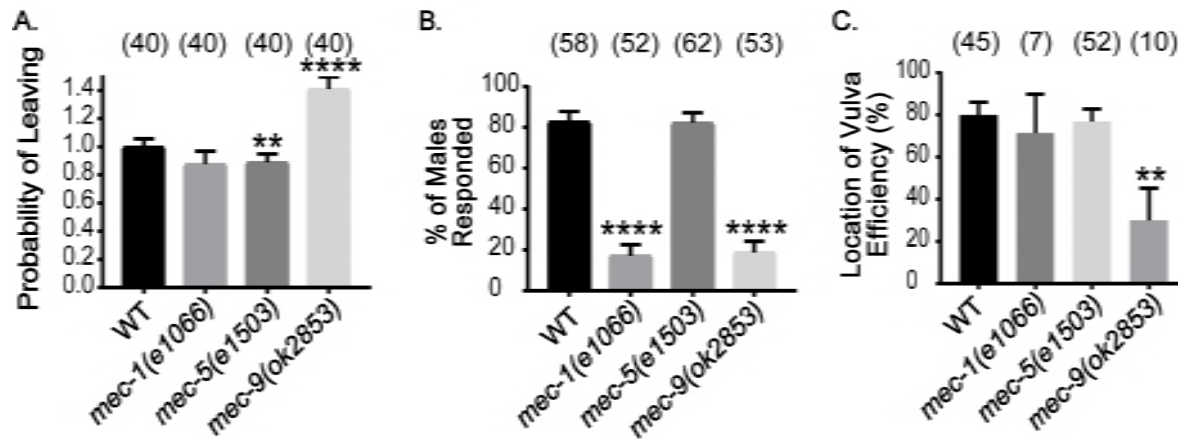
669 Figure 1



671 **Figure 1. *mec-1*, *mec-5*, and *mec-9* regulate PKD-2::GFP localization and**
672 **abundance.** (B-H) Images were compiled from a 630x maximum intensity Z-series
673 obtained by epifluorescence microscopy. Scale bar is 10 μ m. White arrow, ciliary base;
674 white arrow head, cilium; yellow arrow, cell body; yellow arrowhead, dendrite. (A)
675 Schematic of WT cephalic male neurons in the head of a male (green). (B) A lateral
676 view of WT PKD-2::GFP (translational reporter). PKD-2::GFP localized to CEM cell
677 bodies and cilia. (C-D) Lateral view of PKD-2::GFP in *mec-1* mutant males. (C)
678 Increased PKD-2::GFP at *mec-1(e1066)* CEM cilia and ciliary base. (D) *mec-1(e1292)*
679 was indistinguishable from WT. (E-F) *mec-5* PKD-2::GFP ciliary localization. Extra-
680 dendritic and increased ciliary PKD-2::GFP observed in *mec-5(e1503)* and *mec-5(my2)*
681 male CEMs. (G-H.) PKD-2 localization in *mec-9* mutant males. (G) Increased PKD-
682 2::GFP at *mec-9(e1494)* CEM ciliary base. (H) Extra-dendritic and increased PKD-
683 2::GFP at *mec-9(ok2853)* CEM ciliary base. (I) Graph of the fraction of PKD-2::GFP
684 mislocalization in CEM neurons of wild type and mutant males via blind examination. An
685 animal was scored as “Cil” (ciliary localization defective) if CEM cilia, ciliary base, and/or
686 dendrites in male worms had abnormally increased or mislocalized PKD-2::GFP.
687 Values were reported as fraction of animals that are Cil. Significance was determined by
688 Kruskal Wallace test with Dunn’s multiple comparisons test performed to compare
689 groups. ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. In WT, we observed occasional Cil
690 animals (~16% of males). *mec-1*, *mec-5* and *mec-9* all had mutant alleles that
691 mislocalized PKD-2::GFP. (J) Ratio (cilium/cell body) of maximum intensity showed that
692 PKD-2::GFP abundance in CEM cilia is increased in comparison with the cell bodies
693 only in *mec-9* ECM gene mutants; however, *mec-1* and *mec-5* alleles also affected

694 PKD-2 abundance (Supplemental Table 1). Background measurements were
695 subtracted from cilium and cell body values for standardization of images and we
696 expressed the measurements in ratio of cilia to cell body FI. Significance was measured
697 by Kruskal-Wallis test, comparisons made using Dunn's multiple
698 comparisons. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$. WT values were
699 normalized to 100. The *mec-9* mutants had a brighter maximum FI (~1.75x) than WT
700 (Figure 1J).

701 Figure 2



702

703

704 **Figure 2. *mec-1*, *mec-5*, and *mec-9* are required for male mating behaviors. (A-C)**

705 *mec-1(e1066)* males were response defective, *mec-5(e1503)* males were leaving assay

706 defective, *mec-9(ok2853)* males were defective in all three mating behaviors. (A)

707 Leaving assay measured the probability of males leaving food to search for a mate. WT

708 values normalized to 1. *mec-5(e1503)* males were leaving assay defective when

709 compared to WT. *mec-9(ok2853)* males left food more readily than WT. (B) *mec-*

710 *1(e1066)* and *mec-9(ok2853)* males were defective in responding to hermaphrodite

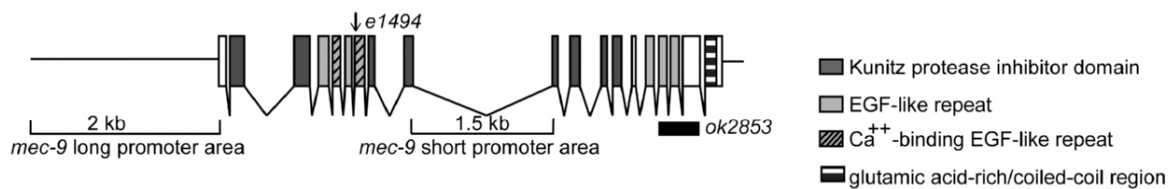
711 contact. (C) *mec-9(ok2853)* males were location of vulva defective. Significance

712 determined by Kruskal-Wallis test (Mann-Whitney test for WT vs. *mec-5(e1503)* only); p

713 values compared by Dunn's multiple comparisons test. ** p<.01; **** p<0.0001.

714 Figure 3

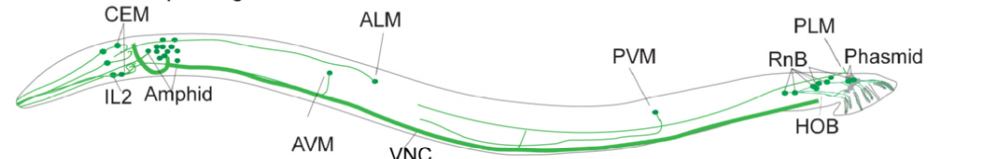
A. *mec-9* long isoform



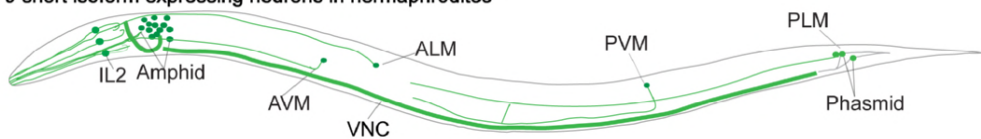
B. *mec-9* short isoform



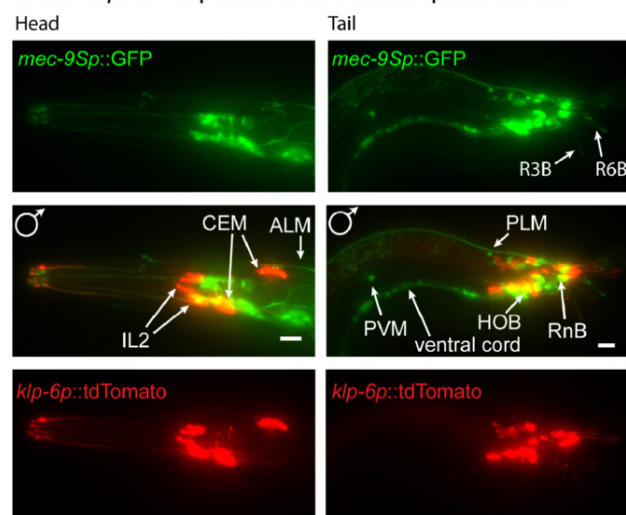
C. *mec-9* short isoform expressing neurons in males



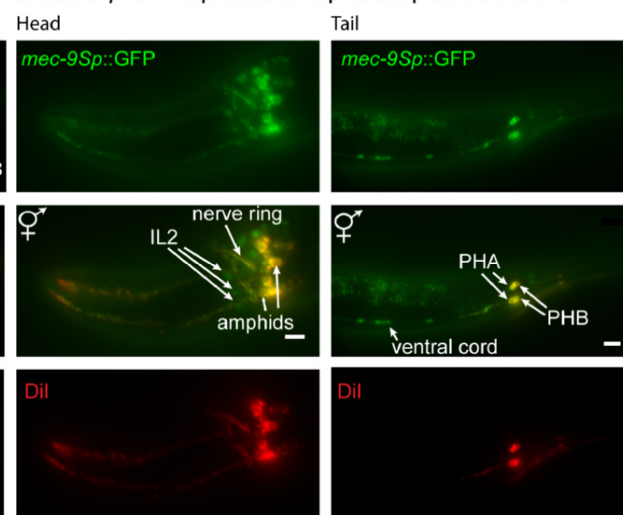
mec-9 short isoform expressing neurons in hermaphrodites



D. *mec-9Sp::GFP* expresses in IL2 and male specific neurons



E. *mec-9Sp::GFP* expresses in amphid and phasmid neurons

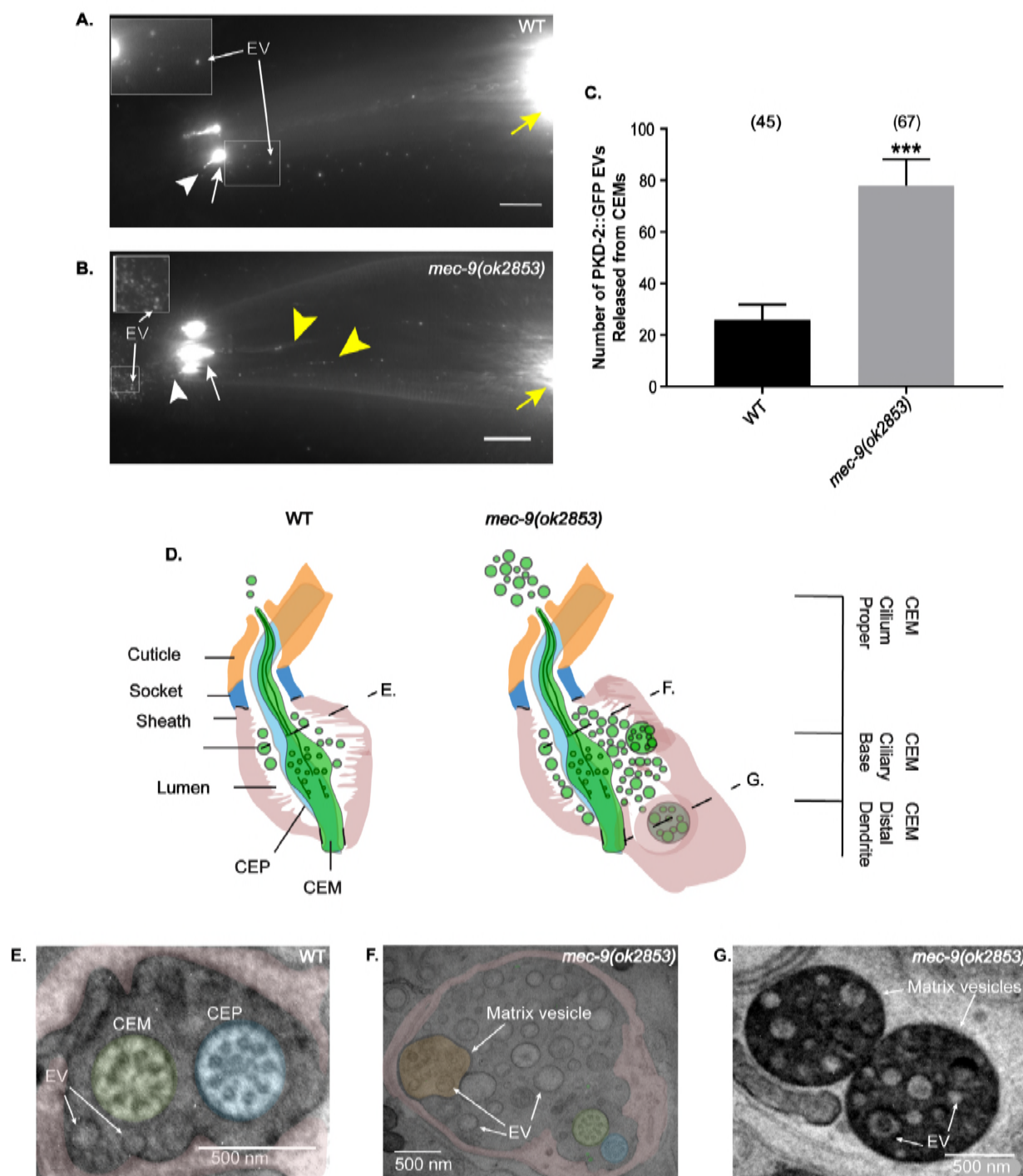


715

716

717 **Figure 3 *mec-9Sp::GFP* is expressed in ciliated sensory neurons.** (A and B) *mec-9*
718 long and short transcripts. (A) *mec-9* long isoform encodes a secreted ECM protein that
719 has 5 Kunitz domains, 7 EGF repeats, and a glutamic acid-rich/coiled-coil region (Du et
720 al., 1996). *e1494* is a point mutation in the first set of EGF domains and *ok2853*
721 perturbs the second set of EGF domains via base pair deletions. (B) *mec-9* short
722 transcript is 502 aa (Du et al., 1996): the promoter for the *mec-9* short transcript located
723 in *mec-9* long 9th intron. We used the 2kb upstream sequence with a C terminal GFP
724 tag to construct *mec-9Lp::GFP* transcriptional reporter. We used the 1.5kb *mec-9* long
725 intron 9 sequence with a C terminal GFP tag to construct *mec-9Sp::GFP* transcriptional
726 reporter. (C) Ciliated sensory neurons expressed *mec-9Sp::GFP*: CEMs, IL2, amphids,
727 RnB, HOB, phasmids. Male (top), hermaphrodite (bottom). Ventral nerve cord (non-
728 ciliated neurons) expression also observed and shown. (D) *mec-9Sp::GFP* coexpressed
729 with the kinesin-3 gene, *klp-6p::tdTomato* transcriptional reporter. *klp-6p::tdTomato*
730 expressed in male-specific CEM and IL2 neurons in the head and RnB and HOB male-
731 specific neurons in the male tail. Left column is lateral view of male head. *mec-*
732 *9Sp::GFP* expressed in CEMs and IL2 neurons. Right column is lateral view of male
733 tail. *mec-9Sp::GFP* expressed in HOB and RnB neurons. (E) Some *mec-9Sp::GFP*
734 expressing ciliated neurons filled with Dil. Dil is a lipophilic dye taken up by amphids
735 and phasmids. Left column is lateral view of hermaphrodite head. *mec-9Sp::GFP*
736 expresses in amphids in the hermaphrodite and male head. Right column is lateral view
737 of hermaphrodite tail. *mec-9Sp::GFP* expressed in phasmids.

738 Figure 4



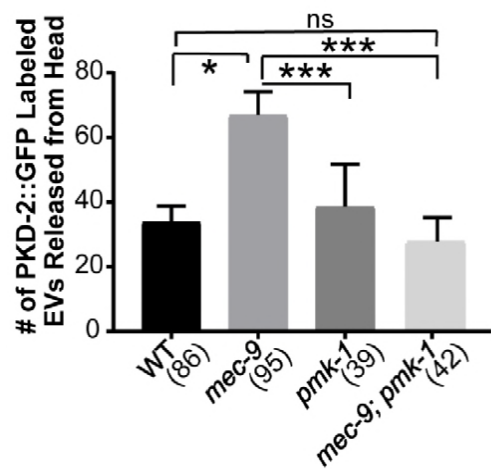
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740

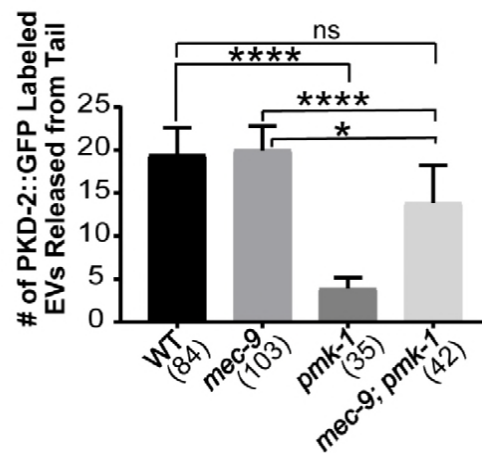
741 **Figure 4 *mec-9(ok2853)* males produce excessive EVs and matrix-filled vesicles.**
742 (A-B) Head images of WT and *mec-9(ok2853)* mutant males show EVs were released.
743 Image brightness was increased and inset was magnified to visualize EVs. Scale bars
744 10 μ m. White arrow, ciliary base; white arrow head, cilium; yellow arrow, cell body;
745 yellow arrowhead, dendrite. (B) *mec-9(ok2853)* males produced significantly more PKD-
746 2::GFP tagged EVs than WT (A). (C) *mec-9(ok2853)* released significantly more PKD-
747 2::GFP-tagged EVs. Significance determined by Mann-Whitney test; *** $p < 0.001$ (D)
748 Schematics of WT and *mec-9* mutant cephalic sensilla. In WT, EVs are released from
749 cilium and are stored in lumen created by the sheath glial cell. Dashed lines (E-G)
750 denote cross section level observed in images 4E, F, and G. *mec-9(ok2853)* mutants
751 store and release excess EVs and contain dark and light matrix vesicles that contain
752 EVs. (E-F) Cross section of the cephalic sensillum at the level of the transition zone in
753 WT and *mec-9(ok2853)* males. CEM neurons shaded green, CEP neurons shaded
754 blue. Scale bars 500 nm (E) Two EVs (arrows) observed in WT; one in the lumen and
755 one in CEM cilium. (F) *mec-9(ok2853)* had a distended lumen with a significant
756 increase of EVs and a lightly shaded matrix filled vesicle itself containing EVs. (G) Dark
757 electron-dense matrix filled vesicles contained EVs. These vesicles were located at the
758 level of the distal dendrite (See dotted line marked G in Figure 4D).

759 Figure 5

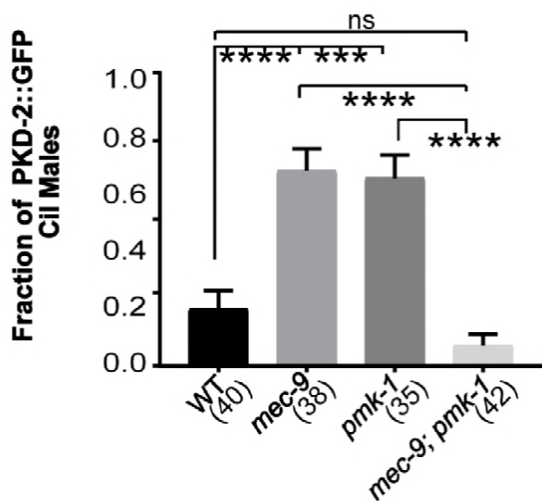
A.



B.

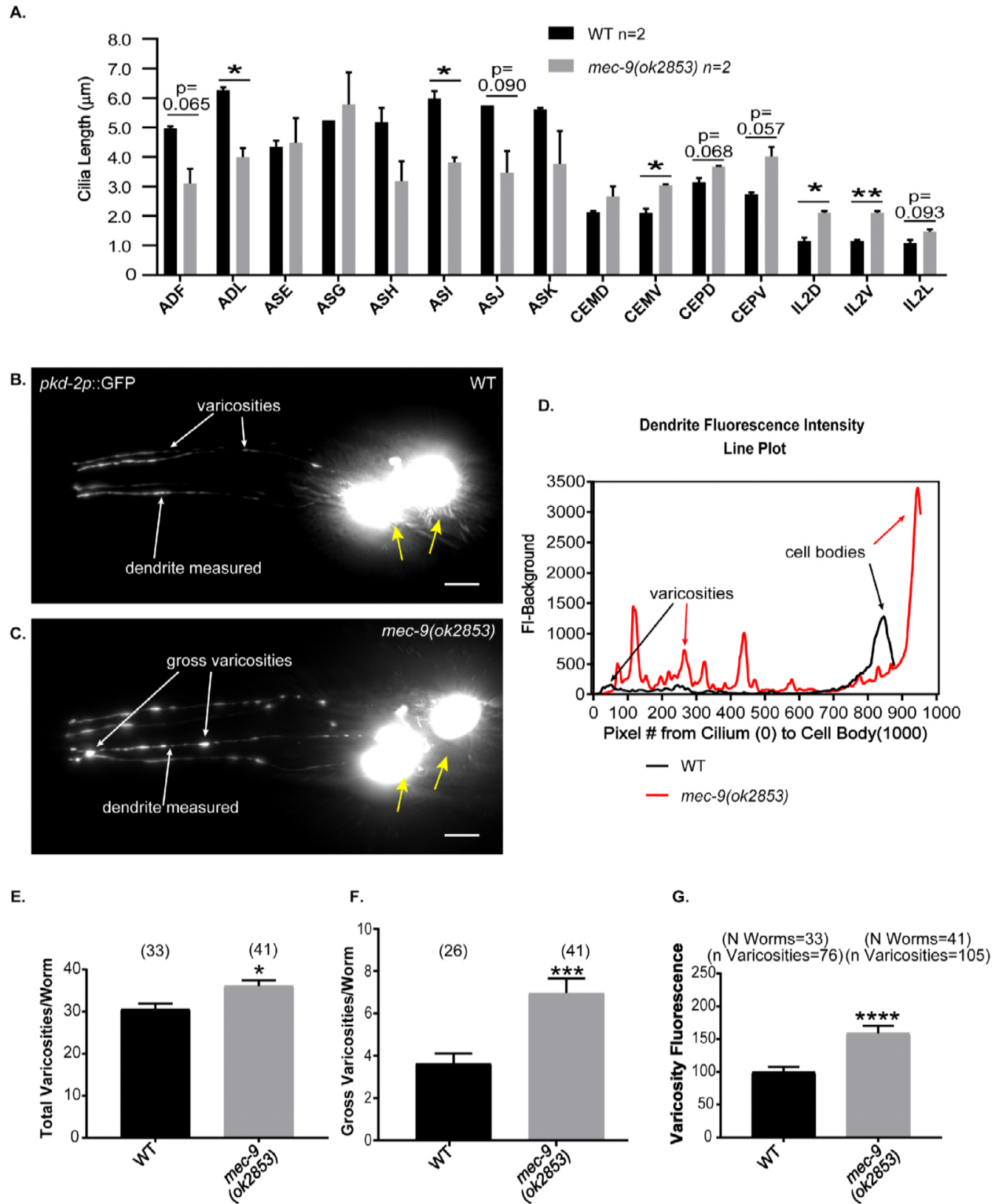


C.



761 **Figure 5. *mec-9* and *pmk-1* double mutant genetically suppressed single mutant**
762 **EV biogenesis/release and PKD-2::GFP Cil phenotypes.** (A) p38 MAPK *pmk-*
763 *1(km25)* mutants are defective in RnB EV release (Wang et al., 2015). *pmk-1* mutation
764 suppressed the *mec-9(ok2853)* abnormal EV hypersecretion in the head. (B) *mec-9*
765 suppressed the *pmk-1* RnB EV release defect. (C) *pmk-1; mec-9* double mutant
766 suppressed PKD-2::GFP Cil defect observed in both *mec-9* and *pmk-1* single mutant
767 males. Significance was determined by Kruskal-Wallace test and Dunn's multiple
768 comparisons test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

769 Figure 6



771 **Figure 6. *mec-9* regulated neuron ciliary and dendritic anatomy** *mec-9* regulated
772 CEM, IL2, and amphid channel ciliary lengths and maintained CEM neuronal integrity.
773 (A) Amphid, double ciliated (ADL, ADF), amphid single ciliated (ASE, ASG, ASI, ASJ,
774 ASK), cephalic (CEP) and male-specific CEM (ventral and dorsal), and inner labial (IL2)
775 neurons were measured using serial sectioned electron micrographs (each layer~70
776 nm). Lengths were determined by counting number of 70 nm layers from transition zone
777 to ciliary tip of each cilium. *mec-9(ok2853)* amphid cilia were shorter than WT and *mec-*
778 *9(ok2853)* CEM, CEP and IL2 cilia were longer than WT cilia. Significance measured by
779 Kruskal-Wallis test and Dunn comparisons test. * $p < 0.05$, ** $p < 0.01$ (B-D.) *mec-9*
780 maintained dendritic integrity. Images are maximum intensity Z-stacks of WT and *mec-*
781 *9(ok2853)* males that expressed a *pkd-2p::GFP* transcriptional reporter. Images were
782 brightened to observe dendritic morphology. Scale bars are 10 μm . Cell bodies denoted
783 by yellow arrow. (B) Varicosities were observed in WT male CEMs, examples denoted
784 by arrows. (C) *mec-9* mutant males have more varicosities. Large, “gross” varicosities
785 denoted by arrows. (D) A line plot of WT (black) and *mec-9(ok2853)* (red) dendritic
786 fluorescent intensity disclosed increased number of varicosities (E), increased gross
787 varicosities (F), and that varicosities had a greater fluorescence. FI measured here
788 using transcriptional reporter which allows for observation of neuron morphology only,
789 not protein abundance measurements (G). Total varicosities and gross varicosities
790 measured by blind study. Significance measured by Mann-Whitney test. Varicosity
791 Fluorescence (G) normalized to 100. * $p < 0.05$, *** $p < 0.001$, **** $p < 0.0001$.

792 **Table 1**

793 ***mec-9* regulated PKD-2::GFP particle abundance and velocity in CEM dendrites**

	MEAN ± SEM	n	N	P VALUE	DIFF. FROM WT
TOTAL PARTICLES/μM					
WT	82.66 ± 5.199	48	18		
<i>mec-9(ok2853)</i>	63.97 ± 2.976	83	23	0.0241	-18.68 ± 5.559
ANTEROGRADE VELOCITY					
WT	0.8215 ± 0.007	4654	18		
<i>mec-9(ok2853)</i>	0.7496 ± 0.005	4765	23	<0.0001	-0.07186 ± 0.009
RETROGRADE VELOCITY					
WT	-0.7607 ± 0.006	4971	18		
<i>mec-9(ok2853)</i>	-0.6551 ± 0.004	5019	23	<0.0001	-0.1056 ± 0.008

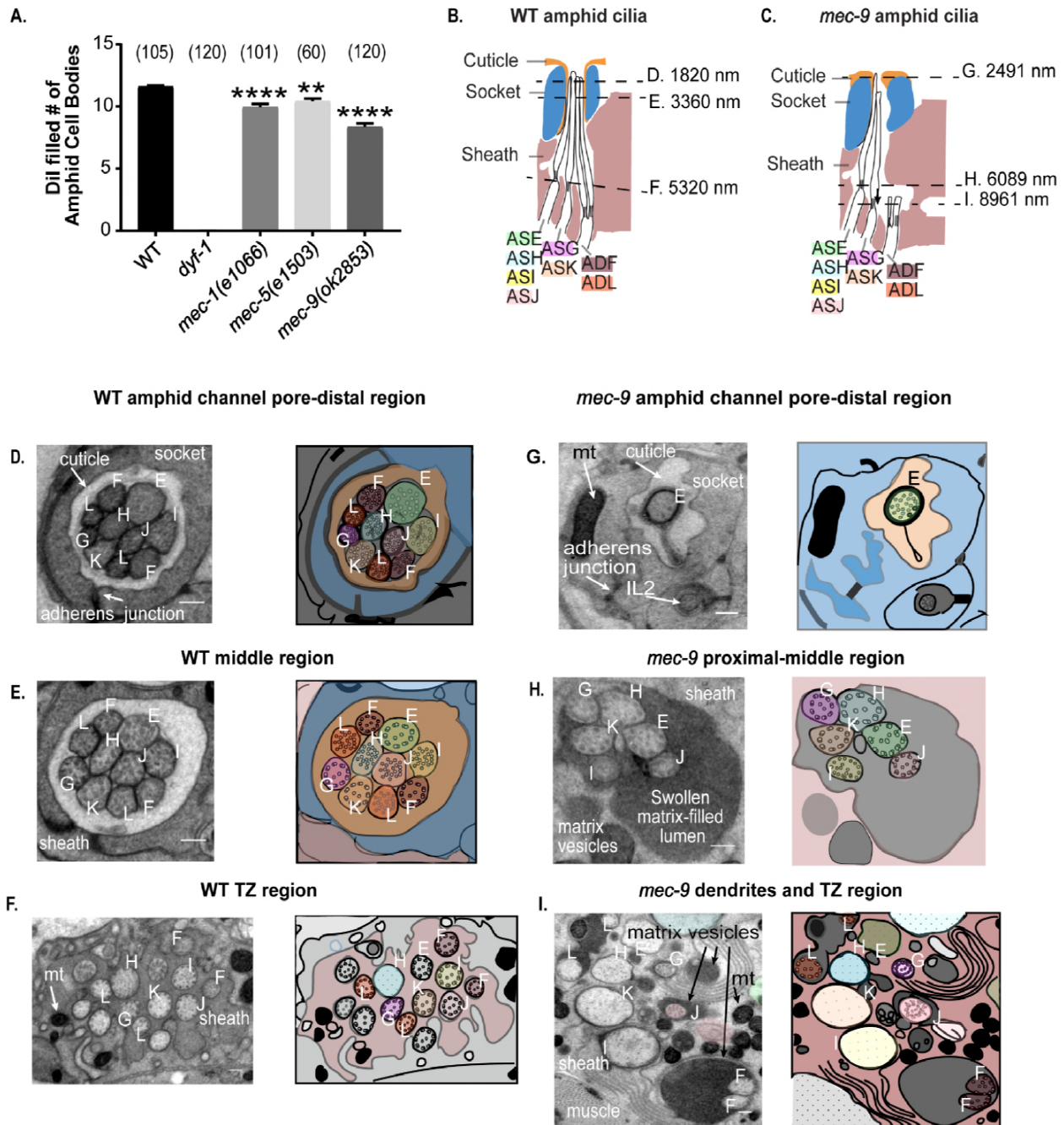
794

795 **Table 1. *mec-9* regulated PKD-2::GFP particle abundance and velocity in CEM**

796 **dendrites** The number and velocity of PKD::GFP particles was measured using time
 797 lapse fluorescent videos. TOTAL PARTICLES/μM: The average number of anterograde
 798 and retrograde PKD-2::GFP particles moving along the dendrite are reported here in
 799 particles/μm. *mec-9(ok2853)* mutants have significantly fewer particles than WT.

800 ANTEROGRADE AND RETROGRADE VELOCITY: Anterograde and retrograde PKD-
 801 2::GFP particles velocities were measured in microns per second along the entire CEM
 802 dendrite. In *mec-9(ok2853)* males, PKD-2::GFP overall dendritic anterograde and
 803 retrograde particle velocity was slower than WT. Time lapse exposure per frame: 300
 804 ms. Significance measured using Mann-Whitney test for particle number and Kruskal-
 805 Wallace test with Dunn's comparison test for velocities.

806 Figure 7



807

808 **Figure 7. *mec-1*, *mec-5*, and *mec-9* mutants are dye filling defective and *mec-9* is**

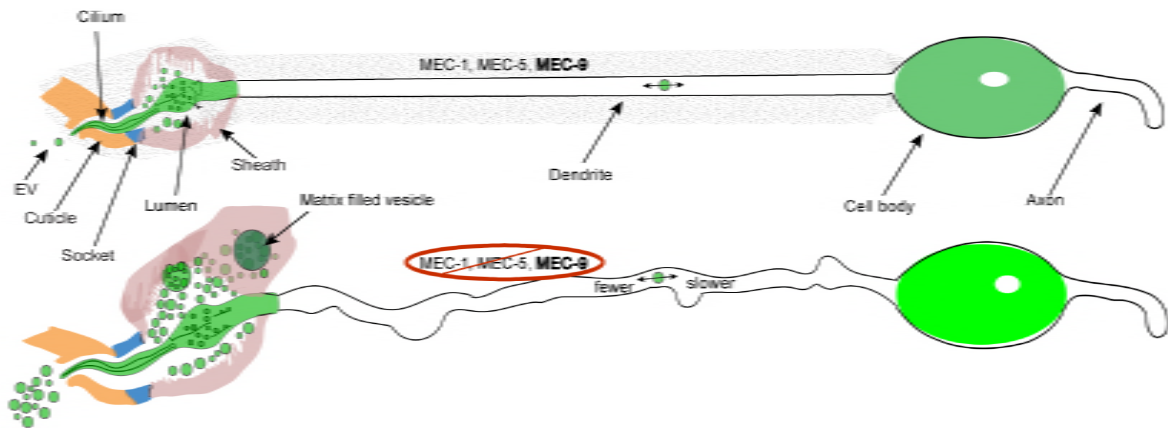
809 **necessary for amphid cilia organization and ECM deposition. (A) Number of**

810 **amphid neurons out of 12 that filled with dye. (B-C) Schematics of WT and *mec-***

811 *9(ok853)* amphid sensory organ (not all neurons shown). Proximal and medial sections
812 of WT amphid cilia were in the sheath. Distal cilia were surrounded by socket and
813 inverted cuticle with cilia tips exposed to the environment. Dashed lines denote levels
814 of cross sections shown in (D-I). (C) *mec-9(ok2853)* amphid cilia were short and
815 displaced with few ciliary tips exposed to the environment. (D) Left amphid channel
816 pore and its schematic showing ten cilia at level of singlet microtubules. WT double
817 ciliated amphids were found dorsally and ventrally in the pore with the ADF (medial) and
818 ADL (lateral). The WT single ciliated amphids are found in the center two rows of
819 amphids. The top row of single ciliated amphid channel neurons from medial to lateral
820 are ASE, ASH, and ASG. The bottom row: ASI, ASJ, and ASK. WT left and right (not
821 shown) amphids are mirror images of each other. Neurons are labeled by final amphid
822 letter. (E) WT middle segment contained 10 cilia with microtubules arranged
823 concentrically. (F) We observed the transition zones of most WT amphid cilia at this
824 level and they were embedded within the amphid sheath. (G) Only one cilium out of ten
825 was visible in *mec-9* mutant channel pore. Socket and sheath had abnormal
826 morphology as compared to WT. (H) *mec-9* mutant middle segment: six out of ten cilia
827 were visible in the lumen and there was increased matrix. *mec-9* mutant amphids were
828 abnormally arranged in three rows of two cilia. The top row: ASG and ASH, the second
829 row: ASK and ASE, and the bottom row: ASI and ASJ, all of which are single ciliated
830 amphids. Increased numbers of matrix filled vesicles were observed. (I) The double
831 ciliated amphids (ADFs and ADLs) were in adjacent, aberrant electron dense spaces
832 (increased matrix). Scale bar 200 nm.

833 Figure 8

834



835

836

837

838 **Figure 8. MEC-1, MEC-5, and MEC-9 ECM influence ciliated neuronal structure,**

839 **neuronal transport, and neuronal function.** The ECM genes *mec-1*, *mec-5*, and *mec-*

840 *9* regulate PKD-2::GFP localization and male mating behaviors (Figure 1 and 2). *mec-9*

841 also is necessary for PKD-2::GFP dendritic transport (Table 1), negative regulation of

842 EV biogenesis, storage, and release (Figure 4), and neuron anatomy, such as dendritic

843 integrity (Figure 6), cilia length (Figure 6) and organization (Figure 7), and matrix

844 deposition (Figures 4G and 7H-I).

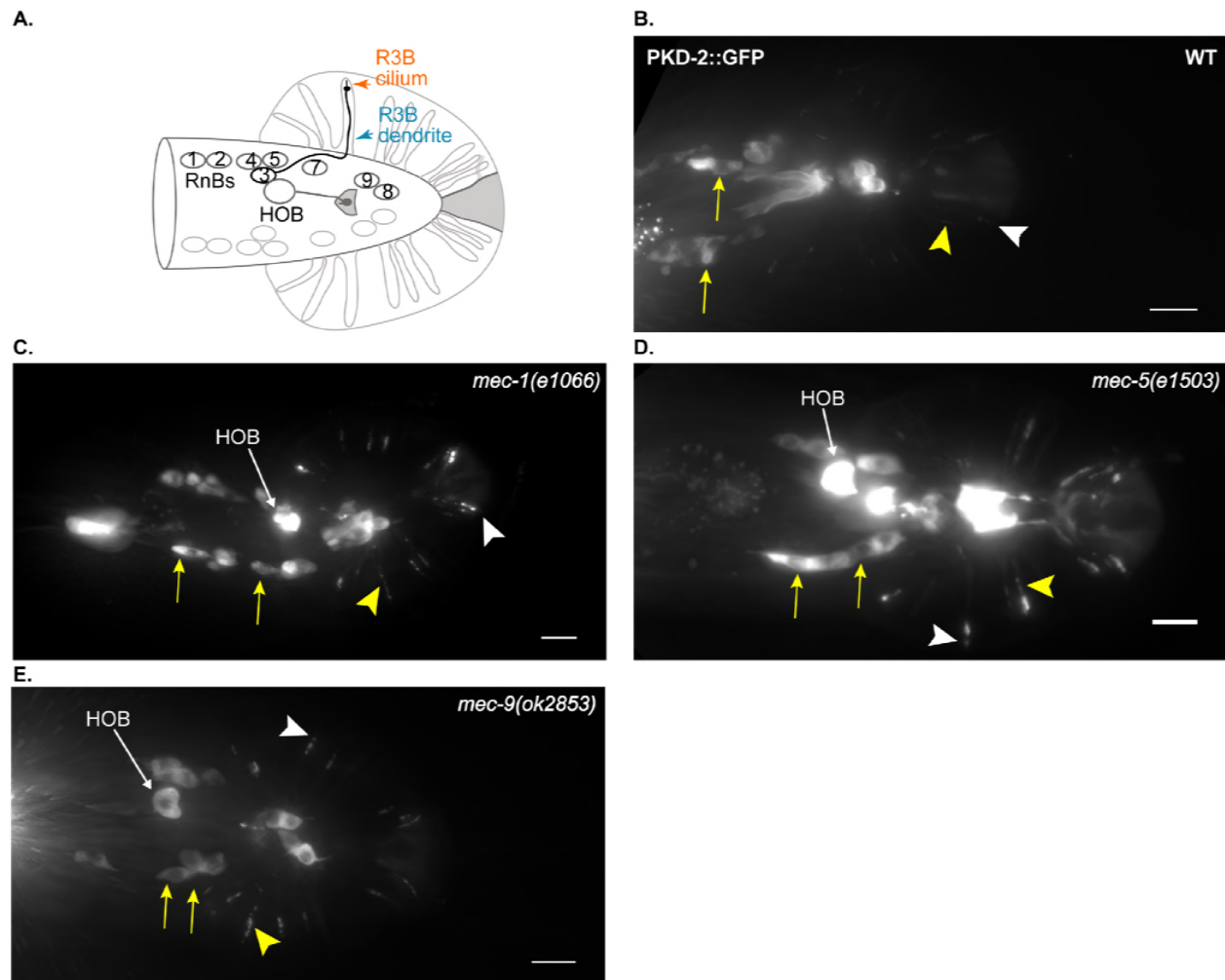
845 Supplemental Figures-

846 **Table S1 ECM Mutant Analyses of Polycystin Fluorescence Intensity (FI)**

847 (↑ Increased FI, ↓ Decreased FI, *= p value)

	WT n=54	Cil=cilium CB=cell body Den=dendrite BG=background	<i>mec-1</i> (<i>e1066</i>) n=72	<i>mec-5</i> (<i>e1503</i>) n=31	<i>mec-9</i> (<i>ok2853</i>) n=59	
CEM (head)	cilium/cell body	Cil/CB mean	↓ *	ns	↑ ****	
		Cil/CB max	↓ *	ns	↑ **	
		Den/CB mean	↓ *	↑ *	↓ ****	
		Den/CB max	↑ *	↑ **	ns	
	FI-background	Cil-BG mean	ns	ns	↑ ****	
		Cil-BG max	ns	ns	↑ ****	
		Den-BG mean	ns	ns	↑ ****	
		Den-BG max	↑ ***	ns	↓ ****	
		CB-BG mean	↑ *	↓ *	↑ ****	
		CB-BG max	ns	↓ ****	↑ ****	
	RnB (tail)	cilium/cell body	Cil/CB mean	ns	ns	ns
			Cil/CB max	↓ **	ns	↑ *
Den/CB mean			↓ *	↑ *	↓ *	
Den/CB max			ns	ns	ns	
FI-background		Cil-BG mean	ns	ns	↑ *	
		Cil-BG max	↓ *	↓ ****	↑ ***	
		Den-BG mean	ns	ns	ns	
		Den-BG max	ns	ns	↑ **	
		CB-BG mean	ns	ns	↑ *	
		CB-BG max	ns	ns	↑ **	

848 **Table S1 *mec-1*, *mec-5* and *mec-9* mutants regulate PKD-2::GFP abundance in**
849 **male specific neurons of the head (CEM) and tail (RnB).** CEM and RnB ratio
850 (cilium/cell body or part of neuron specified) of maximum or mean fluorescence intensity
851 (as denoted in table) showed that PKD-2::GFP abundance in *mec-1*, *mec-5* and *mec-9*
852 mutants was variable. For example (cilium/cell body) of maximum intensity showed that
853 PKD-2::GFP abundance in CEM cilia is increased in comparison with the cell bodies
854 only in *mec-9* ECM gene mutants (Figure 1J); however, *mec-1* and *mec-5* alleles also
855 affected PKD-2 abundance. Background measurements were subtracted from cilium
856 and cell body values for standardization of images and we expressed the
857 measurements in ratio of cilia to cell body FI. Significance was measured by Kruskal-
858 Wallace test, comparisons made using Dunn's multiple comparisons. Wild type animal
859 values were normalized to 100. The *mec-9* mutants had the brightest maximum FI
860 when compared than WT * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

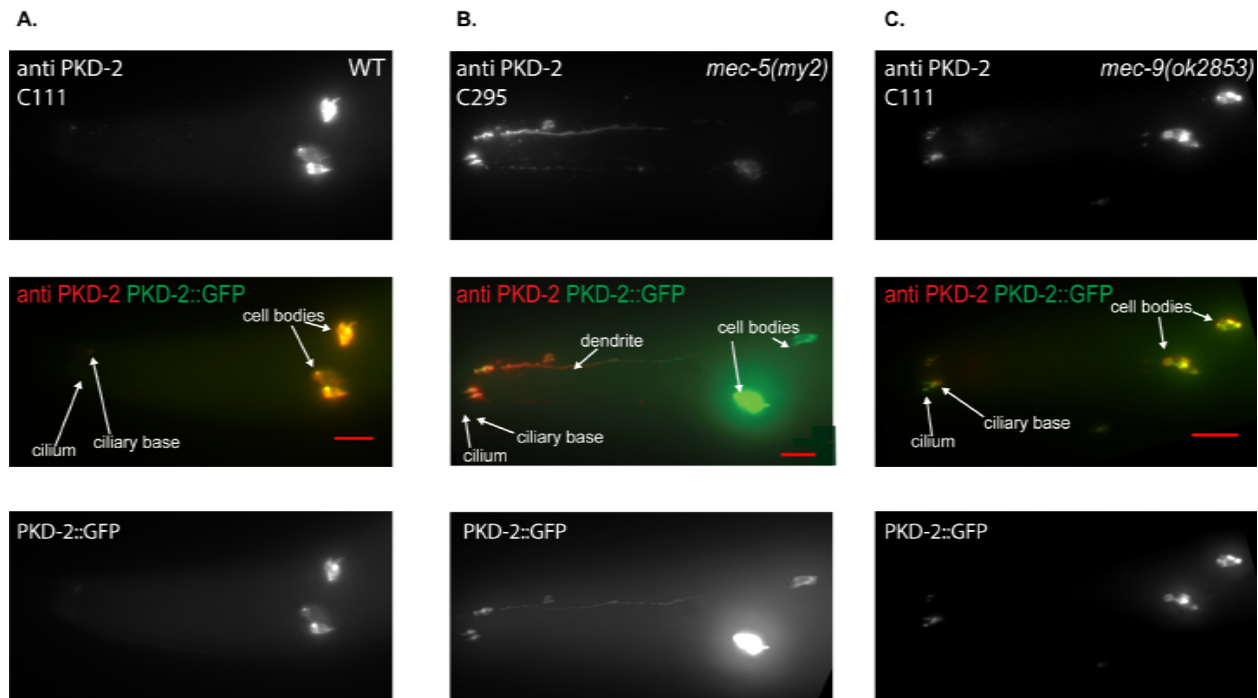


861

862 **Figure S1 Alleles of *mec-1*, *mec-5* and *mec-9* regulate PKD-2::GFP localization**
863 **and abundance in RnBs.** A. Schematic of WT RnBs and HoB male neurons in the tail
864 of a male (green). (B-E) Images were compiled from a 630x maximum intensity Z-
865 series obtained by fluorescent microscope. Scale bar is 10 μ m. White arrow head,
866 cilium; cell body, yellow arrow; dendrite, yellow arrowhead. (B) A ventral view of WT
867 PKD-2::GFP (translational reporter). GFP localized to RnB and HOB cell bodies and
868 cilia. (C) Dorsal view of *mec-1(e1066)* here showed increased PKD-2::GFP at RnB cilia
869 and ciliary base but statistically there was decreased FI (Supplemental Table 1). (D)
870 *mec-5(e1503)* PKD-2::GFP ciliary localization showed increased PKD-2::GFP at RnB

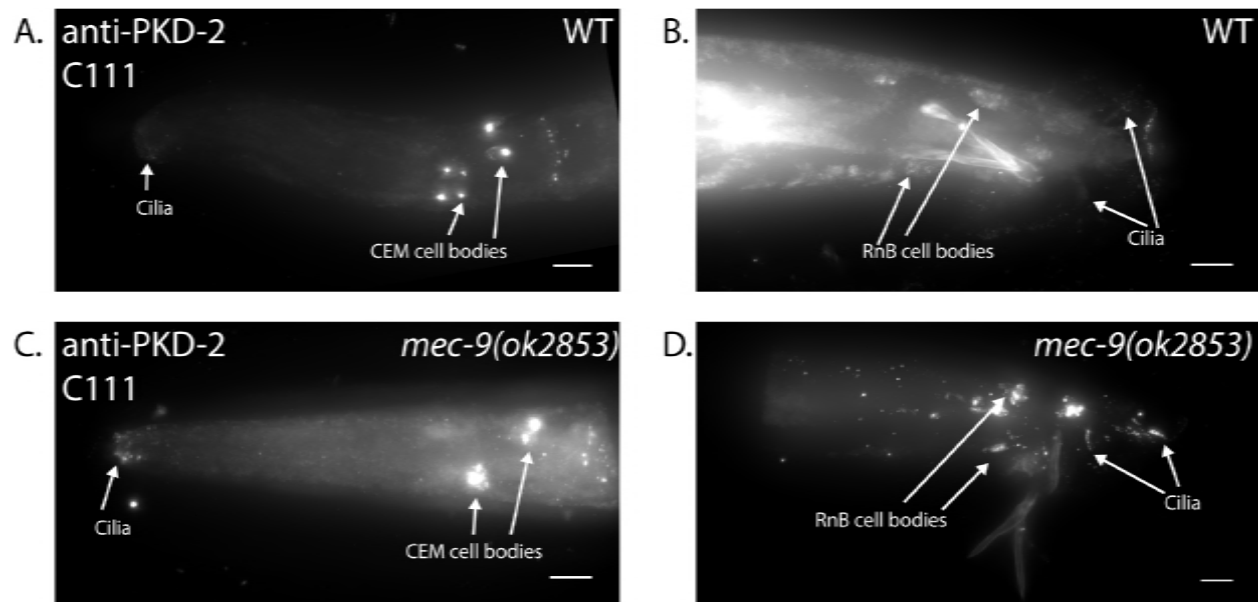
871 cilia and ciliary base but overall there was no statistical difference from WT
872 (Supplemental Table 1). (E) We observed increased PKD-2::GFP at *mec-9(ok2853)* at
873 RnB cilia and ciliary base and a significant increase in FI (Supplemental Table 1).

874



875

876 **Figure S2. Anti-PKD-2 antibody stained CEM neurons.** Antibody fluoresces with
877 PKD-2::GFP in CEM neuron. Anti-PKD-2 in *mec-5(my2)* males have characteristic
878 extradendritic accumulation seen in both antibody and PKD-2::GFP. Shown here are
879 two different antibodies. Clone C111 is generated to an epitope at the N-terminus of
880 PKD-2. This PKD-2 antibody aggregated in the cell body of WT (A) and *mec-9(ok2853)*
881 (B). Clone C295, was also generated to an epitope at the N-terminus. C295 is a non-
882 aggregating PKD-2 antibody that was used in *mec-5(my2)* (B). We observed no overt
883 differences in PKD expression or intensity in *mec-9(ok2853)* when anti-PKD-2 and
884 PKD-2::GFP are compared. Scale bar is 10 μ m.



885

886

887 **Figure S3. N-terminal anti-PKD-2 (C111) antibody showed increased, endogenous**

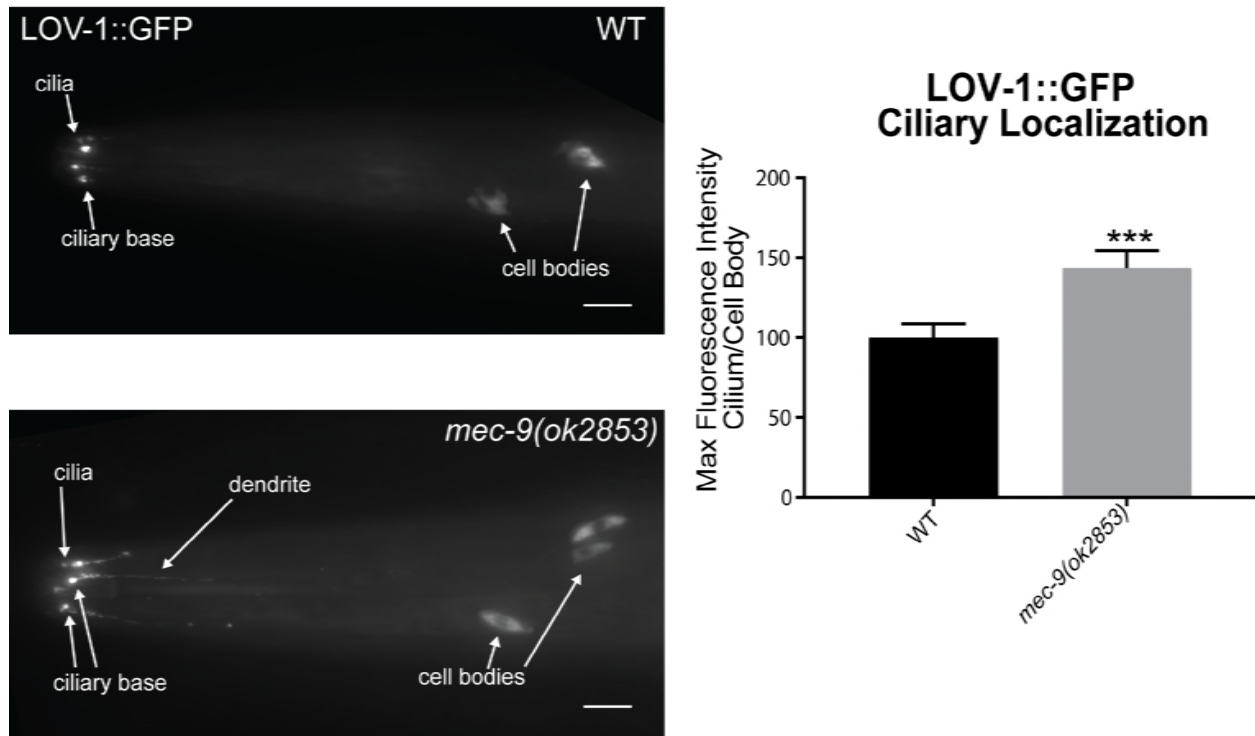
888 **localization at male specific CEM and RnB cilia.** In WT, endogenous PKD-2 was

889 limited to the cell bodies and cilia of CEM head neurons (A) and ray RnB and hook HOB

890 tail neurons (B). *mec-9(ok2853)* males are Cil (C) and endogenous PKD-2 mislocalized

891 to dendrites and (D). Scale bar 10 μm

892



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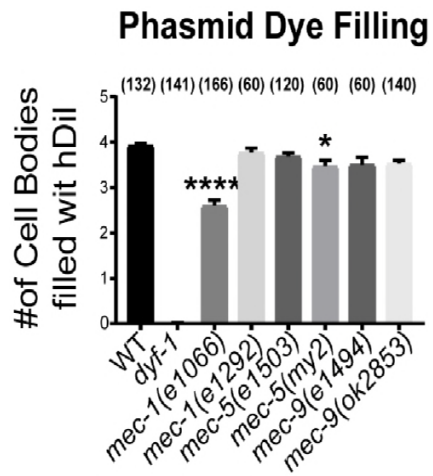
896 **Figure S4 MEC-9 regulates LOV-1::GFP ciliary localization** LOV-1::GFP

897 mislocalized to CEM distal dendrites in mutant animals. Scale bar is 10 μ m. Quantitative

898 measurement of fluorescence intensity in *mec-9* mutants revealed an abundance of

899 LOV-1::GFP at the cilia and cilium proper of male specific CEM neurons when in ratio to

900 CEM cell body. Significance measured by Mann-Whitney test. ** $p < 0.01$



901

902

903

904 **Figure S5 *mec-1(e1066)* and *mec-5(e1503)* phasmids are Dyf.** In *mec-1(e1066)* and

905 *mec-5(e1503)*, one-two out of four phasmid cells did not fill. Significance measured by

906 Kruskal-Wallis test with Dunn comparisons. ****= <0.0001 , * $p<0.05$.

907

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