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2 **Meiosomes, folded membrane platforms, link the epidermis to**
3 **the cuticle in *C. elegans***

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19 **Abstract**

20 Apical extracellular matrices (aECMs) form a physical barrier to the environment. In *C. elegans*, the
21 epidermal aECM, the cuticle, is composed mainly of different types of collagen, associated in circumferential
22 ridges separated by furrows. Here, we show that in mutants lacking furrows, the normal intimate connection
23 between the epidermis and the cuticle is lost, specifically at the lateral epidermis, where, in contrast to the
24 dorsal and ventral epidermis, there are no hemidesmosomes. At the ultrastructural level, there is a profound
25 alteration of structures that we term “meiosomes”, in reference to eisosomes in yeast. We show that
26 meiosomes are composed of stacked parallel folds of the epidermal plasma membrane, alternately filled with
27 cuticle. We propose that just as hemidesmosomes connect the dorsal and ventral epidermis, above the
28 muscles, to the cuticle, meiosomes connect the lateral epidermis to it. Moreover, furrow mutants present
29 marked modifications of the biomechanical properties of their skin and exhibit a constitutive damage response
30 in the epidermis. As meiosomes co-localise to macrodomains enriched in phosphatidylinositol (4,5)
31 biphosphate, they might act, like eisosomes, as signalling platforms, to relay tensile information from the
32 aECM to the underlying epidermis, as part of an integrated stress response to damage.

33 Introduction

34 Apical extracellular matrices (aECMs) are associated with all epithelia and are essential for animal life. In
35 *C. elegans*, a collagen-rich aECM covers the entire surface of the worm, and is called the cuticle. It is a
36 complex multilayer structure that acts as an exoskeleton, to which body-wall muscles are connected via
37 structures called hemidesmosomes that traverse the intervening epidermis (Davies & Curtis, 2011; Johnstone,
38 2000; Page & Johnstone, 2007). Specific subsets of the more than 170 collagens are enriched in the different
39 layers of the cuticle. Some are involved in the formation of distinct structures, including the circumferential
40 parallel furrows that cover the entire animal (Cox & Hirsh, 1985; Cox et al., 1980; McMahon et al., 2003;
41 Page & Johnstone, 2007; Thein et al., 2003), and the longitudinal lateral alae. The latter have been proposed
42 to be involved in facilitating the traction of *C. elegans* to its substrate during locomotion, although, notably
43 they are not present from the L2 through the L4 larval stages (Cox et al., 1981; Katz et al., 2022).

44 The cuticle also constitutes a physical barrier, protecting the underlying epidermal syncytium from biotic and
45 abiotic stresses. When the cuticle is damaged, mechanically or through infection, the epidermis reacts,
46 activating an immune response, reflected by the increased expression of antimicrobial peptide (AMP) genes,
47 including those of the *nlp-29* cluster (Belougne et al., 2020; Pujol, Cypowyj, et al., 2008; Taffoni et al., 2020).
48 An elevated level of AMP gene expression is also observed in a subset of mutants that affect the cuticle,
49 specifically those that lack furrows (Dodd et al., 2018; Pujol, Zugasti, et al., 2008; Zugasti et al., 2016). These
50 6 known furrowless mutants (*dpy-2, 3, 7, 8, 9, and 10*) exhibit other characteristic physiological alterations,
51 including an activation of detoxification genes, dependent on the Nrf transcription factor SKN-1, and the
52 induction of genes required for osmolyte accumulation (Dodd et al., 2018).

53 If the pathway leading to AMP induction in the epidermis is well described (reviewed in (Martineau et al.,
54 2021)), exactly how the epidermis senses cuticular damage remains obscure. Part of the induction seen in
55 *dpy-10* mutants is the consequence of an increase in the levels of hydroxyphenyllactic acid (HPLA). This
56 metabolite, derived from tyrosine by transamination and reduction, activates the G-protein coupled receptor
57 (GPCR) DCAR-1 (Zugasti et al., 2014), switching on a signalling cascade that leads to AMP gene expression
58 (Polanowska et al., 2018; Zugasti et al., 2016). What provokes elevated HPLA levels in *dpy-10* mutants is,
59 however, currently unknown. Further, the HPLA/DCAR-1 signalling pathway only accounts for part of the
60 elevated *nlp-29* expression seen in furrow collagen mutants (Zugasti et al., 2014). We have therefore proposed
61 that a hypothetical, cuticle-associated, damage sensor exists that would control, in an as yet undefined
62 manner, AMP gene expression. This damage sensor would also function to coordinate antimicrobial responses
63 with the distinct detoxification and hyperosmotic responses that are simultaneously activated in furrow
64 collagen mutants (Dodd et al., 2018; Rohlfing et al., 2010; Wheeler & Thomas, 2006).

65 In yeast, eisosomes, single invaginations of the plasma membrane underneath the aECM, the cell wall, are
66 responsible for detecting changes in nutrient availability, but also cell wall integrity and membrane tension.

67 They relay information primarily via BAR domain proteins to induce the appropriate responses to
68 environmental changes (Appadurai et al., 2020; Lanze et al., 2020; Moseley, 2018). While nematodes lack
69 eisosomes, the apical plasma membrane of the epidermis, which is in direct contact with the aECM (the
70 cuticle), is characterised by localised regions of folds that can be observed by electron microscopy (Wood,
71 1988). Given their superficial similarity, we refer to these structures as meisosomes, for multifold-eisosomes.
72 In this study, we undertook a detailed ultrastructural analysis of meisosomes in adults, as well as
73 characterising them during development, and in furrowless mutants. This mutant analysis proposed a role for
74 meisosomes in maintaining the structural integrity of the cuticle and the epidermis, and has opened the way to
75 future, more detailed, characterisation of their function.

76 Results

77 Meisosomes: epidermal plasma membrane folds interfacing the apical ECM

78 The stacked organelles that we refer to as meisosomes were mentioned during early electron microscopy
79 characterisation of *C. elegans* (Wood, 1988). A survey of the long transverse transmission electron
80 microscopy (TEM) series “N2U” from the MRC archive (White et al., 1986), which is of a 4-5 day old adult
81 hermaphrodite, found hundreds of meisosomes across the 400 odd available transverse prints in the midbody.
82 As a first step in the detailed investigation of meisosomes, we undertook a focused TEM study to determine
83 their 2D organization and their 3D structure. Meisosomes are repeated folded structures at the interface of the
84 aECM (the cuticle) and the epidermis (Figure 1). They can be found in similar locales at all larval stages,
85 predominantly in the epidermal syncytium hyp7 at the lateral, dorsal and ventral ridges, and in the tail tip
86 epidermal cells. They are not present on the basal side of the epidermis, nor in the seam cells, the rectal
87 epithelia, nor in the pharynx (Figure 1 & Figure 1-figure supplement 1). In adults, meisosomes typically
88 comprised 4 to 10 closely apposed parallel folds of the plasma membrane, although we observed some with
89 up to 30 folds (Figure 1C). Most folds formed an indentation 200-400 nm deep (Figure 1C-G). The folds were
90 regularly spaced. The gap between each cytoplasmic-facing plasma membrane fold was 35 nm, 75 % wider
91 than that between the folds made from cuticle-facing plasma membrane (20 nm) (Figure 1G). The
92 cytoplasmic faces of the folds were free of ribosomes but contained dense material close to the plasma
93 membrane, separated by a thin less electron-dense area (Figures 1G). Meisosomes were frequently found in
94 close proximity to mitochondria (85 %, n=355) (Figures 1C-E). On their apical side, some folds were found
95 close to a furrow (Figure 1C&E). Although very variable in a single worm, meisosomes of similar dimensions
96 were observed in both transverse and longitudinal sections (Table 1), consistent with a random orientation
97 relative to the animal’s antero-posterior axis. This random orientation was clearly visible in electron
98 micrographs of freeze-fractured samples (Figure 1-figure supplement 2A-B). It contrasted with a much more
99 regular pattern in moulting larvae, in which meisosomes were in-between the position of circumferential
100 furrows, (Figure 1-figure supplement 2C-D). As described below, a similar organisation could be observed

101 through moulting using *in vivo* markers. Some much smaller meisosomes, typically with only 2-4 shallow
102 folds were seen in the thin epidermal tissue that lies between body-wall muscles and the cuticle (dorsal and
103 ventral epidermis, see Figure 1A) and that is largely devoid of cytoplasmic content (Figure 1-figure
104 supplement 3).

105 To understand meisosomes' 3D structure, we undertook a tomographic analysis on serial 350 nm-thick
106 sections. This approach confirmed the existence of groups of parallel folds, all found in continuity with the
107 plasma membrane (Figure 2). The tomographic analysis also revealed variability in the geometry of the folds.
108 Although most groups of folds were perpendicular to the apical surface, some were tilted. The folds had a
109 relatively uniform depth, but were deeper at the centre of each stack. No break in the plasma membrane was
110 observed on neither the apical nor the basal side of the meisosomes. Despite their close apposition with
111 mitochondria, no membrane continuity was observed between meisosomes and mitochondria (Figure 2A-D,
112 Figure 2 - Video 1).

113 In order to evaluate not just the topology, but also the distribution of these organelles, we developed a fixation
114 protocol for serial block-face scanning electron microscopy (SBF-SEM) of *C. elegans* samples. Starting with
115 protocols previously described (Deerinck et al., 2010; D.H. Hall et al., 2012; D. H. Hall et al., 2012), we
116 adapted the solvents and temperatures for each contrasting agent, including lead and uranyl acetate, to
117 maximize sample contrast (see Materials and Methods). We acquired series of electron micrographs of the
118 lateral epidermis as transversal views along 12 μm . We produced a voxel-based classification within the
119 Waikato Environment for Knowledge Analysis (WEKA in Fiji) and then used its machine learning algorithms
120 to perform semi-automated organelle recognition. This revealed that the meisosomes were irregularly spaced
121 at the apical surface of the lateral epidermis, with various sizes and orientations and confirmed their frequent
122 apposition to mitochondria (Figure 2-figure supplement 1).

123 **VHA-5 is a marker of meisosomes**

124 VHA-5, one of four α -subunits of the transmembrane V0 complex of the vacuolar ATPase (V-ATPase) (Oka
125 et al., 2001; Pujol et al., 2001), and RAL-1, ortholog of human RALA (RAS like proto-oncogene A) (Frische
126 et al., 2007), are the only known markers of meisosomes, with both proteins being also associated with
127 multivesicular bodies (MVB) that play a role in exosome secretion (Hyenne et al., 2015; Liegeois et al.,
128 2006). The expression pattern of VHA-5 is the better characterised of the two proteins. A TEM/immunogold
129 staining study showed that more than 85 % of the VHA-5 signal can be attributed to meisosomes (Liegeois et
130 al., 2006).

131 We have used several VHA-5 reporter strains, including one expressing a GFP-tagged version of VHA-5 from
132 a CRISPR/Cas9 engineered genomic locus [KI], a single copy insertion under an epidermis promoter [Si], or
133 classic multi-copy integrated [Is] or extrachromosomal [Ex] transgenic arrays. In all the strains, and in line

134 with previous reports (Liegeois et al., 2006), we observed the same punctate fluorescence at the apical surface
135 of the lateral epidermis, and in the ventral and dorsal ridges, from the head to the tail. As expected, it was
136 almost completely absent from the dorsal and ventral epidermis above the body-wall muscles, and underneath
137 the seam cells (Figure 3A-C). By combining a MUP-4::GFP (Suman et al., 2019) and a VHA-5::RFP reporter
138 (Liegeois et al., 2006), we confirmed the complementary pattern in the epidermis of hemidesmosomes above
139 the muscles, and meisosomes in the lateral epidermis and dorso/ventral ridges (Figure 3D). Higher resolution
140 analysis in the lateral epidermis revealed the VHA-5-marked structures to have an irregular shape in no
141 preferred direction in the lateral epidermis, consistent with the TEM and SBF analyses (Figure 3E).

142 When we examined worms co-expressing VHA-5::GFP and HGRS-1::mScarlet, a known marker of MVBs
143 and the endosome degradation pathway (Liegeois et al., 2006; Norris et al., 2017; Roudier et al., 2005;
144 Serrano-Saiz et al., 2020), we observed essentially no co-localisation. Further, the patterns of the two markers
145 were distinct, with HGRS-1::mScarlet labelling structures that were less apical, larger and more scattered than
146 those labelled by VHA-5::GFP (Figure 3F & Figure 3-figure supplement 1A). We also did not observe any
147 co-localisation between VHA-5::GFP and SNX-1::mScarlet, a marker for the recycling endosomes, nor with
148 LGG-1::mScarlet, an autophagolysosome marker (Serrano-Saiz et al., 2020) (Figure 3-figure supplement
149 1B&C). We have previously shown that the plasma membrane of the epidermis in young adult worms
150 contains heterogeneous macrodomains that can be revealed with a prenylated, or a pleckstrin homology
151 domain-tagged form of GFP (GFP::CAAX and GFP::PH-PLC1 δ , respectively) (Taffoni et al., 2020).
152 Interestingly, in worms co-expressing VHA-5::RFP and either one of these membrane probes, we observed a
153 high degree of co-localisation (Figure 3G&H & Figure 3-figure supplement 1D-F). This further reinforces the
154 notion that VHA-5 is primarily a marker of subdomains of the plasma membrane.

155 The structures labelled with both VHA-5::GFP and CAAX::GFP or PH-PLC δ ::GFP in the adult epidermis
156 were similar in size and spatial distribution to the meisosomes reconstituted from the SBF data (Figure 2-
157 figure supplement 1D). To confirm that the observable fluorescence signal from VHA-5::GFP indeed
158 primarily originated from meisosomes, we performed Correlation Light and Electron Microscopy (CLEM)
159 using the VHA-5::GFP [Si] strain in which the strong and potentially confounding excretory canal GFP signal
160 is absent, due to the use of an heterologous epidermis-specific promoter. As we used a different fixation
161 technique to preserve the GFP signal (Johnson et al., 2015) and we worked on semi-thin section, meisosomes
162 were revealed by electron tomography. After alignment of the confocal and TEM images, we could show that
163 the fluorescence foci matched meisosomes (Figure 3I-J & Figure 3-figure supplement 2). Together with
164 previous CLEM observations in the excretory duct (Kolotuev et al., 2009), these results indicate that the
165 VHA-5::GFP signal that we observe at the apical membrane in the epidermis corresponds to meisosomes and
166 that VHA-5::GFP can be used *in vivo* as a *bona fide* meisosome marker for this study.

167 Meisosomes align in between furrows before moulting

168 VHA-5 has been shown to have an essential role in alae formation and secretion of Hedgehog-related peptides
169 through exocytosis via MVBs, but not to be involved in secretion of the collagen DPY-7, nor in meisosome
170 morphology (Liegeois et al., 2006). Indeed, to date, no gene has been assigned a role in determining
171 meisosome structure. As a path to understanding their function, we first observed their morphology during
172 development. Consistent with previous reports (Liegeois et al., 2007), we observed that VHA-5::GFP aligns
173 parallel to the actin fibres in animals entering the L4/adult moult, a stage we refer to here as “late L4”. We
174 refined this observation by precisely staging the worms throughout the L4 stage on the basis of vulval
175 morphology and the shape of the lumen as previously described in (Cohen et al., 2020; Mok et al., 2015)
176 (Figure 4A & 5). The parallel circumferential alignment of VHA-5::GFP could be observed at the beginning
177 of the L4 stage, in L4.1 worms, but was then lost at the L4.2 stage. It reappeared progressively starting in the
178 L4.3 stage, culminating between the L4.7 and L4.9 stages, just before the moult. This is consistent with the
179 EM observations of meisosome alignment before moulting (Figure 1-figure supplement 2C-D).

180 As meisosomes, like the rest of the apical plasma membrane, are in direct contact with the aECM, the cuticle,
181 we investigated the relation of meisosomes to different cuticle components. Different classes of cuticular
182 collagen exist that form either the circumferential constricted furrows, or the cuticle in the regions between
183 the furrows, called the annuli (Cox & Hirsh, 1985; Cox et al., 1980; McMahan et al., 2003; Page & Johnstone,
184 2007; Thein et al., 2003). As revealed with one marker of a furrow collagen, DPY-7::GFP (Miao et al., 2020),
185 in combination with VHA-5::RFP, meisosomes align in between the furrows at the late L4 stage (Figures 4B).
186 Notably, during the L4.7 stage, some DPY-7::GFP can be observed in small vesicles on the apical side of the
187 epidermis, that could represent the ongoing secretion of furrow collagen at that stage. Interestingly, these
188 vesicles do not co-localise with VHA-5::RFP (Figure 4B, right panel). We further show that the CAAX and
189 PH-PLC1 δ markers that co-localise with VHA-5 in young adult animals (Figure 3G-H) also align during the
190 L4 stage, but other vesicular components, like the one marked by HGRS-1, SNX-1 or LGG-1 do not (Figure
191 4C-D). Thus, meisosomes together with specific membrane subdomains align in between the furrow before
192 moulting.

193 **Furrow collagens determine the organisation of the cytoskeleton and meisosomes in L4 larvae**

194 Before moulting, there is profound reorganisation of the cytoskeleton in the lateral epidermis. Microtubules
195 and actin fibres align in a series of circumferential bands that are not present in adults (Castiglioni et al., 2020;
196 Costa et al., 1997; Taffoni et al., 2020). Interestingly, meisosomes exhibited the same sequence of dynamic
197 changes in alignment as microtubules and actin. After the moult, there was a concomitant loss of alignment of
198 VHA-5, actin and microtubules, so that in wild-type adult animals, as described above, there was no clear
199 overall pattern to the organisation of meisosomes, actin or microtubules (Figures 5).

200 We then examined the consequence of knocking down the expression of one of the furrow collagen genes,
201 *dpy-7*, on the organised VHA-5::GFP pattern of late L4s worms. Compared to the control, RNAi of *dpy-7*

202 provoked a loss of meiosis alignment (Figures 5 and Figure 5-figure supplement 1). A similar phenotype
203 was observed in *dpy-3* mutant worms; DPY-3 is another furrow collagen (Figure 5-figure supplement 1B).
204 Strikingly, this loss of expression of furrow collagens was also associated with a disruption of the normal
205 organized pattern of both actin fibres and microtubules from the L4.3 stage (Figure 5). It was previously
206 proposed that the formation of actin fibres and microtubules in apposed circumferential bands plays an
207 instructive role in positioning the furrows (Costa et al., 1997). Our results suggest, on the contrary, that furrow
208 collagens in the cuticle govern the alignment of the underlying cytoskeleton as development progresses into
209 the final moult. Thus, furrow collagens appear to be required to align both meiosis and the actin-
210 microtubule cytoskeleton.

211 **Abnormal meiosis in adult furrow mutant worms**

212 As previously mentioned, different classes of cuticular collagen exist that are expressed and form either
213 furrows or annuli (Figure 6A) (Cox & Hirsh, 1985; Cox et al., 1980; McMahon et al., 2003; Page &
214 Johnstone, 2007; Thein et al., 2003). While mutant in these collagens all have a Dumpy (i.e. short and fat;
215 Dpy) phenotype, only the furrow-less mutants, in contrast to mutants of annuli collagens, exhibit an increased
216 expression of the AMP reporter *nlp-29p::GFP* (Dodd et al., 2018; Pujol, Zugasti, et al., 2008; Zugasti et al.,
217 2014; Zugasti et al., 2016). This is one reason that furrow collagens have been proposed to be part of a
218 damage sensor that relays information about cuticle integrity to the epidermis (Dodd et al., 2018).
219 Interestingly, this reporter is also induced at the late L4 stage in the wild type before the last moult when the
220 cuticle is reshaping (Figure 6-figure supplement 1A; (Miao et al., 2020)).

221 We examined the consequences of knocking down the expression of all these different collagen genes on the
222 pattern of meiosis in the adult. Collagen inactivation was always confirmed by observing the effect on
223 body size, as well as the change in the expression of *nlp-29p::GFP* in parallel experiments (Figure 6-figure
224 supplement 1B-C). Compared to control RNAi or to annuli collagen inactivation (*dpy-4*, *dpy-5* and *dpy-13*),
225 inactivation of all 6 furrow collagen genes (*dpy-2*, *dpy-3*, *dpy-7*, *dpy-8*, *dpy-9* and *dpy-10*) provoked a marked
226 and specific alteration in the pattern of VHA-5::GFP. The meiosis' normal reticulated pattern was
227 fragmented, as reflected by a decrease in their average size and Feret's diameter, and a >25 % increase in their
228 density (Figures 6B-C). A similar fragmentation was observed with the different VHA-5 reporter strains, and
229 either following inactivation of the furrow collagen gene's expression with RNAi or in null mutants (Figure 6-
230 Source data file 1).

231 To test if the fragmentation was associated with a change in VHA-5's relation to other organelles, we
232 inactivated furrow collagen genes in strains combining VHA-5::GFP and different mScarlet-tagged vesicular
233 membrane markers, LGG-1 for autophagosomes, HGRS-1 for multivesicular bodies and SNX-1 for recycling
234 endosomes. In contrast to VHA-5::GFP, we observed no marked alteration in their patterns, and there was still
235 no overlap between the signal from VHA-5::GFP (Figure 6-figure supplement 2A). Further, neither *dpy-3* nor

236 *dpy-7* inactivation had any effect on the size and density of the vesicular pattern of EEA-1::GFP (Figure 6-
237 figure supplement 2B), a marker of early endosomes (Shi et al., 2009). Thus, loss of furrow collagen gene
238 expression leads to a substantial fragmentation of meisosomes, without affecting vesicular organelles in the
239 epidermis. This suggests that furrow collagens play an important and specific role in maintaining meisosome
240 integrity.

241 **Furrow mutants, with small meisosomes, display a detached cuticle**

242 The cuticle is connected, through the epidermis, to the underlying body-wall muscles via hemidesmosomes.
243 The maintenance of hemidesmosome integrity is vital; their complete loss causes a fully penetrant lethality.
244 On the other hand, partial loss of the hemidesmosome component MUA-3 causes the cuticle to detach above
245 the muscles (Bercher et al., 2001). We have shown above that hemidesmosomes and meisosomes are present
246 in complementary and non-overlapping regions of the epidermis (Figure 3B-D). This suggest that
247 hemidesmosomes cannot play a role in the attachment of the lateral epidermis and the dorso/ventral ridge to
248 the cuticle. As the meisosomes, containing numerous folds of the plasma membrane, increase the surface
249 contact between the epidermis and the cuticle, we asked whether meisosome fragmentation could impact the
250 attachment of the epidermis to the cuticle. Examination by TEM first confirmed that the meisosomes are
251 significantly smaller in furrow collagen mutants compared with another Dpy mutant (*dpy-13*) or the wild-
252 type, irrespective of the direction of the section, as both longitudinal and sagittal sections show the same
253 phenotype (Figure 7A-D, Table 1 and Figure 1 for the wild type). In all furrow collagen mutants examined,
254 there was a frequent disruption of the contact between the epidermal plasma membrane and the cuticle, either
255 in the lateral epidermis or the dorso or ventral ridge, but not above the muscle quadrants (Figures 7B&G and
256 Figure 7-figure supplement 1). This detachment is clearly distinct from what is observed in so-called Blister
257 mutants where, due to the absence of the connective struts, the detachment happens between the 2 main layers
258 of the cuticle (Page & Johnstone, 2007). To confirm the phenotype, we analysed by SBF entire transversal
259 worm sections over a length of 21.5 and 34.4 μm , for a wild-type for a *dpy-2* young adult mutant worm,
260 respectively. This confirmed that the detachment between the cuticle and the epidermis was always found in
261 the furrow collagen mutant outside the region of the body-wall muscles (Figures 7H). In furrow collagen
262 mutants, the space between the cuticle and the underlying epidermal cell was often filled with a diverse range
263 of cytoplasmic content, including membrane-bound vesicles with the appearance of endosomes, lysosomes,
264 mitochondria, as well as electron-dense particles the size of ribosomes (Figures 8A).

265 To exclude the remote possibility that this detachment was an artefact linked to the different fixation protocols
266 used for electron microscopy, we carried out live imaging on two independent strains in which the cuticle was
267 labelled with a collagen tagged with mScarlet (ROL-6::mScarlet [KI]) and the epidermal plasma membrane
268 was labelled with GFP::CAAX or GFP::PH-PLC1 δ . Compared to the wild-type, where the GFP signal is
269 restricted to heterogeneous macrodomains in the plasma membrane (Taffoni et al., 2020), in a *dpy-3* furrow

270 collagen mutant, the GFP was seen in numerous brightly-stained vesicular structures that accumulated outside
271 the epidermis at the level of the mScarlet cuticular signal (Figure 8B). Together, these phenotypes suggest that
272 the meisosomes may play an important role in attaching the cuticle to the underlying epidermal cell and that
273 loss of this intimate connection causes a profound alteration of epidermal integrity.

274 **Furrow mutants have abnormal biomechanical properties**

275 We predicted that the changes in cuticle attachment seen in the furrow mutants would impact the
276 biomechanical properties of worms. It was previously shown that furrows are stiffer than the rest of the cuticle
277 in wild-type worms (Essmann et al., 2016). We therefore used atomic force microscopy to measure the
278 resistance to force in wild-type and mutant worms, as previously described (Essmann et al., 2016; Essmann et
279 al., 2020). While topographic AFM imaging (Figures 9A) provided further corroboration of the fact that in the
280 absence of furrow collagens the cuticle has a disorganised aspect with irregular folds, lacking the usual
281 repeated linear pattern of annuli and furrows, force spectroscopy AFM revealed differences in stiffness. In
282 contrast to the non-furrow *dpy-13* mutant that had a rigidity similar to wild-type, the different *dpy* furrow
283 mutants (*dpy-2*, *dpy-3*, *dpy-7* and *dpy-8*) exhibited markedly less steep force-indentation curves (Figure 9B),
284 and hence lower stiffness or Young's Moduli (Figure 9C). This suggests that furrow collagens are required for
285 normal stiffness. While lack of certain collagens in the cuticle could directly affect cuticle stiffness, we
286 hypothesise that the effect on stiffness is a consequence of the fact that furrow collagens are essential for the
287 presence of normal meisosomes.

288 **Discussion**

289 In this study, we undertook the characterisation of structures that link the nematode epidermis to the cuticle.
290 Across species, interfaces exist between flexible and dynamic cell membranes and more rigid extracellular
291 matrices. Because of requirements for growth, signal transduction, and repair, the microstructures of the ECM
292 need to be tightly linked to the plasma membrane and cytoskeleton of the underlying cell (Chebli et al., 2021).
293 In yeast, eisosomes are single membrane invaginations located under the cell wall that bridge this boundary
294 and fulfil this function. They can disassemble in minutes to buffer changes in membrane tension, protecting
295 cells from osmotic shock (Lemiere et al., 2021). Eisosomes are specific to yeast; there are no orthologues for
296 core components, such as LSP-1, in animals. Conversely, the meisosomes that we describe here in *C. elegans*,
297 with their multiple membrane invaginations that individually are similar in appearance to eisosomes, are, to
298 the best of our knowledge, distinct from interfacial structures in non-nematode species. Interestingly, we show
299 here that they are enriched in a PH-PLC δ marker, which is known to bind phosphatidylinositol 4,5-
300 bisphosphate (PIP₂) (Lemmon et al., 1995). PIP₂ has a major role in signal transduction and in regulating
301 cellular processes including actin cytoskeleton and membrane dynamics (Katan & Cockcroft, 2020).
302 Moreover, we have previously shown that the same PH-PLC δ marker rapidly reorganises upon wounding of

303 the lateral epidermis (Taffoni et al., 2020). So, it is tempting to propose that analogous to eisosomes,
304 meisosomes could have a role as a signalling platform in response to stress.

305 While the presence of meisosomes had been noted in earlier studies (Hyenne et al., 2015; Liegeois et al.,
306 2006), we have been able to go beyond their previous characterisation, in part because of improvements in
307 electron microscopy techniques. Specifically, we adapted the fixation protocol after high pressure freezing to
308 have a better membrane contrast in serial block scanning electron microscopy, allowing semi-automated *in*
309 *silico* segmentation of meisosomes. Moreover, adapting a CLEM protocol, we were able to match the
310 VHA-5::GFP observed by fluorescence microscopy to meisosomes revealed by tomography. VHA-5, together
311 with RAL-1, are currently the only known meisosome components. In contrast to the well-defined roles of
312 these two proteins in alae formation and exosomes biogenesis (Hyenne et al., 2015; Liegeois et al., 2006),
313 their function in meisosomes remains to be characterised. Notably, inactivation of *ral-1* did not eliminate
314 VHA-5::GFP fluorescence in the epidermis (Hyenne et al., 2015), and knocking down the expression of *vha-5*
315 did not affect the secretion of DPY-7 (Liegeois et al., 2006). This suggests that the V-ATPase on meisosomes
316 is not involved in cuticle synthesis. Further study will be required to determine the catalogue of proteins that
317 are needed for meisosome formation and maintenance.

318 Notably, a recent study reported the isolation of mutants with an abnormal pattern of VHA-5::RFP in the
319 epidermis but attributed this to a change in MVBs (Shi et al., 2022), despite a lack of substantial co-
320 localisation with HGRS-1, a well characterised MVBs marker, part of the ESCRT-0 complex that sorts
321 endosomes to MVBs (Babst, 2011). Since previous studies (Liegeois et al., 2006), and the results presented
322 here, show that VHA-5 is predominantly a marker of meisosomes, more so than of MVBs, we hypothesise
323 that the one gene that Shi *et al.* characterised in detail, *fln-2*, which encodes the F-actin cross-linking protein
324 filamin (Zhao et al., 2019), could actually be involved in the formation and/or maintenance of meisosomes.
325 Interestingly, a *fln-2* loss of function mutation has been serendipitously found in several *C. elegans* strains
326 originating from a different wild-type stocks (Zhao et al., 2019), so careful attention to genotypes will be
327 needed in future work. Regardless, *fln-2* may represent an important tool to investigate meisosome function.

328 Setting this issue aside, by taking an ultrastructural approach, we were able to build up a detailed picture of
329 the organisation of meisosomes. One of their defining features is the constant 25 nm spacing of their
330 constituent plasma membrane folds. This raises the question of how the membrane folds with such precision.
331 One possibility is that the striking electron-dense material that is apposed to each side of the membrane on the
332 cytoplasm-facing folds, spaced less than 10 nm apart, will contain specific structural protein that maintain the
333 uniform width of each meisosome fold, and influence their mechanical properties. These structures will
334 require more precise characterisation. We equally have yet to establish whether the frequent proximity of
335 meisosomes to mitochondria, with a close apposition of membranes, has a functional significance.

336 Contrary to the cuticle of many adult insects, the nematode cuticle is flexible enough to allow bending during
337 locomotion. It is also thought to stretch to accommodate growth between moults. When the old cuticle is shed,
338 it leaves in its place the new cuticle that had been moulded underneath it. The circumferential furrows of the
339 new cuticle thus appear exactly in register with the position of old furrows. Before moulting, the cytoskeleton
340 aligns in the apical epidermis, underneath and parallel to each furrow. Although this had been proposed to be
341 important for positioning the furrows of the new cuticle (Costa et al., 1997; McMahon et al., 2003; Page &
342 Johnstone, 2007), a recent study found unexpectedly, that actin is dispensable for the alignment of furrows
343 (Katz et al., 2018). On the other hand, we found that the furrows are required for the alignment of actin fibres
344 before the last moult. We propose therefore that only the old furrows are required to pattern the new furrows.
345 Consistent with such a model, the LPR-3 protein that is part of the transient pre-cuticle that is formed between
346 the old and the new cuticles before each moult is absent from the region of the furrows (Forman-Rubinsky et
347 al., 2017). We have shown that furrow determines the regular parallel and circumferential positioning of
348 meisosomes. It is not yet clear whether this alignment of meisosomes is functionally important. It could result
349 from steric constraints during moulting, in the limited space between nascent furrows of the new cuticle and
350 the closely apposed circumferential actin fibres. It should, however, be noted that this alignment is not seen
351 for vesicular organelles like MVBs, endosome or autophagosomes.

352 As adults, furrowless collagen mutants have fragmented meisosomes and a detached cuticle. We hypothesise
353 that this fragmentation causes the detachment, and that the multiple folds of plasma membrane normally
354 increase its contact surface with the cuticle thus ensuring a more robust connection of the aECM to the lateral
355 epidermis. While the lateral epidermis is rich in meisosomes, it is devoid of hemidesmosomes. Conversely, in
356 the dorsal and ventral quadrants, there are essentially no meisosomes, but abundant hemidesmosomes. These
357 latter structures secure the muscles to the cuticle through epidermis and are indispensable for worm
358 development and viability. Above the muscles, the epidermis is extremely thin, with the apical and basal
359 plasma membranes juxtaposed, linked via intermediate filaments that bridge apical and basal hemidesmosome
360 protein complexes (Zhang & Labouesse, 2010). MUA-3 is an hemidesmosome transmembrane protein in
361 direct contact with the cuticle. In hypomorphic *mua-3* mutants, large gaps form between the apical epidermal
362 surface and the cuticle in the dorso-ventral quadrants, reflecting a loss of attachment of apical
363 hemidesmosomes to the cuticle. Unlike the cytoplasm-filled gaps we observed in furrowless mutants, in *mua-*
364 *3(rh195)* worms, these spaces appear devoid of contents, and the apical epidermal membrane is intact
365 (Bercher et al., 2001). So, in contrast to the loss of hemidesmosomes, fragmentation of meisosomes in
366 furrowless mutants affects the integrity of the apical epidermal membrane in the lateral epidermis, potentially
367 explaining the permeability phenotype of furrowless mutants (Sandhu et al., 2021). Despite these differences,
368 both meisosomes in the lateral epidermis, and hemidesmosomes in the dorso-ventral quadrants, do appear to
369 have an analogous function, ensuring the attachment of the apical plasma membrane to the cuticle.

370 In animals, ECMs provide mechanical support for tissue assembly and organ shape. During embryogenesis in
371 *C. elegans*, the aECM is essential during elongation as it not only maintains embryonic integrity, but also
372 relays the mechanical stress produced by the actomyosin cytoskeleton and the muscles (T. T. Vuong-Brender
373 et al., 2017; T. T. K. Vuong-Brender et al., 2017). In the adult, the mechanical properties of the aECM have
374 only recently started to be explored. Atomic force microscopy revealed that the furrows have a higher
375 stiffness than the annuli (Essmann et al., 2016). Here, we show that loss of specific furrow collagens, but not
376 of non-furrow collagens, decreases stiffness. Part or all of this could be a direct consequence of the altered
377 cuticle morphology, an analogy being the increased stiffness that corrugation provides. Furrow Dpy mutants
378 are known to have a higher internal concentration of glycerol (Wheeler & Thomas, 2006), which will decrease
379 their internal hydrostatic pressure. We propose that this decreased hydrostatic pressure is a consequence of the
380 decrease in the stiffness of the cuticle. It would ensure the necessary balance of inward and outward pressures
381 required for body integrity. Since we used a 10 micrometer diameter AFM probe to indent the worm, and the
382 indentation depth was greater than the thickness of the cuticle (ca. 800 nm compared to 500 nm for the
383 cuticle), our measurements did not directly assess the cuticle stiffness, so further investigations will be needed
384 to confirm our hypothesis. It is interesting to note, however, that a decrease in stiffness and an increase in the
385 activity of innate immune signalling pathways in the epidermis are signatures of ageing in *C. elegans* (E et al.,
386 2018; Essmann et al., 2020). How physiological and pathological modifications of the biomechanical
387 properties of the aECM are surveyed by the epidermis remains an open question for future studies.

388 **Materials and Methods**

389 **Nematode strains**

390 All *C. elegans* strains were maintained on nematode growth medium (NGM) and fed with *E. coli* OP50, as
391 described (Stiernagle, 2006), the wild-type N2, IG274 *frIs7[col-12p::DsRed, nlp-29p::GFP]* IV (Pujol,
392 Cypowyj, et al., 2008), IG1697 *dpy-2(e8) II; frIs7[nlp-29p::GFP, col-12p::DsRed]* IV, IG1685 *dpy-3(e27) X;*
393 *frIs7[nlp-29p::GFP, col-12p::DsRed]* IV, IG1689 *dpy-7(e88) X; frIs7[nlp-29p::GFP, col-12p::DsRed]* IV
394 (Dodds et al., 2018), IG1699 *dpy-8(e130) X; frIs7[nlp-29p::GFP, col-12p::DsRed]* IV, IG344 *dpy-13(e184)*
395 *frIs7[nlp-29p::GFP, col-12p::DsRed]* IV, RT424 *pwIs126[eea-1p::GFP::EEA-1]* (Shi et al., 2009), RT3657
396 *pwSi46[hyp7p::mScarlet::HGRS-1+G418-R]*, RT3640 *pwSi65[hyp7p::mScarlet::SNX-1+G418-R]*, RT3635
397 *pwSi62[hyp7p::mScarlet::LGG-1+G418-R]* (Serrano-Saiz et al., 2020), ML2113
398 *mcls67[dpy-7p::LifeAct::GFP; unc-119(+)] V; stIs10088[hhlh-1::his-24::mCherry, unc-119(+)]* (Lardennois
399 et al., 2019) to visualise actin in larval stages, IG1813 *frSi9[pNP151(col-62p::Lifeact::mKate_3'c-nmy), unc-*
400 *119(+)* *tTi5605]/+ II; tbb-2(tj26[GFP::TBB-2]) III* (Taffoni et al., 2020) to visualise actin in the adult,
401 IG1935 *frSi9[pNP151(col-62p::Lifeact::mKate_3'c-nmy), unc-119(+)* *tTi5605]* II; *Is[wrt-2p::GFP::PH-*
402 *PLC1δ, wrt-2p::GFP::H2B, lin-48p::mCherry]*, XW18042 *qxSi722[dpy-7p::DPY-7::sfGFP; tTi5605]* II

403 (Miao et al., 2020) and MBA365 *Ex[dpy-7p::GFP::CAAX, myo-2p::GFP]* kindly provided by M. Barkoulas
404 (UCL).

405 Extrachromosomal transgenic strain [Ex] containing GFP tagged version of VHA-5 were generated by
406 microinjection in N2 worms of the *vha-5pVHA-5::GFP* construct pML670 (Liegeois et al., 2006) kindly
407 provided by M. Labouesse (LBD/IBPS) at 3 ng/μl together with *unc-122p::GFP* at 100 ng/μl to generate
408 IG1930. The transgene *frSi26* is a single copy insertion on chromosome II (ttTi5605 location) of pNP165
409 (*dpy-7p::VHA-5::GFP*) by CRISPR using a self-excising cassette (SEC) (Dickinson et al., 2015). pNP165 was
410 obtained by insertion of the *dpy-7* promoter, which leads to an epidermal specific expression, in front of
411 VHA-5::GFP into the pNP154 vector. pNP154 was made from a vector containing the SEC cassette for single
412 insertion on Chromosome II at the position of ttTi5605 (pAP087, kindly provided by Ari Pani) (Watts et al.,
413 2020). Constructs were made using Gibson Assembly (NEB Inc., MA) and confirmed by sequencing. pNP165
414 was injected in N2 at 20 ng/μl together with pDD122 (*eft-3p::Cas9*) at 50 ng/μl, pCFJ90 (*myo-2p::mCherry*)
415 at 2 ng/μl, and #46168 (*eef-1A.Ip::CAS9-SV40-NLS::3'tbb-2*) at 30 ng/ml. pCFJ90 was a gift from Erik
416 Jorgensen (Addgene plasmid # 19327; <http://n2t.net/addgene:19327>; RRID:Addgene_19327) (Frokjaer-
417 Jensen et al., 2008). Non-fluorescent roller worms were selected then heat shocked to remove the SEC by
418 FloxP as described in (Dickinson et al., 2015) to generate IG2118 *frSi26[pNP165(dpy-7p::VHA-5::GFP)*
419 *ttTi5605] II*. Fluorescent knock-in [KI] reporter strains were generated through CRISPR editing
420 (SunyBiotech) at the C-terminus of the gene to generate PHX5715 *vha-5(syb5715[VHA-5::sfGFP]) IV*, that
421 was further outcrossed 2 times to generate IG2144, and PHX2235 *rol-6(syb2235[ROL-6::mScarlet]) II*. All
422 the multiple reporter strains generated in this study were obtained by conventional crosses (see Supplementary
423 File 1 for a list of all strains).

424 **Transmission Electron Microscopy (TEM)**

425 Day 1 adult worms were frozen in NaCl 50 mM medium containing 5 % of BSA and *E. coli* bacteria using
426 Leica EM Pact 2 high pressure freezer. After freezing, samples were freeze-substituted at -90 °C in acetone
427 containing 2 % OsO4 for 96 hours. The temperature was gradually increased to -60 °C and maintained for 8
428 hours. The temperature was then raised to -30 °C and maintained for 8 hours, before to be raised again to RT.
429 Samples were finally washed in acetone and embedded in Epoxy resin. Resin was polymerised at 60 °C for 48
430 hours. 70 nm ultrathin and 350 nm semithin sections were performed using a Leica UC7 ultramicrotome and
431 post-stained with 2 % uranyl acetate and Reynolds' lead citrate. Images were taken with a Tecnai G2
432 microscope (FEI) at 200 kV. For tomography acquisitions, tilted images (+60°/-60° according to a Saxton
433 scheme) were acquired using Xplorer 3D (FEI) with a Veleta camera (Olympus, Japan). Tilted series
434 alignment and tomography reconstruction was performed using IMOD (Mastrorade, 1997).

435 **Freeze fracture**

436 Wild-type adults were fixed in buffered 2.5 % glutaraldehyde, then cryoprotected in 30 % glycerol overnight
437 prior to freezing. Fixed animals were positioned between two gold discs, and plunge frozen in liquid nitrogen-
438 chilled isopentane. Frozen worms were placed into a double replica holder for a Balzer's 301 freeze etch
439 device. Samples were cleaved within the freeze etch device by free breaks, then shadowed with Pt/C to form a
440 metal replica. Replicas were washed in bleach to remove all tissue prior to mounting on slot grids for
441 examination by TEM.

442 Scanning electron microscopy by Serial Block Face (SBF)

443 After freezing in the aforementioned conditions, samples were incubated at -90 °C in acetone containing 2 %
444 OsO₄ for 106 hours. The temperature was gradually increased to 0 °C and samples were washed over 1h in
445 acetone at RT. Samples were then incubated in acetone containing 0.1 % TCH for 60 min, washed over 1h in
446 acetone, and incubated in acetone containing 2 % OsO₄ for 1 hour at RT. After rehydration in ethanol
447 decreasing graded series, samples were incubated ON in 1 % aqueous uranyl acetate at 4 °C and in 30 nM
448 lead aspartate for 30 min at 60 °C. Samples were finally dehydrated in graded series of ethanol baths and pure
449 acetone and embedded in Durcupan resin. Resin was polymerised at 60 °C for 48 hours. For the segmentation
450 of meisosomes, regions of the lateral epidermis were acquired over a length of 12 µm, with a resolution of 10
451 nm par pixels. For scanning the cuticle detachment, entire transversal sections were acquired over a length of
452 21.5 and 34.4 µm, for a wild-type and a *dpy-2* mutant worm, respectively, with a resolution of 10 nm par
453 pixels.

454 Correlative Light Electron Microscopy (CLEM)

455 Sample for CLEM experiments were treated as in (Johnson et al., 2015). Briefly, the worms were high
456 pressure frozen (EMPACT2, Leica) and then freeze-substituted (AFS2, Leica) for 20 hours from -130 °C to -
457 45 °C in an acetone-based cocktail containing 0.2 % uranyl acetate, 0.1 % tannic acid and 5 % H₂O. After 2 h
458 of acetone washes at -45 °C, the samples were infiltrated with gradients of HM20 resin over 9 h, with pure
459 resin for 18 h at -45 °C and the resin was polymerized under UV for 24 h at -45 °C and for 12 h at 0 °C. 350
460 nm semithin sections were processed as described for TEM tomography above. TEM grids were first analysed
461 at by confocal imaging where a bright field image is overlaid with the fluorescent image, then analysed in
462 TEM at low magnification. Brightfield, GFP confocal and TEM images were aligned using AMIRA. Several
463 positions with 2 or 3 GFP spots were chosen to do a high magnification tomography, as described above, to
464 reveal the meisosomes.

465 Segmentations and 3D Image analysis

466 For electron tomography datasets, a binned version of the reconstructed tomogram was segmented using the
467 Weka 3D segmentation plugin in Fiji/ImageJ to visualize the mitochondria and the meisosomes. The cuticle

468 was visualized by the Amira-embedded Volume Rendering plugin from a manually segmented mask. A
469 cropped area of interest of the full resolution electron tomogram was segmented in iLastik to visualize a
470 representative portion of the organelle. For Serial Block-face datasets, the segmentation of the meisosome and
471 mitochondria was generated using the Weka 3D segmentation plugin in Fiji/ImageJ. Animations and
472 snapshots were generated in Amira.

473 RNA interference

474 RNAi bacterial clones were obtained from the Ahringer or Vidal libraries and verified by sequencing (Kamath
475 et al., 2003; Rual et al., 2004). RNAi bacteria were seeded on NGM plates supplemented with 100 µg/ml
476 ampicillin and 1 mM Isopropyl-β-D-thiogalactopyranoside (IPTG). Worms were transferred onto RNAi plates
477 as L1 larvae and cultured at 20 °C or 25 °C until L4 or young adult stage. In all our experiments, we are using
478 *sta-1* as our control, as we have shown over the last decade that it does not affect the development nor any
479 stress or innate response in the epidermis (Dierking et al., 2011; Lee et al., 2018; Taffoni et al., 2020; Zhang
480 et al., 2021; Zugasti et al., 2014; Zugasti et al., 2016).

481 Fluorescent Image acquisition

482 Live young adult worms were placed on a 2 % agarose pad containing 0.25 mM levamisole in NaCl to
483 immobilize the worms. Images were acquired using a confocal laser scanning microscopy: Zeiss LSM780 and
484 its acquisition software Zen with a Plan-Apochromat 40 X/1.4 Oil DIC M27 objective with a zoom 2 to 4, a
485 Plan-Apochromat 63 X/1.40 Oil DIC M27 with a zoom 1. Spectral imaging combined with linear unmixing
486 was used in most confocal images to separate the autofluorescence of the cuticle.

487 Airyscan super-resolution microscopy

488 Airyscan imaging were performed using a commercial Zeiss confocal microscope LSM 880 equipped with an
489 Airyscan module (Carl Zeiss AG, Jena, Germany) and images were taken with a 63x/1.40NA M27 Plan
490 Apochromat oil objective. In this mode, emission light was projected onto an array of 32 sensitive GaAsP
491 detectors, arranged in a compound eye fashion. The Airyscan processing was done with Zen Black 2.3
492 software by performing filtering, deconvolution and pixel reassignment to improve SNR. The Airyscan
493 filtering (Wiener filter associated with deconvolution) was set to the default filter setting of 6.1 in 2D.

494 Fluorescent Image analysis

495 To extract the morphological properties of meisosomes, we devised an automatic Fiji segmentation procedure
496 (GitHub https://github.com/centuri-engineering/BD_BlobsSeg). We first restricted the analysis to manually
497 drawn ROIs and isolated organelles (foreground image) from the background by using the "remove outliers"
498 function of Fiji (radius = 30 pixels and threshold = 30). We next applied a Gaussian blur (sigma = 1 pixel) on

499 the foreground image and automatically defined a threshold value to binarize the newly blurred image. This
500 threshold was determined automatically by multiplying the background value (retrieved by averaging the
501 fluorescent levels of the background image) by a constant coefficient. This allowed us to normalize the
502 segmentation, since the expression levels of fluorescent proteins may vary from one animal to another.
503 Finally, after filtering out smaller objects (less than $\sim 0.15 \mu\text{m}^2$), we measured the averaged organelles area,
504 Feret's diameter (longest axis) and density in the different conditions. Unpaired t test was used to compare the
505 samples which passed the normality test (Shapiro-Wilk normality test) and with homogeneity variances
506 (Fisher test) and unpaired nonparametric Mann-Whitney test for the others. For co-localisation analysis, we
507 counted the percentage of segmented objects in a given channel, green (G) or red (R), whose centroid is
508 located in an object of the other channel. We then averaged these percentages across images, each
509 representing a different worm (n=10 for each strain analysed).

510 Atomic Force Microscopy (AFM)

511 Worms were prepared as described before (Essmann et al., 2016). Briefly, staged 1-day-old young adult
512 worms were paralysed in 15 mg/ml 2, 3-butanedione monoxime (Sigma) for 2 h at room temperature and
513 transferred to a ~ 2 mm thick 4 % agarose bed in a petri dish (30 mm). Heads and tails were fixed with tissue
514 glue (Dermabond, Ethicon) and the dish filled with a 2.5 ml M9 buffer. AFM data of worms were obtained
515 using a NanoWizard3 (JPK) under aqueous conditions. Type qp-CONT-10 (0.1 N/m; nanosensors) cantilevers
516 were used for imaging in contact-mode at setpoint 0.3 V and 0.5 Hz scanning speed, and NSC12 tipless
517 cantilevers (7.5 N/m; MikroMash) with a 10 μm borosilicate bead attached (produced by sQUBE
518 www.sQUBE.de) were used in force spectroscopy mode to obtain force-indentation measures at 450 nN force
519 setpoint and 0.5 $\mu\text{m/s}$ indentation speed. Cantilever sensitivity and stiffness (k) were calibrated using the JPK
520 system calibration tool before each experiment. AFM raw data were analysed using the JPK analysis
521 software. All force curves were processed to zero the baseline, to determine the tip-sample contact point and
522 to subtract cantilever bending. The Young's Modulus was calculated within the software by fitting the
523 Hertz/Sneddon model respecting the indenter shape (10 μm bead) to each curve. All topographical images are
524 flattened using the plane fitting option of the JPK software at 1-2 degree to correct for sample tilt and natural
525 curvature of the worm.

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548

549 **Figure legends**

550 **Figure 1: Meisosomes are membrane folded structures on the apical side of the lateral epidermis.**

551 (A) Schematic view of the outer tissues of an adult *C. elegans* worm near the mid-body. The different regions
552 of the epidermis containing meisosomes are boxed in blue (lateral, and dorsal and ventral ridges), the regions
553 above the muscles that are extremely thin are boxed in red. (B) Schematic view of the connection between the
554 cuticle and the plasma membrane (pm) of the lateral epidermis highlighting the position of meisosomes
555 relative to multivesicular bodies (mvb) and mitochondria. (C-G) TEM images of longitudinal sections of
556 young adult wild-type hermaphrodite worms reveal epidermal structures, meisosomes (m), contacting the
557 cuticle (cut), composed of parallel plasma membrane (pm) folds. (C-F) Meisosomes typically comprise less
558 than 10 folds, but can have more than 30, as shown in (C) where the meisosome is 4 μm wide, and extends on
559 both sides of a furrow (f). Some folds are apposed to mitochondria (mit) (D), can vary in size and orientation
560 (C-F) and can appear not to be in direct contact with the cuticle in some EM sections (F); scale bar, 200 nm.
561 (G) High magnification view of plasma membrane (pm) folds. The “internal” folds (i) are 35 nm wide,
562 contain a ribosome-free cytoplasm but ladder-like banding (indicated by the black arrowheads), and are less

563 electron dense in their middle (*). The “outer” folds (o) on the cuticle side are 20 nm wide. Epidermis (epi);
564 scale bar, 50 nm.

565 Figure 1-figure supplement 1: Meisosomes are present in epidermis at all development stages.

566 (A) Schematic view of the tissues of an adult *C. elegans* worm. MRC archival transversal TEM images
567 showing that meisosomes (red arrow) are present in different body regions in the epidermis (hyp7, B) and in
568 the tail (hyp9/10, C-D), as well as in different larval stages L1 (E), L2 (F), L3 (G), L4 (H), adult 5 days (I),
569 adult 15 days (J), dauer stage (K).

570

571 Figure 1-figure supplement 2: Meisosomes are randomly orientated except before moulting.

572 (A) Schematic view of the outer tissues of an adult *C. elegans* worm near the mid-body. The plane of
573 longitudinal EM sections and the *en face* view obtained by freeze fracture are shown in blue and red dotted
574 lines, respectively. (B) Freeze fracture view of adult epidermal plasma membrane (anterior to the top right)
575 shows meisosome with random orientation vs the body axis, highlighted by red arrows. MRC archival image.
576 (C-D) Lengthwise TEM view of the epidermis at pre-moult stages reveals an alignment of meisosomes in
577 between the position of the furrows (f) facing the annuli (a) of the newly synthesised cuticle (nc), while the
578 old cuticle (oc) lies to the outside, on its way to be shed. (C) Late L4 stage, scale bar, 500 nm. (D) Late L1
579 stage, MRC archival image, scale bar, 1 μ m.

580 Figure 1-figure supplement 3: Smaller meisosomes can be found over the muscle quadrants.

581 (A) Schematic view of the outer tissues of an adult *C. elegans* worm near the mid-body. The different regions
582 of the epidermis analysed are boxed: either the lateral side as in Figure 1 (blue), or the ventral (or dorsal) side
583 overlaying the muscle quadrants (red). (B) TEM image of longitudinal section of young adult wild-type worm
584 reveals small meisosomes in the epidermis above the body-wall muscle; the thickness of the epidermal cell is
585 less than 500 nm at these positions. Scale bar, 500 nm. (C) A tomogram of a transversal section reveals
586 several smaller meisosomes (green) with typically not more than 4 folds; other small organelles lie in this
587 region, including multivesicular bodies (pink), ribosomes (red), and a possible small mitochondrion (dark
588 blue). (D) One selected TEM image of the same tomogram, red arrows point to small meisosomes. Scale bars
589 in C&D, 200 nm.

590

591 Figure 2: Meisosomes are formed by epidermal plasma membrane facing the apical ECM.

592 Adjacent meisosomes in two serial thick (300 nm) sections were analysed with by electron tomography. (A)
593 Selected virtual image from the serial reconstruction. (B-E) Segmentation of membranes and mitochondria
594 reveal their 3D topology. Meisosomes (in yellow) are in close apposition to, but not in continuity with,

595 mitochondria (orange) and are formed by epidermal plasma membrane folds, as observed in an *en face* view
596 after removing the cuticle *in silico* (E). (F) Two folds were extracted and manually filled for a schematic
597 view. Cuticle (cut), epidermis (epi), furrow (f), mitochondria (mit), meisosomes (m); scale bar, 200 nm.

598 **Figure 2-figure supplement 1: Meisosome distribution.**

599 (A) Schematic view of the outer tissues of an adult *C. elegans* worm near the mid-body. The plane of
600 transverse EM sections and the orthogonal *en face* view for EM and fluorescence microscopy are indicated
601 with the blue and red dashed rectangles, respectively. (B-C) Representative image from an SBF acquisition,
602 the boxed area in (B) is shown at a higher magnification in (C). Scale bar, 500 nm. (D) SBF segmentation and
603 3D reconstruction revealed the *en face* spatial distribution of meisosomes (yellow) compared to mitochondria
604 (orange). Scale bar, 1 μ m.

605 **Figure 3: VHA-5 is a marker of plasma membrane containing meisosomes**

606 (A) Schematic view of the outer tissues of an adult *C. elegans* worm near the mid-body, with the boxed *en*
607 *face* view (red) or z projection (blue) for fluorescence microscopy. (B-H) Confocal images of young adult
608 worms using different strains expressing either a single copy insertion of VHA-5::GFP under an epidermis
609 promoter [SI], or a classic multi-copy integrated VHA-5::RFP [Is] or extrachromosomal VHA-5::GFP [Ex]
610 transgenic array. (B) In a young adult worm, VHA-5::GFP fluorescence from a multicopy transgene [Ex] is
611 observed in the lateral epidermis, as well as in the excretory canal (exc), but largely excluded from the ventral
612 (v) and dorsal (d) regions above the muscle quadrants, known to contain hemidesmosomes, as well as the
613 region above the seam cell (sc), as observed in *en face* (left panel), and orthogonal (right) projections of
614 reconstructed confocal stacks. (C) VHA-5::GFP is also expressed in the ventral ridge, as observed in a ventral
615 view. (D) High resolution imaging on Airyscan mode revealed VHA-5::GFP from a single copy insertion [Si]
616 to be associated with long and convoluted apical membranous structures. (D) Meisosomes (m), visualised
617 using a VHA-5::RFP integrated transgene [Is] (magenta), are complementary with hemidesmosomes (hd)
618 visualised in a MUP-4::GFP KI (green), autofluorescence, in white, is from intestinal granules. Tiled image
619 acquired with the spectral mode z projection of 6 section of 1 μ m; exc, excretory canal. Attachment of
620 different structures to the epidermis with hd are highlighted: vulval muscles (vm), ALM and PLM neurones,
621 anal muscles (a). (F-H) Confocal images of young adult worms expressing VHA-5::GFP [Ex] (green) together
622 with an HGRS-1::mScarlet marker (magenta) (F), a VHA-5::RFP [Is] (magenta) together with a CAAX::GFP
623 (G) or PH-PLC1 δ ::GFP marker (green) (H); see associated Figure 3-figure supplement 1 for the individual
624 channel as well as the co-localisation quantifications. Scale bar, 10 μ m. (I-J) Correlative Light Electron
625 Microscopy (CLEM) aligns the position of 3 meisosomes revealed by tomography (I) with 3 spots of
626 VHA-5::GFP signal imaged by confocal imaging (J), see associated Figure 3-figure supplement 2 for the
627 detailed procedure and another example. Epidermis is pseudo-coloured in beige. Scale bar, 1 μ m.

**628 Figure 3-figure supplement 1: VHA-5::FP colocalise with membrane markers but not with vesicular
629 markers in the epidermis.**

630 (A-C) Confocal images of young adult worms expressing VHA-5::GFP [Ex] (green, left panel) together with
631 an mScarlet marker (magenta, middle panel) in the epidermis; right panel overlay; in A, HGRS-1, in B,
632 SNX-1 and in C, LGG-1. (D-E) Confocal images of young adult worms expressing VHA-5::RFP [Is]
633 (magenta, middle panel) together with a GFP marker (green, left panel) in the epidermis; right panel overlay;
634 in D, CAAX, and in E, PH-PLC1 δ . Scale bar, 10 μ m. (F) Quantification of VHA-5::RFP co-localisation with
635 either the CAAX::GFP or PH-PLC1 δ ::GFP marker. Segmentation of objects in each channel define green (G)
636 and red (R) objects (left panels), the G objects whose centroid is located in a R object are represented in white
637 and counted as 1, the G objects whose centroid is located outside a R object are represented in green and
638 counted as 0, the R objects whose centroid is located outside a G object are represented in magenta and
639 counted as 0. The average % of all objects is represented, for each reverse situation, with each dot being an
640 analysed ROI in one worm, n=10 for each strain.

641 Figure 3-figure supplement 2: Correlative Light Electron Microscopy (CLEM).

642 Two different VHA-5::GFP [Si] worms (A-D and E-H) fixed and sliced for TEM were first analysed by
643 confocal imaging where a bright field view is overlaid with the green GFP signal (A&E). The same
644 preparation was then analysed in TEM at low magnification and both the GFP confocal and the TEM images
645 were aligned using AMIRA (B&F). Then a position with 2 or 3 GFP spots was chosen to do a high
646 magnification tomography TEM acquisition to reveal the meisosomes, 2 meisosomes (indicated by the thin
647 yellow lines) in C correlate with 2 GFP spots in D, 3 meisosomes in G correlate with 3 GFP spots in H. Scale
648 bar, 1 μ m.

649 Figure 4: Meisosome aligned in between furrow before moulting.

650 (A) Confocal images of worms expressing VHA-5::GFP [Si] from early L4 to young adult (YA) stage. To
651 define the precise L4 stage of all observed worms, the vulva was observed and worms classified according to
652 (Cohen et al., 2020; Mok et al., 2015). (B) Confocal images of worms expressing both DPY-7::GFP and
653 VHA-5::RFP, red channel on the left, merge channel on the right. The inserts show selected regions at a 1.6 X
654 higher magnification. (C) Merged red and green channel confocal images of lateral epidermis of late L4
655 worms expressing: upper panel, a VHA-5::RFP [Is] with a CAAX::GFP (left panel) or a PH-PLC1 δ ::GFP
656 (right panel); lower panel, a HGRS-1::mScarlet (left panel), SNX-1::mScarlet (middle panel) or
657 LGG-1::mScarlet (right panel) with VHA-5::GFP [Ex]. Scale bar, 10 μ m.

**658 Figure 5: Furrow collagen inactivation provokes a loss of meisosome and cytoskeleton alignment during
659 the L4 stage.**

660 Confocal images of worms expressing VHA-5::GFP [Ex] (upper paired panels), TBB-2::GFP (middle paired
661 panels), LIFEACT::GFP (lower paired panels) from early L4 to young adult (YA) stage, treated with the
662 control (*sta-1*) or furrow Dpy (*dpy-7*) RNAi clones, n>4. Scale bar, 5 μ m. To define the precise L4 stage, the
663 vulva was observed and worms classified according to (Cohen et al., 2020). A representative example of the
664 vulva at each stage is shown on the top row in worms expressing the marker TBB-2::GFP.

**665 Figure 5-figure supplement 1: Furrow collagen inactivation provokes a loss of meisosomes alignment
666 during the L4 stage.**

667 (A) Confocal images of worms expressing VHA-5::GFP [KI] at the L4.6 (upper panels) and L4.8 stages
668 (lower panels) treated with a control (*sta-1*; left panels) or a *dpy-7* RNAi (right panels), n>5. (B) A selection
669 of confocal images of selected regions on the lateral epidermis of late L4 wild-type worms expressing
670 VHA-5::GFP [Ex] and treated with a control RNAi (*sta-1*; left panels), a *dpy-7* RNAi (middle panels) or in a
671 *dpy-3(e27)* mutant background (right panels). Scale bar, 5 μ m.

672 Figure 6: Furrow collagen inactivation provokes meiosome fragmentation.

673 (A) Schematic view of the outer tissues of an adult *C. elegans* worm near the mid-body, highlighting the
674 furrows that separate the annuli, the different collagen genes in the annuli (green) or furrow (blue) and
675 indicating the region analysed with the red dashed rectangle. (B, C) L1 larvae expressing VHA-5::GFP [Si]
676 were treated with the indicated RNAi clones and analysed at the young adult stage: control (*sta-1*), annuli
677 collagens *dpy-4*, *dpy-5* and *dpy-13* (green) and the furrow collagens: *dpy-2*, *dpy-3*, *dpy-7*, *dpy-8*, *dpy-9* or
678 *dpy-10* (blue). (B) Confocal images of selected regions on the lateral epidermis, at a constant position relative
679 to the vulva, scale bar, 5 μ m. (C) Quantification of VHA-5 signal average size, Feret's diameter and density.
680 All statistics are in Figure 6-Source data file 1, ****p<0.0001 and ***p<0.001. Control: *sta-1* (n= 17), *dpy-4*
681 (n= 11), *dpy-5* (n= 10), *dpy-13* (n= 11); furrow Dpy: *dpy-2* (n= 11) *dpy-3* (n= 10), *dpy-7* (n= 14), *dpy-8*
682 (n=13), *dpy-9* (n=13) or *dpy-10* (n= 11).

**683 Figure 6-figure supplement 1: Worms increase AMP gene expression at the late L4 stage and in furrow
684 collagen mutants.**

685 IG274 worms carrying the *frIs7* reporter transgene constitutively express the *col-12p::DsRed* reporter and
686 express *nlp-29p::GFP* only under certain conditions, red and green fluorescence were visualized
687 simultaneously in all images. (A) Compared to young adults, in late L4 animals entering the moult, the
688 expression of the *nlp-29p::GFP* reporter is markedly increased. Scale bar, 200 μ m (left). Quantification of
689 relative green fluorescence, L4 stage (n=260), young adult stage (n=149) (right). (B-C) RNA inactivation of
690 selected collagen genes leads to the induction of *nlp-29p::GFP* in young adult worms. Compared to wild-type
691 or annuli Dpy mutants, in furrow Dpy mutants, the expression of the *nlp-29p::GFP* reporter is markedly
692 increased (left panels). Quantification of relative green fluorescence in worms carrying *frIs7* (middle panel)

693 and the length of the worm (TOF, right panel). Note that these 2 phenotypes (induction of the *nlp-29p::GFP*
694 reporter and short size) serve as controls for the correct inactivation of the selected genes, and were always
695 run in parallel to RNAi inactivation on other reporter strains, as the one used in all Figure 5,6 & 8. (B)
696 Control: *sta-1* (n= 539), *dpy-13* (n= 492); furrow Dpy: *dpy-3* (n= 680), *dpy-7* (n= 688). (C) Control: *sta-1* (n=
697 195), *dpy-4* (n= 68), *dpy-5* (n=81); furrow Dpy: *dpy-3* (n= 72) or *dpy-7* (n= 95).

698 **Figure 6-figure supplement 2: Inactivation of furrow Dpy do not change the VHA-5 relationship to**
699 **endosomes and MVB, nor affect early endosomes.**

700 (A) Confocal images of selected regions on the lateral epidermis of young adult epidermis expressing
701 LGG-1::mScarlet (left panel), SNX-1::mScarlet (middle panel) or HGRS-1::mScarlet (right panel) with
702 VHA-5::GFP [Ex] and treated with the indicated RNAi clones. Control: *sta-1*, *dpy-4* and *dpy-5*; furrow Dpy:
703 *dpy-3* or *dpy-7*, n>7. Scale bar, 5 μ m. (B) Confocal images and segmentation views (left panels), with the
704 corresponding quantification (right panels) of selected regions on the lateral epidermis of young adults
705 expressing EEA-1::GFP and treated with the indicated RNAi clones. Scale bar, 5 μ m. Control: *sta-1* (n= 9),
706 *dpy-13* (n=10); furrow Dpy: *dpy-3* (n=10) or *dpy-7* (n=9).

707 **Figure 7: Furrow collagen inactivation leads to smaller and abnormal meisosomes and detachment of**
708 **the cuticle.**

709 TEM images of young adult worms. Compared to wild-type (Figure 1) or *dpy-13* (A), *dpy-2* (B) *dpy-7* (C-D)
710 have abnormally small meisosomes with irregular spacing between the membrane folds (D); number of
711 worms analysed are given in Table 1. Compared to wild-type (E) or *dpy-13* (F), lower magnification reveals
712 detachment of the cuticle (cut) from the plasma membrane (pm) in *dpy-3* mutant worms over the whole lateral
713 surface of the epidermal cell, on both sides of the alae (al) (G). (H) Compared to wild-type (left), SBF
714 analysis of the entire transversal worm also reveals the detachment (red arrow) in a *dpy-2* mutant of the
715 cuticle from the lateral epidermis, contrary to the regions above the muscles, delineated in beige and green,
716 respectively (one representative slice per animal, entire transversal sections were acquired over a length of
717 21.5 and 34.4 μ m, for a wild-type and a *dpy-2* mutant worm, respectively.). Scale bar 500 nm in A&B, 250
718 nm in insert in B, 200 nm in C&D, 5 μ m in E-G and 10 μ m in H.

719 **Figure 7-figure supplement 1: Furrow collagen inactivation leads to detachment of the cuticle in lateral**
720 **and ventral/dorsal ridges.**

721 TEM images of sagittal sections of young adult worms. Compared to *dpy-13* (A), *dpy-7* (B-C) and *dpy-8* (D)
722 present detachment of the cuticle (cut) from the plasma membrane, and have abnormally small meisosomes
723 (C, and inset), in the lateral epidermis (C, D) or the dorsal cord in between muscles (mus) (B). Scale bar, 500
724 nm in all including C inset, except in C, 1 μ m.

725 **Figure 8: Furrow collagen inactivation provokes extrusion of membrane and cytoplasmic contents into**
726 **the cuticle.**

727 (A) TEM images of *dpy-2* (left) and *dpy-7* (right) young adult mutant worms revealing the presence of
728 membranous organelles (*) and cytoplasmic content, including ribosome-like particles (#), between the
729 cuticle and the plasma membrane, epidermis is pseudo-coloured in beige. Scale bar, 500 nm. (B) Confocal
730 images of wild-type (left) and *dpy-3* mutant (right) young adult worms expressing ROL-6::mScarlet and
731 VHA-5::GFP, CAAX::GFP or PH-PLC1 δ ::GFP, one confocal plane was selected at the level of the cuticle
732 using the ROL-6::mScarlet. Scale bar, 10 μ m. (C) Quantification of the percentage of worm presenting the
733 abnormal presence of GFP extrusion at the level of the cuticle for the different markers; number of worms
734 observed is noted in parenthesis.

735 **Figure 9: Furrow collagen inactivation provokes a reduction in stiffness of the cuticle.**

736 (A) AFM topography of the cuticle in wild-type, *dpy-13*, *dpy-2*, *dpy-3*, *dpy-7* and *dpy-8* mutant adult worms.
737 Scale bar, 1 μ m. (B) Mean force-indentation curves of wild-type and collagen mutants acquired by AFM. (C)
738 Young's Modulus estimation from force curves by applying the Hertz model for contact mechanics. Data are
739 from 3 independent experiments with a total of n= 30, 25, 16, 40, 34 and 32, for wild-type, *dpy-13(e184)*,
740 *dpy-2(e8)*, *dpy-3(e27)*, *dpy-7(e88)* and *dpy-8(e130)* mutant worms, respectively.

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<i>genotype</i>	<i>cut</i>	<i>n</i> (<i>worms</i>)	<i>n</i> (<i>meisosome</i>)	<i>mean</i> <i>length</i> (μm)	<i>smallest</i> <i>length</i> (μm)	<i>longest</i> <i>length</i> (μm)
<i>wild-type</i>	Transv.	5	22	0,71	0,2	2,6
<i>wild-type</i>	Longit.	4	41	0,77	0,1	4
<i>dpy-13(e184)</i>	Transv.	3	31	0,77	0,3	4
<i>dpy-13(e184)</i>	Longit.	1	11	1,07	0,2	4
<i>dpy-2(e8)</i>	Transv.	1	45	0,39	0,05	1
<i>dpy-2(e8)</i>	Longit.	4	6	0,28	0,1	0,6
<i>dpy-3(e8)</i>	Longit.	1	16	0,49	0,1	1
<i>dpy-7(e88)</i>	Transv.	2	24	0,28	0,05	0,7
<i>dpy-7(e88)</i>	Longit.	4	37	0,37	0,1	1,5

745

746 **Table 1: Quantification of the length of the meisosomes on TEM images in young adult wild type and**
747 **different collagen mutants.** The orientation of the section, transversal or longitudinal, the number of
748 different worms observed and the number of meisosomes analysed are reported.

749 **Supplementary File 1: *C. elegans* strains used in this study.**

750 **Figure 2 - Video 1: Visualisation of the electron tomography and the 3D segmentation of meisosomes.**

751 Two serial tomograms were stitched and segmentations of the plasma membrane, mitochondria and cuticle
752 were made (see material and methods for details). The continuity of the plasma membrane could be revealed
753 by semi-automated segmentation, except under certain orientation due the high membrane curvature of the
754 meisosomes and the missing wedge artefact, which is inherent to electron tomography. At the end of the
755 video, the segmentation of two folds was completed manually to present a schematic view.

756 **Figure 6 - Source data file 1: Quantification of the fragmentation of the meisosomes.** Using different
757 VHA-5 reporter strains in wild type and different collagen RNA inactivation or mutants, VHA-5 positive
758 objects were segmented and three parameters were quantified, average size, Feret's diameter and density; the
759 number of worms, total surface analysed per condition are presented, together with statistical analysis (see
760 material and methods for details).

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1012

FIGURE 1

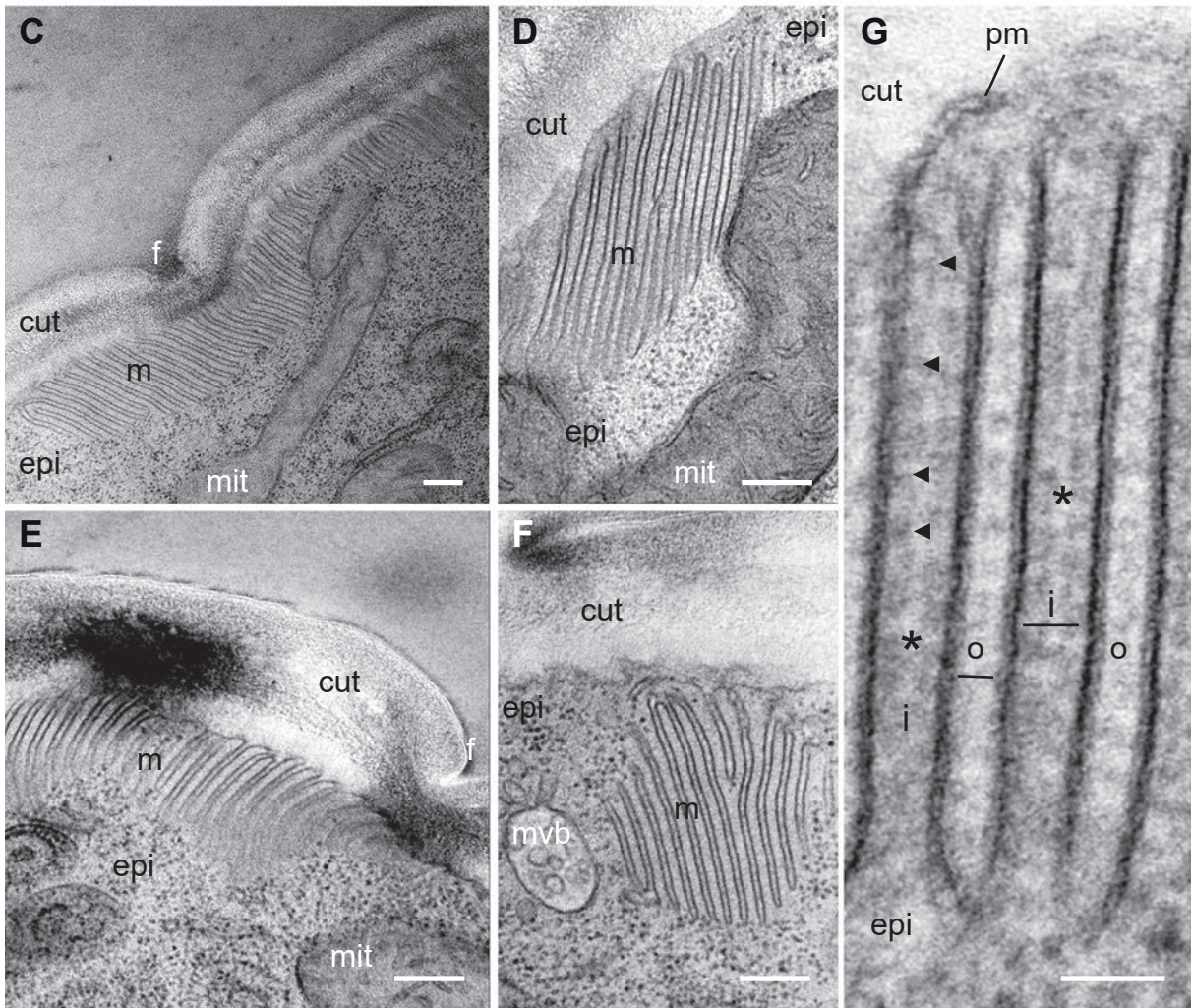
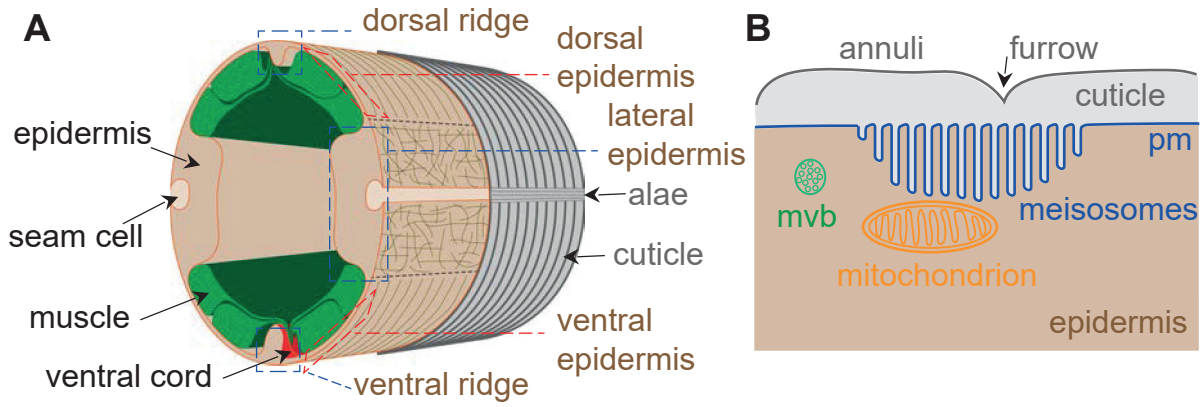


FIGURE 1-Supplement 1

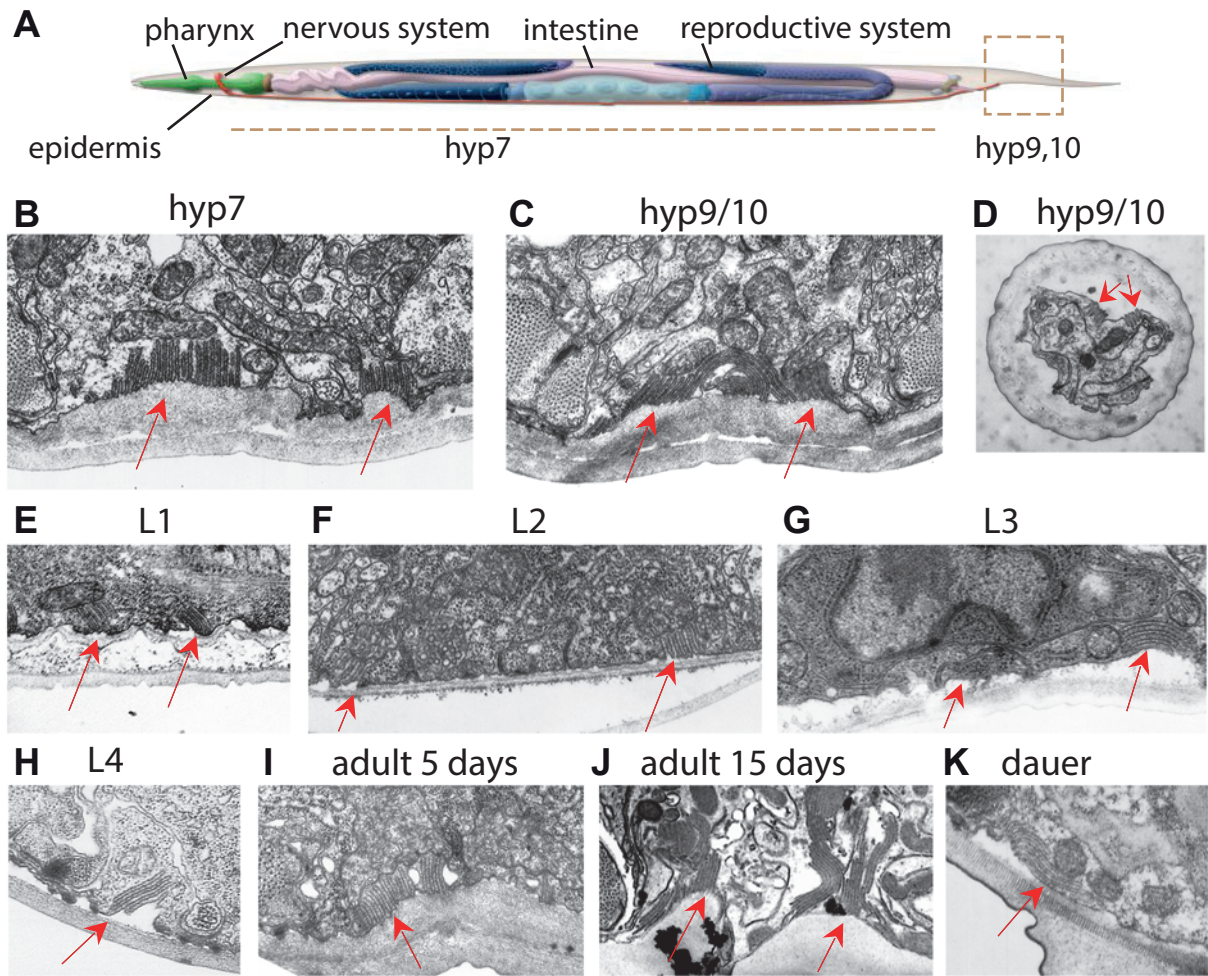


FIGURE 1-Supplement 2

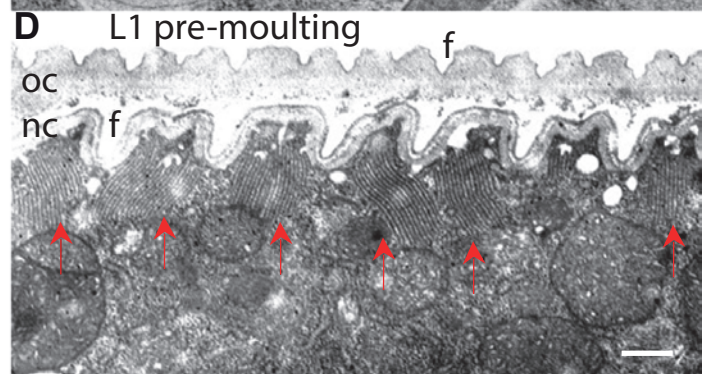
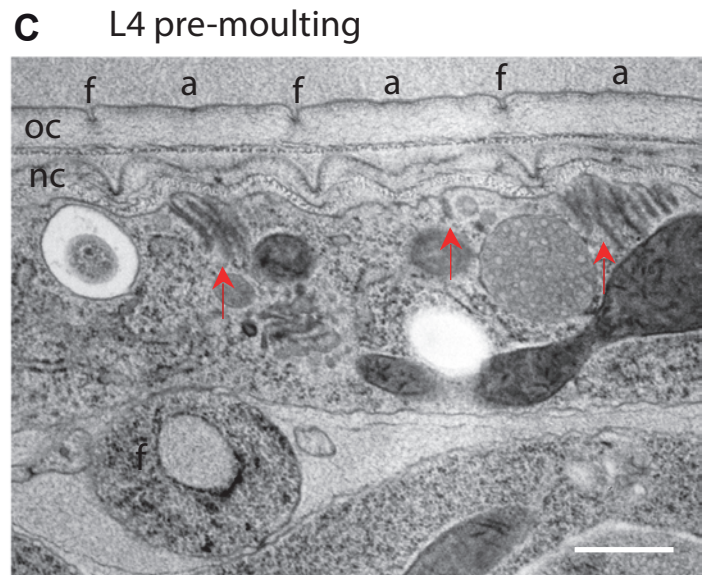
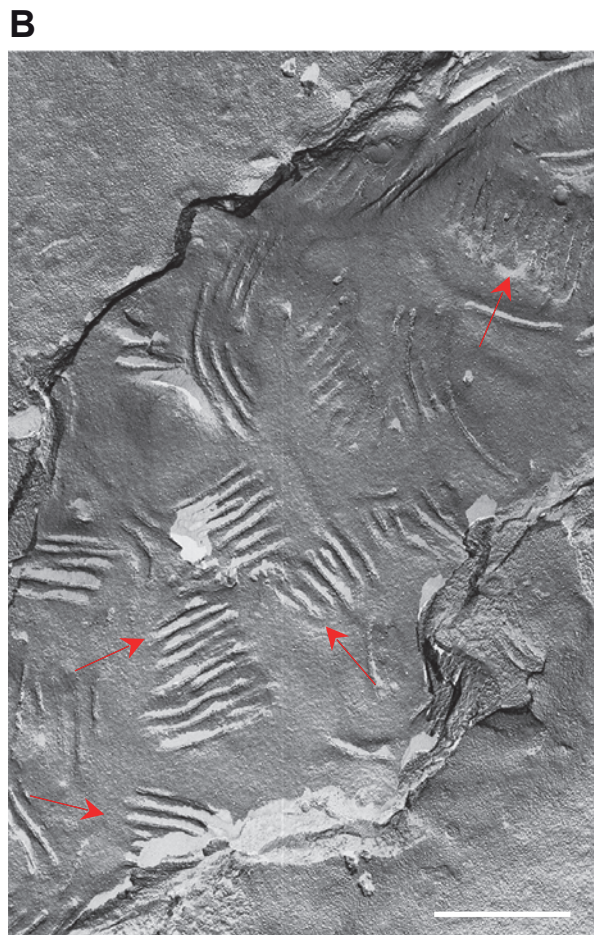
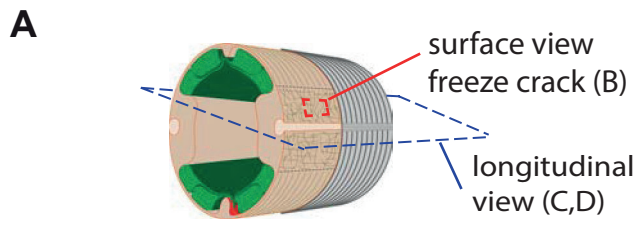


FIGURE 1-Supplement 3

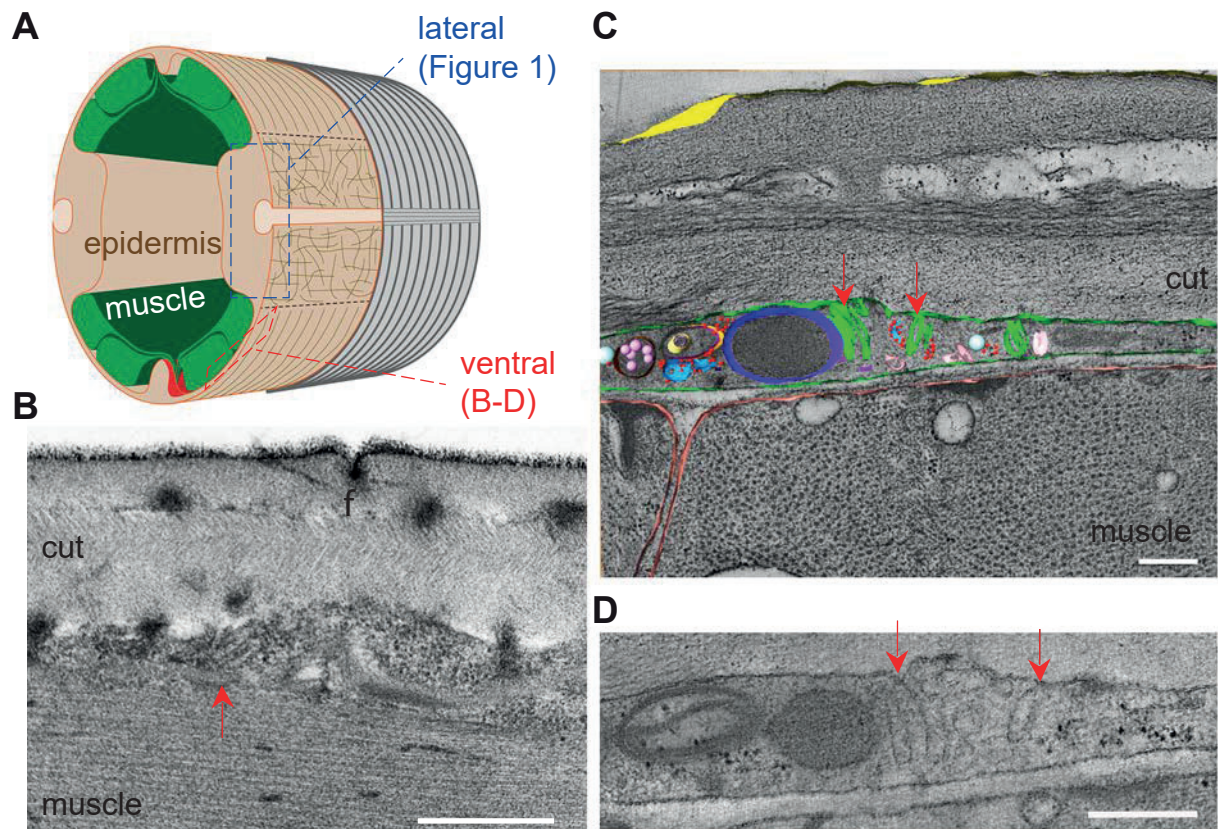


FIGURE 2

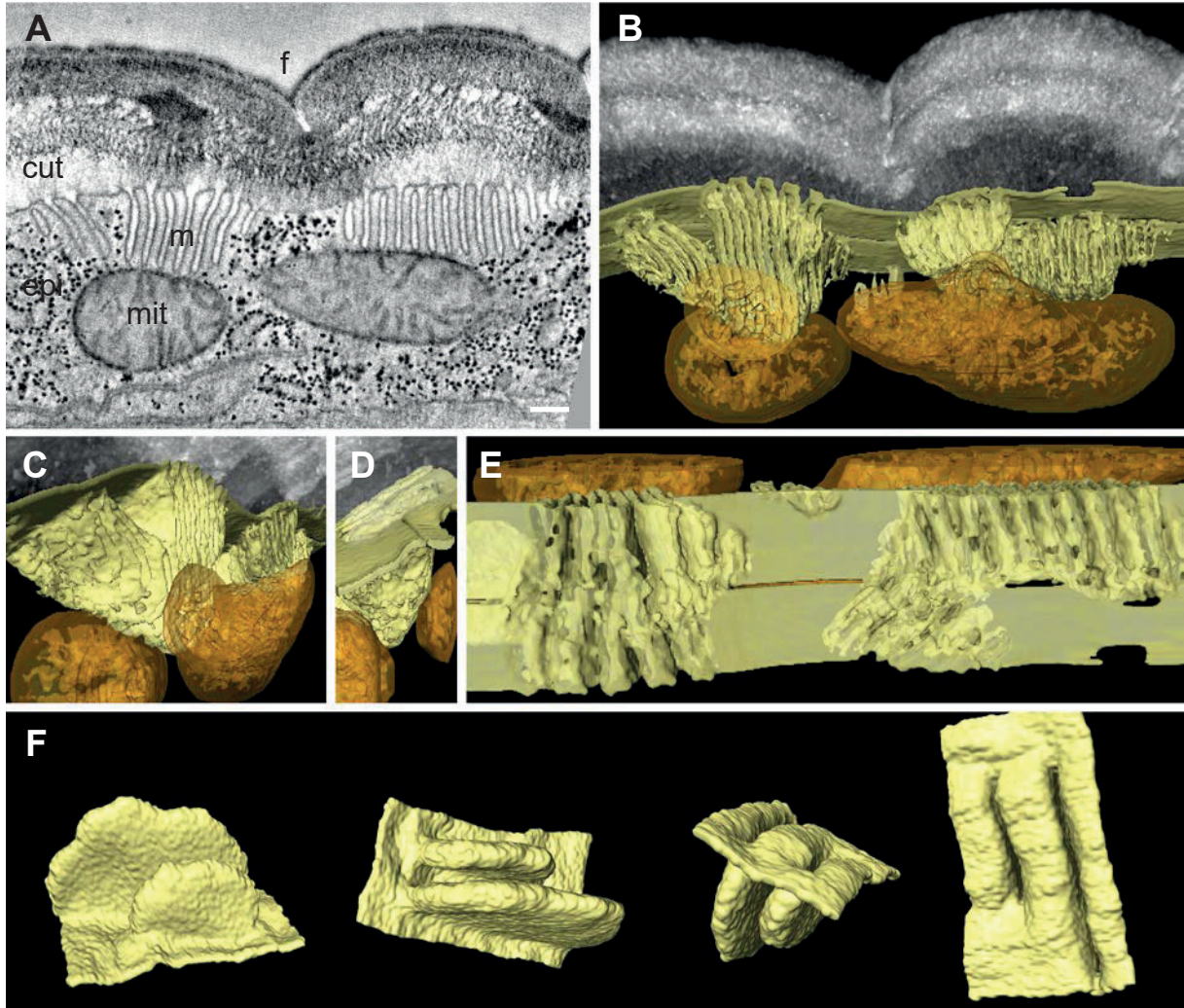


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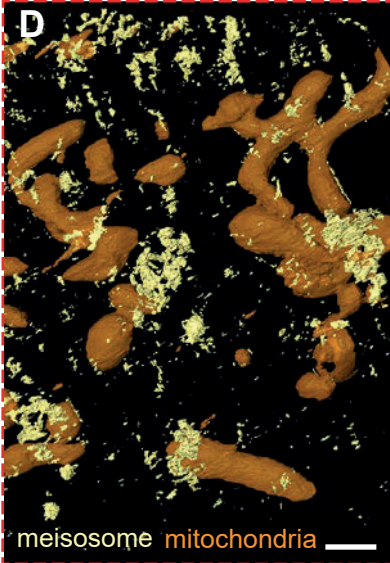
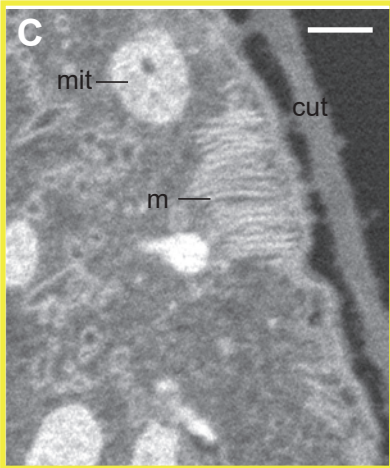
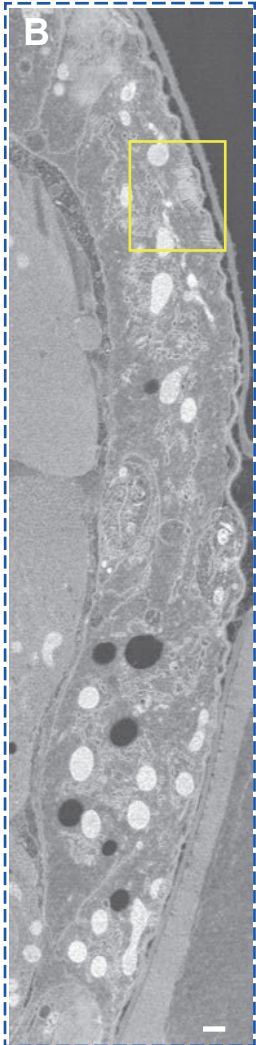
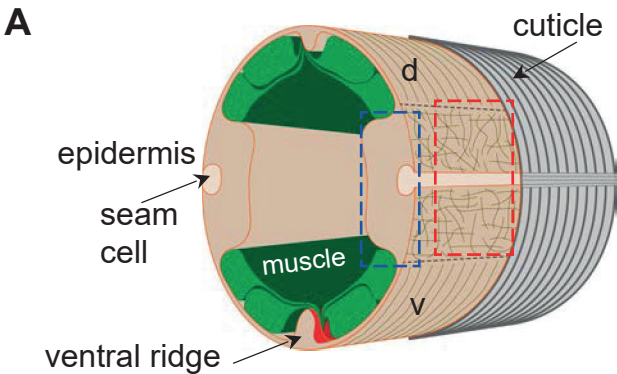


FIGURE 3

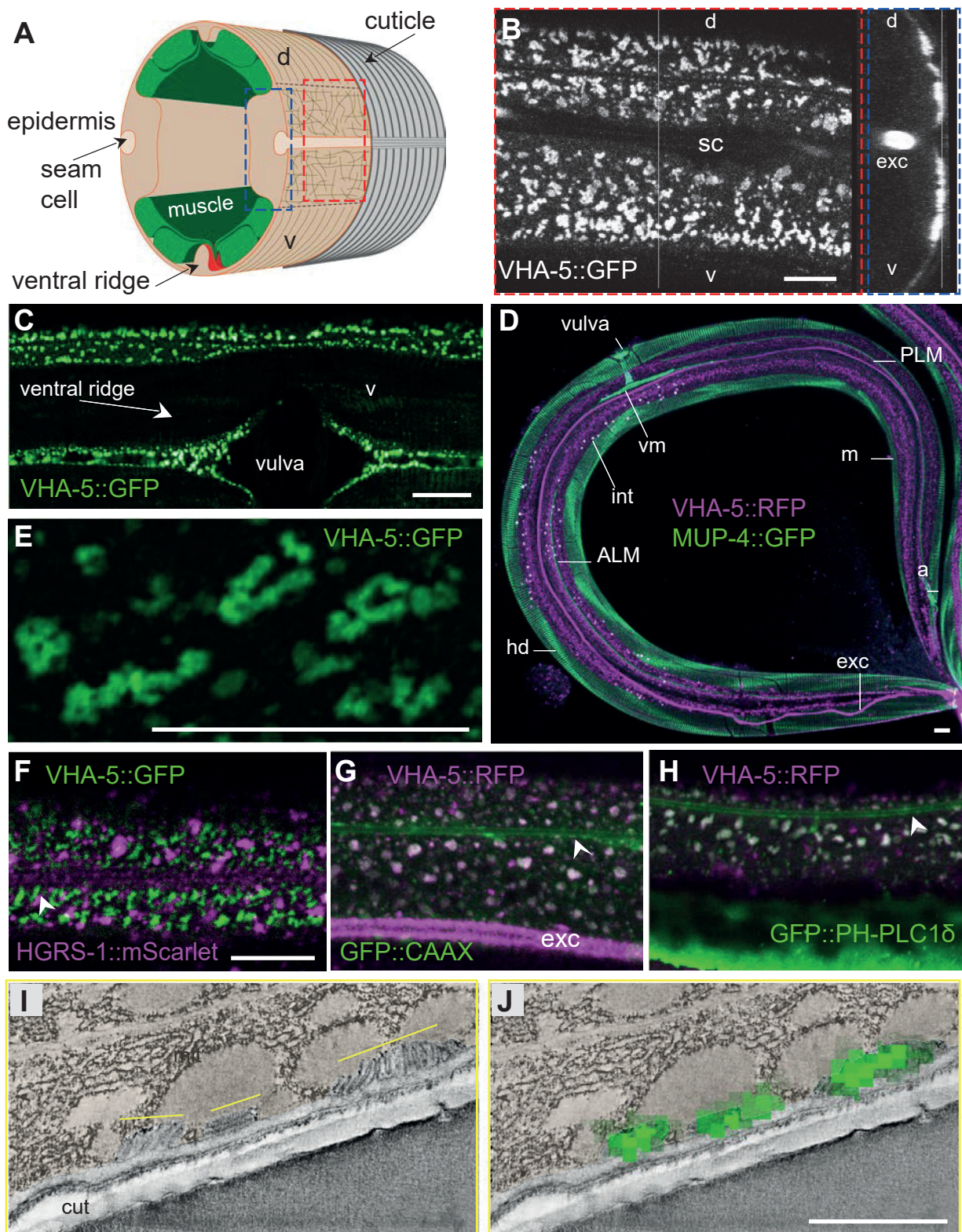


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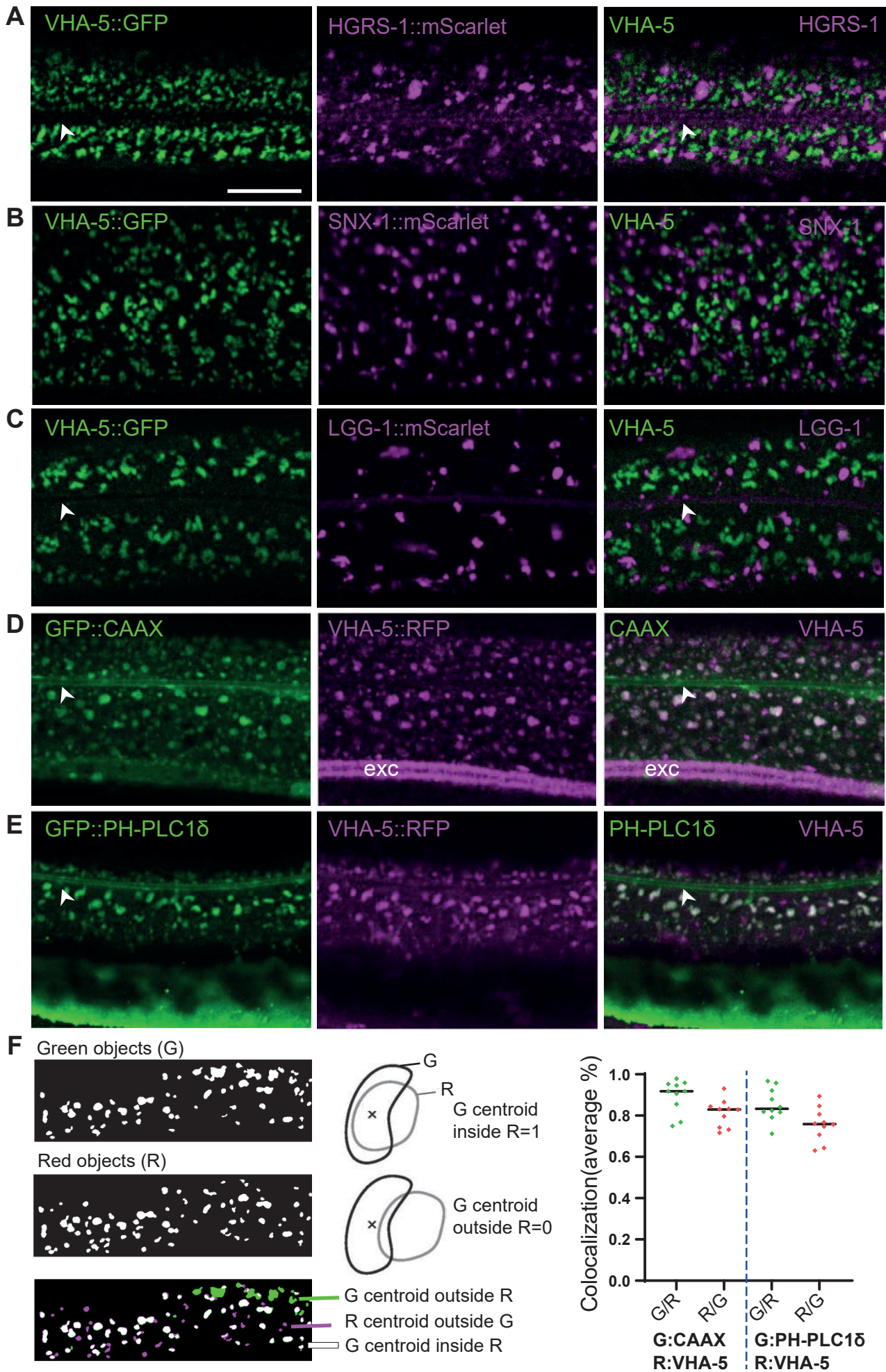


FIGURE 3-Supplement 2

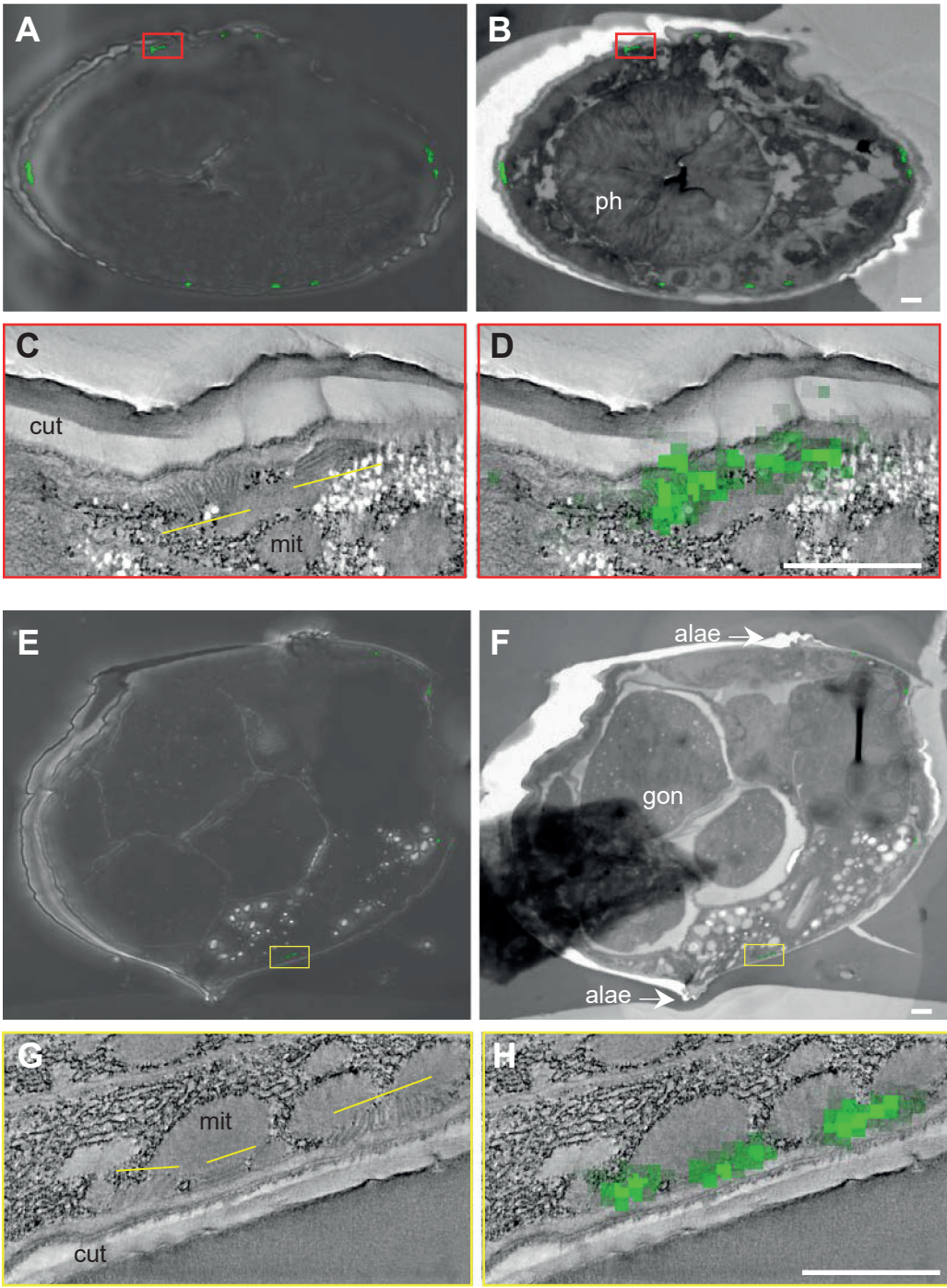


FIGURE 4

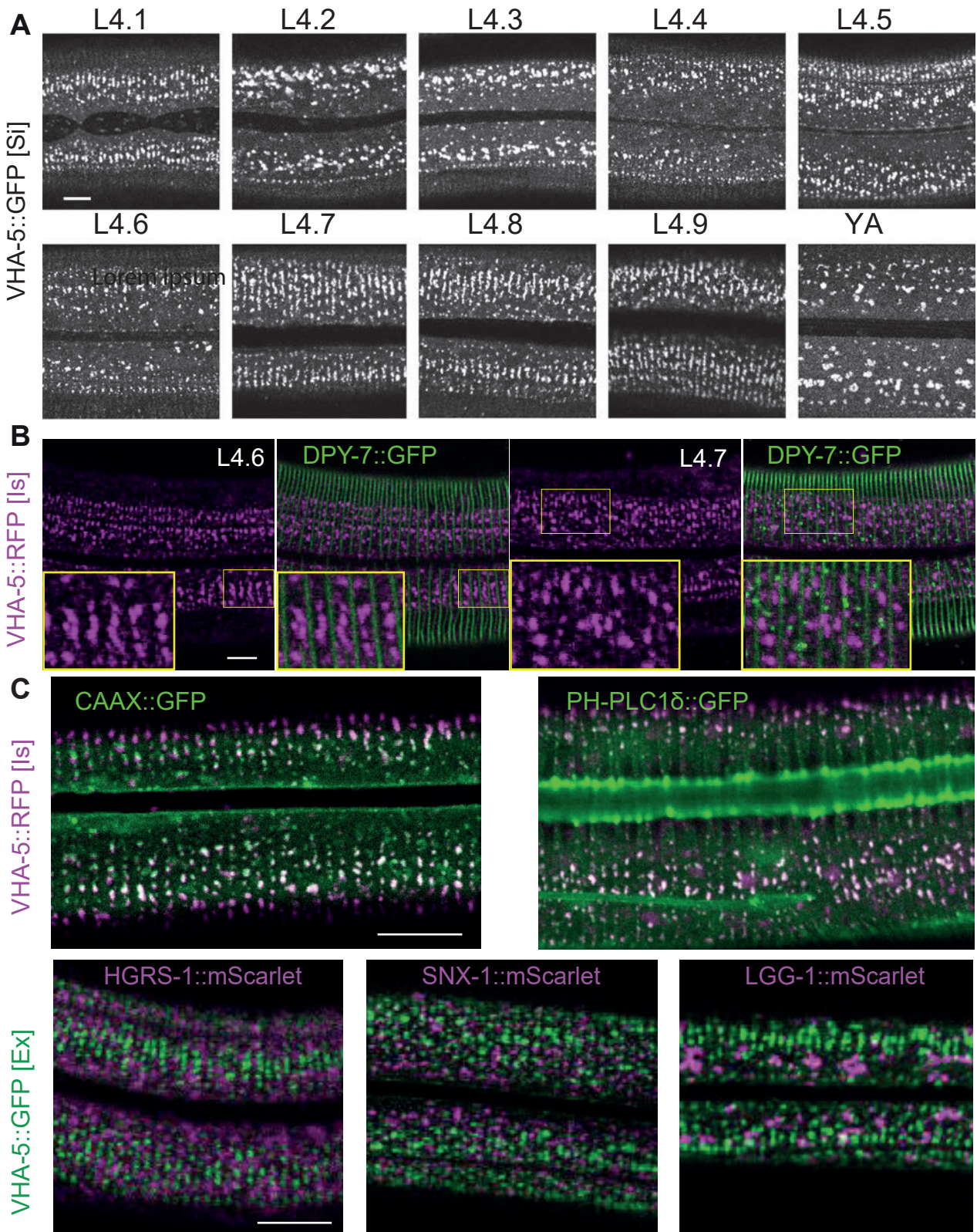


FIGURE 5

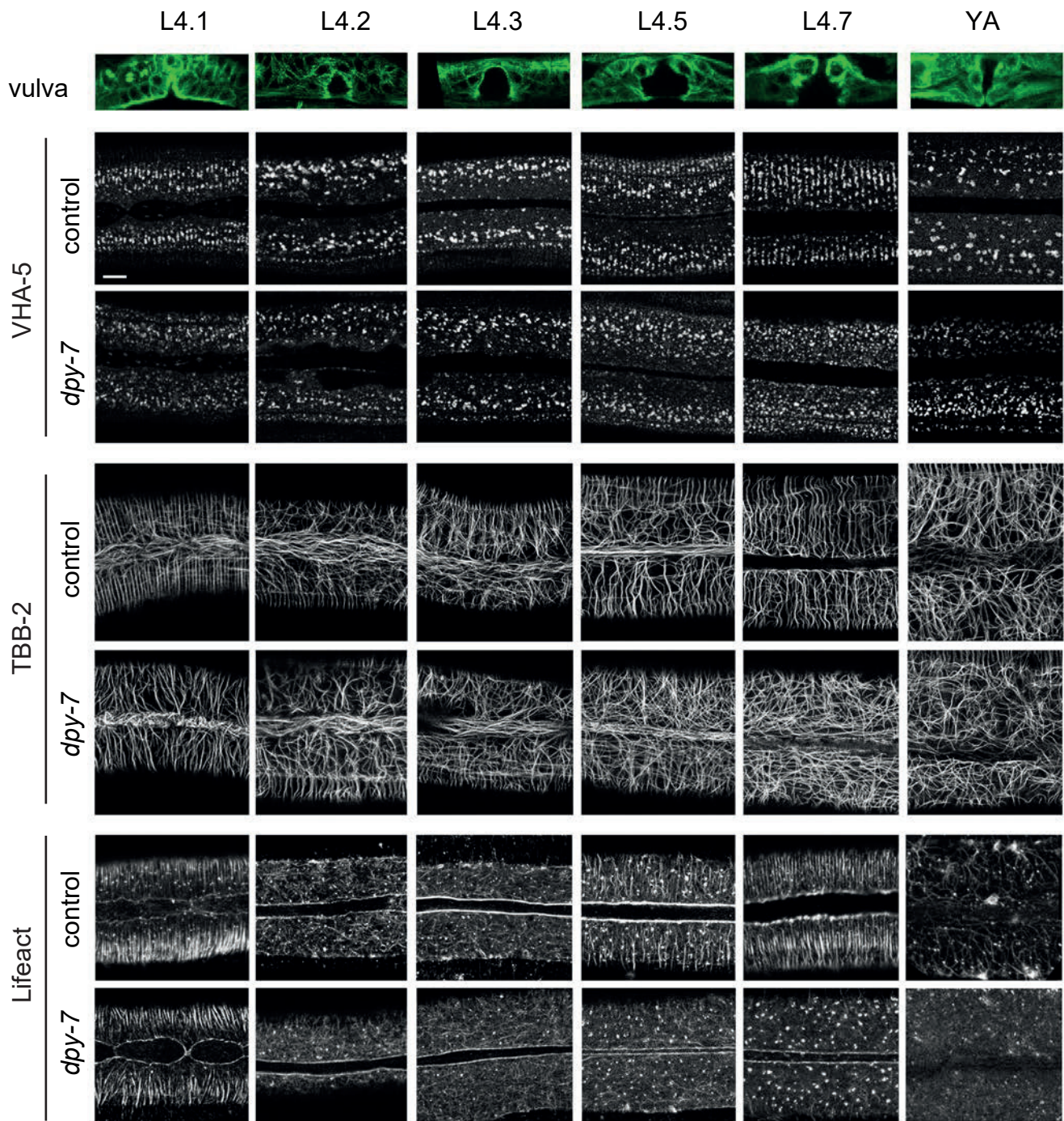


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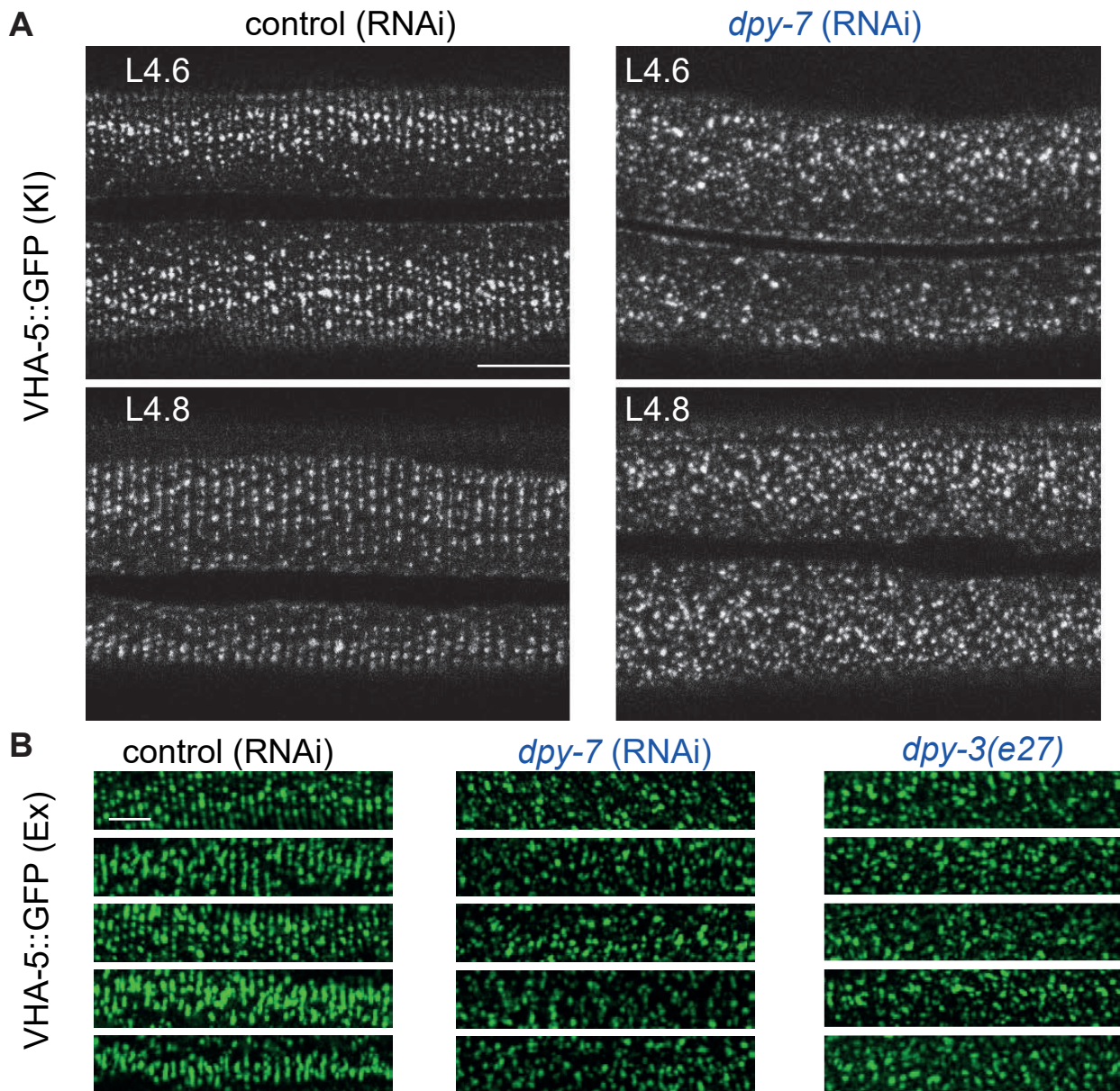


FIGURE 6

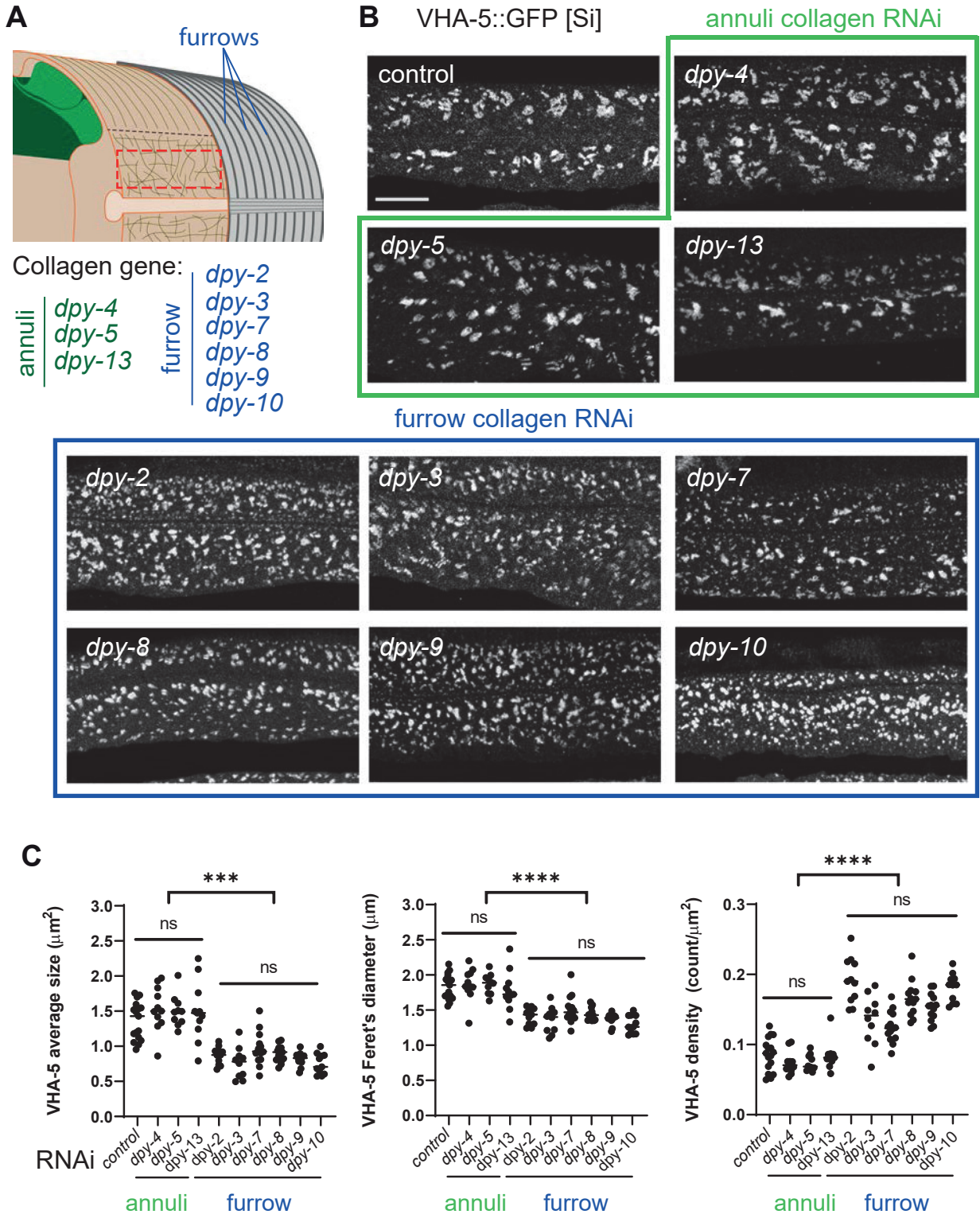
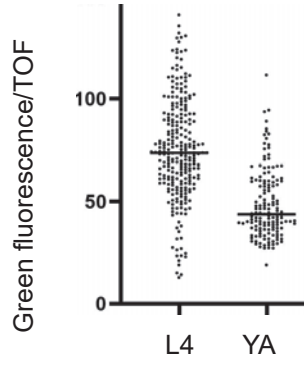
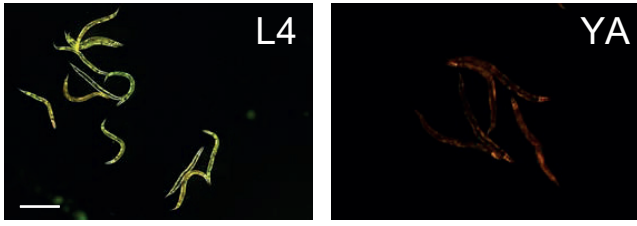
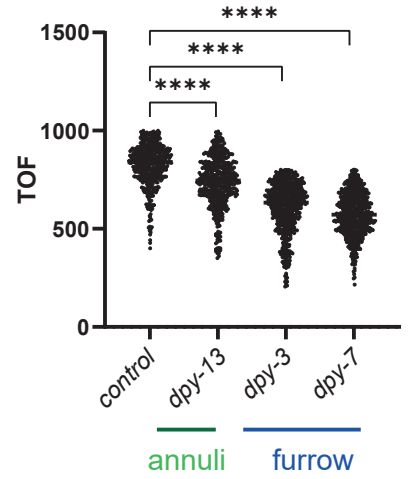
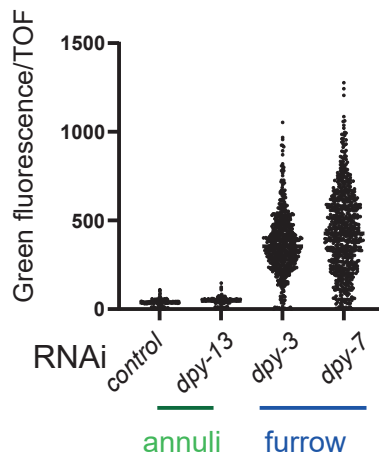
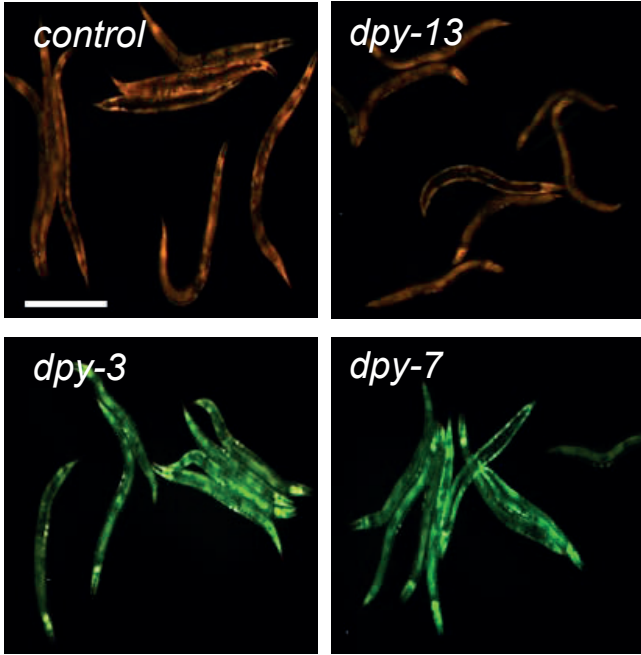


FIGURE 6-Supplement 1

A *nlp-29p::GFP;col-12p::DsRed*



B



C

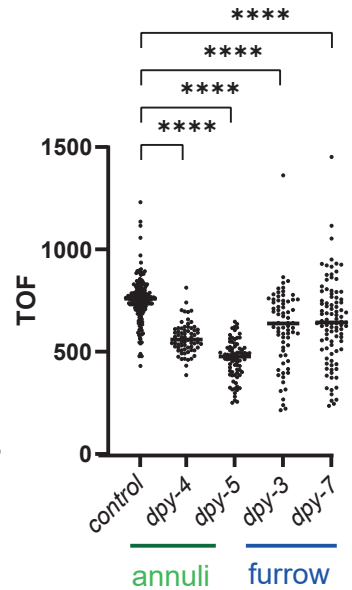
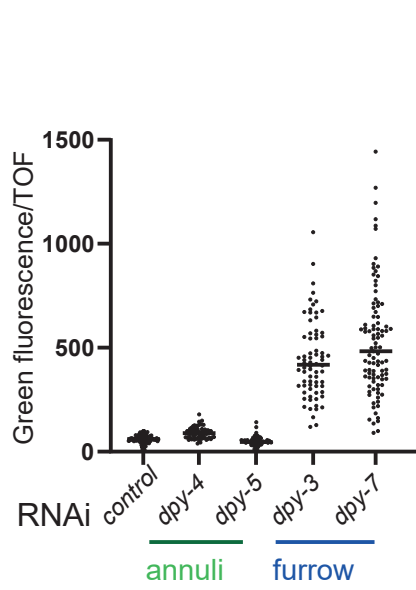
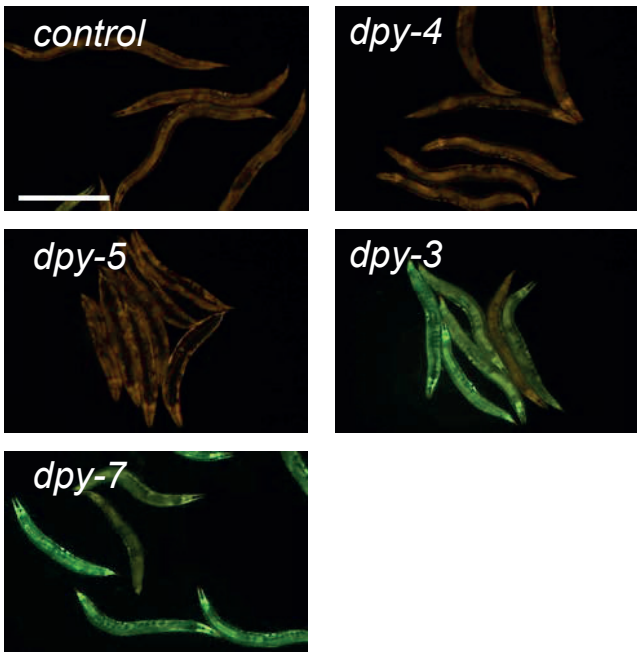


FIGURE 6-Supplement 2

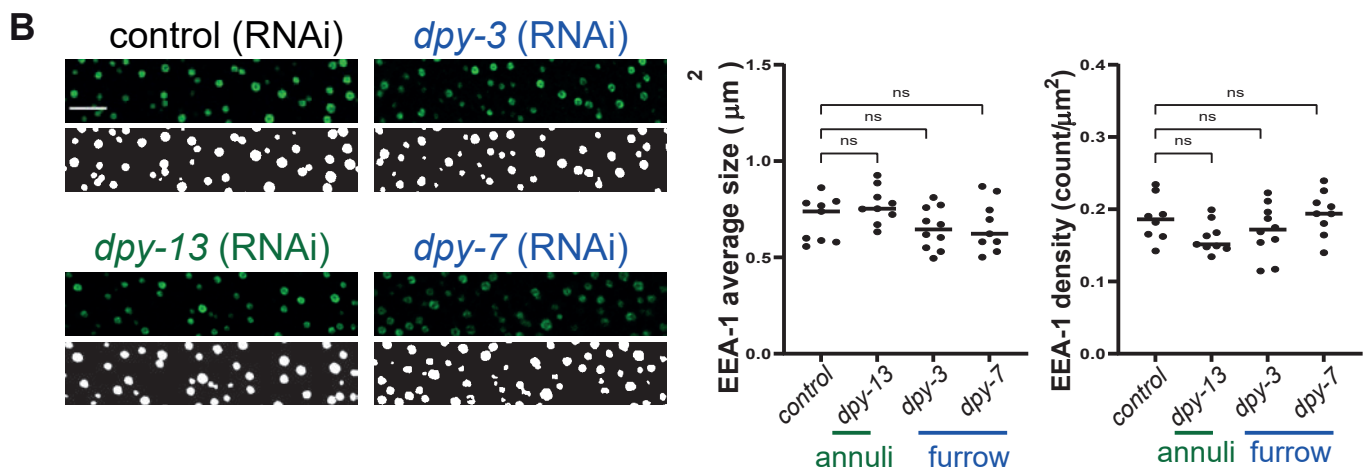
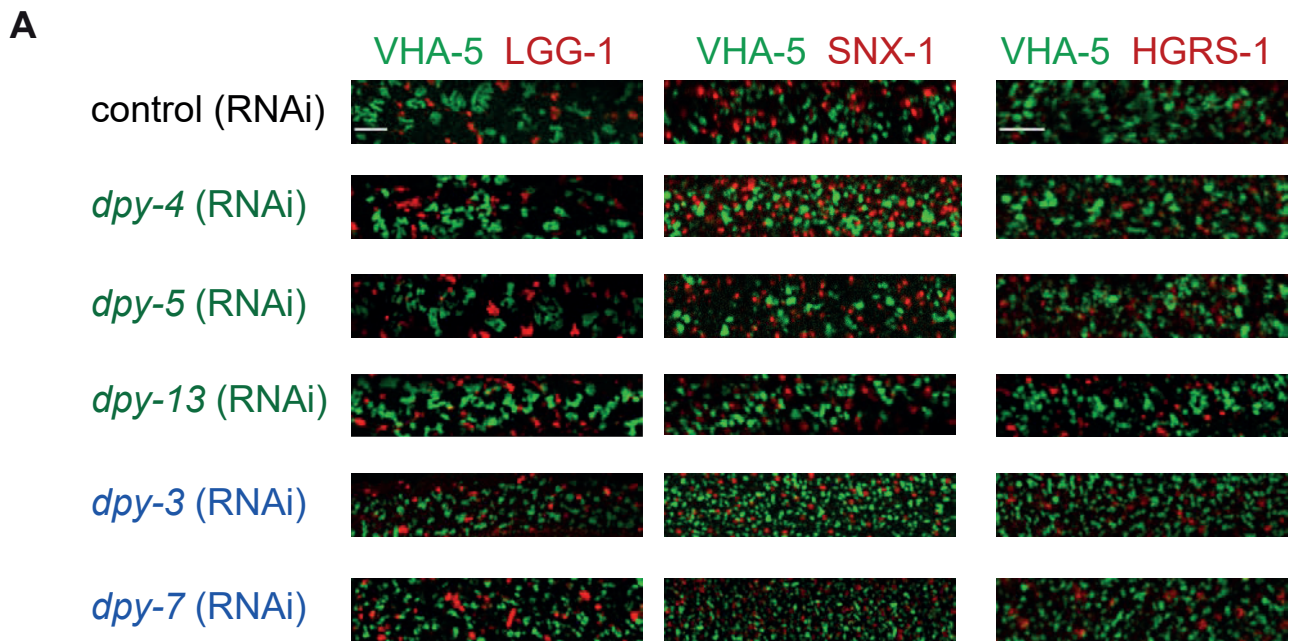


FIGURE 7

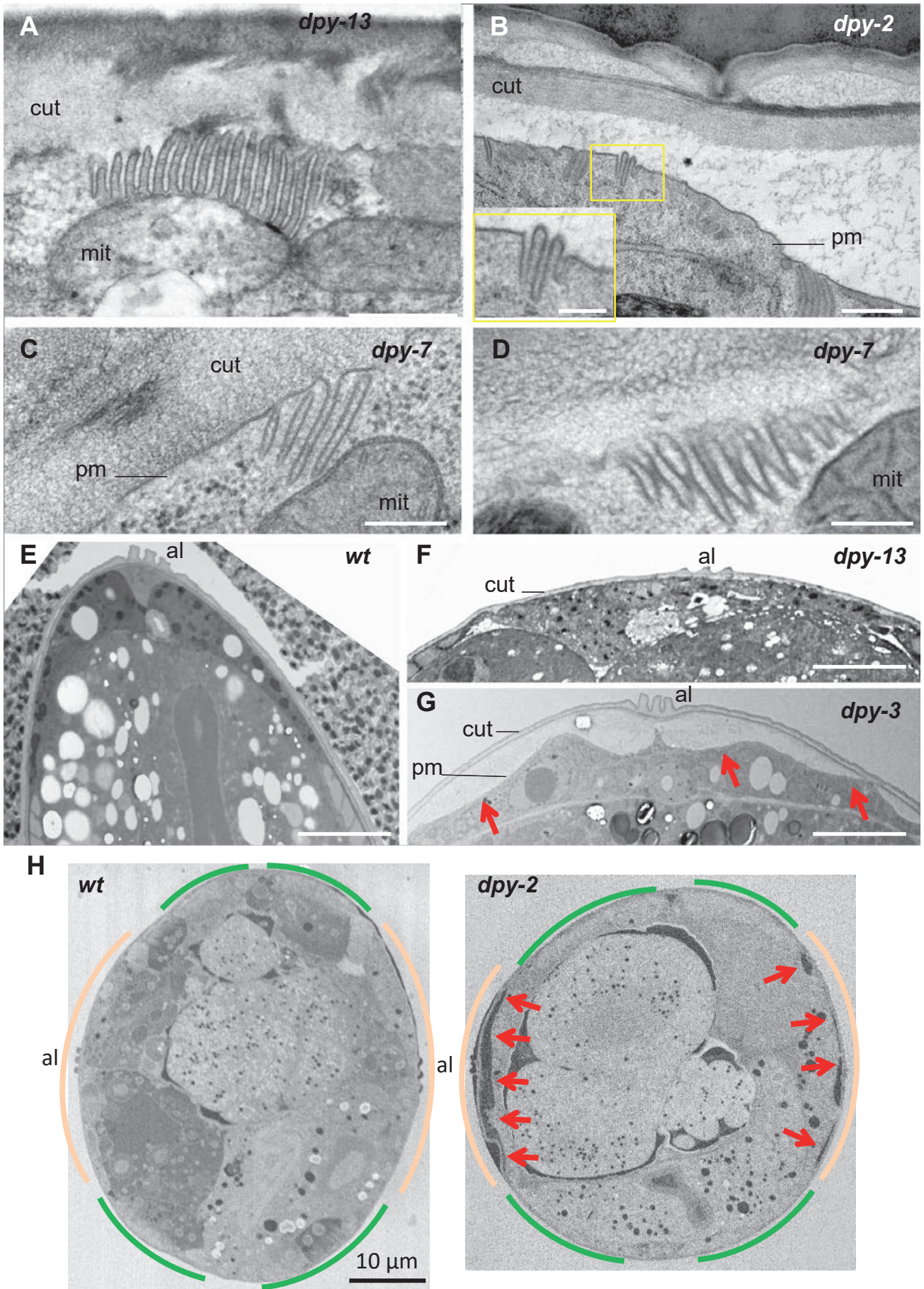


FIGURE 7-Supplement 1

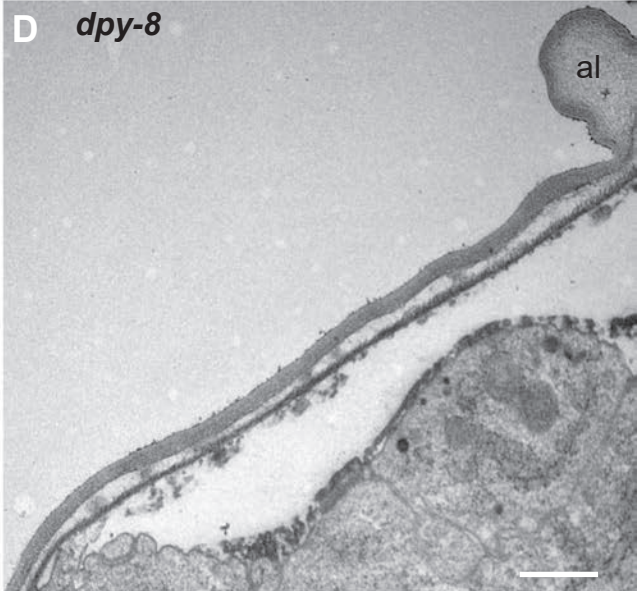
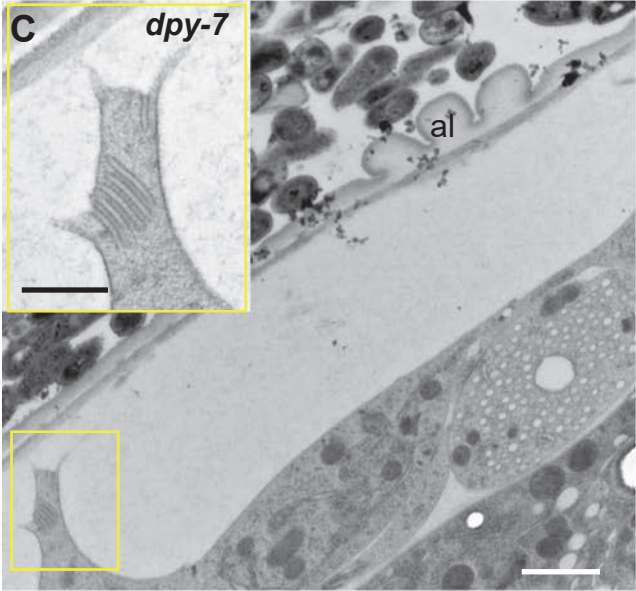
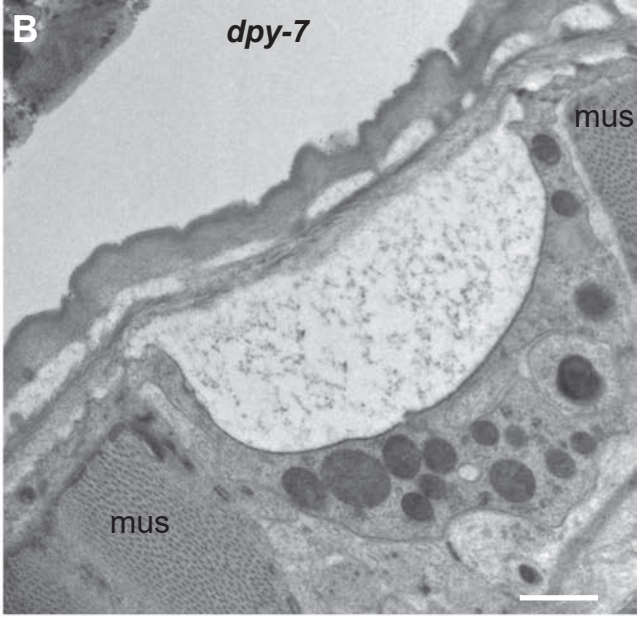
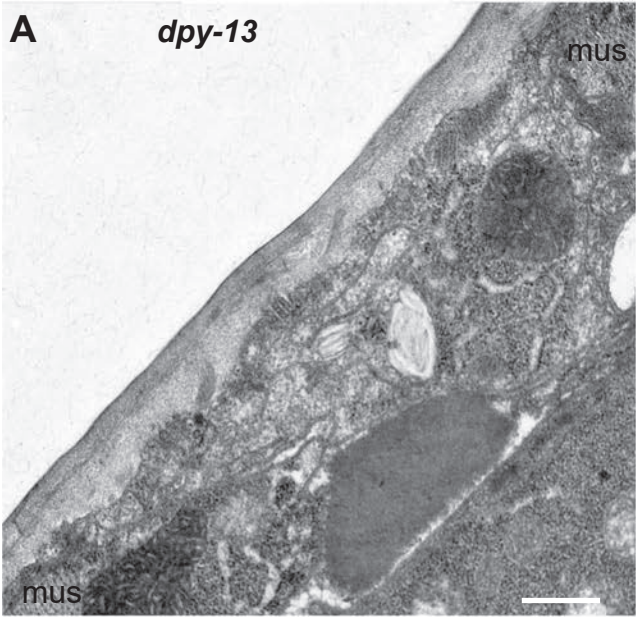
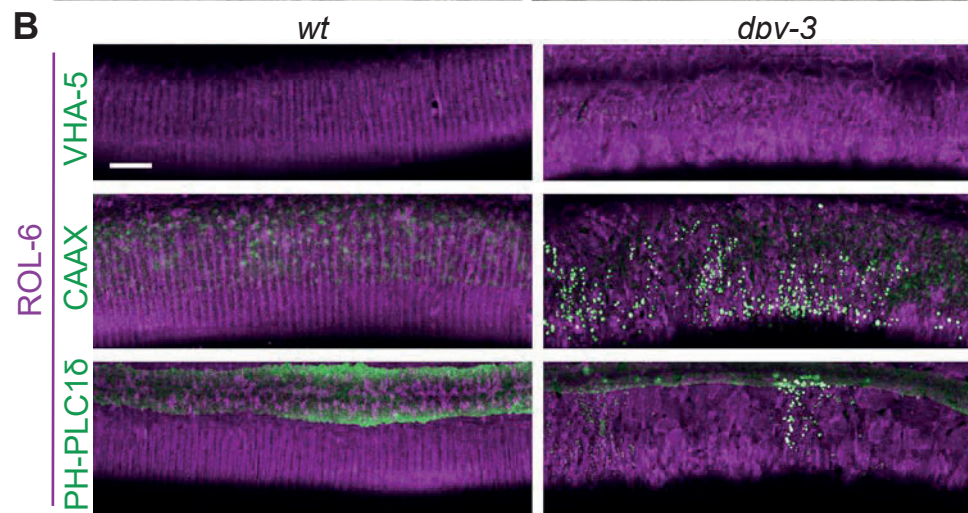
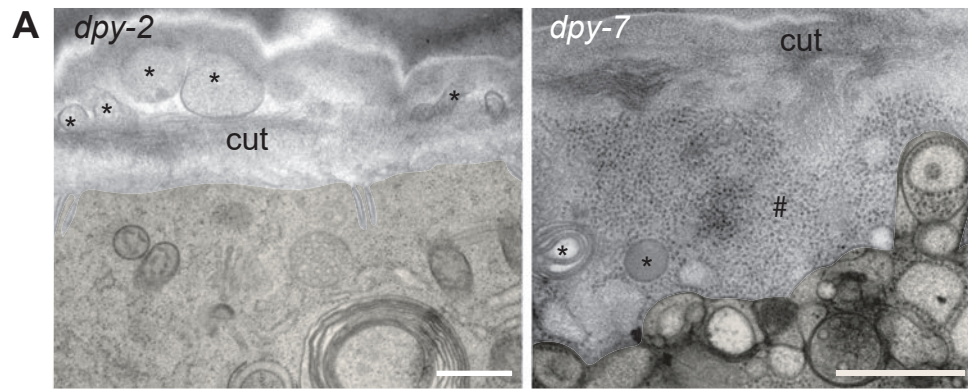


FIGURE 8



% of worms with GFP at the level of the cuticle

	<i>wt</i>	<i>dpy-3</i>
VHA-5::GFP	0 % (n>50)	0 % (n>50)
GFP::CAAX	0 % (n=20)	100 % (n=21)
GFP::PH-PLC1 δ	0 % (n=27)	89,6 % (n=29)

FIGURE 9

