

1 Full Title:

2 The uptake of avermectins in *Caenorhabditis elegans* is dependent on Intra-Flagellar Transport and
3 other protein trafficking pathways

4 Short Title:

5 Uptake of avermectins in *C. elegans* use IFT and other protein trafficking pathways

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21 Abstract:

22 Parasitic nematodes are globally important and place a heavy disease burden on infected humans,
23 crops and livestock, while commonly administered anthelmintics used for treatment are being
24 rendered ineffective by increasing levels of resistance. Although the modes of action and resistance
25 mechanisms caused by detoxification and target site insensitivity for these compounds is well
26 documented, the mechanisms for uptake, which can also cause resistance, are still poorly defined. It
27 has recently been shown in the model nematode *Caenorhabditis elegans* that the avermectins or
28 macrocyclic lactones such as ivermectin and moxidectin gain entry through the sensory cilia of the
29 amphid neurons. This study interrogated the molecular mechanisms involved in the uptake of
30 avermectins using a combination of forward genetics and targeted resistance screening approaches
31 along with visualising a BODIPY labelled ivermectin analog and confirmed the importance of
32 intraflagellar transport in this process. This approach also identified the protein trafficking pathways
33 used by the downstream effectors and the components of the ciliary basal body that are required for
34 effector entry into these non-motile structures. Mutations in many of the genes under investigation
35 also resulted in resistance to the unrelated anthelmintic drugs albendazole and levamisole, giving
36 insights into the potential mechanisms of multidrug resistance observed in field isolates of the
37 parasitic nematodes that are a scourge of ruminant livestock. In total 50 novel *C. elegans*
38 anthelmintic survival associated genes were identified in this study, three of which (*daf-6*, *rab-35*
39 and *inx-19*) are associated with broad spectrum cross resistance. When combined with previously
40 known resistance genes, there are now 53 resistance associated genes which are directly involved in
41 amphid, cilia and IFT function.

42 Author Summary:

43 Nematodes represent significant pathogens of man and domestic animals and control relies heavily
44 on limited classes of Anthelmintic drugs. Single and multi-drug resistance is a growing problem
45 however mechanisms of anthelmintic drug resistance and drug uptake by nematodes remain to be
46 clearly elucidated. In *Caenorhabditis elegans* there has been an association between amphid and dye

47 filling defects with resistance to avermectins however the effector and causal mechanisms remain
48 elusive. This study uses a combination of fluorescently labelled anthelmintics and anthelmintic
49 resistance screens to probe the uptake mechanisms for these drugs. The role of the sensory amphids
50 in the uptake of avermectins was confirmed. The avermectins enter the distal segment of the cilia
51 using an effector which is delivered by the UNC-119 and UNC-33/UNC-44 transport systems to the
52 base of the cilia, followed by distal appendage dependent entry and transport along the cilia by the
53 intraflagellar transport pathway. Of the genes investigated, three (*daf-6*, *rab-35* and *inx-19*) were
54 linked to cross resistance against all the anthelmintics tested (Ivermectin, Moxidectin, Albendazole
55 and Levamisole). This study gives further insight into how important classes of anthelmintics enter
56 nematodes and highlights the potential for this process to give rise to anthelmintic resistance.

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58 Introduction:

59 Parasitic nematodes place a highly significant and heavy disease burden on infected plants and
60 animals causing annual global yield and productivity losses in excess of \$100 billion (1, 2) and in
61 addition requires over \$20 billion annually to treat with anthelmintics (3). Currently available broad
62 spectrum anthelmintics are from a limited range of chemical families (3) and resistance to one or
63 more classes is becoming widespread in field populations (4) jeopardizing food security and human
64 health. Therefore, until new anthelmintic classes are developed, it is necessary to prolong the
65 efficacy of existing drugs by finding ways to suppress resistance.

66 The avermectins or macrocyclic lactones such as ivermectin and moxidectin are the most
67 commonly administered anthelmintics due to their low cost and high persistent efficacy (5) however
68 the rapid spread of resistance is beginning to render them ineffective (4). Avermectins function by
69 paralysing the central nervous system, which eventually leads to death, through interaction with
70 multiple subunits of the glutamate gated chloride channel primary target, as well as multiple
71 secondary targets, thereby resulting in constitutive activation (6). The target binding specificity of

72 avermectins is determined by saccharide groups on C-13 (eg. ivermectin), methoxime on C-23 (eg.
73 moxidectin) and alkyl groups on C-25 of the lactone ring (6, 7). Since subunit interactions vary
74 between different avermectins, commonly occurring target site insensitivity mutations in one
75 subunit binding site do not necessarily confer cross resistance to other macrocyclic lactones (8). As
76 nematodes have limited capacity for phase I detoxification of macrocyclic lactones (9, 10), resistance
77 relies on increased phase II conjugation and efflux (11), target site insensitivity or reduced drug
78 uptake (12). However, all identified and candidate resistance genes that interact directly with
79 avermectins or their metabolites function downstream of macrocyclic lactone uptake (11-13). The
80 macrocyclic lactones lack the chemical properties that would allow them to spontaneously cross
81 biological membranes (14) meaning that uptake is dependent on the ability of biological systems of
82 the organism to accumulate appropriate concentrations in the target tissues however, the
83 mechanism and associated genes involved in uptake are still unknown or poorly defined.

84 There is a high degree of conservation in the layout of the central nervous system between
85 nematode species, which consists of around 200-300 neurons, with sensory inputs from sensilla
86 being processed by the nerve ring to output motor neuron mediated responses (15-17). The amphid
87 sensilla function as the primary sensory organ for environmental stimuli (chemical, ion and osmotic
88 gradients, temperature, pheromones and noxious compounds). The sensilla consist of two pairs of
89 12-13 neurons (12 in *Caenorhabditis elegans*) which have non-motile cilia enriched in G protein-
90 coupled receptors on the dendrites that are exposed to the environment through pores in the cuticle
91 (16, 18-20). Ciliogenesis of sensory cilia utilise assembly pathways that are conserved throughout
92 Eukaryota where a centriole derived basal body anchors to the cell membrane restricting the local
93 diffusion of proteins and lipids and organises microtubules (21). These microtubules are then used
94 for the delivery of lipids and proteins to the growing cilia by intraflagellar transport (IFT) complexes
95 that travel along the microtubules using dynein and kinesin motors (21).

96 There has been an observed correlation between macrocyclic lactone resistance caused by
97 reduced uptake and defects in amphid morphology in *Caenorhabditis elegans* with several causative
98 genes being associated with dye-filling, chemosensation, osmosensation, dauer formation and
99 mechanosensation defective phenotypes (12, 22, 23). Amphid morphology and dye filling defects
100 have also been noted in field populations of *Haemonchus contortus* that are resistant to avermectins
101 (22, 24). This current study uses a mechanistic approach to investigate cellular processes associated
102 with previously discovered resistance genes, in combination with targeted resistance screens and a
103 BODIPY labelled ivermectin analog in *C. elegans*, to identify the roles played by anterograde and
104 retrograde intraflagellar transport in the ciliary distal segment of the amphid neurons in the uptake
105 of avermectins (ivermectin and moxidectin). Pathways involved in trafficking cilia proteins to and
106 from the ciliary gate of the basal body were also investigated, revealing that the UNC-101 and UNC-
107 119 mediated secretion pathways and the polarisers of axon-dendrite protein sorting UNC-33 and
108 UNC-44 are important components involved in avermectin uptake, whereas the RAB-35 recycling
109 pathway plays a role downstream of uptake. Candidate avermectin resistance genes were also
110 checked for cross resistance to other anthelmintics, revealing the IFT and the SEC-24 secretion
111 pathways as being important for susceptibility to the benzimidazole drug, albendazole, while
112 susceptibility to the imidazothiazole levamisole is not dependent on any of the tested secretion
113 pathways. A whole genome sequencing approach was applied to map candidates from a forward
114 genetic screen for resistance to avermectins, and in combination with a targeted resistance screen,
115 50 novel anthelmintic-survival associated genes were uncovered in *C. elegans* including: 16
116 avermectin resistant, 7 avermectin and albendazole resistant, 9 albendazole resistant, 7 levamisole
117 resistant, 1 avermectin and levamisole resistant and 3 genes (*daf-6*, *rab-35* and *inx-19*) which cause
118 broad spectrum cross resistance to all four anthelmintics tested.

119 Methods:

120 Chemicals

121 Suppliers and catalogue numbers of all reagents used are listed in the Supplementary Methods.

122 Nematode strains

123 Putative orthologs of key basal body genes for which there was no primary literature were chosen
124 using a combination of Protein BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) and the Marrvel (25)
125 and AceView (26) databases.

126 TM prefixed strains were obtained from the National BioResource Project, Japan while all other
127 strains used were purchased from the *C. elegans* Genetics Centre, USA. All strains were maintained
128 on *Escherichia coli* OP50-1 inoculated Nematode Growth Medium (NGM) plates following standard
129 protocols (http://www.wormbook.org/toc_wormmethods.html). Strains used in this study are listed
130 in the Supplementary Methods.

131 Anthelmintic resistance assays

132 Anthelmintic stock solutions were prepared as follows: 10 μ M ivermectin stock was made by the
133 serial dilution of a 10mM stock using DMSO as a solvent for both stocks; 10 μ M moxidectin stock was
134 prepared using the same procedure as ivermectin; 50mM albendazole stock was made by dissolving
135 in DMSO at 31°C with vigorous agitation; 1M levamisole stock was made by dissolving in sterile
136 distilled water. Stock solutions were dispensed into 1ml aliquots and stored at -20°C.

137 NGM plates containing anthelmintics were produced by adding volumes of anthelmintic
138 stock solution to cooled molten NGM agar (50°C) before mixing and pouring onto 3cm petri dishes.
139 The volume of anthelmintic stock solution added never exceeded 0.3% of the final volume.
140 Anthelmintic plate concentrations used were 10nM ivermectin, 5nM and 10nM moxidectin, 100 μ M
141 and 150 μ M albendazole and 0.2mM and 0.8mM levamisole. Plates were inoculated with 50 μ l OP50-
142 1 24 hours before starting assays.

143 To determine ivermectin and moxidectin resistance, survival assays were performed by
144 picking 5 L4 worms of the strain to be tested onto each plate with two biological and two technical
145 replicates. Growth and mortality were inspected every 48 hours using a light microscope. A strain
146 was considered resistant if it could produce an F2 generation before the plates desiccated compared
147 to susceptible strains which showed paralysis and growth arrest with the F1 generation failing to

148 reach adulthood. The wild type N2 strain was used as a susceptible negative control and
149 DA1316(*ad1305; vu227; pk54*) was used as a resistant positive control. The strength of resistance for
150 ivermectin exposure was categorised as weak (+ (w)) if the population only reached F2, moderate (+)
151 if it reached F3 or F4 and strong (++) if growth was visually indistinguishable from NGM plates
152 without anthelmintics. Moxidectin resistance strength was categorised as weak (+ (w)) if the
153 population only reached F2 on 5nM plates, moderate (+) if it reached F3 or F4 on 5nM plates and
154 strong (++) if it reached F3 or F4 on 10nM plates.

155 Albendazole and levamisole resistance was determined using uncoordinated phenotype
156 assays by picking 5 adult worms of the strain to be tested onto each plate with two biological and
157 two technical replicates. At day 3 and day 6 a random sample of 20 worms per plate were poked on
158 their head with a platinum wire and scored for the ability to reverse backwards (an inability to
159 reverse corresponds to an Unc or uncoordinated phenotype). For mutant strains which innately
160 show an uncoordinated phenotype (Unc), a different scoring criteria was used; with worms being
161 scored as resistant if any muscle movement was shown in response to being poked and scored as
162 sensitive if they were completely paralysed. N2 was used as a negative (sensitive) control and
163 CB3474(*e1880*) (for albendazole resistance), ZZ1(*x1*) or ZZ15(*x15*) (for levamisole resistance) were
164 used as positive controls. Strains were considered resistant if over 50% of sampled worms were
165 unaffected by the anthelmintic; categorised as moderately resistant (+) at the lower dose and
166 strongly resistant (++) at the higher dose. If at the higher dose a strain was 100% unaffected it was
167 classed as extremely resistant (+++). Susceptible strains which had less than 50% of the sampled
168 population unaffected at the lower dose (-) were categorised as highly susceptible (--) by comparing
169 for impaired growth and reproduction relative to controls on NGM plates without anthelmintics.
170 Strains were deemed extremely susceptible (---) if mortality was observed at either anthelmintic
171 concentration.

172 Synthesis and evaluation of BODIPY labelled anthelmintic analogs

173 Details of chemical synthesis, purification and analysis of fluorescent analogs of ivermectin and
174 albendazole (Fig 1) are listed in Supplementary Methods. Fatty-BODIPY-Ivermectin (FBI) was
175 synthesised in 11 steps as shown in Fig S3A. BODIPY-Albendazole (BABZ) was synthesised in 5 steps
176 as shown in Fig S3B.

177 The acute toxicity of parent anthelmintic compounds and fluorescent analogs were
178 compared by bleaching worms to synchronise larval development before immediate use for
179 ivermectin and FBI or rearing to L4 for albendazole and BABZ. Worms suspended in M9 were
180 transferred to Eppendorfs in batches of 300 and made up to 184-188 μ l with M9 before adding 10 μ l
181 OP50-1 culture and 2-6 μ l of anthelmintic solution (200 μ l total volume) and incubated for 24 hours at
182 21°C. The worms were then washed and split onto 3 NGM plates and for ivermectin and FBI the
183 number of living worms were counted at 0 and every 48 hours after transfer until death or
184 adulthood while for albendazole and BABZ all worms were immediately assessed for an
185 uncoordinated phenotype using the same method as used to check for albendazole resistance.
186 Ivermectin and FBI stock solutions were diluted in methanol while albendazole and BABZ stock
187 solutions were diluted in DMSO. The range of doses tested on N2 were 10-50nM ivermectin, 1,000-
188 15,516(1% of stock solution)nM FBI, 10-500 μ M albendazole and 88.45-265.35(3% stock solution) μ M
189 BABZ. For the ivermectin resistant strain DA1316, 10-3,443 μ M ivermectin and 9,000-15,516(1% of
190 stock solution)nM FBI were used for the dose ranges. The albendazole resistant strain Ben-1(*e1880*)
191 was exposed to 88.45-265.35(3% stock solution) μ M BABZ. Biological replicates for each dose were
192 performed in triplicate. Mortality and uncoordination percentages for each dose underwent a
193 Grubb's test for outliers (28) and was corrected against solvent only controls using the Schneider-
194 Orelli variant of Abbott's formula (29) before applying Probit analysis (30) to establish the lethal
195 dose (50%)(LD₅₀) for ivermectin and FBI and effective dose (50%)(ED₅₀) for albendazole and BABZ.
196 The statistical significance of LD/ED₅₀ differences between strains and compounds was determined
197 using the Litchfield & Wilcoxon method (31).

198 **Dil dye-filling, FBI and BABZ assays and microscopy**

199 Worms were washed from populated plates using M9 buffer (3g KH₂PO₄, 6g Na₂HPO₄, 5g NaCl and
200 1mM MgSO₄ per litre) and collected in 1.5ml Eppendorfs. Samples were pelleted by centrifugation at
201 7,000 rpm for 10 secs to allow removal of the supernatant. Two washes with M9 were performed
202 before applying 10µg/ml Dil (1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate)
203 dye in M9 buffer for 30 mins. Samples were then washed twice with M9 before incubating at 21°C
204 for 2 hours to allow worms to clear their gut of bacteria and dislodge Dil adhered to the cuticle
205 before performing two more washes in M9. Worms were pelleted and supernatant removed before
206 transfer to an empty petri dish using a pipette and then picking 20-30 specimens onto prepared
207 microscope slides. Slides were coated with a pad of 2% agar with 1% sodium azide and wet with 10µl
208 of M9 containing 0.2% sodium azide, then coverslips were sealed with a thin layer of petroleum jelly.

209 Plates for FBI and BABZ assays were prepared by drying 3cm NGM plates in a laminar air
210 flow cabinet for 40 mins before applying 100µl of 5µM FBI, 150µM BABZ or 150µM 1,3,5,7-
211 tetramethyl-8-pent-4-ene-BODIPY (control for cleavage of the BODIPY containing side chain of BABZ)
212 diluted in methanol and left for 1-3 hours prior to applying 50µl of OP50-1 culture. The next day, 40
213 worms of the strain to be tested were then picked onto the plates before incubation at 16°C. After
214 the defined incubation period, 20-30 specimens were picked into droplets of M9 to wash off excess
215 BODIPY labelled compound before picking onto prepared agarose pads on microscope slides.

216 Slides were viewed using a Zeiss Axioskop 2 Plus microscope fitted with a Zeiss Mercury HBO
217 100 Lamphouse and Zeiss AxioCam camera with images taken using the accompanying Axiovision
218 software. Control images of worms were taken using a Differential Interference Contrast (DIC) filter,
219 0.5 secs exposure time and the minimum setting for the internal light source while Dil, BABZ and
220 1,3,5,7-tetramethyl-8-pent-4-ene-BODIPY staining was viewed and imaged using a Fluorescein
221 Isothiocyanate (FITC) filter, 1 sec exposure time and illumination by the mercury lamp. FITC images
222 of FBI exposed worms used a 2 sec exposure time. A minimum of 10 individuals of each strain were
223 observed under FITC conditions to score the average intensity of Dil, FBI and BABZ staining (negative

224 (-), weak positive (+ (w)) or strong positive (+)). Representative DIC and FITC images for Dil, FBI and
225 BABZ staining patterns in each category are shown in Fig 2 while images for individual strains are
226 available upon request.

227 EMS mutagenesis and whole genome sequencing

228 *C. elegans* L4 stage N2 strain worms were exposed to 50 mM ethyl methanesulfonate (EMS) for 4 h
229 at 20°C following standard mutagenesis procedures (32), then allowed to recover on OP50-1 seeded
230 NGM plates overnight. Worms were then handled according to Page, 2018 (23) selecting for 10nM
231 moxidectin resistance (see Supplementary Methods for details). Lines were then characterised for
232 Dil dye-filling and ivermectin, albendazole and levamisole cross resistance.

233 From the 14 resulting moxidectin resistant lines 5 were selected and together with
234 uncharacterised ivermectin resistant lines TP236(ka30), TP241(ka35), TP272(ka64) and TP274(ka66)
235 from a previous study (23) were processed for single nucleotide polymorphism (SNP) mapping. SNP
236 mapping was carried out as described in Doitsidou, 2010 (33) using MiModD tools on the public
237 instance of the Galaxy platform (<https://usegalaxy.org>)(34) (see Supplementary Methods for details).
238 Genomic DNA was extracted using a Genra Puregene Core Kit A (Qiagen, UK) kit before clean up and
239 concentration using a Genomic DNA Clean & Concentrator-25 (Zymo Research, US) kit. Samples were
240 sent for whole genome sequencing to the Glasgow Polyomics facility, University of Glasgow where
241 libraries were prepared with a TruSeq® Nano DNA LT Sample Prep Kit (Illumina), quality controlled
242 on a 2100 Bioanalyzer (Agilent) and run on an Illumina MiSeq platform using 300bp paired end
243 reads.

244 Results:

245 Outcomes of dye-filling, survival and uncoordinated phenotype assays are listed in Table 1. Strains
246 tested that did not show a phenotype of interest are included in Table S1.

247 **Table 1. Many *C. elegans* mutants for ciliary proteins are resistant to ivermectin, moxidectin,**
 248 **albendazole and levamisole.**

249 **Underline** = previously identified mutants that are resistant to one of the anthelmintics tested. **Dyf** = Dil
 250 amphid dye filling; **IVM R** = Ivermectin resistance; **MOX R** = Moxidectin resistance; **ABZ R** =
 251 Albendazole resistance; **LEV R** = Levamisole resistance; **+++** = Extreme resistance; **++** = Strong
 252 resistance; **+** = Dye filling/Moderate resistance; **-** = Dye filling defective/Susceptible; **--** = Highly
 253 susceptible; **---** = Extremely susceptible; **w** = weak phenotype; **v** = High variability in phenotype (**-/+**:
 254 phenotypes range across the entire spectrum). **IFT** = intraflagellar transport component homology. *H.*
 255 *contortus* homologues investigated by BLASTP using WormBaseParaSite (WBPS14, WS269), hits
 256 shown as % identity over specified amino acid length.

Gene (homology)	Strain (allele)	DYF	IVM R	MOX R	ABZ R	LEV R
Transcription Factor Mutants						
<i>daf-19</i> (RFX transcription factor)	DR86(<i>m86</i>)	-	+	++	-	-
<i>hlh-4</i> (achaete-scute transcription factor)	TM604(<i>tm604</i>)	+ (w)	+	+	-	---
Cell Migration/Adhesion Defect Mutants						
<i>dyf-7</i> (ZP protein)	SP1735(<i>m537</i>)	-	++	++	++	-
<i>mec-8</i> (RRM domain/splice factor)	CB398(<i>e398</i>)	+	++	+	+	-
Amphid Channel Morphology Mutants						
<i>daf-6</i> (PTCHD-1/4 ortholog)	CB1377(<i>e1377</i>)	-	++	++	+	+
Protein Secretion/Trafficking Defect Mutants						
<i>aex-4</i> (SNAP23 ortholog, syntaxin)	JT5244(<i>sa22</i>)	+	-	-	+	-
<i>arl-13</i> (ARL13B ortholog)	TM1745(<i>tm1745</i>)	+	-	+ (w)	-	-
<i>che-14</i> (DISP1 ortholog)	CB3687(<i>e1960</i>)	-/+ (v)	-	-	-	---
<i>che-14</i> (DISP1 ortholog)	ML514(<i>ok193</i>)	-/+ (v)	-	-	-	---
<i>dnc-1</i> (p150(glued) ortholog)	EU1006(<i>or404</i>)	+	-	-	++	---
<i>dyf-5</i> (map kinase)	SP1745(<i>mn400</i>)	+ (w)	+	+	-	-
<i>dyf-18</i> (CDK-8/19/20 ortholog)	ET100(<i>ok200</i>)	+ (w)	-	+	-	-
<i>mvb-12</i> (MVB12A ortholog, ESCRT I Complex)	RB2514(<i>ok3482</i>)	+	-	-	+	-
<i>osta-1</i> (SLC51A ortholog)	TM5255(<i>tm5255</i>)	+	-	+	-	-
<i>pamn-1</i> (PAM ortholog)	VC2129(<i>ok2681</i>)	+	-	-	-	---
<i>rab-3</i> (RAB family)	NM210(<i>y250</i>)	+	-	-	++	-
<i>rab-35</i> (RAB family)	RT206(<i>b1013</i>)	+	+	+	+	+
<i>rpi-2</i> (RP2 ortholog)	RB1550(<i>ok1863</i>)	+	-	-	+	-
<i>sedl-1</i> (TRAPPC2 ortholog)	RB1912(<i>ok2485</i>)	+	-	-	-	+
<i>snpn-1</i> (SNAPIN ortholog, BLOC-1 complex)	TM1892(<i>tm1892</i>)	+	-	-	-	---
<i>t06g6.3</i> (myosin heavy chain like)	VC1188(<i>gk546</i>)	+	-	-	-	+
<i>unc-18</i> (syntaxin)	CB234(<i>e234</i>)	+	-	-	-	---
<i>unc-33</i> (CRMP1 ortholog, filamin binding)	CB1193(<i>e1193</i>)	+	+	+	-	-
<i>unc-44</i> (ANK2/ANK3 ortholog)	CB1197(<i>e1197</i>)	- (v)	++	++	-	-
<i>unc-101</i> (AP1M1 ortholog)	PS529(<i>sy108</i>)	-	+	+	-	-
<i>unc-119</i> (HRG4 ortholog)	CB4845(<i>e2498</i>)	+ (w)	+	+	-	---
<i>vamp-7</i> (VAMP8 ortholog, SNAP receptor)	TM6588(<i>tm6588</i>)	+	-	-	++	-
<i>vps-36</i> (VPS36 ortholog, ESCRT II complex)	VC947(<i>gk427</i>)	+	-	-	-	+ (w)
Cilia Nucleation and Region Identity Mutants						
<i>b0432.8</i> (TATDN3 ortholog, has FAM92 homology)	TM6737(<i>tm6737</i>)	+	-	-	-	---
<i>c14h10.2</i> (JAKMIP3 ortholog, putative CEP123 ortholog)	TM10737(<i>tm10737</i>)	- (v)	+ (w)	+	+	---
<i>che-10</i> (rootelin, IFT)	CB3329(<i>e1809</i>)	+ (w)	+	++	-	-
<i>che-12</i> (TOGARAM1 ortholog, IFT)	CB3332(<i>e1812</i>)	-	+	+	-	---
<i>dpy-6</i> (putative OFD1 ortholog)	CB5542(<i>e2762</i>)	+	-	-	-	---
<i>dyf-17</i> (MAGEL2 like)	EG175(<i>ox175</i>)	- (v)	++	++	++	-

<i>dyf-19</i> (FBF1 ortholog)	ZP541(<i>jhu455</i>)	-	+	++	-	-
<i>f59g1.4</i> (ARMC9/JBTS-30 ortholog)	VC3981(<i>gk5058</i>)	+	-	-	++	-
<i>gasr-8</i> (GAS8 ortholog)	VC2343(<i>gk1232</i>)	+	-	-	-	++
<i>hyls-1</i> (hydrolethalus syndrome ortholog)	TM3067(<i>tm3067</i>)	+ (w)	++	++	-	-
<i>mks-5</i> (RPGRIP1L ortholog)	RB2574(<i>ok3582</i>)	+	-	-	-	+
<i>nphp-4</i> (nephrocystin 4 ortholog)	TM925(<i>tm925</i>)	+ (w)	+ (v)	+ (v)	-	-
<i>tag-278</i> (putative OFD1 ortholog)	VC934(<i>gk382</i>)	-/+ (v)	-	-	-	-
<i>unc-15</i> (has ODF2/Cenexin homology, myosin heavy chain)	CB1402(<i>e1402</i>)	+	-	-	-	---
<i>unc-54</i> (has SCLT1 homology, myosin heavy chain)	CB1201(<i>e1201</i>)	+	-	-	-	---
<i>yap-1</i> (WWTR1 ortholog, has CEP164 homology)	TM1416(<i>tm1416</i>)	+	-	+ (w)	-	-
Microtubule Mutants						
<i>ben-1</i> (β-tubulin homolog)	CB3474(<i>e1880</i>)	+	-	-	+++	-
<i>dyf-10</i> (α-tubulin homolog)	SP1709(<i>e1383</i>)	-	++	++	-	-
Dynein and Kinesin Motor Mutants						
<i>che-3</i> (Dynein HC avr-1, IFT)	CB1124(<i>e1124</i>)	-	+	++	-	-
<i>dhc-3</i> (Dynein HC, IFT)	TP239(<i>ka33</i>)	-	+	++	-	-
<i>klp-7</i> (KIF2A ortholog)	TM7884(<i>tm7884</i>)	+	-	-	++	-
<i>osm-3</i> (kinesin family, IFT)	PR802(<i>p802</i>)	-	+	++	-	-
<i>xbx-1</i> (DYNC2LI1 ortholog)	JT11069(<i>ok279</i>)	-/+ (v)	++	++	++	-
IFT-A Complex Mutants						
<i>che-11</i> (IFT140 homolog, IFT)	CB3330(<i>e1810</i>)	-	++	++	+	-
<i>daf-10</i> (IFT122A homolog, WD repeat, IFT)	CB1387(<i>e1387</i>)	-	++	++	++	---
<i>dyf-2</i> (IFT144 homolog, WRD19, IFT)	SP1234(<i>m160</i>)	-	+	++	-	-
<i>ift-43</i> (IFT43 homolog, IFT)	TM8137(<i>tm8137</i>)	+	-	+ (w)	-	-
<i>ifta-1</i> (IFT122B homolog, WDR35, IFT)	MX124(<i>nx61</i>)	+ (w)	++	+	-	-
<i>zk328.7</i> (IFT139 homolog)	VC1130(<i>gk508</i>)	+	-	-	++	-
IFT-B Complex Mutants						
<i>che-2</i> (IFT80 homolog, G-protein, WD repeat)	CB1033(<i>e1033</i>)	-	+	++	-	-
<i>che-13</i> (IFT57/Hippi)	CB3323(<i>e1815</i>)	-	++	+	+	---
<i>dyf-1</i> (IFT70 homolog, IFT)	SP1205(<i>mn335</i>)	-	+	++	-	-
<i>dyf-3</i> (IFT38 homolog, CLUAP protein, IFT)	SP1603(<i>m185</i>)	-	++	++	+	-
<i>dyf-6</i> (IFT46 homolog, IFT)	SP1712(<i>m175</i>)	-	+	++	-	-
<i>dyf-11</i> (IFT54 homolog, IFT)	SP1713(<i>mn392</i>)	-	+	++	-	-
<i>dyf-13</i> (IFT56 homolog, IFT)	SP1678(<i>mn396</i>)	+	+	++	-	-
<i>ift-20</i> (IFT20 homolog, IFT)	RB2353(<i>ok3191</i>)	-	+ (w)	++	-	-
<i>ift-74</i> (IFT72/74 homolog, IFT)	VC2140(<i>ok2866</i>)	+	+ (w)	+	++	-
<i>osm-1</i> (IFT172 homolog, WD repeat, IFT)	PR808(<i>p808</i>)	-	+	+	-	-
<i>osm-1</i> (IFT172 homolog, WD repeat, IFT)	PR816(<i>p816</i>)	-	+	++	++	-
<i>osm-5</i> (IFT88 homolog, polaris, IFT)	PR813(<i>p813</i>)	-	++	++	++	-
<i>osm-6</i> (IFT52 homolog, IFT)	PR811(<i>p811</i>)	-	+	+	-	-
<i>rab-28</i> (RAB family, IFT27 homolog)	RB2484(<i>ok3424</i>)	-	++	++	-	+
Bardet-Biedl Syndrome Complex Mutants						
<i>bbs-1</i> (BBS1 ortholog, IFT)	VC837(<i>ok1111</i>)	+ (w)	++	++	++	---
<i>bbs-2</i> (BBS2 ortholog, IFT)	VC1569(<i>ok2053</i>)	-	++	++	++	-
<i>bbs-5</i> (BBS5 ortholog, IFT)	VC1316(<i>gk537</i>)	+	-	-	+	-
<i>bbs-8</i> (BBS8 ortholog, TPR protein, IFT)	MX52(<i>nx77</i>)	+ (w)	+	+	-	---
<i>bbs-9</i> (BBS9 ortholog, IFT)	VC1062(<i>gk471</i>)	+ (w)	++	++	+	-
<i>k07c11.10</i> (BBS10 ortholog)	TM3304(<i>tm3304</i>)	+	++	++	++	-
<i>osm-12</i> (<i>bbs7</i> , IFT)	MT3645(<i>n1606</i>)	+ (w)	+	+	-	---
IFT Cargo Mutants						
<i>npr-24</i> (SSTR5 ortholog)	VC2421(<i>ok3192</i>)	+	-	-	-	+
<i>osm-9</i> (TRPV5/6 family)	CX10(<i>ky10</i>)	+	++	+	-	-
OSM-9 Interacting/Associated Proteins						
<i>npr-1</i> (NPY1R ortholog)	CX4148(<i>ky13</i>)	+	-	+	-	-
Other Cilia Membrane Protein Mutants						
<i>cil-7</i> (myristoylated coiled-coil protein, leucine zipper domain)	TM5848(<i>tm5848</i>)	+	-	-	-	+
Other Sensory Mutants						
<i>inx-19</i> (innexin homolog)	CX6161(<i>ky634</i>)	-	++	++	++	+
<i>unc-7</i> (innexin homolog)	CB5(<i>e5</i>)	+	+ (w)	+	-	-
<i>unc-9</i> (innexin homolog)	CB101(<i>e101</i>)	+	+ (w)	+ (w)	-	-

Miscellaneous						
<i>f59f5.7</i> (KNK ortholog, DOMON domain protein)	TM7257(<i>tm7257</i>)	+	-	-	--	---

257

258 Intraflagellar transport complex subunits

259 Of the previously 34 identified ivermectin resistance genes (12, 22, 23), 16 encode for proteins of
260 the IFT-A complex, IFT-B complex and the BBSome, all of which are interacting multiprotein
261 complexes involved in intraflagellar transport. Therefore orthologs of the remaining 14 known, but
262 untested, subunits of these complexes and an ortholog of the chaperone protein BBS10, were
263 investigated for anthelmintic resistance. Out of the 15 genes tested, mutant alleles for 8 showed
264 resistance to ivermectin. Within the IFT-A complex mutants, the IFTA-1 dynein interacting protein
265 was found to be strongly resistant to ivermectin, while mutants for the dynein loading proteins IFT-
266 43 and ZK328.7 remained susceptible. From the IFT-B complex mutants, the Golgi vesicle sorting
267 protein IFT-20 and the tubulin delivery protein IFT-74 were only weakly resistant to ivermectin
268 whereas the IFT27 ortholog RAB-28 was highly resistant. Mutants for the core BBSome proteins BBS-
269 2 and BBS-9 and the BBS10 ortholog K07C11.10 all displayed strong ivermectin resistance while
270 those for the cargo adapter subunits BBS-4 and BBS-5 were susceptible.

271 Known IFT cargoes

272 As the primary function of IFT is the delivery of ciliary proteins, genes for known IFT cargo proteins
273 were tested for ivermectin resistance to identify downstream effectors of resistance. Of the 14
274 cargo-protein encoding genes tested, only the CX10(*ky10*) mutant of *osm-9* was found to exhibit
275 resistance however this finding was not replicated with the VC1262(*ok1677*) and JY190(*yz6*) *osm-9*
276 mutant strains indicating that perhaps resistance is caused by an unrelated, uncharacterised,
277 mutation in the CX10(*ky10*) strain. The ciliary membrane protein cargo adaptor Tub-1(*ok1972*)
278 mutant was found to be susceptible to ivermectin, supporting the hypothesis that the downstream
279 effector for ivermectin resistance must be delivered by another secretion pathway.

280 Protein trafficking pathways

281 To gain insight into the trafficking of the downstream effectors for ivermectin resistance, known
282 ciliary protein secretion pathways upstream of the IFT and ciliary membrane protein removal
283 pathways were investigated. The clathrin adapter protein-1 ortholog involved in Golgi vesicle

284 secretion UNC-101, the CRMP1 ortholog involved in polarizing axon-dendrite sorting UNC-33, the
285 ANK2/ANK3 ortholog involved in polarizing axon-dendrite sorting UNC-44, UNC-119 which inserts
286 myristoylated proteins into the cell membrane and RAB-35 which regulates early endosome
287 recycling were all involved in ivermectin resistance. Mutants for the two SNAP25 family protein
288 encoding genes *aex-4* and *ric-4* were found to be susceptible, supporting the contention that the
289 downstream effector for ivermectin resistance must be delivered via vesicle fusion using the
290 essential SNAP-29 protein. All the genes so far tested that are involved in endocytosis, designation to
291 lysosomal degradation, early endosome maturation, extracellular vesicle formation, synaptic vesicle
292 fusion and other post-Golgi transport complexes did not confer ivermectin resistance. Intriguingly,
293 mutants for the RAB-8 and RAB-10 exocytosis regulators, which have roles in crossing the ciliary
294 gate, were likewise susceptible to this drug.

295 Dyneins and kinesins play an important role in protein trafficking and IFT with *Osm-3(p802)*,
296 *Che-3(e1124)* and *Dhc-3(ka33)* already being associated with ivermectin resistance (12, 23),
297 therefore additional members of these families were investigated. Of the 20 genes tested, only
298 mutations in the dynein light-intermediate chain *xbx-1* resulted in ivermectin resistance. Mutant
299 alleles for all three genes encoding the IFT heteromeric kinesin (*kap-1*, *k1p-11* and *k1p-20*) and the
300 axonal kinesin *unc-104* had no impact on ivermectin resistance.

301 **The ciliary gate**

302 The ciliary gate of the basal body acts as a physical barrier at the base of the cilia that selectively
303 allows the passage of ciliary proteins. Components of the ciliary gate (some putative) were therefore
304 investigated to uncover those required to deliver downstream effectors associated with ivermectin
305 resistance. The MAGEL2 like protein DYF-17, the distal appendage interacting subunit of the basal
306 body HYLS-1, the FBF1 ortholog DYF-19, the transition fibre subunit NPHP-4 and the JAKMIP3
307 ortholog with CEP123 homology C14H10.2 were all found to be involved in maintaining ivermectin
308 susceptibility although some *Nphp-4(tm925)* individuals showed incomplete penetrance of the
309 resistance phenotype. Mutants for all other transition fibre genes, putative subdistal appendage

310 proteins, putative ESCRT complex, Exocyst vesicle, TRAPP complex and Rab interacting basal body
311 subunits and orthologs of the ARMC9/TOGARAM1 complex were all tested and found to have no
312 impact on ivermectin resistance.

313 Cell migration, amphid formation, ciliogenesis and ciliated neuron enriched genes 314 tested

315 As gross morphological defects to amphid neurons, their cilia and the amphid channel invariably
316 cause ivermectin resistance, some transcription factors that determine amphid neuron cell fate and
317 the proteins involved in axon guidance and lumen formation were assessed for a role in ivermectin
318 resistance. Of the 5 genes tested only mutant alleles for the ADL neuron determining transcription
319 factor *hlh-4* and the lumen endocytosis regulator *daf-6* were found to cause resistance to
320 ivermectin.

321 Some genes involved in gap junction formation (*unc-7* and *unc-9*), mechanosensation (*mec-1*
322 and *mec-8*) and osmotic avoidance (*osm-1*, *osm-3*, *osm-5*, *osm-6* and *osm-12*) cause ivermectin
323 resistance (12, 23), so additional genes in those categories along with several cilia enriched
324 membrane proteins (35, 36) were likewise investigated. Of the genes from this grouping that have
325 been tested only the gap junction innexin *Inx-19(ky634)* mutant displayed resistance to ivermectin.

326 Amphidal dye-filling defect correlation with ivermectin resistance

327 It has previously been found that there is a correlation between ivermectin resistance and dye-filling
328 defects (23), so the full extent of this relationship was examined. Of previously known ivermectin
329 resistance genes, the mutant alleles *Daf-19(m86)*, *Dyf-7(m537)*, *Che-12(e1812)*, *Dyf-10(e1383)*, *Che-*
330 *3(e1124)*, *Dhc-3(ka33)*, *Osm-3(p802)*, *Che-11(e1810)*, *Daf-10(e1387)*, *Dyf-2(m160)*, *Che-2(e1033)*,
331 *Che-13(e1815)*, *Dyf-1(mn335)*, *Dyf-3(m185)*, *Dyf-6(m175)*, *Dyf-7(m537)*, *Dyf-10(e1383)*, *Dyf-*
332 *11(mn392)*, *Osm-1(p808)*, *Osm-1(p816)*, *Osm-3(p802)*, *Osm-5(p813)* and *Osm-6(p811)* were dye-
333 filling negative; *Bbs-8(nx77)*, *Che-10(e1809)*, *Bbs-1(ok1111)* and *Osm-12(n1606)* and *Dyf-5(mn400)*
334 exhibited weak dye-filling; *Che-1(p672)*, *Che-1(ot75)*, *Che-6(e1126)*, *Dyf-13(mn396)*, *Mec-1(e1066)*,
335 *Mec-8(e398)*, *Unc-7(e5)* and *Unc-9(e101)* were dye-filling positive; and *Che-14(e1960)* exhibited

336 highly variable degrees of dye-filling between individuals. Among the novel ivermectin resistance
337 genes identified in the present study, the mutant alleles *Unc-101(sy108)*, *Daf-6(e1377)*, *Ift-*
338 *20(ok3191)*, *Rab-28(ok3424)*, *Bbs-2(ok2053)*, *Dyf-19(jhu455)* and *Inx-19(ky634)* were all dye-filling
339 negative; *Hlh-4(tm604)*, *Unc-119(e2498)*, *Hyls-1(tm3067)*, *Nphp-4(tm925)*, *Ifta-1(nx61)* and *Bbs-*
340 *9(gk471)* displayed weak dye-filling; *Rab-35(b1013)*, *Unc-33(e1193)*, *Ift-74(ok2866)* and
341 *K07c11.10(tm3304)* were dye-filling positive; and *C14h10.2(tm10737)*, *Dyf-17(ox175)*, *Unc-44(e1197)*
342 and *Xbx-1(ok279)* had highly variable degrees of dye-filling between individuals, with
343 *C14h10.2(tm10737)*, *Dyf-17(ox175)* and *Unc-44(e1197)* being predominantly dye-filling negative. The
344 *Tag-278(gk382)* mutant also showed highly variable degrees of dye-filling between individuals but
345 showed no resistance to any of the tested anthelmintics. Processes that are essential for ciliogenesis
346 and cilia maintenance showed a strong correlation between the extent of dye-filling defects and the
347 strength of ivermectin resistance although *Mec-8(e398)*, *Hyls-1(tm3067)*, *Ifta-1(nx61)* and *Bbs-*
348 *9(gk471)* defied the trend by showing strong resistance despite having weak dye-filling. Mutants for
349 proteins which are involved in trafficking cilia membrane proteins along the axon such as *UNC-33*
350 and proteins which function downstream of IFT, including *RAB-35* and helper/regulatory proteins
351 like *K07C11.10* showed no correlation. This indicates that although Dil dye-filling and ivermectin
352 uptake require effector delivery to the cilia through shared pathways, both processes do not
353 necessarily use the same effector.

354 **Observed resistances to other anthelmintics and roles of IFT in cross resistance**

355 Candidate genes were also tested for moxidectin (an avermectin), albendazole and levamisole (both
356 non-avermectins) resistance to examine possible cross resistance. Mutants for all genes that were
357 ivermectin resistant were also resistant to moxidectin, indicating as expected, shared mechanisms
358 but also similar levels of resistance to the two drugs. Mutants for the kinase *DYF-18* which plays a
359 role in ciliogenesis and IFT, a regulator of ciliary protein trafficking *OSTA-1*, the small GTPase
360 nucleotide exchange factor involved in ciliogenesis *ARL-13*, a *WWTR1* ortholog with *CEP164*
361 homology *YAP-1* and the IFT-A complex dynein loading protein *IFT-43* however, showed moxidectin

362 resistance but not ivermectin resistance. The CX4148(*ky13*) mutant strain of the OSM-9 interacting
363 protein NPR-1 showed moxidectin resistance, however this finding was not reproduced for a second
364 allele using the RB1330(*ok1447*) strain indicating that it is most probably caused by an unrelated,
365 uncharacterised, mutation in the CX4148(*ky13*) strain.

366 Cross resistance to the unrelated benzimidazole drug albendazole was observed for genes
367 involved in cell migration and IFT with *daf-6*, *dyf-17*, *rab-35*, *c14h10.2* and *inx-19* also showing cross
368 resistance. Within the IFT-B complex, resistance was limited to the tubulin interacting protein IFT-
369 74, the BBSome interacting DYF-3/CHE-13 dimer and the SEC-24 (COPII ortholog) pathway
370 interacting protein OSM-5. The BBSome cargo adapter proteins which are important for albendazole
371 susceptibility differed from those for avermectins with *Bbs-5(gk537)* mutants showing resistance
372 while *Bbs-8(nx77)* mutants were susceptible. Several other genes whose mutation confers
373 albendazole resistance were identified including the IFT-A complex dynein loading protein *zk328.7*,
374 the SNAP25 family member *aex-4*, the VAMP8 ortholog SNAP receptor *arl-7*, the SEC-24 (COPII
375 ortholog) pathway interacting p150^{glued} ortholog *dnc-1*, the ARL-3 activating kinase *rpi-2*, the
376 negative regulator of microtubule length *f59g1.4*, the ESCRTI complex subunit *mvp-12*, the regulator
377 of vesicle trafficking for endocytosis and exocytosis *rab-3*, and the kinesin *klp-7*. Of all genes tested
378 only the KNK ortholog *f59f5.7* was found to have increased albendazole susceptibility showing a
379 greatly reduced population growth, in comparison to controls, that was unable to clear the plates of
380 OP50-1 within 144 hours. None of the mutants tested showed resistance as strong as the well
381 characterised albendazole resistant mutant control Ben-1(*e1880*) (37).

382 Levamisole, represents the third unrelated class of anthelmintic examined for cross
383 resistance. Levamisole resistance and susceptibility had no obvious connection to the other
384 anthelmintics tested, however *Rab-35(b1013)*, *Daf-6(e1377)* and *Inx-19(ky634)* mutants displayed
385 moderate broad-spectrum cross resistance and *Rab-28(ok3424)* had resistance to both avermectins
386 and levamisole. The uncharacterised gene T06g6.3(*gk546*), the TRAPP-I/II complex subunit Sedl-

387 1(*ok2485*), nexin link protein Gasr-8(*gk1232*), transition fibre subunit Mks-5(*ok3582*), the IFT cargo
388 Npr-24(*ok3192*), the amphid exosome export protein Cil-7(*tm5848*) and the ESCRT-II complex
389 subunit Vps-36(*gk427*) mutants were however all found to confer resistance to levamisole. Mutants
390 for Bbs-1(*ok1111*), Bbs-8(*nx77*), Che-13(*e1815*), Che-14(*e1960*), Dnc-1(*or404*), Daf-10(*e1387*), the
391 putative OFD1 ortholog Dpy-6(*e2764*), Hlh-4(*tm604*), Osm-12(*n1606*), the PAM ortholog Pamn-
392 1(*ok2681*), C14h10.2(*tm10737*), and the myosin heavy chain with cenexin homology Unc-15(*e1402*)
393 all showed greatly reduced population growth compared to controls and the other tested
394 susceptible strains, indicating an increased susceptibility to levamisole. Although levamisole
395 exposure usually does not kill *C. elegans*, even at doses as high as 10mM (determined from
396 preliminary dose ranging experiments using N2), high mortality was observed in the syntaxin Unc-
397 18(*e234*), the BLOC1 complex subunit Snpn-1(*tm1892*), the uncharacterised protein with FAM92
398 homology B0432.8(*tm6737*), the myosin heavy chain with SCLT1 homology Unc-54(*e1201*), Unc-
399 119(*e2498*), the TOGARAM1 ortholog Che-12(*e1812*) and F59f5.7(*tm7257*) mutants at the 0.2mM
400 and 0.8mM doses used for resistance assays.

401 **Visualisation of anthelmintic uptake using BODIPY labelled analogs**

402 To visualise the route of ivermectin and albendazole uptake, chemical analogs which were linked to
403 a BODIPY fluorophore were constructed and applied to live worms. The ivermectin probe was
404 named FBI and the albendazole probe called BABZ. It was confirmed that the analogs retained some
405 of their target binding using acute toxicity assays to compare the LD/ED₅₀s of analogs to the parent
406 compounds in sensitive N2 and target site insensitive DA1316, for ivermectin and FBI, and Ben-
407 1(*e1880*), for albendazole and BABZ, backgrounds. Ivermectin was found to have an LD₅₀ of 25.98nM
408 (95%CI = 24.49-27.56 nM; N = 3,330) for N2 and 496.33μM (95%CI = 432.52-569.56μM; N = 8,024)
409 for DA1316 while FBI had an LD₅₀ of 6.35 μM (95%CI = 5.76-7 μM)(N = 2,098) for N2 and 83.59μM
410 (95%CI = 35.43-197.18μM; N = 1,996) for DA1316. This indicates the primary toxicity, caused by
411 target binding, of FBI is 244 times lower than the parent compound while secondary toxicity, caused
412 by off target effects, is 5.9 times higher. The ED₅₀ for albendazole was found to be 51.63μM (95%CI =

413 43.71-60.98 μ M; N = 3,607) for N2 and estimated to be 1.68mM (95%CI = 1.53-1.85mM; N = 2,492)
414 for Ben-1(*e1880*) while the ED₅₀ for BABZ was estimated to be 252.28 μ M (95%CI = 216.96-
415 293.35 μ M; N = 3,307) for N2 and 471.39 μ M (95%CI = 424.18-523.85 μ M; N = 1,646) for Ben-
416 1(*e1880*). This suggests that the primary toxicity of BABZ is 4.9 times lower than the parent
417 compound while secondary toxicity is 3.6 times higher.

418 Time course experiments were performed on N2 worms to characterise uptake progression
419 and establish the optimum incubation time with the probes (see Supplementary Results and
420 Supplementary Figs S1 and S2 for details). FBI uptake was found to be restricted to the amphid
421 neurons with limited spread to the adjacent nerve ring while BABZ was primarily absorbed in the
422 hind gut but showed progressive systemic spread. A 72 hour incubation period was selected to be
423 used for all subsequent assays.

424 The uptake of the ivermectin probe FBI and the albendazole probe BABZ in different
425 genotypes was then examined (Table 2). The susceptible strain CB4856 and target site insensitivity
426 mutants DA1316 and Ben-1(*e1880*) stained in an identical manner to N2 indicating no differences in
427 uptake between those strains. Next a selection of resistant mutants from the moxidectin forward
428 genetic screen and the targeted resistance screen were exposed to FBI and/or BABZ, depending on
429 the resistances of the strain, to determine if resistance is being caused by changes in flux. Uptake of
430 FBI by the amphids occurred in Rab-35(*b1013*), Unc-7(*e5*) and Unc-9(*e101*) but not in Daf-6(*e1377*),
431 Dyf-19(*jhu455*), Inx-19(*ky634*), Osm-5(*p813*) Osm-9(*ky10q*), TP236(*ka30*), TP241(*ka35*), TP272(*ka64*),
432 TP274(*ka66*) and TP388(*ka204*) worms while Hyls-1(*tm3067*), Unc-44(*e1197*) and TP378(*ka201*)
433 showed weak uptake. The distribution of absorbed BABZ in most tested resistant strains was limited
434 to the gut with Dnc-1(*or404*), Osm-5(*p813*), TP272 and TP384 showing weak uptake while Daf-
435 6(*e1377*), Inx-19(*ky634*), Rab-35(*b1013*) and TP386 showed almost no uptake. TP236 and TP375
436 were exceptions in that weak BABZ uptake was observed while still retaining systemic spread
437 throughout the whole worm. TP241 showed positive uptake comparable to the controls suggesting

438 the observed resistance to albendazole may be caused by target site insensitivity or enhanced phase
439 I detoxification.

440 **Table 2. Uptake patterns of BODIPY labelled anthelmintic analogs across different strains. FBI**

441 = Fatty-BODIPY-Ivermectin; **BABZ** = BODIPY-Albendazole; + = Positive uptake; **+(w)** = Weak uptake; -

442 = No or barely visible uptake; **NT** = Not tested.

Strain	N2	CB4856	DA1316	Ben-1	Rab-35	Daf-6	Inx-19	Dyf-19	Osm-5	Dnc-1	Osm-9	Hyls-1
FBI	+	+	+	+	+	-	-	-	-	NT	-	+(w)
BABZ	+	+	+	+	-	-	-	NT	+(w)	+(w)	NT	NT
Strain	Unc-7	Unc-9	Unc-44	TP236	TP241	TP272	TP274	TP375	TP378	TP384	TP386	TP388
FBI	+	+	+(w)	-	-	-	-	-	+(w)	-	-	-
BABZ	NT	NT	NT	+(w)	+	+(w)	+	+(w)	+	+(w)	-	+

443

444 Whole genome sequencing of mutants from forward genetic screens

445 Extensive EMS genetic screens for ivermectin and abamectin resistant mutants were carried out

446 previously and limited mapping identified two IFT-related mutants (*Che-3(ka32)* and *Dhc-3(ka33)*)

447 (23). In this current study, a new forward genetic screen to identify moxidectin resistant strains was

448 performed. Together these screens identified 31 mutants resistant to avermectins which also had

449 their albendazole and levamisole resistance and Dil dye-filling phenotypes characterised (Table 3).

450 Based on phenotype TP236(*ka30*), TP241(*ka35*), TP272(*ka64*) and TP274(*ka66*) from the previous

451 abamectin screen along with TP375(*ka200*), TP378(*ka201*), TP384(*ka202*), TP386(*ka203*), and

452 TP388(*ka204*) from the current moxidectin screen were selected for backcrossing, whole genome

453 sequencing and SNP mapping. Of the selected strains all were resistant to ivermectin and moxidectin

454 with TP272(*ka64*), TP274(*ka66*) and TP384(*ka202*) having cross resistance to levamisole and

455 TP241(*ka35*), TP375(*ka200*) and TP386(*ka203*) having cross resistance to albendazole and levamisole

456 with TP375(*ka200*) showing strong cross resistance to all three. TP388(*ka204*) was dye-filling positive

457 while all others were dye-filling negative. The whole genome sequencing and mapping data (aligned

458 reads available at <https://www.ncbi.nlm.nih.gov/sra/PRJNA768320>) identified novel alleles of *osm-3*,

459 *che-3* (4 different alleles), *osm-1*, *dhc-3*, *dyl-2* and *ifta-1* (Fig 3) as the causative genes for resistance
 460 to avermectins. Details of identified alleles are listed in Table S2.

461 **Table 3. Resistance profiles and causative genes for resistance to avermectins in EMS**
 462 **generated mutant strains.**

463 **Dyf** = Dil amphid dye filling; **IVM R** = Ivermectin resistance; **MOX R** = Moxidectin resistance; **ABZ R** =
 464 Albendazole resistance; **LEV R** = Levamisole resistance; **++** = Strong resistance; **+** = Dye
 465 filling/Moderate resistance; **-** = Dye filling defective/Susceptible.

Strain (selection screen used for isolation)	Assigned allele	Causal gene	Mutation/effect	DYF	IVM R	MOX R	ABZ R	LEV R
TP236 (10nM Ivermectin)	<i>ka30</i>	<i>osm-3</i>	Substitution/Nonsense	-	++	+	-	-
TP241 (50nM Abamectin)	<i>ka35</i>	<i>che-3</i>	Deletion/Coding	-	++	+	+	+
TP272 (10nM Ivermectin)	<i>ka64</i>	<i>che-3</i>	Substitution/Nonsense	-	++	+	-	+
TP274 (10nM Ivermectin)	<i>ka66</i>	<i>che-3</i>	Substitution/Missense	-	++	+	-	+
TP375 (10nM Moxidectin)	<i>ka200</i>	<i>osm-1</i>	Substitution/Nonsense	-	+	++	++	++
TP378 (10nM Moxidectin)	<i>ka201</i>	<i>dhc-3</i>	Deletion/Frameshift	-	++	++	-	-
TP384 (10nM Moxidectin)	<i>ka202</i>	<i>dyl-2</i>	Substitution/Nonsense	-	+	++	-	+
TP386 (10nM Moxidectin)	<i>ka203</i>	<i>che-3</i>	Splice site substitution	-	++	++	+	+
TP388 (10nM Moxidectin)	<i>ka204</i>	<i>ifta-1</i>	Substitution/Nonsense	+	++	++	-	-

466 Discussion:

467 **IFT protein resistances and redundancies**

468 IFT is highly conserved throughout eukaryota, being required for the import and transport of ciliary
 469 proteins to their correct localisations within the cilia (38) (Fig 4A). Loss of IFT impacts on cell motility,
 470 cell migration, cell signalling, cell division and the ability to sense environmental stimuli with
 471 mutants for mammalian orthologs of IFT proteins being responsible for 16 of the 35 known disease
 472 causing ciliopathies (38-40). IFT mutants have also been recently linked to ivermectin resistance in *C.*
 473 *elegans* (12, 22, 23). The protein-protein interactions of the IFT-A and IFT-B complexes (38, 41-46)
 474 and the BBSome (46-48) are well documented however not all interactions have been verified in *C.*
 475 *elegans*. When the identified resistance causing genes are overlaid with known and predicted
 476 interactions (Fig 4B) potential mechanisms for resistance become apparent.

477 In *C. elegans* the kinesins responsible for anterograde IFT are redundant in the intermediate
 478 segment of the cilia, with both the homomeric kinesin OSM-3 and the heteromeric kinesin (KAP-1,
 479 KLP-11 and KLP-20) being sufficient to build and maintain the intermediate segment. The distal
 480 segment however, is dependent solely on OSM-3 function (38). Mutants for the heteromeric kinesin

481 had no impact on ivermectin resistance while those for *osm-3* were resistant (23) indicating that the
482 downstream effectors for ivermectin susceptibility are transported to the distal segment of the cilia.
483 There is further indication that the effector protein localises to the distal segment through analysis
484 of the *Unc-101(sy108)* and *Unc-119(e2498)* mutants, both of which lack distal segments (49) and the
485 *Dyf-5(mn400)* and *Dyf-18(ok200)* mutants which have elongated middle segments that invade the
486 distal segment (50-52), since all these mutants show resistance. There is evidence that the
487 downstream effector for ivermectin resistance needs to be transported back to the base of the cilia
488 by retrograde IFT for ivermectin susceptibility, since mutation of the ciliary dynein heavy chains CHE-
489 3 and its paralogue DHC-3 together with their interacting light-intermediate chain XBX-1 (53) cause
490 resistance. The importance of effector protein retrieval from the cilia for ivermectin susceptibility is
491 highlighted by the resistance seen in mutants for DYF-6, OSM-1 and DYF-13 which are involved in
492 dynein import into cilia and the turning around of IFT complexes at the distal tip (54-57).

493 The cilia axoneme is composed of polarised tubulins (including DYF-10 and DYF-12 (58)) that
494 grow distally from the basal body and require a high local concentration of tubulins to polymerise,
495 confirming the requirement for their delivery to the tip of the cilium (59). Tubulin heterodimers are
496 imported into the cilia via the redundant IFT-B proteins IFT-74 and IFT-81 (60). Consequently Ift-
497 74(*ok2866*) mutants only show weak resistance to ivermectin indicating that unlike other species,
498 the *C. elegans* dimer subunits, do not have an equal role in tubulin import. The distance that the IFT
499 cargoes are transported on the axoneme is determined by the stability of the interaction between
500 the complexes and the microtubule rail, with the ability to form IFT trains through IFT-80 (CHE-2)
501 interaction (44) and the microtubule interacting DYF-11/IFT-20 dimers playing an important role in
502 this process (61). The interactions of the three gene products is reflected in their shared ivermectin
503 resistance, while the low level of resistance observed in IFT-20 mutants can be attributed to a known
504 inequality in functional redundancy of the two heterodimer subunits (61). The tubulin interacting
505 IFT-B subunit IFT-74 was also found to play a role in albendazole resistance, potentially through the
506 transport of poisoned BEN-1 subunits to the plus end of microtubules or delivery of unexposed BEN-

507 1 to the interface with albendazole. The OSM-6/DYF-6 dimer physically bridges between the two IFT-
508 B core subcomplexes that contain the albendazole resistance causing subunits IFT-74 and OSM-5,
509 DYF-3 and CHE-13 respectively (62). The OSM-6/DYF-6 dimer is also essential for IFT-B targeting to
510 the basal body and for stability during transport (63-65) and mutants are susceptible to albendazole
511 indicating either the dimer subunits have a redundancy in *C. elegans* or the IFT-B interactions with
512 albendazole are occurring outside of IFT.

513 RAB-28 is a prenylated protein that functions both as an IFT27 ortholog and a GTPase, with
514 mutants causing dye-filling defects through amphid pore malformations (66, 67). As the interaction
515 of RAB-28 with IFT is dependent on PDL-1 mediated prenylation and BBS-3 interaction (66, 68), the
516 results suggest that the ivermectin resistance observed in the *rab-28* mutant is being caused by the
517 BBS-8 mediated interaction with the periciliary membrane (66, 67). The farnesylated-protein
518 converting enzymes FCE-1 and FCE-2 process proteins for prenylation (69). FCE-1 and FCE-2 play no
519 role in ivermectin resistance and therefore it is likely that the downstream effectors and proteins for
520 all essential ciliary protein delivery pathways do not require prenylation to function. In
521 *Chlamydomonas reinhardtii* BBS-3 is known to interact with another IFT-B subunit, IFT-22 (IFTA-2)
522 (70), where they both play an important role for BBSome recruitment, however evidence from our
523 results suggest that this interaction either does not occur in *C. elegans* or is not essential for IFT as
524 neither gene had a role in dye-filling or ivermectin resistance.

525 Within the BBSome, loss of function induced ivermectin resistance was observed in DYF-3 in
526 the IFT-B complex which interacts with OSM-12 (71) and across the core BBSome proteins (BBS-1,
527 BBS-2, BBS-9 and OSM-12) to the BBS-1 interacting protein in the IFT-A complex, DYF-2 (72). This
528 indicates the important role the BBSome plays in bridging the IFT-A and IFT-B complexes during IFT.
529 The BBSome acts as a carrier for protein cargoes by both delivering proteins to the correct location
530 in the cilia and by retrieving ciliary membrane proteins for recycling or degradation (73). During
531 transport, the IFT cargoes interact with subsets of BBS-1, BBS-4, BBS-5 and BBS-8 (74). As BBS-8 is

532 important for ivermectin susceptibility (23) it can be deduced that the downstream effector for
533 ivermectin susceptibility is probably an IFT transported BBSome cargo which interacts with one or
534 more of the adapter subunits, however the role of BBS-4 and BBS-5 was not clearly defined, a fact
535 that may relate to their functional redundancy for some cargoes (75). In the case of albendazole
536 susceptibility, a strong interaction between the BBSome and IFT-A and IFT-B complexes does not
537 seem to be required as *Dyf-2(m160)* and *Osm-12(n1606)* mutants are both susceptible to this drug,
538 perhaps indicating redundancy in their interactions and the BBSome having a role upstream of IFT.

539 Core subunits within the IFT-A complex showed ivermectin resistance up to the dynein motor
540 interacting IFTA-1 subunit. Mutants for the IFTA-1 interacting dynein docking proteins ZK328.7 and
541 IFT-43 were susceptible, an observation explained by the known redundancy these proteins have
542 when interacting with specific dyneins (45, 76, 77). Moxidectin resistance shows a similar pattern to
543 ivermectin resistance except the resistance seen with IFT-43 loss which indicates an inequality in
544 functional redundancy with ZK328.7. Interestingly, albendazole susceptibility did not require IFTA-1,
545 however ZK328.7 was necessary for susceptibility indicating that ZK328.7 interacts with other core
546 IFT-A subunits in *C. elegans*. Such interactions have been supported by other studies (77) and
547 ZK328.7 is probably interacting via the IFT-144 ortholog DYF-2 (45).

548 Benzimidazoles, such as albendazole, function by binding to the colchicine-binding domain
549 of β -tubulins resulting in the premature capping of microtubules leading to microtubule
550 depolymerisation and a loss of cellular structure (78). BEN-1 is the only albendazole sensitive tubulin
551 in *C. elegans* and acts redundantly with other β -tubulins in microtubule formation (37). This
552 redundancy explains why loss of function alleles like *Ben-1(e1880)* are resistant to albendazole
553 without causing morphological defects or cross resistances to the other anthelmintics tested. There
554 is also evidence that BEN-1 is not a true ciliary tubulin (79) suggesting that the albendazole
555 resistance observed in the IFT and BBSome complex mutants are occurring through a loss of
556 interaction with BEN-1 outside of the cilia and potentially, outside the ciliated neurons.

557 Secretion pathways used by cilia proteins and the ciliary gate

558 Cilia proteins are delivered via the SEC-24(COPII), TRAPP^{II}, ESCRT, exocyst, BLOC-1 and PDL-
559 1(PDE6D)/UNC-119 secretion pathways (80-83) (summarised in Fig 5A) with many being secreted
560 from the endoplasmic reticulum via Golgi vesicles in a clathrin adapter protein-1 (UNC-101)
561 dependent pathway (84). These proteins would include those for ciliogenesis, IFT and the
562 downstream effectors for ivermectin susceptibility thereby providing an explanation for the
563 ivermectin resistance seen in *Unc-101(sy108)* mutants. The SEC-24(COPII) pathway is an essential
564 pathway making it difficult to probe directly, however orthologs of *Osm-5(p813)* and *Dnc-1(or404)*,
565 which are both resistant and showed reduced labelled albendazole (BABZ) uptake, are known to
566 interact with the vesicle coat proteins of this pathway (80, 85) suggesting a role in albendazole
567 susceptibility. As *Osm-5(p813)* and *Dnc-1(or404)* showed reduced but not abolished BABZ
568 fluorescence compared to the wild type there is an indication for either redundancy in the functions
569 of OSM-5 and DNC-1 or the existence of additional routes for albendazole uptake. In multicellular
570 organisms the ESCRT complexes are essential for development due to their role in controlling cell
571 surface receptor populations through facilitating endocytosis, endosome maturation and fusion of
572 vesicles to the lysosome and by having direct roles in establishing cell polarity and cleavage during
573 cell division (86, 87) meaning that only non-essential subunits could be investigated for roles in
574 anthelmintic resistance. The ESCRT-I complex is known to have a genetic interaction with the
575 BBSome (88) and both are involved in the removal of ubiquitylated receptors (86, 89) so the
576 albendazole resistance seen in the ESCRT-I complex subunit *Mvb-12(ok3482)* indicates that the
577 ESCRT pathway may be functioning downstream of the BBSome to facilitate albendazole uptake and
578 the effector may be a monoubiquitinated protein. The results associated both the ESCRT-II complex
579 subunit VPS-36 and TRAPP complex subunit *SEDL-1* with levamisole resistance indicating that this
580 compound may be gaining entry through multiple routes. Mutation in the exocyst pathway genes
581 *exoc-7* and *exoc-8* have been shown to cause weak levamisole resistance during acute exposure (90)
582 so it was surprising that these phenotypes were unable to be replicated in mutants for any of the

583 exocyst complex genes tested (*exoc-7*, *exoc-8* and *sec-6*) indicating this pathway only plays a minor
584 role during chronic exposure. Despite having a known interaction with IFT-20 (82), the BLOC-1
585 complex was not associated with survival against any of the anthelmintics tested. The UNC-119
586 secretion pathway of myristoylated and laurylated acyl-anchored membrane proteins (91) is known
587 to deliver ARL-3 and ARL-13 to the cilia facilitating regulation of the assembly and disassembly of the
588 IFT complexes (92). The observed resistance to avermectins in the *Unc-119(e2498)* and *Arl-*
589 *13(tm1745)* mutants may therefore be explained by the impaired delivery of proteins involved in
590 cilia maintenance resulting in truncated cilia (49).

591 The ciliary gate of the basal body (Fig 5B) acts as an impermeable barrier to proteins and
592 macromolecules over 70 kDa in size (93) meaning that cargo delivery pathways for ciliary proteins
593 need to interact with the basal body. To allow passage of the IFT-B complex, along with associated
594 proteins and complexes, through the basal body MKSR-2 interacts with DYF-1 (94), DYF-19 with DYF-
595 11 (95) and the CCDC41 ortholog C18C4.7 with IFT-20 (96). The BBSome gains entry to the cilia by
596 interacting with the distal appendage interacting protein NMY-3 (*Dzip1* ortholog)(97) while the
597 subdistal appendage component T04F8.6 (*ninein* ortholog) allows passage of the dynactin complex
598 associated cargo via the interaction with the p150^{glued} ortholog DNC-1 (98) and through cargo
599 delivered by the exocyst pathway (99). Vesicles delivered by the exocyst pathway originate from the
600 UNC-101 secretion pathway and have their entry regulated by RAB-8 and RAB-10 GTPases (100,
601 101). The RAB-8 and RAB-10 GTPases in turn localize to the basal body via interaction with cenexin
602 and the CBY1 ortholog NFYB-1 (102, 103). The ESCRT complex mediated cargoes are potentially
603 delivered via T28D6.6, as orthologs (*DRG1* and *SPI1*) interact with both centrin from the basal body
604 and the ESCRT-III complex (104, 105). Of all these gating proteins only those from the distal
605 appendage (DYF-19 and possibly the essential protein C18C4.7) are important for resistance to
606 avermectins indicating that the downstream effector for resistance to avermectins is delivered into
607 the cilia as part of IFT particles and not independently via the exocyst or ESCRT secretion pathways.
608 The protein DYF-19 is known to play an important role in facilitating the passage of IFT components

609 across the transition zone of the basal body (95) a fact that explains why both Dyf-19(*jhu455*)
610 mutants and those for Hyls-1(*tm3067*), which connects DYF-19 to the mother centriole of the basal
611 body (106, 107), were also found to be resistant to avermectins and showed impaired uptake of FBI.
612 YAP-1 is an ortholog of a transcription factor from the Hippo pathway with a role in cell cycle
613 regulation, thermotolerance and neuronal development (108, 109) however it also has homology
614 with the distal appendage protein CEP164 meaning that the cause of the weak moxidectin resistance
615 observed in Yap-1(*tm1416*) needs further investigation as it could be the result of either changes in
616 amphid neuron or cilia morphology or the upregulation of stress response pathways. The predicted
617 CEP123 ortholog, *c14h10.2*, was found to be a novel gene associated with a dye filling defective
618 phenotype (Dyf) and the resistance of C14h10.2(*tm10737*) mutants to avermectins and albendazole
619 support it having a role in the cilia and suggest that it might directly interact with the BBSome or one
620 of the IFT complexes. As T04f8.6(*tm4830*) mutants were susceptible to albendazole it can be
621 deduced that resistance seen in Dnc-1(*or404*) is being caused by a function of the protein unrelated
622 to cilia and is probably fusing using the AEX-4 tSNARE and VAMP-7 vSNARE proteins.

623 **Transition fibres and the TOGARAM1 complex**

624 The transition fibres attach at the basal body between the axoneme and the ciliary membrane.
625 Through the interaction with nucleoporins (93, 110) and highly redundant interactions among
626 transition fibre proteins (111-113), the transition zone plays a key role in maintaining the
627 impermeability of the ciliary gate. Mutations in transition fibre genes are associated with multiple
628 ciliopathies in vertebrates (112) however, it was surprising that of the transition fibre genes tested
629 only Nphp-4(*tm925*) mutants showed resistance to the avermectins. These findings are consistent
630 with observations of dye-filling defects in *C. elegans* transition fibre mutants, where the high degree
631 of redundancy requires the loss of multiple transition fibre proteins to cause any significant ciliary
632 defects (114). Both the transition fibres and basal body interact with a protein complex involved in
633 the post-translational modification of axoneme tubulin, with CHE-12 being an ortholog of the
634 TOGARAM1 subunit (115). When this complex was investigated only the ARMC9/JBTS-30 ortholog

635 *f59g1.4* was found to cause albendazole resistance, and none of the tested subunit mutants were
636 resistant to ivermectin/moxidectin as observed in Che-12(*e1812*). This suggests highly specialised
637 roles for the core subunits in ciliary maintenance while the periphery subunits exhibit redundancy
638 between the basal body and transition fibre interactions.

639 There are several transition fibre associated proteins whose specific protein-protein
640 interactions with the basal body have yet to be determined, however our results identified four
641 proteins which have roles in anthelmintic resistance. OSTA-1 is known to cause minor ciliary distal
642 segment length defects, especially in AWB neurons, through RAB-5 mediated regulation of IFT (116).
643 This reduction in distal segment surface area in the OSTA-1 mutant would lead to lower moxidectin
644 uptake and hence may explain the low level of moxidectin resistance. The transition fibre associated
645 protein GASR-8 is orthologous to proteins that form ciliary nexin links through microtubule
646 interaction and bundling in other organisms (106, 117) and it was therefore surprising to find strong
647 levamisole but not albendazole resistance in the *Gasr-8(gk1232)* mutant suggesting that this protein
648 has additional functions in *C. elegans*. The cause of levamisole resistance seen in T06g6.3(*gk546*)
649 mutants remains elusive since little is known about this protein other than it is enriched in the cilia
650 (35) and interacts with the following proteins: AFD-1, GEI-4, LET-413, LIN-15A, LIN-37, NHR-11 and
651 VAB-3 (118, 119). The transition fibre protein DYF-17 has an as-yet undefined role in distal segment
652 assembly (51) however, orthologs are known to interact with BBS-4 and the axon guidance protein
653 UNC-76 (120) suggesting that it may function through facilitating the gating of the BBSome.

654 **Exosomes, recycling and degradation pathways**

655 In neurons, proteins are synthesised in the soma and require kinesins for anterograde transport
656 along the axon to their destination, whereas retrograde transport is carried out by the dyneins (121,
657 122). The direction of transport is determined by the polarity of the axonal microtubules and is
658 dependent on UNC-33 and UNC-44 (123). This polarity requirement provides an explanation for the
659 observed resistance to ivermectin in *Unc-33(e1193)* and *Unc-44(e1197)* mutants, as the delivery of
660 amphid cilia proteins and the downstream effectors for ivermectin susceptibility would become

661 disorganised in these mutants. In an attempt to identify which kinesins are responsible for delivering
662 these proteins to the end of the axon, the axonal kinesins KLC-1, KLP-6 and UNC-104 (121, 124, 125)
663 were investigated but found not to influence ivermectin tolerance, indicating either functional
664 redundancy or that ciliary proteins are transported by other cytoplasmic kinesin family members.
665 Unlike the other kinesins tested, the kinesin-13 family, of which KLP-7 is a member, has roles in
666 microtubule depolymerisation and primary ciliogenesis (126). As *Klp-7(tm7884)* mutants showed no
667 ivermectin resistance or dye-filling defects it can be concluded that it plays a negligible role in
668 ciliogenesis in *C. elegans* and the observed albendazole resistance is probably being caused by
669 reduced microtubule/free tubulin cycling resulting in a reduction in available unhindered
670 albendazole binding sites.

671 Similarly, protein degradation also requires the retrieval of damaged and superfluous
672 proteins across the axon from where they are localized. The results indicated that the downstream
673 effectors for ivermectin susceptibility needs to be returned from the cilia for ivermectin efficacy. The
674 GTPase RAB-35 is involved in the recycling of endosomes which can contain ciliary membrane
675 proteins (127), and resistance to avermectins in this mutant may infer an important role for
676 endocytosis in the efficacy of this class of anthelmintics. Several proteins commonly involved in
677 membrane protein endocytosis were also investigated, however both *Cav-1(ok2089)* and *Dpy-*
678 *23(e840)* (an AP-2 subunit) mutants remained susceptible to the tested anthelmintics. Surprisingly
679 *Chc-1(b1025)* mutants, for the clathrin heavy chain, were likewise susceptible to all anthelmintics
680 tested. *CHC-1* plays an important role in *UNC-101*, *DPY-23* and *CAV-1* mediated vesicle formation
681 therefore indicating that vesicle formation for IFT and the downstream effector for ivermectin
682 susceptibility may be occurring via one or more of the clathrin-independent pathways (128).

683 The Rab GTPases, of which *C. elegans* has 31 members (129), play important roles in
684 regulating vesicle trafficking and membrane fusion. As RAB-35 was found to have roles in
685 anthelmintic resistance, a selection of key Rabs which function upstream and downstream were

686 investigated to clarify which endosome recycling routes were important for each class of
687 anthelmintic. RAB-2 (also known as UNC-108) is involved in deciding if late endosomes are sent for
688 degradation in the lysosome (130) and RAB-11.1 and RAB-11.2 which facilitate the slow endosome
689 recycling pathway (127) had no impact on anthelmintic resistance indicating that the observed
690 resistances are being caused by defects in the fast recycling pathway. Interestingly Rab-3(y250)
691 which regulates the exocytosis of secretory vesicles (131) showed resistance to albendazole despite
692 being a neuron specific RAB in *C. elegans* (132) suggesting that the Unc phenotype observed during
693 exposure in wild types is being caused by microtubule disruption in the nervous system rather than
694 the muscles.

695 Ciliated neurons in *C. elegans* release protein and RNA containing secreted vesicles called
696 exosomes (also known as ectosomes or extracellular vesicles) (133). Exosomes have a role in inter-
697 organism signalling (134, 135) that can cause resistance by becoming decoys, with proteins being
698 used to take up or bind toxic compounds and pathogens into discarded vesicles (136). The
699 endocytosis of released exosomes can also potentially increase susceptibility by increasing the
700 surface area available for uptake. The impairment of IFT or lysosomal degradation pathways are
701 known to stimulate the release of exosomes that contain cilia proteins (137) and ivermectin resistant
702 Rab-28(ok3424) mutants are known to have impaired exosome release (68). Components of the *C.*
703 *elegans* exosome release/uptake pathway were therefore investigated for their roles in anthelmintic
704 resistance. Exosomes are exported from the cilia in a KLP-6 and CIL-7 dependent manner (133), so if
705 exosomes are important for resistance, mutants would either be more resistant due to a reduction
706 in environmental exosomes or would be more susceptible due to the uptake of accumulated
707 exosomes in the amphids. The Lamp1 ortholog *Imp-1* plays a role in exosome uptake (138) as well as
708 being important for lysosome formation and fusion between endosomes and autophagosomes (139,
709 140). The Cil-7(tm5848) and Klp-6(tm8587) mutants were found not to be resistant to the
710 avermectins or albendazole and did not have a mortality rate which was discernible from the other
711 susceptible strains tested. The Lmp-1(ok3228) and Rab-2(e713) mutants were also susceptible to all

712 anthelmintics tested indicating that neither exosomes or the lysosomal degradation pathway are
713 important for anthelmintic uptake. As *Cil-7(tm5848)* mutants were resistant to levamisole it can be
714 deduced that the protein may have additional functions outwith exosome export.

715 **Genes that cause broad spectrum cross resistance**

716 In this study, three genes were identified (*daf-6*, *inx-19* and *rab-35*) that when mutated caused
717 broad spectrum cross resistance to the avermectins, albendazole and levamisole. The protein DAF-6
718 plays an important role in lumen formation and the morphogenesis of the amphidial sheath but is
719 also present in other tubular lumens, such as the intestine, which would also potentially be exposed
720 to anthelmintics (141, 142). DAF-6 is predicted to function by inhibiting endocytosis of the
721 extracellular matrix (142), a determining factor in apical-basal polarity of the lumen (143). Although
722 there is strong evidence that the resistance to the avermectins is being caused by amphidial sheath
723 defects, the resistance towards the other anthelmintics seen in *Daf-6(e1377)* mutants is probably
724 being caused by the reduction in polarity, thus resulting in downstream effectors for anthelmintic
725 uptake being mislocalised on membrane surfaces. The reduced fluorescence observed when
726 exposed to FBI and BABZ compared to the N2 wild type further supports a reduction in uptake as the
727 cause of resistance. The Rab GTPase RAB-35 determines whether to send early endosomes for
728 recycling as opposed to the lysosomal degradation pathway and plays an important role in
729 maintaining membrane receptor populations (127, 144). This function alone could cause the cross
730 resistance noted by reducing the number of downstream effector proteins available for anthelmintic
731 uptake or restricting the number of primary targets. However, RAB-35 also plays roles in cell
732 migration, neurite outgrowth and cell polarity (143, 145), all of which could reduce target access or
733 uptake for anthelmintics. It was surprising that *Rab-35(b1013)* showed FBI uptake comparable to the
734 wild type while having greatly impaired BABZ uptake (Fig 2J) suggesting that the observed
735 resistances are being caused by more than just one mechanism. The innexins form intercellular
736 channels that function as gap junctions in neurotransmission, with members such as *unc-7* and *unc-9*
737 being involved in ivermectin resistance through what is believed to be a reduction in the

738 transmission of erroneous excitations caused by neurotoxic anthelmintics (12). This was reflected in
739 their uptake of Dil and FBI which was comparable to the wild type. INX-19 is however functioning
740 through a different mechanism since mutants exhibited dye-filling defects and impaired FBI uptake
741 which would indicate structural abnormalities of the ciliated amphid neurons. The INX-19 gap
742 junctions allow the passage of nucleotide signalling molecules and other small compounds between
743 cells (146, 147) potentially facilitating the neural distribution of lipophilic dyes and anthelmintics.
744 There is also a role that INX-19 plays in determining neural cell fate (147, 148) which could be
745 important for the differentiation into cells involved in dye and anthelmintic uptake. As *Inx-19(ky634)*
746 showed greatly reduced BABZ uptake in the gut there is indication that INX-19 might also have a role
747 outside the nervous system. If the above three genes maintain the same roles in parasitic nematode
748 species of economic or medical importance, then it would be possible for a single mutation to
749 render three of the most widely used anthelmintic families ineffective.

750 BODIPY labelled anthelmintic analogs

751 BODIPY labelled probes have been shown to be biocompatible and successfully applied to a variety
752 of biologically relevant compounds (149) and the results show that they are also applicable to
753 anthelmintics. The use of a fluorescently labelled ivermectin probe allowed the hypothesis that the
754 amphids are the tissue responsible for ivermectin uptake to be visually confirmed while the labelled
755 albendazole probe has shown that albendazole enters via the gut. Differences in the intensity of
756 absorbed probe fluorescence compared to wild type also corresponded well with observed
757 resistance in the tested mutant strains. As the intensity of fluorescence is proportional to
758 concentration at the steady state the question remains open as to whether a reduction in uptake or
759 increase in efflux is responsible for the reduced intensity observed in many of the tested mutants,
760 however, given the functions of the mutated genes it is probably being caused by reduced uptake.

761 The reduced primary toxicity observed in the FBI probe was to be expected as even small
762 structural changes to the 4'' position can have large effects on the potency of avermectins (150) and
763 the BODIPY fluorophore is a comparatively bulky chemical group. Given the low solubility of

764 benzimidazoles in aqueous/DMSO emulsions (14) relative to their EC₅₀s in susceptible strains it is
765 challenging to assay resistant strains and labelled compounds with reduced toxicity as precipitation
766 occurs before the majority of physiologically relevant doses. This means that at higher doses
767 additional routes of exposure occur through ingestion of and direct contact with the precipitate and
768 that identified EC₅₀s are not always practically attainable. As the effectors for ivermectin and
769 albendazole uptake are still unknown it was not possible to design and use self-quenching probes
770 and the BODIPY fluorophore, despite having high fluorescence efficiency, is prone to
771 photodegradation at the meso carbon in the presence of environmental oxygen when in polar (eg.
772 aqueous) solutions (149) meaning that videoing the progressive uptake within individuals was not
773 feasible. Still the approach shows promise for identifying the routes and tissues involved in
774 anthelmintic uptake and testing BODIPY labelled analogs of other classes of anthelmintics will be the
775 subject of future work.

776 **Whole genome sequencing of forward genetics screen mutants**

777 The avermectin resistance causing genes uncovered by random mutagenic screens and identified by
778 whole genome sequencing were all determined to be involved in IFT. The dynein heavy chains *che-3*
779 and *dhc-3* were found to be mutated more commonly than other genes. This is similar to
780 TP238(*ka32*) and TP239(*ka33*) from the previous study looking for ivermectin resistance (23) and
781 other studies looking for dye-filling defects (49, 151). This overrepresentation of dyneins in forward
782 genetic screens is probably caused by dynein heavy chains having very long coding sequences
783 (12,516nt and 9,828nt for *che-3* and *dhc-3* respectively) making them more prone to mutation by
784 EMS as the rate of mutation for a loss of function mutation is proportional to gene size (152). Having
785 found genes involved in ciliogenesis and IFT is not surprising as it is a complex process requiring the
786 interaction of multiple genes to produce a functional structure and lacks redundancy. Therefore, for
787 the aforementioned reasons, genes involved in IFT are statistically more likely to be mutated by EMS
788 than single downstream effectors that rely on functional cilia.

789 Conclusion:

790 The findings of this study not only provide strong evidence that the avermectin compounds
791 ivermectin and moxidectin are taken up via the amphid cilia as has been shown previously (23) but
792 refines the location of the effectors to the distal segment of the cilia. This study also uncovers the
793 pathways used to deliver the effectors and other ciliary proteins in *C. elegans*. Due to the strong
794 correlation between IFT function with dye-filling defects and resistance to avermectins it may be
795 possible to use resistance phenotypes to identify if novel dye-filling mutants from forward genetic
796 screens are upstream or downstream of IFT. There is also evidence that the three complexes
797 associated with IFT have additional roles in protein trafficking outside of IFT and these may be
798 responsible for resistance to albendazole. Levamisole was not the primary focus of this study
799 however several genes associated with ciliary processes were found to cause resistance or increased
800 susceptibility indicating a sharing of proteins by other pathways, including those needed to
801 compensate for the loss of controlled cholinergic neurotransmission. Although the downstream
802 effectors for ivermectin uptake remains elusive it can be deduced from the chemical properties of
803 ivermectin (153) that such effectors must associate with the extracellular membrane. The results
804 from this study suggest that the effectors localise to the distal segment of the amphid cilia and
805 possess either a transmembrane domain or are anchored via a myristoyl or palmitoyl group.
806 Whether the effectors are functioning as a carrier protein or transporter remains to be determined.
807 If the resistance causing genes uncovered in this study have the same functions in other nematode
808 species, then there would be important implications for resistance monitoring strategies.

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815 References:

- 816 1. Singh S, Singh B, Singh AP. Nematodes: A Threat to Sustainability of Agriculture. *Procedia*
817 *Environmental Sciences*. 2015;29:215-6.
- 818 2. Grisi L, Leite RC, Martins JR, Barros AT, Andreotti R, Cancado PH, et al. Reassessment of the
819 potential economic impact of cattle parasites in Brazil. *Rev Bras Parasitol Vet*. 2014;23(2):150-6.
- 820 3. Abongwa M, Martin RJ, Robertson AP. A brief review on the mode of action of
821 antinematodal drugs. 2017;67(2):137.
- 822 4. Crook EK, O'Brien DJ, Howell SB, Storey BE, Whitley NC, Burke JM, et al. Prevalence of
823 anthelmintic resistance on sheep and goat farms in the mid-Atlantic region and comparison of in
824 vivo and in vitro detection methods. *Small Ruminant Research*. 2016;143:89-96.
- 825 5. McArthur MJ, Reinemeyer CR. Herding the U.S. cattle industry toward a paradigm shift in
826 parasite control. *Vet Parasitol*. 2014;204(1):34-43.
- 827 6. Chen IS, Kubo Y. Ivermectin and its target molecules: shared and unique modulation
828 mechanisms of ion channels and receptors by ivermectin. *J Physiol-London*. 2018;596(10):1833-45.
- 829 7. Prichard R, Ménez C, Lespine A. Moxidectin and the avermectins: Consanguinity but not
830 identity. *International Journal for Parasitology: Drugs and Drug Resistance*. 2012;2:134-53.
- 831 8. Ardelli BF, Stitt LE, Tompkins JB, Prichard RK. A comparison of the effects of ivermectin and
832 moxidectin on the nematode *Caenorhabditis elegans*. *Vet Parasitol*. 2009;165(1-2):96-108.
- 833 9. Yilmaz E, Gerst B, McKay-Demeler J, Krücken J. Minimal modulation of macrocyclic lactone
834 susceptibility in *Caenorhabditis elegans* following inhibition of cytochrome P450 monooxygenase
835 activity. *Experimental Parasitology*. 2019;200:61-6.
- 836 10. Vokral I, Jedlickova V, Jirasko R, Stuchlikova L, Bartikova H, Skalova L, et al. The metabolic
837 fate of ivermectin in host (*Ovis aries*) and parasite (*Haemonchus contortus*). *Parasitology*.
838 2013;140(3):361-7.
- 839 11. James CE, Davey MW. Increased expression of ABC transport proteins is associated with
840 ivermectin resistance in the model nematode *Caenorhabditis elegans*. *International Journal for*
841 *Parasitology*. 2009;39(2):213-20.
- 842 12. Dent JA, Smith MM, Vassilatis DK, Avery L. The genetics of ivermectin resistance in
843 *Caenorhabditis elegans*. *P Natl Acad Sci USA*. 2000;97(6):2674-9.
- 844 13. Ménez C, Alberich M, Kansoh D, Blanchard A, Lespine A. Acquired Tolerance to Ivermectin
845 and Moxidectin after Drug Selection Pressure in the Nematode *Caenorhabditis elegans*.
846 *Antimicrobial Agents and Chemotherapy*. 2016;60(8):4809-19.
- 847 14. Escher BI, Berger C, Bramaz N, Kwon J-H, Richter M, Tsinman O, et al. Membrane–Water
848 partitioning, membrane permeability, and baseline toxicity of the parasiticides ivermectin,
849 albendazole, and morantel. *Environmental Toxicology and Chemistry*. 2008;27(4):909-18.
- 850 15. Cook SJ, Jarrell TA, Brittin CA, Wang Y, Bloniarz AE, Yakovlev MA, et al. Whole-animal
851 connectomes of both *Caenorhabditis elegans* sexes. *Nature*. 2019;571(7763):63-71.
- 852 16. Hong RL, Riebesell M, Bumbarger DJ, Cook SJ, Carstensen HR, Sarpolaki T, et al. Evolution of
853 neuronal anatomy and circuitry in two highly divergent nematode species. *eLife*. 2019;8.
- 854 17. Schafer W. Nematode nervous systems. *Current Biology*. 2016;26(20):R955-R9.
- 855 18. Perkins LA, Hedgecock EM, Thomson JN, Culotti JG. Mutant sensory cilia in the nematode
856 *Caenorhabditis elegans*. *Developmental biology*. 1986;117(2):456-87.
- 857 19. Vidal B, Aghayeva U, Sun H, Wang C, Glenwinkel L, Bayer EA, et al. An atlas of *Caenorhabditis*
858 *elegans* chemoreceptor expression. *PLoS biology*. 2018;16(1):e2004218.
- 859 20. Brear AG, Yoon J, Wojtyniak M, Sengupta P. Diverse cell type-specific mechanisms localize G
860 protein-coupled receptors to *Caenorhabditis elegans* sensory cilia. *Genetics*. 2014;197(2):667-84.
- 861 21. Sung CH, Leroux MR. The roles of evolutionarily conserved functional modules in cilia-
862 related trafficking. *Nature cell biology*. 2013;15(12):1387-97.
- 863 22. Urdaneta-Marquez L, Bae SH, Janukavicius P, Beech R, Dent J, Prichard R. A *dyf-7* haplotype
864 causes sensory neuron defects and is associated with macrocyclic lactone resistance worldwide in

- 865 the nematode parasite *Haemonchus contortus*. *International Journal for Parasitology*.
866 2014;44(14):1063-71.
- 867 23. Page AP. The sensory amphidial structures of *Caenorhabditis elegans* are involved in
868 macrocyclic lactone uptake and anthelmintic resistance. *International Journal for Parasitology*.
869 2018;48(13):1035-42.
- 870 24. Freeman AS, Nghiem C, Li J, Ashton FT, Guerrero J, Shoop WL, et al. Amphidial structure of
871 ivermectin-resistant and susceptible laboratory and field strains of *Haemonchus contortus*. *Vet*
872 *Parasitol*. 2003;110(3-4):217-26.
- 873 25. Wang J, Al-Ouran R, Hu YH, Kim SY, Wan YW, Wangler MF, et al. MARRVEL: Integration of
874 Human and Model Organism Genetic Resources to Facilitate Functional Annotation of the Human
875 Genome. *Am J Hum Genet*. 2017;100(6):843-53.
- 876 26. Thierry-Mieg D, Thierry-Mieg J. AceView: a comprehensive cDNA-supported gene and
877 transcripts annotation. *Genome Biology*. 2006;7(1):S12.
- 878 27. Wu Z, Lee D, Joo J, Shin J-H, Kang W, Oh S, et al. CYP2J2 and CYP2C19 Are the Major Enzymes
879 Responsible for Metabolism of Albendazole and Fenbendazole in Human Liver Microsomes and
880 Recombinant P450 Assay Systems. *Antimicrobial Agents and Chemotherapy*. 2013;57(11):5448-56.
- 881 28. Grubbs FE. Sample Criteria for Testing Outlying Observations. *Ann Math Statist*.
882 1950;21(1):27-58.
- 883 29. Schneider-Orelli O. *Entomologisches Praktikum: Einführung in die land-und*
884 *forstwirtschaftliche Insektenkunde: Sauerländer*; 1947.
- 885 30. Bliss CI. SOME PRINCIPLES OF BIOASSAY. *American Scientist*. 1957;45(5):449-66.
- 886 31. LITCHFIELD JT, WILCOXON F. A SIMPLIFIED METHOD OF EVALUATING DOSE-EFFECT
887 EXPERIMENTS. *Journal of Pharmacology and Experimental Therapeutics*. 1949;96(2):99-113.
- 888 32. Brenner S. Genetics of *Caenorhabditis-Elegans*. *Genetics*. 1974;77(1):71-94.
- 889 33. Doitsidou M, Poole RJ, Sarin S, Bigelow H, Hobert O. *C. elegans* mutant identification with a
890 one-step whole-genome-sequencing and SNP mapping strategy. *PloS one*. 2010;5(11):e15435.
- 891 34. Jalili V, Afgan E, Gu Q, Clements D, Blankenberg D, Goecks J, et al. The Galaxy platform for
892 accessible, reproducible and collaborative biomedical analyses: 2020 update. *Nucleic Acids Res*.
893 2020;48(W1):W395-w402.
- 894 35. Blacque OE, Perens EA, Boroevich KA, Inglis PN, Li C, Warner A, et al. Functional genomics of
895 the cilium, a sensory organelle. *Current biology : CB*. 2005;15(10):935-41.
- 896 36. Kunitomo H, Uesugi H, Kohara Y, Iino Y. Identification of ciliated sensory neuron-expressed
897 genes in *Caenorhabditis elegans* using targeted pull-down of poly(A) tails. *Genome Biology*.
898 2005;6(2):R17.
- 899 37. Driscoll M, Dean E, Reilly E, Bergholz E, Chalfie M. Genetic and molecular analysis of a
900 *Caenorhabditis elegans* beta-tubulin that conveys benzimidazole sensitivity. *The Journal of cell*
901 *biology*. 1989;109(6 Pt 1):2993-3003.
- 902 38. Taschner M, Lorentzen E. The Intraflagellar Transport Machinery. *Cold Spring Harbor*
903 *perspectives in biology*. 2016;8(10).
- 904 39. Boehlke C, Janusch H, Hamann C, Powelske C, Mergen M, Herbst H, et al. A Cilia
905 Independent Role of Ift88/Polaris during Cell Migration. *PloS one*. 2015;10(10).
- 906 40. Reiter JF, Leroux MR. Genes and molecular pathways underpinning ciliopathies. *Nature*
907 *reviews Molecular cell biology*. 2017;18(9):533-47.
- 908 41. Haycraft CJ, Schafer JC, Zhang Q, Taulman PD, Yoder BK. Identification of CHE-13, a novel
909 intraflagellar transport protein required for cilia formation. *Experimental cell research*.
910 2003;284(2):251-63.
- 911 42. Funabashi T, Katoh Y, Okazaki M, Sugawa M, Nakayama K. Interaction of heterotrimeric
912 kinesin-II with IFT-B-connecting tetramer is crucial for ciliogenesis. *Journal of Cell Biology*.
913 2018;217(8):2867-76.

- 914 43. Vuong LT, Iomini C, Balmer S, Esposito D, Aaronson SA, Mlodzik M. Kinesin-2 and IFT-A act as
915 a complex promoting nuclear localization of β -catenin during Wnt signalling. *Nature*
916 *Communications*. 2018;9(1):5304.
- 917 44. Taschner M, Lorentzen A, Mourao A, Collins T, Freke GM, Moulding D, et al. Crystal structure
918 of intraflagellar transport protein 80 reveals a homo-dimer required for ciliogenesis. *Elife*. 2018;7.
919 45. Behal RH, Miller MS, Qin HM, Lucker BF, Jones A, Cole DG. Subunit Interactions and
920 Organization of the *Chlamydomonas reinhardtii* Intraflagellar Transport Complex A Proteins. *J Biol*
921 *Chem*. 2012;287(15):11689-703.
- 922 46. Nakayama K, Katoh Y. Architecture of the IFT ciliary trafficking machinery and interplay
923 between its components. *Critical reviews in biochemistry and molecular biology*. 2020;55(2):179-96.
924 47. Woodsmith J, Apelt L, Casado-Medrano V, Ozkan Z, Timmermann B, Stelzl U. Protein
925 interaction perturbation profiling at amino-acid resolution. *Nature methods*. 2017;14(12):1213-21.
926 48. Klink BU, Zent E, Juneja P, Kuhlee A, Raunser S, Wittinghofer A. A recombinant BBSome core
927 complex and how it interacts with ciliary cargo. *eLife*. 2017;6:e27434.
- 928 49. Ou G, Koga M, Blacque OE, Murayama T, Ohshima Y, Schafer JC, et al. Sensory ciliogenesis in
929 *Caenorhabditis elegans*: assignment of IFT components into distinct modules based on transport and
930 phenotypic profiles. *Molecular biology of the cell*. 2007;18(5):1554-69.
- 931 50. Burghoorn J, Dekkers MP, Rademakers S, de Jong T, Willemsen R, Jansen G. Mutation of the
932 MAP kinase DYF-5 affects docking and undocking of kinesin-2 motors and reduces their speed in the
933 cilia of *Caenorhabditis elegans*. *Proc Natl Acad Sci U S A*. 2007;104(17):7157-62.
- 934 51. Phirke P, Efimenko E, Mohan S, Burghoorn J, Crona F, Bakhoun MW, et al. Transcriptional
935 profiling of *C. elegans* DAF-19 uncovers a ciliary base-associated protein and a CDK/CCRK/LF2p-
936 related kinase required for intraflagellar transport. *Developmental biology*. 2011;357(1):235-47.
- 937 52. Maurya AK, Rogers T, Sengupta P. A CCRK and a MAK Kinase Modulate Cilia Branching and
938 Length via Regulation of Axonemal Microtubule Dynamics in *Caenorhabditis elegans*. *Current*
939 *biology : CB*. 2019;29(8):1286-300 e4.
- 940 53. Hao L, Efimenko E, Swoboda P, Scholey JM. The retrograde IFT machinery of *C. elegans* cilia:
941 two IFT dynein complexes? *PloS one*. 2011;6(6):e20995.
- 942 54. Ahmed NT, Gao C, Lucker BF, Cole DG, Mitchell DR. ODA16 aids axonemal outer row dynein
943 assembly through an interaction with the intraflagellar transport machinery. *Journal of Cell Biology*.
944 2008;183(2):313-22.
- 945 55. Ishikawa H, Ide T, Yagi T, Jiang X, Hirono M, Sasaki H, et al. TTC26/DYF13 is an intraflagellar
946 transport protein required for transport of motility-related proteins into flagella. *Elife*. 2014;3.
947 56. Williamson SM, Silva DA, Richey E, Qin HM. Probing the role of IFT particle complex A and B
948 in flagellar entry and exit of IFT-dynein in *Chlamydomonas*. *Protoplasma*. 2012;249(3):851-6.
- 949 57. Yang H, Huang K. Dissecting the Vesicular Trafficking Function of IFT Subunits. *Front Cell Dev*
950 *Biol*. 2020;7:352-.
- 951 58. Hao L, Thein M, Brust-Mascher I, Civelekoglu-Scholey G, Lu Y, Acar S, et al. Intraflagellar
952 transport delivers tubulin isoforms to sensory cilium middle and distal segments. *Nature cell biology*.
953 2011;13(7):790-8.
- 954 59. Craft JM, Harris JA, Hyman S, Kner P, Lehtreck KF. Tubulin transport by IFT is upregulated
955 during ciliary growth by a cilium-autonomous mechanism. *Journal of Cell Biology*. 2015;208(2):223-
956 37.
- 957 60. Kubo T, Brown JM, Bellve K, Craige B, Craft JM, Fogarty K, et al. Together, the IFT81 and
958 IFT74 N-termini form the main module for intraflagellar transport of tubulin. *Journal of cell science*.
959 2016;129(10):2106-19.
- 960 61. Zhu X, Liang Y, Gao F, Pan J. IFT54 regulates IFT20 stability but is not essential for tubulin
961 transport during ciliogenesis. *Cellular and Molecular Life Sciences*. 2017;74(18):3425-37.
- 962 62. Taschner M, Kotsis F, Braeuer P, Kuehn EW, Lorentzen E. Crystal structures of IFT70/52 and
963 IFT52/46 provide insight into intraflagellar transport B core complex assembly. *Journal of Cell*
964 *Biology*. 2014;207(2):269-82.

- 965 63. Richey EA, Qin H. Dissecting the sequential assembly and localization of intraflagellar
966 transport particle complex B in *Chlamydomonas*. *PLoS one*. 2012;7(8):e43118.
- 967 64. Lv B, Wan L, Taschner M, Cheng X, Lorentzen E, Huang K. Intraflagellar transport protein
968 IFT52 recruits IFT46 to the basal body and flagella. *Journal of cell science*. 2017;130(9):1662-74.
- 969 65. Hou Y, Qin H, Follit JA, Pazour GJ, Rosenbaum JL, Witman GB. Functional analysis of an
970 individual IFT protein: IFT46 is required for transport of outer dynein arms into flagella. *Journal of*
971 *Cell Biology*. 2007;176(5):653-65.
- 972 66. Akella JS, Carter SP, Nguyen K, Tsiropoulou S, Moran AL, Silva M, et al. Ciliary Rab28 and the
973 BBSome negatively regulate extracellular vesicle shedding. *eLife*. 2020;9.
- 974 67. Jensen VL, Carter S, Sanders AA, Li C, Kennedy J, Timbers TA, et al. Whole-Organism
975 Developmental Expression Profiling Identifies RAB-28 as a Novel Ciliary GTPase Associated with the
976 BBSome and Intraflagellar Transport. *PLoS Genet*. 2016;12(12):e1006469.
- 977 68. Akella JS, Carter SP, Rizvi F, Nguyen KCQ, Tsiropoulou S, Moran AL, et al. A ciliary BBSome-
978 ARL-6-PDE6D pathway trafficks RAB-28, a negative regulator of extracellular vesicle biogenesis.
979 *bioRxiv*. 2019:715730.
- 980 69. Cadinanos J, Schmidt WK, Fueyo A, Varela I, Lopez-Otin C, Freije JM. Identification, functional
981 expression and enzymic analysis of two distinct CaaX proteases from *Caenorhabditis elegans*. *The*
982 *Biochemical journal*. 2003;370(Pt 3):1047-54.
- 983 70. Xue B, Liu YX, Dong B, Wingfield JL, Wu MF, Sun J, et al. Intraflagellar transport protein
984 RABL5/IFT22 recruits the BBSome to the basal body through the GTPase ARL6/BBS3. *P Natl Acad Sci*
985 *USA*. 2020;117(5):2496-505.
- 986 71. Murayama T, Toh Y, Ohshima Y, Koga M. The *dyf-3* gene encodes a novel protein required
987 for sensory cilium formation in *Caenorhabditis elegans*. *Journal of molecular biology*.
988 2005;346(3):677-87.
- 989 72. Wei Q, Zhang Y, Li Y, Zhang Q, Ling K, Hu J. The BBSome controls IFT assembly and
990 turnaround in cilia. *Nature cell biology*. 2012;14(9):950-7.
- 991 73. Ye F, Nager AR, Nachury MV. BBSome trains remove activated GPCRs from cilia by enabling
992 passage through the transition zone. *The Journal of cell biology*. 2018;217(5):1847-68.
- 993 74. Su X, Driscoll K, Yao G, Raed A, Wu M, Beales PL, et al. Bardet-Biedl syndrome proteins 1 and
994 3 regulate the ciliary trafficking of polycystic kidney disease 1 protein. *Human Molecular Genetics*.
995 2014;23(20):5441-51.
- 996 75. Xu Q, Zhang Y, Wei Q, Huang Y, Li Y, Ling K, et al. BBS4 and BBS5 show functional redundancy
997 in the BBSome to regulate the degradative sorting of ciliary sensory receptors. *Scientific Reports*.
998 2015;5(1):11855.
- 999 76. Yi P, Li W-J, Dong M-Q, Ou G. Dynein-Driven Retrograde Intraflagellar Transport Is Triphasic
1000 in *C. elegans* Sensory Cilia. *Current Biology*. 2017;27(10):1448-61.e7.
- 1001 77. Scheidel N, Blacque OE. Intraflagellar Transport Complex A Genes Differentially Regulate
1002 Cilium Formation and Transition Zone Gating. *Current biology : CB*. 2018;28(20):3279-87 e2.
- 1003 78. Lacey E. Mode of action of benzimidazoles. *Parasitol Today*. 1990;6(4):112-5.
- 1004 79. Hurd DD, Miller RM, Nunez L, Portman DS. Specific alpha- and beta-tubulin isotypes optimize
1005 the functions of sensory cilia in *Caenorhabditis elegans*. *Genetics*. 2010;185(3):883-96.
- 1006 80. Ding J, Shao L, Yao Y, Tong X, Liu H, Yue S, et al. DGK δ triggers endoplasmic reticulum release
1007 of IFT88-containing vesicles destined for the assembly of primary cilia. *Scientific Reports*.
1008 2017;7(1):5296.
- 1009 81. Nachury MV, Seeley ES, Jin H. Trafficking to the ciliary membrane: how to get across the
1010 periciliary diffusion barrier? *Annual review of cell and developmental biology*. 2010;26:59-87.
- 1011 82. Monis WJ, Faundez V, Pazour GJ. BLOC-1 is required for selective membrane protein
1012 trafficking from endosomes to primary cilia. *Journal of Cell Biology*. 2017;216(7):2131-50.
- 1013 83. Mukhopadhyay S, Badgandi HB, Hwang SH, Somatilaka B, Shimada IS, Pal K. Trafficking to the
1014 primary cilium membrane. *Molecular biology of the cell*. 2017;28(2):233-9.

- 1015 84. Sato K, Norris A, Sato M, Grant BD. *C. elegans* as a model for membrane traffic. In:
1016 Community TCeR, editor. WormBook: WormBook.
- 1017 85. Zong M, Satoh A, Yu MK, Siu KY, Ng WY, Chan HC, et al. TRAPPC9 Mediates the Interaction
1018 between p150(Glued) and COPII Vesicles at the Target Membrane. *PloS one*. 2012;7(1).
- 1019 86. Michelet X, Djeddi A, Legouis R. Developmental and cellular functions of the ESCRT
1020 machinery in pluricellular organisms. *Biology of the Cell*. 2010;102(3):191-202.
- 1021 87. Stoten CL, Carlton JG. ESCRT-dependent control of membrane remodelling during cell
1022 division. *Seminars in Cell & Developmental Biology*. 2018;74:50-65.
- 1023 88. Leitch CC, Lodh S, Prieto-Echague V, Badano JL, Zaghoul NA. Basal body proteins regulate
1024 Notch signaling through endosomal trafficking. *Journal of cell science*. 2014;127(Pt 11):2407-19.
- 1025 89. Shinde SR, Nager AR, Nachury MV. Ubiquitin chains earmark GPCRs for BBSome-mediated
1026 removal from cilia. *Journal of Cell Biology*. 2020;219(12).
- 1027 90. Jiu Y, Jin C, Liu Y, Holmberg CI, Jantti J. Exocyst subunits Exo70 and Exo84 cooperate with
1028 small GTPases to regulate behavior and endocytic trafficking in *C. elegans*. *PloS one*.
1029 2012;7(2):e32077.
- 1030 91. Cromm PM, Adihou H, Kapoor S, Vazquez-Chantada M, Davey P, Longmire D, et al. Lipidated
1031 Stapled Peptides Targeting the Acyl Binding Protein UNC119. *Chembiochem*. 2019.
- 1032 92. Zhang Q, Li Y, Zhang Y, Torres VE, Harris PC, Ling K, et al. GTP-binding of ARL-3 is activated by
1033 ARL-13 as a GEF and stabilized by UNC-119. *Scientific Reports*. 2016;6(1):24534.
- 1034 93. Endicott SJ, Brueckner M. NUP98 Sets the Size-Exclusion Diffusion Limit through the Ciliary
1035 Base. *Current Biology*. 2018;28(10):1643-+.
- 1036 94. Zhao CT, Malicki J. Nephrocystins and MKS proteins interact with IFT particle and facilitate
1037 transport of selected ciliary cargos. *Embo Journal*. 2011;30(13):2532-44.
- 1038 95. Wei Q, Xu Q, Zhang Y, Li Y, Zhang Q, Hu Z, et al. Transition fibre protein FBF1 is required for
1039 the ciliary entry of assembled intraflagellar transport complexes. *Nature Communications*.
1040 2013;4(1):2750.
- 1041 96. Joo K, Kim CG, Lee M-S, Moon H-Y, Lee S-H, Kim MJ, et al. CCDC41 is required for ciliary
1042 vesicle docking to the mother centriole. *Proceedings of the National Academy of Sciences*.
1043 2013;110(15):5987-92.
- 1044 97. Zhang BY, Wang G, Xu XW, Yang SS, Zhuang TH, Wang GP, et al. DAZ-interacting Protein 1
1045 (Dzip1) Phosphorylation by Polo-like Kinase 1 (Plk1) Regulates the Centriolar Satellite Localization of
1046 the BBSome Protein during the Cell Cycle. *J Biol Chem*. 2017;292(4):1351-60.
- 1047 98. Mazo G, Soplop N, Wang WJ, Uryu K, Tsou MF. Spatial Control of Primary Ciliogenesis by
1048 Subdistal Appendages Alters Sensation-Associated Properties of Cilia. *Developmental cell*.
1049 2016;39(4):424-37.
- 1050 99. Naharros IO, Gesemann M, Mateos JM, Barmettler G, Forbes A, Ziegler U, et al. Loss-of-
1051 function of the ciliopathy protein Cc2d2a disorganizes the vesicle fusion machinery at the periciliary
1052 membrane and indirectly affects Rab8-trafficking in zebrafish photoreceptors. *PloS Genetics*.
1053 2017;13(12).
- 1054 100. Kaplan OI, Molla-Herman A, Cevik S, Ghossoub R, Kida K, Kimura Y, et al. The AP-1 clathrin
1055 adaptor facilitates cilium formation and functions with RAB-8 in *C. elegans* ciliary membrane
1056 transport. *Journal of cell science*. 2010;123(Pt 22):3966-77.
- 1057 101. Babbey CM, Bacallao RL, Dunn KW. Rab10 associates with primary cilia and the exocyst
1058 complex in renal epithelial cells. *Am J Physiol Renal Physiol*. 2010;299(3):F495-F506.
- 1059 102. Yoshimura S, Egerer J, Fuchs E, Haas AK, Barr FA. Functional dissection of Rab GTPases
1060 involved in primary cilium formation. *The Journal of cell biology*. 2007;178(3):363-9.
- 1061 103. Li FQ, Chen X, Fisher C, Siller SS, Zelikman K, Kuriyama R, et al. BAR Domain-Containing
1062 FAM92 Proteins Interact with Chibby1 To Facilitate Ciliogenesis. *Molecular and cellular biology*.
1063 2016;36(21):2668-80.
- 1064 104. Kilmartin JV. Sfi1p has conserved centrin-binding sites and an essential function in budding
1065 yeast spindle pole body duplication. *The Journal of cell biology*. 2003;162(7):1211-21.

- 1066 105. Bowers K, Lottridge J, Helliwell SB, Goldthwaite LM, Luzio JP, Stevens TH. Protein-protein
1067 interactions of ESCRT complexes in the yeast *Saccharomyces cerevisiae*. *Traffic*. 2004;5(3):194-210.
- 1068 106. Wei Q, Zhang Y, Schouteden C, Zhang Q, Dong J, Wonesch V, et al. The hydrolethalus
1069 syndrome protein HYLS-1 regulates formation of the ciliary gate. *Nat Commun*. 2016;7:12437.
- 1070 107. Dammermann A, Pemble H, Mitchell BJ, McLeod I, Yates JR, 3rd, Kintner C, et al. The
1071 hydrolethalus syndrome protein HYLS-1 links core centriole structure to cilia formation. *Genes &
1072 development*. 2009;23(17):2046-59.
- 1073 108. Iwasa H, Maimaiti S, Kuroyanagi H, Kawano S, Inami K, Timalina S, et al. Yes-associated
1074 protein homolog, YAP-1, is involved in the thermotolerance and aging in the nematode
1075 *Caenorhabditis elegans*. *Experimental cell research*. 2013;319(7):931-45.
- 1076 109. Lee H, Kang J, Lee J. Involvement of YAP-1, the Homolog of Yes-Associated Protein, in the
1077 Wnt-Mediated Neuronal Polarization in *Caenorhabditis elegans*. *G3 (Bethesda)*. 2018;8(8):2595-602.
- 1078 110. Blasius TL, Takao D, Verhey KJ. NPHP proteins are binding partners of nucleoporins at the
1079 base of the primary cilium. *PloS one*. 2019;14(9):e0222924.
- 1080 111. Lambacher NJ, Bruel AL, van Dam TJ, Szymanska K, Slaats GG, Kuhns S, et al. TMEM107
1081 recruits ciliopathy proteins to subdomains of the ciliary transition zone and causes Joubert
1082 syndrome. *Nature cell biology*. 2016;18(1):122-31.
- 1083 112. Garcia-Gonzalo FR, Reiter JF. Open Sesame: How Transition Fibers and the Transition Zone
1084 Control Ciliary Composition. *Cold Spring Harbor perspectives in biology*. 2017;9(2):a028134.
- 1085 113. Li C, Jensen VL, Park K, Kennedy J, Garcia-Gonzalo FR, Romani M, et al. MKS5 and CEP290
1086 Dependent Assembly Pathway of the Ciliary Transition Zone. *PLoS biology*. 2016;14(3):e1002416.
- 1087 114. Warburton-Pitt SR, Jauregui AR, Li C, Wang J, Leroux MR, Barr MM. Ciliogenesis in
1088 *Caenorhabditis elegans* requires genetic interactions between ciliary middle segment localized
1089 NPHP-2 (inversin) and transition zone-associated proteins. *Journal of cell science*. 2012;125(Pt
1090 11):2592-603.
- 1091 115. Latour BL, Van De Weghe JC, Rusterholz TDS, Letteboer SJF, Gomez A, Shaheen R, et al.
1092 ARMC9 and TOGARAM1 define a Joubert syndrome-associated protein module that regulates
1093 axonemal post-translational modifications and cilium stability. *bioRxiv*. 2019:817213.
- 1094 116. Olivier-Mason A, Wojtyniak M, Bowie RV, Nechipurenko IV, Blacque OE, Sengupta P.
1095 Transmembrane protein OSTA-1 shapes sensory cilia morphology via regulation of intracellular
1096 membrane trafficking in *C. elegans*. *Development*. 2013;140(7):1560-72.
- 1097 117. Porter ME. 10 - Ciliary and flagellar motility and the nexin-dynein regulatory complex. In:
1098 King SM, editor. *Dyneins: Structure, Biology and Disease (Second Edition)*: Academic Press; 2018. p.
1099 298-335.
- 1100 118. Lenfant N, Polanowska J, Bamps S, Omi S, Borg JP, Reboul J. A genome-wide study of PDZ-
1101 domain interactions in *C. elegans* reveals a high frequency of non-canonical binding. *BMC genomics*.
1102 2010;11:671.
- 1103 119. Li S, Armstrong CM, Bertin N, Ge H, Milstein S, Boxem M, et al. A map of the interactome
1104 network of the metazoan *C. elegans*. *Science*. 2004;303(5657):540-3.
- 1105 120. Lee S, Walker CL, Karten B, Kuny SL, Tennese AA, O'Neill MA, et al. Essential role for the
1106 Prader-Willi syndrome protein *neccdin* in axonal outgrowth. *Human Molecular Genetics*.
1107 2005;14(5):627-37.
- 1108 121. Muresan V. One axon, many kinesins: What's the logic? *J Neurocytol*. 2000;29(11-12):799-
1109 818.
- 1110 122. Goldstein LS, Yang Z. Microtubule-based transport systems in neurons: the roles of kinesins
1111 and dyneins. *Annual review of neuroscience*. 2000;23:39-71.
- 1112 123. Maniar TA, Kaplan M, Wang GJ, Shen K, Wei L, Shaw JE, et al. UNC-33 (CRMP) and ankyrin
1113 organize microtubules and localize kinesin to polarize axon-dendrite sorting. *Nature neuroscience*.
1114 2011;15(1):48-56.

- 1115 124. Hoerndli FJ, Maxfield DA, Brockie PJ, Mellem JE, Jensen E, Wang R, et al. Kinesin-1 regulates
1116 synaptic strength by mediating the delivery, removal, and redistribution of AMPA receptors. *Neuron*.
1117 2013;80(6):1421-37.
- 1118 125. Peden EM, Barr MM. The KLP-6 Kinesin Is Required for Male Mating Behaviors and
1119 Polycystin Localization in *Caenorhabditis elegans*. *Current Biology*. 2005;15(5):394-404.
- 1120 126. Kobayashi T, Tsang WY, Li J, Lane W, Dynlacht BD. Centriolar kinesin Kif24 interacts with
1121 CP110 to remodel microtubules and regulate ciliogenesis. *Cell*. 2011;145(6):914-25.
- 1122 127. Grant BD, Donaldson JG. Pathways and mechanisms of endocytic recycling. *Nat Rev Mol Cell*
1123 *Bio*. 2009;10(9):597-608.
- 1124 128. Mayor S, Parton RG, Donaldson JG. Clathrin-independent pathways of endocytosis. *Cold*
1125 *Spring Harbor perspectives in biology*. 2014;6(6).
- 1126 129. Gallegos ME, Balakrishnan S, Chandramouli P, Arora S, Azameera A, Babushekar A, et al. The
1127 *C. elegans* rab family: identification, classification and toolkit construction. *PloS one*.
1128 2012;7(11):e49387-e.
- 1129 130. Chun DK, McEwen JM, Burbea M, Kaplan JM. UNC-108/Rab2 regulates postendocytic
1130 trafficking in *Caenorhabditis elegans*. *Molecular biology of the cell*. 2008;19(7):2682-95.
- 1131 131. van Weering JRT, Toonen RF, Verhage M. The role of Rab3a in secretory vesicle docking
1132 requires association/dissociation of guanidine phosphates and Munc18-1. *PloS one*. 2007;2(7):e616-
1133 e.
- 1134 132. Nonet ML, Staunton JE, Kilgard MP, Fergestad T, Hartwig E, Horvitz HR, et al.
1135 *Caenorhabditis elegans* rab-3 Mutant Synapses Exhibit Impaired Function and Are
1136 Partially Depleted of Vesicles. *The Journal of Neuroscience*. 1997;17(21):8061-73.
- 1137 133. Maguire JE, Silva M, Nguyen KC, Hellen E, Kern AD, Hall DH, et al. Myristoylated CIL-7
1138 regulates ciliary extracellular vesicle biogenesis. *Molecular biology of the cell*. 2015;26(15):2823-32.
- 1139 134. Buck AH, Coakley G, Simbari F, McSorley HJ, Quintana JF, Le Bihan T, et al. Exosomes
1140 secreted by nematode parasites transfer small RNAs to mammalian cells and modulate innate
1141 immunity. *Nature Communications*. 2014;5.
- 1142 135. Wang J, Silva M, Haas LA, Morsci NS, Nguyen KCQ, Hall DH, et al. *C-elegans* Ciliated Sensory
1143 Neurons Release Extracellular Vesicles that Function in Animal Communication. *Current Biology*.
1144 2014;24(5):519-25.
- 1145 136. Keller MD, Ching KL, Liang FX, Dhabaria A, Tam K, Ueberheide BM, et al. Decoy exosomes
1146 provide protection against bacterial toxins. *Nature*. 2020;579(7798):260-+.
- 1147 137. Nager AR, Goldstein JS, Herranz-Perez V, Portran D, Ye F, Garcia-Verdugo JM, et al. An Actin
1148 Network Dispatches Ciliary GPCRs into Extracellular Vesicles to Modulate Signaling. *Cell*. 2017;168(1-
1149 2):252-+.
- 1150 138. McKelvey KJ, Powell KL, Ashton AW, Morris JM, McCracken SA. Exosomes: Mechanisms of
1151 Uptake. *J Circ Biomark*. 2015;4:7-.
- 1152 139. Kostich M, Fire A, Fambrough DM. Identification and molecular-genetic characterization of a
1153 LAMP/CD68-like protein from *Caenorhabditis elegans*. *Journal of cell science*. 2000;113 (Pt
1154 14):2595-606.
- 1155 140. Eskelinen E-L. Roles of LAMP-1 and LAMP-2 in lysosome biogenesis and autophagy.
1156 *Molecular Aspects of Medicine*. 2006;27(5):495-502.
- 1157 141. Oikonomou G, Perens EA, Lu Y, Watanabe S, Jorgensen EM, Shaham S. Opposing activities of
1158 LIT-1/NLK and DAF-6/patched-related direct sensory compartment morphogenesis in *C. elegans*.
1159 *PLoS biology*. 2011;9(8):e1001121.
- 1160 142. Perens EA, Shaham S. *C. elegans* daf-6 encodes a patched-related protein required for lumen
1161 formation. *Developmental cell*. 2005;8(6):893-906.
- 1162 143. Overeem AW, Bryant DM, van IJzendoorn SCD. Mechanisms of apical-basal axis orientation
1163 and epithelial lumen positioning. *Trends in cell biology*. 2015;25(8):476-85.
- 1164 144. Sato M, Sato K, Liou W, Pant S, Harada A, Grant BD. Regulation of endocytic recycling by *C.*
1165 *elegans* Rab35 and its regulator RME-4, a coated-pit protein. *The EMBO journal*. 2008;27(8):1183-96.

- 1166 145. Klinkert K, Echard A. Rab35 GTPase: A Central Regulator of Phosphoinositides and F-actin in
1167 Endocytic Recycling and Beyond. *Traffic*. 2016;17(10):1063-77.
- 1168 146. Voelker L, Upadhyaya B, Ferkey DM, Woldemariam S, L'Etoile ND, Rabinowitch I, et al. INX-
1169 18 and INX-19 play distinct roles in electrical synapses that modulate aversive behavior in
1170 *Caenorhabditis elegans*. *PLOS Genetics*. 2019;15(10):e1008341.
- 1171 147. Schumacher JA, Hsieh YW, Chen S, Pirri JK, Alkema MJ, Li WH, et al. Intercellular calcium
1172 signaling in a gap junction-coupled cell network establishes asymmetric neuronal fates in *C. elegans*.
1173 *Development*. 2012;139(22):4191-201.
- 1174 148. Chuang CF, Vanhoven MK, Fetter RD, Verselis VK, Bargmann CI. An innexin-dependent cell
1175 network establishes left-right neuronal asymmetry in *C. elegans*. *Cell*. 2007;129(4):787-99.
- 1176 149. Bañuelos J. BODIPY Dye, the Most Versatile Fluorophore Ever? *The Chemical Record*.
1177 2016;16(1):335-48.
- 1178 150. Zhang J, Nan X, Yu H-T, Cheng P-L, Zhang Y, Liu Y-Q, et al. Synthesis, biological activities and
1179 structure-activity relationships for new avermectin analogues. *European Journal of Medicinal
1180 Chemistry*. 2016;121:422-32.
- 1181 151. Starich TA, Herman RK, Kari CK, Yeh WH, Schackwitz WS, Schuyler MW, et al. Mutations
1182 affecting the chemosensory neurons of *Caenorhabditis elegans*. *Genetics*. 1995;139(1):171-88.
- 1183 152. Gengyo-Ando K, Mitani S. Characterization of mutations induced by ethyl methanesulfonate,
1184 UV, and trimethylpsoralen in the nematode *Caenorhabditis elegans*. *Biochem Bioph Res Co*.
1185 2000;269(1):64-9.
- 1186 153. Escher BI, Berger C, Bramaz N, Kwon JH, Richter M, Tsinman O, et al. Membrane-water
1187 partitioning, membrane permeability, and baseline toxicity of the parasiticides ivermectin,
1188 albendazole, and morantel. *Environmental Toxicology and Chemistry*. 2008;27(4):909-18.

1189

1190 **Fig 1. Structure of fluorescent ivermectin and albendazole analogs used.**

1191 Chemical structures of the FBI and BABZ probes with the BODIPY fluorophores highlighted by a
1192 green oval.

1193 **Fig 2. Representative images of Dil and BODIPY labelled anthelmintic analog phenotypes.**

1194 Dil = 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate; FBI = Fatty-BODIPY-
1195 Ivermectin; BODIPY-AlBendaZole. (A) N2: Dil dye filling positive, (B) *Ifta-1(nx61)*: Weak Dil dye filling
1196 positive, (C) *Dyf-2(m160)*: Dil dye filling negative (D) *C14h10.2(tm10737)*: Novel *Dyf* mutant which
1197 has variable Dil dye filling with weak positive individuals in a predominantly negative (pictured)
1198 population, (E) N2: FBI uptake positive, (F) *Hyls-1(tm3067)*: Weak FBI uptake positive, (G) *Osm-*
1199 *5(p813)*: FBI uptake negative, (H) N2: BABZ uptake positive, (I) *Dnc-1(or404)* Weak BABZ uptake
1200 positive, (J) *Rab-35(b1013)*: BABZ uptake negative and (K) N2: Staining pattern of 1,3,5,7-
1201 tetramethyl-8-pent-4-ene-BODIPY. Individuals were photographed using a DIC filter (lower right

1202 inset image) to highlight the position and orientation of the worm and a FITC filter (main image) to
1203 visualise fluorescence. Areas of fluorescence for weak phenotypes are highlighted with arrows.

1204 **Fig 3. Position of novel and tested alleles in resistance genes identified by whole genome**
1205 **sequencing.**

1206 Transcript structures and positions of genes were obtained from WormBase (<https://wormbase.org>)
1207 (JBrowse version: WS281; genome build WBcel235). Arrows above alleles point to their location in
1208 the genomic sequence. Red lines above alleles span the length of deletions. Alleles featured (*name* =
1209 chr-number: position nt-change (aa-change)) are **e1124** = I: 8,071,718 G>A (Q>Stop); **ka30** = IV:
1210 3,797,404 G>A (Q>Stop); **ka32** = I: 8,070,133 C>T (G>R); **ka33** = V: 13,150,172-13,150,276 deletion;
1211 **ka35** = I: 8,058,869-8,079,083 deletion; **ka64** = I: 8,075,488 A>T (L>Stop); **ka66** = I: 8,072,572 C>T
1212 (E>K); **ka200** = X: 16,544,813 C>T (Q>Stop); **ka201** = V: 13,150,224 AGG>AG frameshift; **ka202** = III:
1213 13,676,892 G>A (Q>Stop); **ka203** = I: 8,077,873 G>A splice site acceptor change; **ka204** = X:
1214 5,550,502 A>T (C>Stop); **m160** III: 13,686,367 G>A (R>Stop); **nx61** = X: 5,545,532-5,547,540 deletion;
1215 **p802** = IV: 3,797,722 G>A (Q>Stop); **p808** = X: Uncharacterised; **p816** = X: Uncharacterised ~600bp
1216 deletion.

1217 **Fig 4. Intraflagellar transport in *C. elegans* and resistance patterns in the IFT protein-protein**
1218 **interaction network.**

1219 (A) Summary of *C. elegans* intraflagellar transport. Colours used are for the summation of
1220 resistances found in complexes. **IFT-A** = Intraflagellar transport complex A; **IFT-B** = Intraflagellar
1221 transport complex B; **BBSome** = Bardet-Biedl Syndrome complex; **Line** = protein/complex-
1222 protein/complex interaction; **Small Arrow** = Change in protein or complex localisation or interaction;
1223 **Large Arrow** = Direction of IFT particle travel. (B) Predicted IFT protein-protein interaction network
1224 in *C. elegans* showing resistances found in mutants of each node. **Box** = Group of proteins from the
1225 same complex or with the same function; **Line** = predicted protein-protein interaction; **Small Arrow**

1226 = Protein self-interaction; / = Multiple candidate genes with homology to a node found in other
1227 species.

1228 **Fig 5. Ciliary protein trafficking pathways in *C. elegans* and resistance patterns in the ciliary gate**
1229 **protein-protein interaction network.**

1230 (A) Protein trafficking pathways used to deliver and remove ciliary proteins. **Small Arrow** = Show
1231 directionality of protein trafficking between cellular locations or organelles with key proteins and
1232 complexes involved in trafficking listed next to the arrow (placed before junctions if merging into a
1233 common secretion pathway); **Large Arrow** = Directionality of axonal transport or passive diffusion.

1234 (B) Predicted basal body protein-protein interaction network in *C. elegans* showing resistances found
1235 in mutants of each node. **Box** = Group of proteins from the same complex or with the same function;
1236 **Line** = predicted protein-protein interaction; / = Multiple (2-4) candidate genes with homology to a
1237 node found in other species (if gene IDs differ only by the last digit, then only the last digit is shown
1238 to the right of the candidate with a similar ID); **Ce(node name of vertebrate ortholog)** = Multiple
1239 (>4) candidate genes with homology to the node found in other species.

1240 **Fig S1. Timecourse of BODIPY labelled ivermectin analog, FBI, probe localisation and uptake.**

1241 Timecourse of FBI uptake in N2 at (A) 6 hours, (B) 12 hours, (C) 18 hours, (D) 24 hours, (E) 30 hours,
1242 (F) 36 hours, (G) 42 hours, (H) 48 hours, (I) 54 hours, (J) 60 hours, (K) 66 hours and (L) 72 hours.

1243 Individuals were photographed using a DIC filter (lower right inset image) to highlight the position
1244 and orientation of the worm and a FITC filter (main image) to visualise fluorescence. Areas of weak
1245 fluorescence are highlighted with arrows.

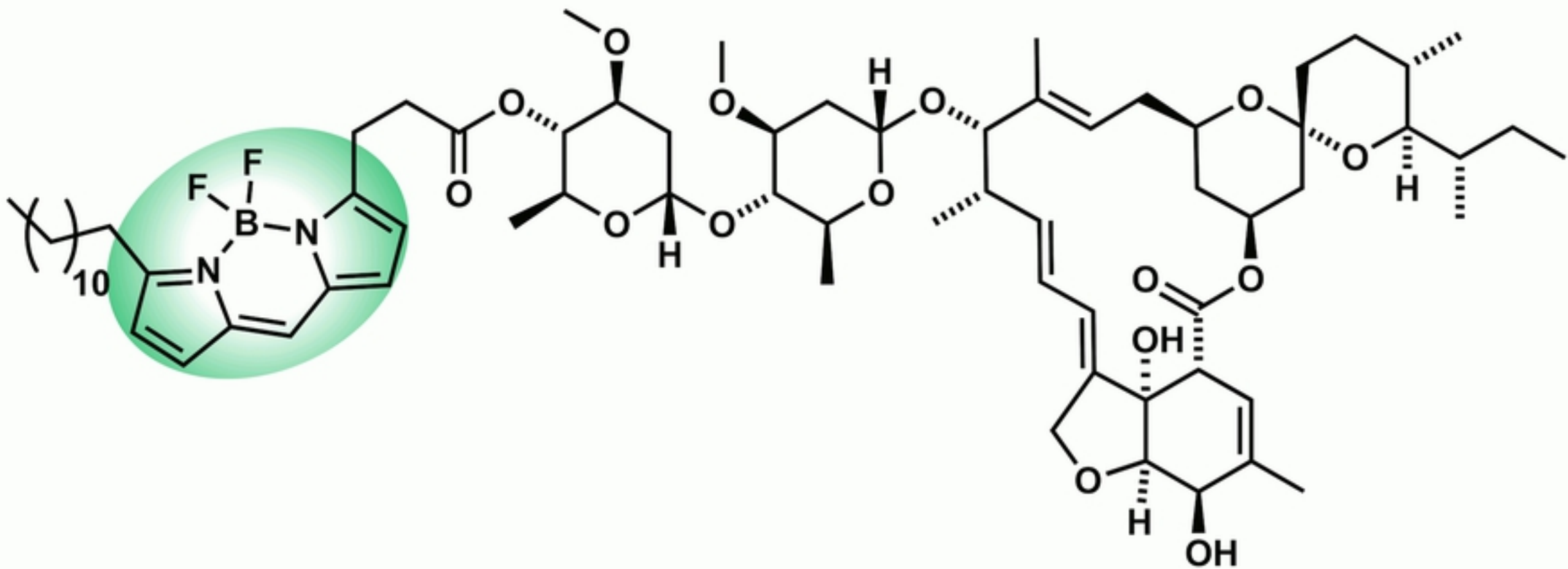
1246 **Fig S2. Timecourse of BODIPY labelled albendazole analog, BABZ, probe localisation and uptake.**

1247 Timecourse of BABZ uptake in N2 at (A) 100x magnification at 1 hours, (B) 250x magnification of gut
1248 at 1 hours, (C) 100x magnification showing transient uptake at 1 hours, (D) 100x magnification at 2
1249 hours, (E) 250x magnification of gut at 2 hours, (F) 100x magnification at 3 hours, (G) 250x

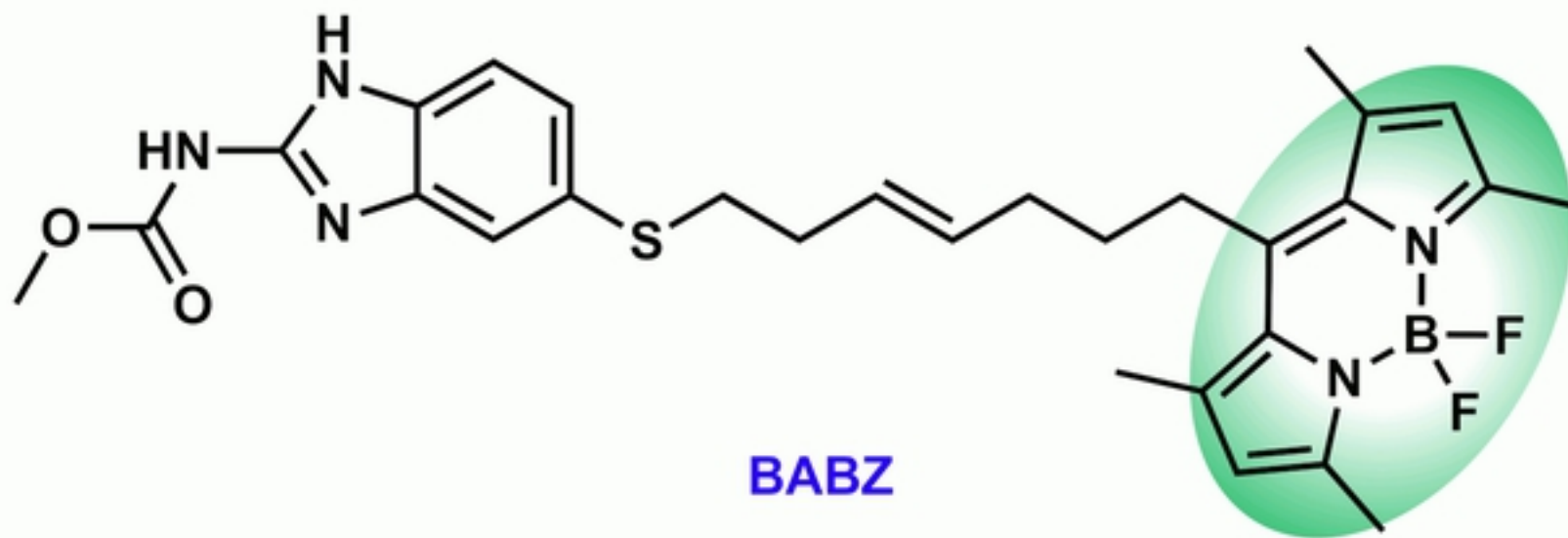
1250 magnification of gut at 3 hours. Arrow highlights observed uptake boundary, (H) 250x magnification
1251 of eggs in the body cavity at 3 hours, (I) 100x magnification at 4 hours, (J) 250x magnification of gut
1252 and eggs in the body cavity at 4 hours, (K) 100x magnification at 5 hours, (L) 250x magnification of
1253 eggs in the body cavity at 5 hours, (M) 100x magnification at 6 hours (N) 250x magnification of gut at
1254 6 hours (O) 250x magnification of eggs in the body cavity at 6 hours, (P) 100x magnification at 7
1255 hours, (Q) 250x magnification of head and gut at 7 hours, (R) 100x magnification at 8 hours, (S) 250x
1256 magnification of head, gut and eggs in the body cavity at 8 hours. Individuals were photographed
1257 using a DIC filter (lower right inset image) to highlight the position and orientation of the worm and
1258 a FITC filter (main image) to visualise fluorescence.

1259 **Fig S3. Schemes for BODIPY labelled anthelmintic analog probe synthesis.**

1260 Schemes for FBI synthesis (A) and BABZ synthesis (B).



FBI



BABZ

Figure 1

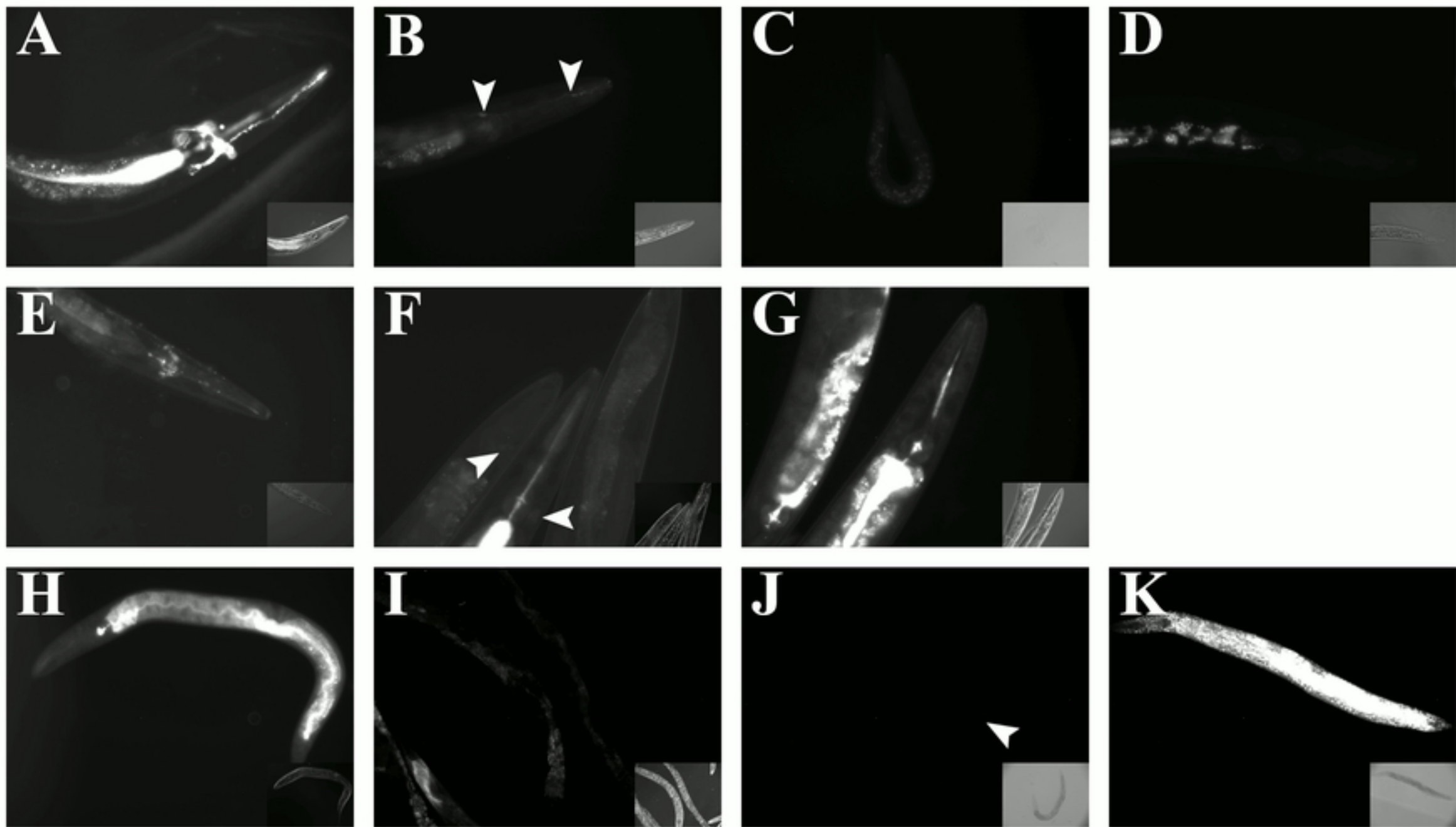
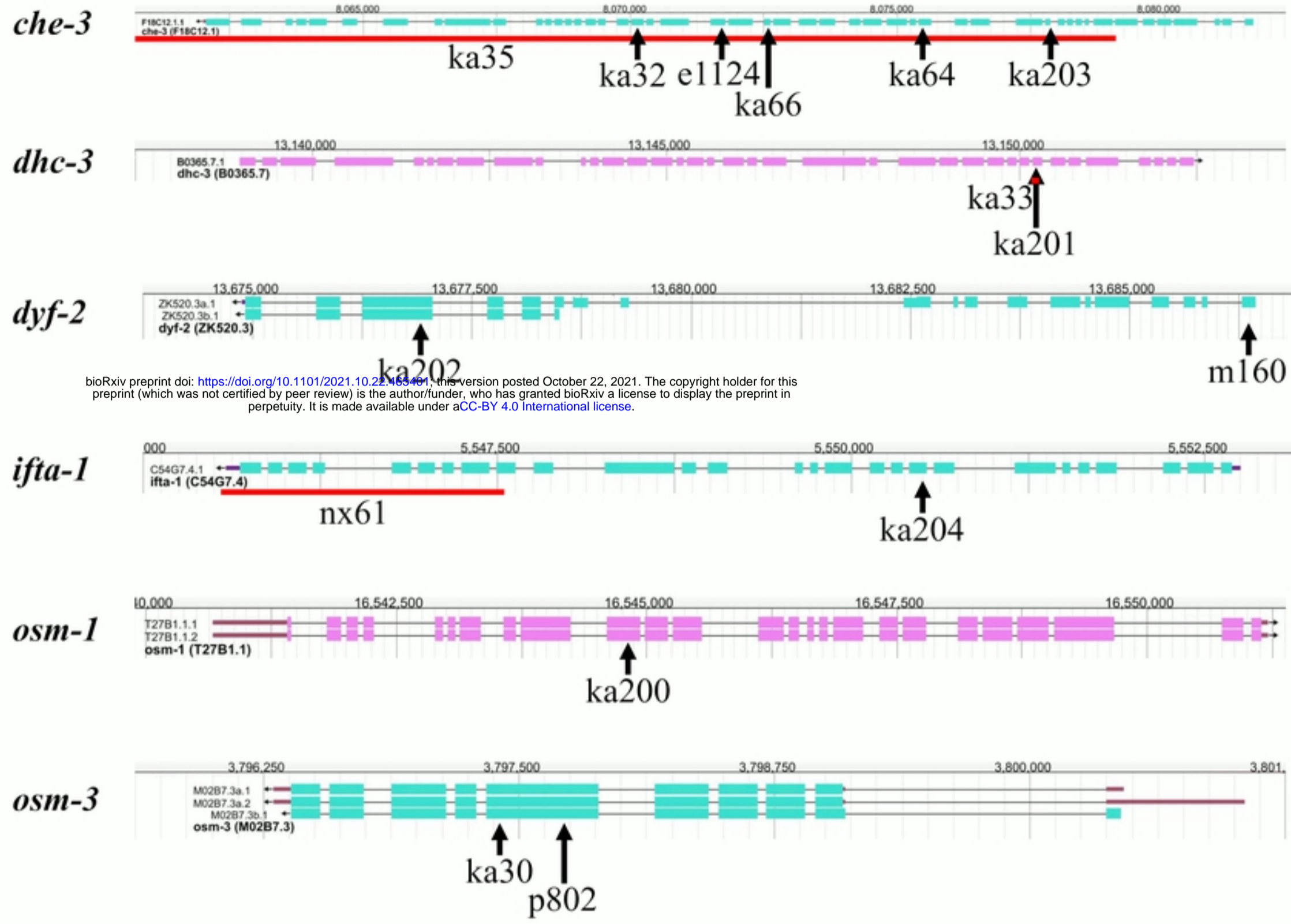


Figure 2



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Figure 3

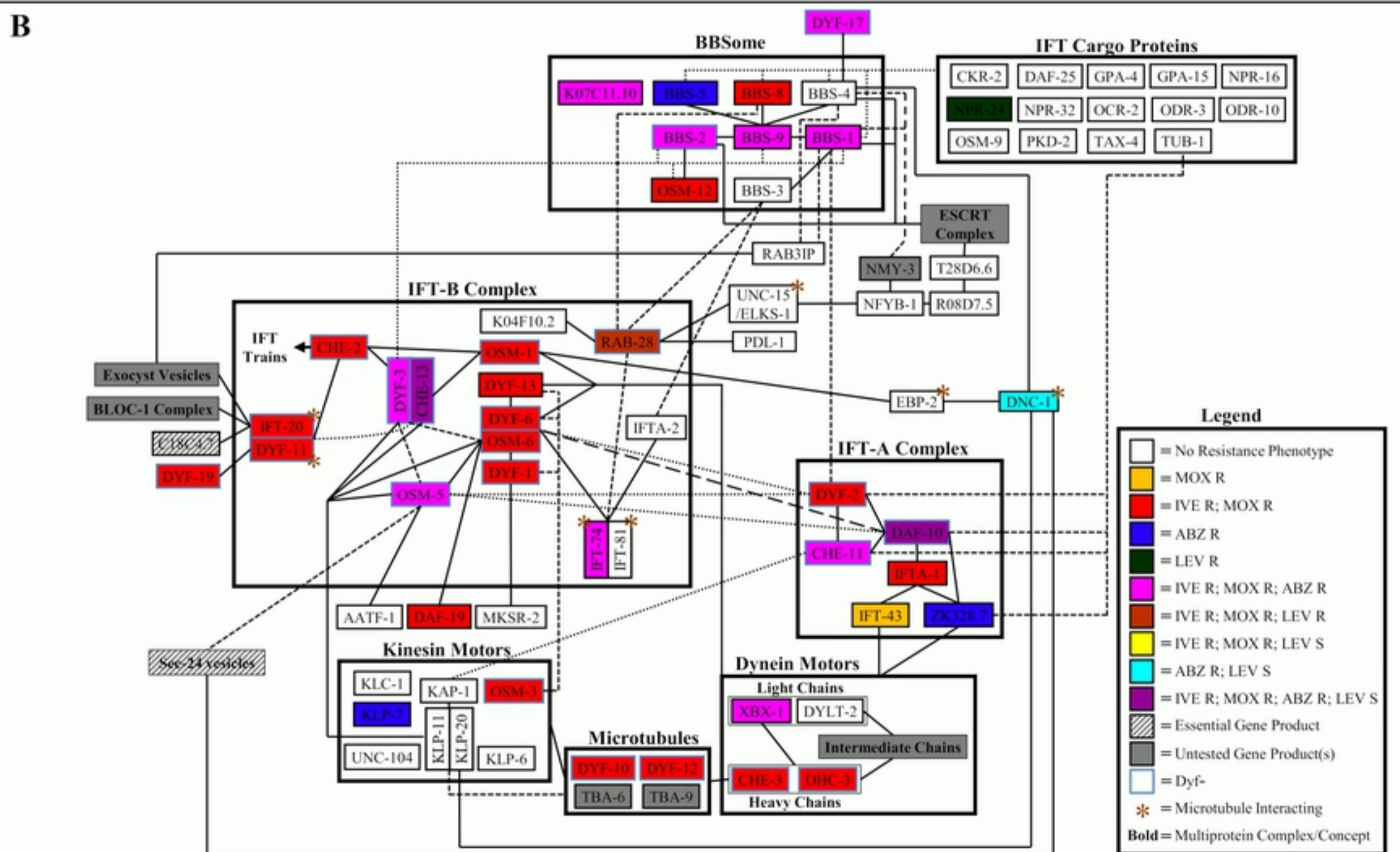
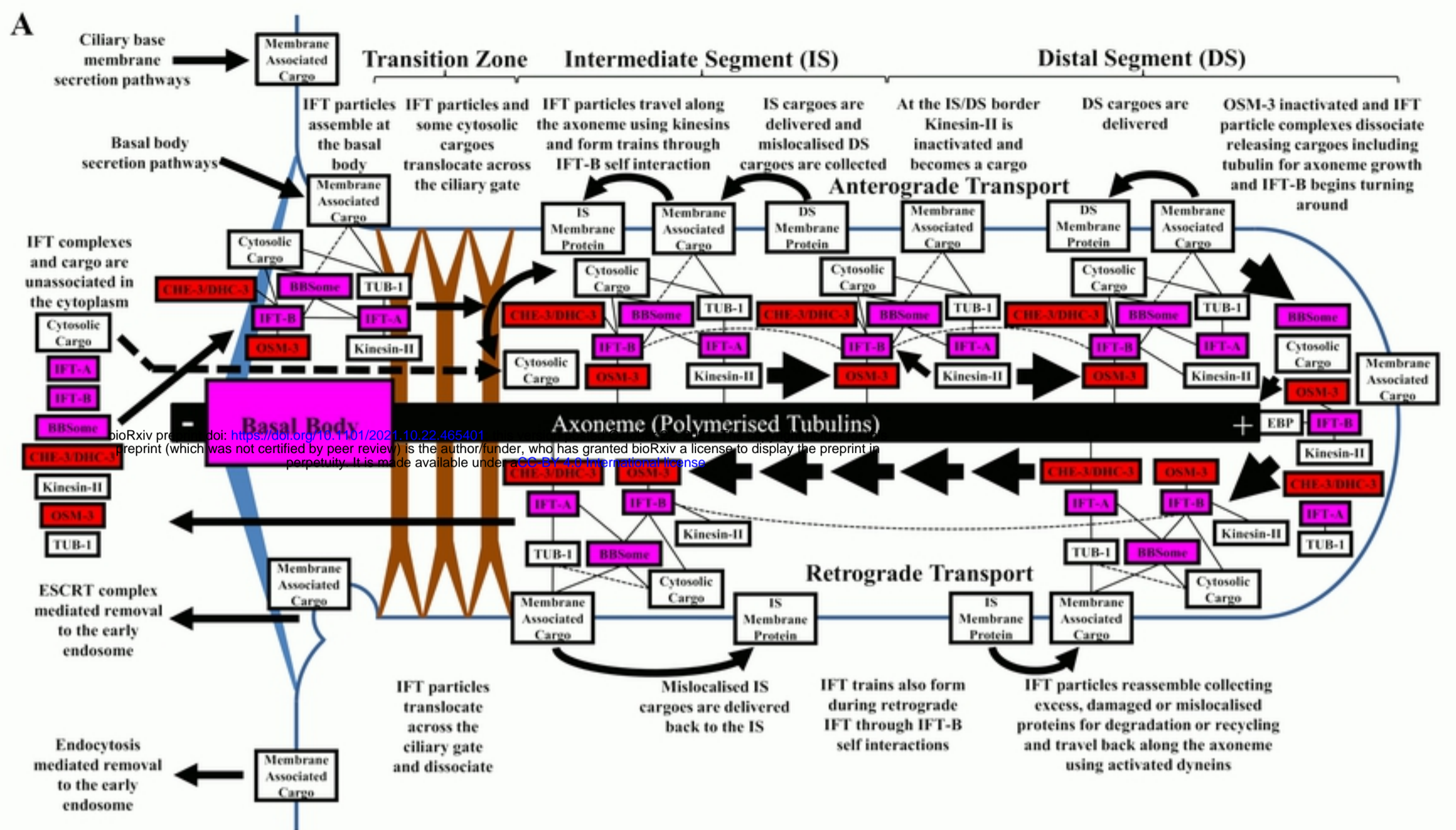
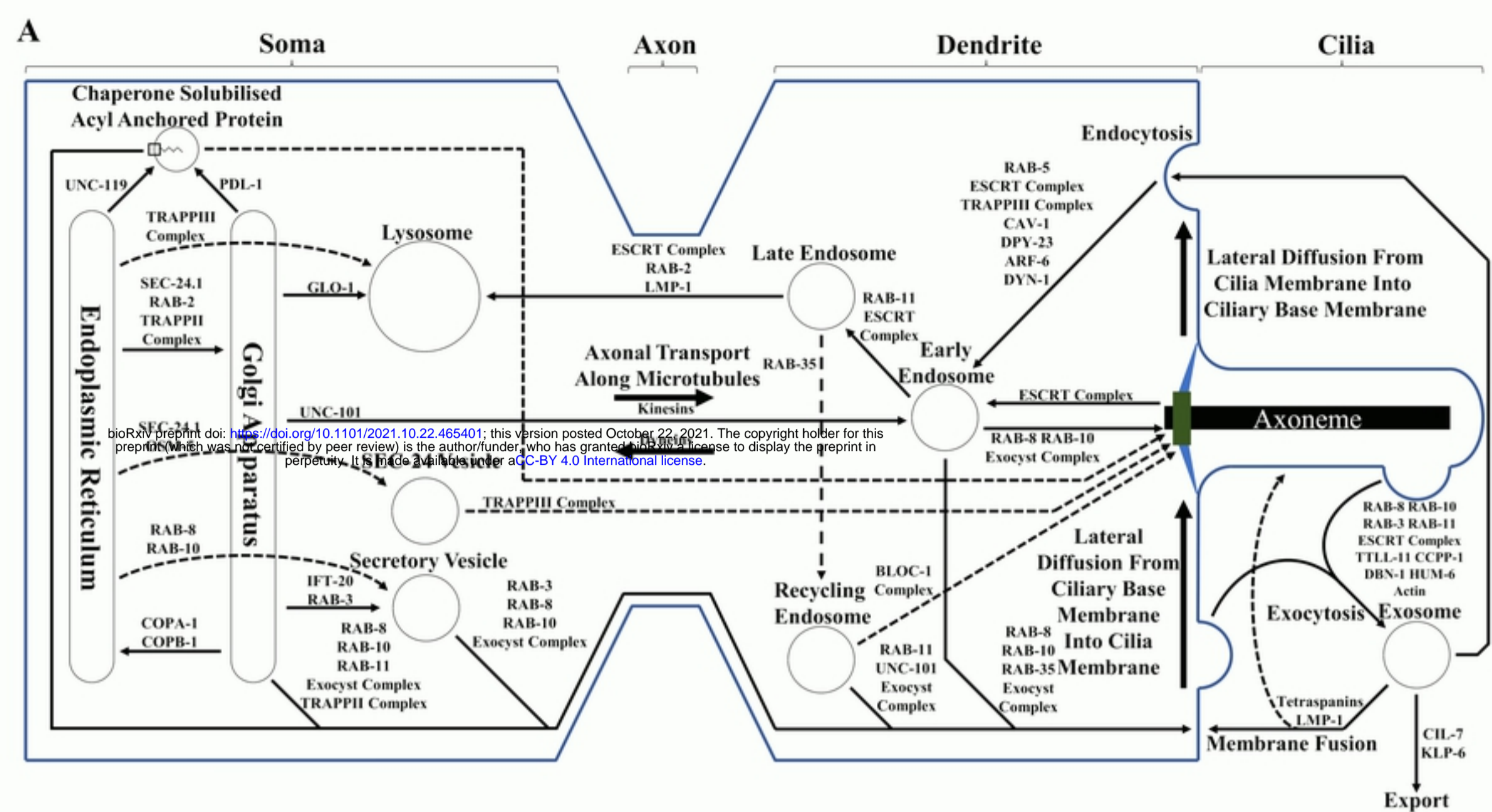


Figure 4



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B

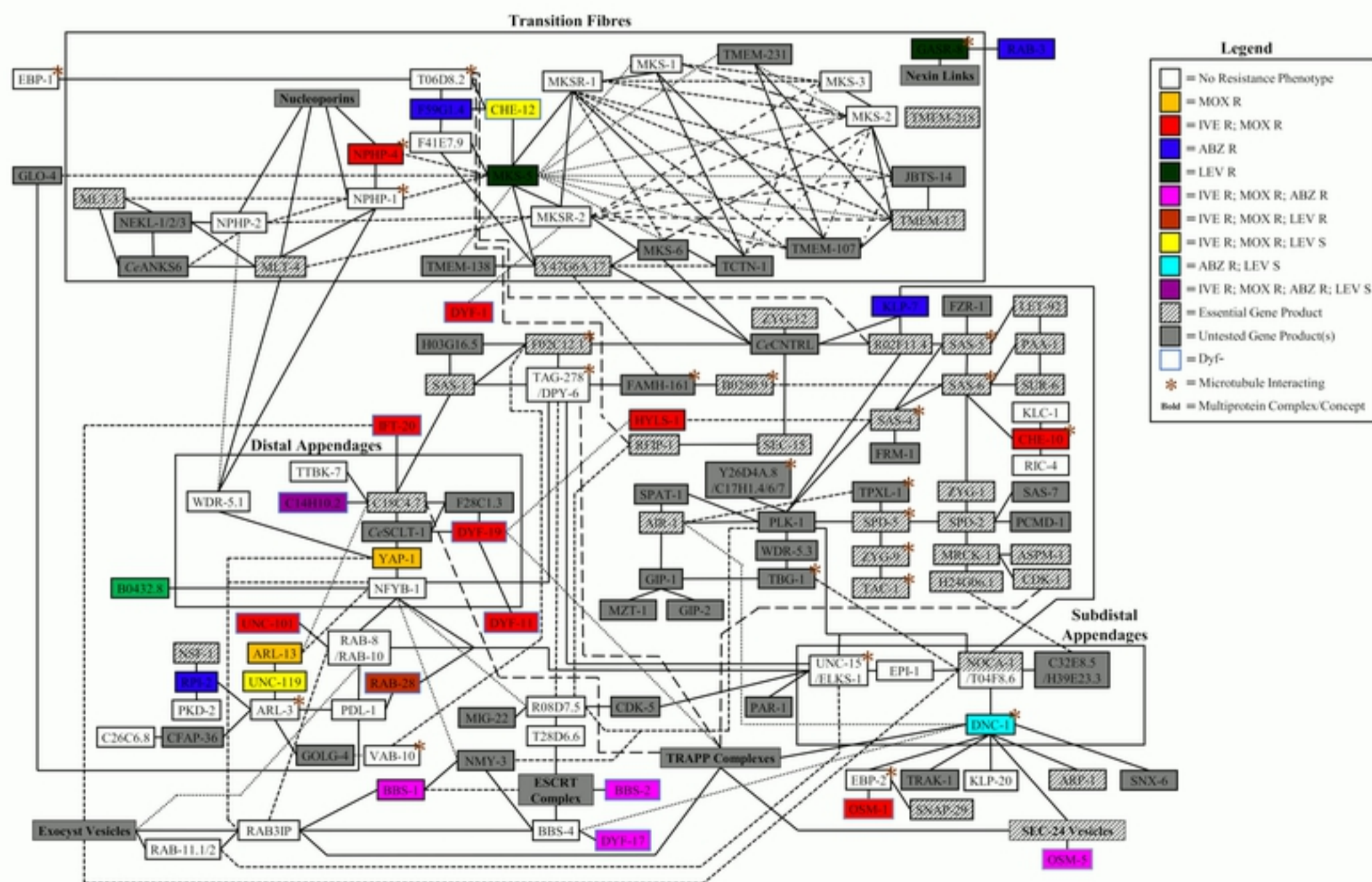


Figure 5