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Helminth genome analysis reveals conservation of extracellular vesicle biogenesis pathways but divergence of RNA loading machinery between phyla.

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1 Succinctus

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3 **Helminth genome analysis reveals conservation of extracellular vesicle**
4 **biogenesis pathways but divergence of RNA loading machinery between phyla**

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32 Note: Supplementary data associated with this article

33 **Abstract**

34 A comparative genomics approach was used to determine whether established extracellular vesicle
35 (EV) biogenesis pathways are conserved in helminths. This revealed conservation of membrane and
36 cytoskeletal-organising proteins as well as the endosomal sorting complex required for transport
37 (ESCRT) previously described in mammalian cells. Domain-level analysis of this complex in
38 helminths, however, indicated that some species may rely on atypical proteins to support subunit
39 interactions and cargo recruitment. Interestingly, helminths displayed phylum-level divergence of
40 proteins associated with loading RNA into EVs. These findings provide a framework for functional
41 studies of helminth EV biogenesis and cargo sorting.

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44 **Keywords:** extracellular vesicle, microvesicle, biogenesis, trematode, nematode, RNA

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46 The secretion of extracellular vesicles (EVs) by cells has now been reported across diverse
47 evolutionary lineages. Less is known, however, about the conservation of EV biogenesis pathways
48 which ultimately determine EV composition, cargo and function. EVs can be generated through an
49 endocytic pathway (exosomes) or bud directly from the plasma membrane (PM) as microvesicles
50 (MVs) (Supplementary Fig. S1A). The generation of both exosomes and MVs requires the
51 formation of membrane microdomains, sequestration of cargo molecules, bending of the lipid
52 bilayer, and finally, membrane scission to release the newly formed vesicle (van Niel et al., 2018).
53 The control of this process is well-described in mammalian systems where exosome formation
54 involves both endosomal complex required for transport (ESCRT)-dependent and ESCRT-
55 independent pathways (Supplementary Fig. S1A). These, along with integral membrane proteins
56 such as tetraspanins, drive the invagination of the endosome-limiting membrane, leading to the
57 formation of intraluminal vesicles (ILVs) within larger membrane-bound multivesicular bodies
58 (MVBs). Small Rab GTPases, together with soluble N-ethylmaleimide-sensitive factor activating
59 protein receptor (SNARE) proteins, promote fusion of the MVB-limiting membrane with the PM
60 and the release of the ILVs into the extracellular environment as exosomes (Hessvik and Llorente,
61 2018) (Supplementary Fig. S1A). The mechanisms governing MV production are less well-
62 understood, but MV formation can be driven by proteins involved in exosome biogenesis, as well as
63 additional proteins with roles in cytoskeletal and membrane restructuring which induce budding of
64 the PM (van Niel et al., 2018) (Supplementary Fig. S1A).

65 Helminth (h)EVs have recently been identified as major players in the host-parasite
66 interaction and provide a mechanism for the delivery of a range of parasite molecules (notably
67 proteins, lipids and nucleic acids) to host cells. Internalisation of hEVs has been shown to modify
68 host immune cell responses and thus contribute to parasite survival (Wu et al., 2018). Therefore,
69 blocking the secretion of hEVs represents a promising strategy for novel anthelmintics. However,
70 designing such interventions is currently hampered by our limited understanding of the machinery
71 controlling EV production in these pathogens.

72 Here, a comparative genomics approach was used to determine whether the pathways
73 described in mammalian systems are conserved in helminths, and thus could operate to produce
74 hEVs. Twenty-five helminth species for which genomic data was available on WormBase ParaSite
75 (parasite.wormbase.org, release WBPS7 (WS254)) were chosen as a representative sample of the
76 Nematoda and Platyhelminthes, with a focus on human pathogens (Supplementary Table S1).
77 Fifteen species were selected from Nematoda Clades I, III, IV and V as defined by Blaxter et al.
78 (1998) (or Clades 2A, 8B, 9A/B 10A/B and 12B as defined by van Megen et al. (2009)) and ten
79 species from the four classes of Platyhelminthes (Cestoda, Trematoda, Monogenea and
80 Rhabditophora). An extensive literature search identified 110 proteins known to function in EV

81 biogenesis and sequences of each isoform (retrieved from UniProt, www.uniprot.org) were used to
82 interrogate the selected genomes on WormBase Parasite using translated nucleotide BLAST
83 (tBLASTn) and protein BLAST (BLASTp) with standard settings. The protein sequence of each
84 primary BLAST hit underwent reciprocal tBLASTn and BLASTp on NCBI BLAST
85 (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) with the relevant query protein being required as the
86 primary hit. The putative parasite orthologues were then analysed for domain structure using
87 InterPro (www.ebi.ac.uk/interpro) with the requirement that at least one functional domain had to
88 match those of the query protein for the helminth protein to be accepted as a valid orthologue.

89 Twenty-five query proteins – notably Rab GTPases, tetraspanins and annexins – listed in
90 Supplementary Table S2 could not be assigned a direct orthologue due to the high level of sequence
91 identity between the various protein family members, so these were excluded from the study.
92 Additionally, 15 protein subfamilies were merged due to identical hits retrieved for each subfamily
93 member. The remaining 70 EV biogenesis proteins were grouped into members of the ESCRT-
94 dependent pathway (21 proteins, Fig. 1), the non-canonical ESCRT-associated pathway (5 proteins,
95 Supplementary Table S3), ESCRT-independent mechanisms (17 proteins, Supplementary Table
96 S4), proteins involved in MV formation (12 proteins, Supplementary Table S5), EV membrane
97 organisers (10 proteins, Supplementary Table S6), and proteins involved in RNA sorting into EVs
98 (5 proteins, Table 1). These proteins were corroborated with all available hEV proteomic data
99 which identified 55 of the 70 proteins (see Supplementary Tables S7-S12 and Fig. 2), supporting
100 their association with the EVs of some parasitic helminths.

101 The proteins which comprise the four subunits (ESCRT-0, I, II and III) of the ESCRT
102 machinery were well-conserved amongst helminths (Fig. 1), complementing earlier work showing
103 the retention of the ESCRT machinery across eukaryotic lineages (Leung et al., 2008). To produce
104 ILVs, the ESCRT subunits act in a sequential manner (Supplementary Fig. S1B) where ESCRT-0, -
105 I and -II sequester proteins onto microdomains on the endosome membrane, then ESCRT-III
106 (recruited through its protein component CHMP6) drives membrane invagination and membrane
107 fission (Christ et al., 2017). Fig. 1 shows that the Cestoda and *Brugia malayi* (a Clade III/8B
108 nematode) have the complete ESCRT machinery which is similarly highly conserved in the free-
109 living species examined (*Caenorhabditis elegans* and *Schmidtea mediterranea*), whilst this is most
110 incomplete in *Heterorhabditis bacteriophora* (a Clade V/9B nematode) and *Protopolystoma*
111 *xenopodis* (Monogenea). Amongst the platyhelminths, ESCRT-0, ESCRT-II and ESCRT auxiliary
112 proteins are almost fully conserved, whereas constituents of ESCRT-I and III are lacking in some
113 trematodes. Conversely, STAMPB (ESCRT-0) is only fully conserved in Clade III of the Nematoda
114 and few orthologues of VPS25 (ESCRT-II) were identified.

115 The interacting regions of the ESCRT-dependent pathway are the most well-defined in
116 terms of EV biogenesis (Christ et al., 2017). To ascertain whether the helminth proteins are likely to
117 retain the functional interactions of their mammalian counterparts, the presence of functionally
118 important domains (retrieved from InterPro) and interacting motifs (retrieved from UniProt) of
119 ESCRT proteins was assessed in helminth orthologues. The domain architecture of the helminth
120 orthologues was predicted using InterPro and compared to the domains of the human protein.
121 Sequence alignments of ESCRT proteins and helminth orthologues were produced using T-Coffee
122 (<https://www.ebi.ac.uk/Tools/msa/tcoffee/>) and manually inspected for interacting motifs. Whilst
123 some helminth orthologues retained the functional domains and motifs of mammalian ESCRT
124 proteins, in some cases these were absent (summarised in Fig. 1, Supplementary Table S13 and
125 Supplementary Fig. S2). Although ESCRT-0 is complete in platyhelminths, several species are
126 predicted to lack interacting motifs required for cargo recognition, as well as the FYVE domain of
127 HGS which targets the ESCRT machinery to the endosomal membrane. In ESCRT-I, the UMA
128 cargo-recognition domain of MVB12 is absent from most platyhelminth MVB12 orthologues and
129 Clade I nematodes lack the VPS36 GLUE domain of ESCRT-II, making these species reliant on
130 earlier ESCRT subunits for cargo recognition. Furthermore, there are predicted differences in the
131 intra-subunit interactions. For instance, *S. mediterranea* lacks the regions required for interactions
132 between HGS, STAM and STAMBP of ESCRT-0, and the regions that direct ESCRT-I formation
133 are absent from some parasitic trematodes.

134 Moreover, motifs required for inter-complex interactions are missing from some helminth
135 orthologues. *Necator americanus* (a Clade V/9B nematode) and *P. xenopodis* (Monogenea) have no
136 orthologue for TSG101 (ESCRT-I) and have also lost the TSG101-interacting motifs from ESCRT-
137 0. The few nematodes that retain STAMBP orthologues lack the MIT domain which binds ESCRT-
138 III subunits. In ESCRT-III, *N. americanus* is missing many of the CHMP protein interacting
139 regions, including those that interact with VPS4 which disassembles the ESCRT machinery. The
140 loss of MIM domains from ESCRT-III subunits in several helminths corresponds to the loss of the
141 MIM-interacting MIT domain in their orthologues of VPS4, although VPS4 itself is conserved. The
142 VPS4 orthologue in *Schistosoma haematobium*, however, lacks catalytic domains and this species is
143 one of the few that also lacks IST1 which regulates VPS4 localisation and assembly (Dimaano et
144 al., 2008). These results indicate that the functions of ESCRT-III may not be as conserved in
145 helminths as indicated by the initial BLAST searches.

146 In several eukaryotic lineages the early ESCRT subunits have been shown to be non-
147 essential for MVB formation (Leung et al., 2008). As the early ESCRT components involved in
148 cargo recognition were found to be the least conserved here, it is possible that these ESCRT
149 subunits also have a reduced importance in helminths. Additionally, Bro1-domain containing

150 proteins such as ALIX (fully conserved) or others found in trematode EV proteomics (Chaiyadet et
151 al., 2015; Cwiklinski et al., 2015) may compensate for the decreased number of ESCRT-I proteins
152 in some trematodes by directly recruiting ESCRT-III to its site of action (Christ et al., 2017). ALIX
153 can also recruit CHMP4 in the absence of CHMP6 (missing from some trematodes) to form ILVs
154 through a syndecan-syntenin-ALIX axis, relying on ESCRT components conserved in
155 platyhelminths (Fig. 1). Once released, these exosomes bear syndecan, syntenin and ALIX (Baietti
156 et al., 2012), all of which have been identified in platyhelminth EV proteomic data (Fig. 2), whilst
157 syntenin and syndecan are absent from nematode EVs (Supplementary Table S7). This suggests that
158 this non-canonical ESCRT-associated exosome biogenesis pathway could function in
159 platyhelminths but not in nematodes where syntenin is poorly conserved.

160 Independently of ESCRT, sphingomyelinases (SMases) generate ILVs by creating lipid
161 microdomains enriched in ceramide which induce membrane curvature (Verderio et al., 2018)
162 (Supplementary Fig. S1A). Furthermore, the conversion of ceramide to sphingosine-1-phosphate by
163 sphingosine kinase (SPHK) activates sphingosine-1-phosphate receptors 1 and 3 (S1PR1/3) that
164 promote MVB maturation and regulate cargo loading (Verderio et al., 2018). Whilst the genes
165 encoding orthologues of these proteins are again well-represented in helminths (Supplementary
166 Table S4), a commonality between the two phyla is the absence of S1PR1/3 and the neutral
167 (n)SMase SMPD3 which has been implicated in both exosome and MV formation (Verderio et al.,
168 2018). The acid (a)SMase SMPD1 is not conserved in platyhelminths and Clade I nematodes are
169 missing the aSMase-like proteins SMPDL3a/b and leukotriene A4 hydrolase (LKHA4). A
170 prominent difference exists between the two phyla in the conservation of lipid transport enzymes
171 which, whilst incomplete in nematodes, are fully conserved in platyhelminths (Supplementary
172 Table S4).

173 A reduced complement of lipid-related enzymes encoded by some helminth genomes could
174 be related to the reliance of parasitic helminths on host lipids due to an inability to synthesise some
175 lipids *de novo* (Frayha and Smyth, 1983). In this regard, it is noteworthy that the only platyhelminth
176 with an orthologue of PLA2 is the free-living *S. mediterranea*. It has been demonstrated that SMase
177 activity requires a neutral pH in some trematodes (Tallima et al., 2011) which is supported by the
178 absence of aSMase (SMPD1) and nSMase (SMPD3) orthologues in platyhelminths and suggests
179 that the conserved nSMase, SMPD2, could be a principal driver of sphingomyelin conversion in
180 these species. Whilst the related SMases SMPDL3a/b are conserved in this phylum and present in
181 *Fasciola hepatica* EVs (Supplementary Tables S4 and S9), direct orthologues of each of these
182 enzymes could not be assigned using the present methodology; therefore, the functional attributes of
183 these helminth enzymes remain to be determined.

184 Tetraspanins and flotillins are used as markers of EVs in mammalian systems and are
185 involved in the formation of membrane microdomains and EV cargo sorting (van Niel et al., 2018).
186 Only the tetraspanin CD63 antigen could be assigned a direct orthologue in this study and was
187 found to be almost universally conserved (Supplementary Fig. S6). Flotillin-1 and flotillin-2 are
188 also conserved in platyhelminths, but poorly represented in nematodes (Supplementary Fig. S6).
189 Notably, the lack of flotillins in hEV proteome data (Supplementary Fig. S11) is a distinguishing
190 feature of EVs released by these parasites. Given that flotillins promote the incorporation of
191 cholesterol into the EV membrane (Strauss et al., 2010), their poor conservation in nematodes could
192 be linked to the finding of a reduced cholesterol content in the EVs of *Heligmosomoides bakeri* (a
193 Clade V/9 nematode) compared to EVs derived from murine cells (Simbari et al., 2016). SNARE
194 proteins are well-conserved in helminths, bar VAMP proteins (Supplementary Fig. S6). VAMPs are
195 challenging to classify in helminths due to divergent sequences (Kloepper et al., 2008) and here a
196 clear orthologue of VAMP7 was found in only three of the sampled species (*Romanomermis*
197 *culicivoxax*, *Trichuris trichiura* and *S. mediterranea*).

198 Similar to the early stages of exosome formation, MV biogenesis involves the initial
199 formation of membrane microdomains which induce outward protrusion of the PM (van Niel et al.,
200 2018). Components of exosome biogenesis pathways also have roles in this process, and 18 of the
201 proteins highlighted in Fig. 1 and Supplementary Tables S3-S4 participate in MV formation. The
202 membrane and cytoskeletal rearrangements that lead to PM budding are additionally driven by
203 scramblases and Ca^{2+} -dependent proteases such as calpain following activation by increases in
204 cytosolic Ca^{2+} levels (van Niel et al., 2018). The MV is then released by further cytoskeletal
205 remodelling, regulated by small GTPases such as ARF6 and RhoA, which constricts the neck of the
206 budding vesicle (van Niel et al., 2018). Both helminth phyla have an almost complete repertoire of
207 proteins involved in MV formation (Supplementary Table S5). This supports the hypothesis that
208 MVs are released by helminths as membranous ‘blebs’ from their outer surface in a process
209 utilising the same cellular machinery as described in other metazoans (de la Torre-Escudero et al.,
210 2016). One poorly conserved protein is ARRDC1 which generates a specific subset of MVs in T
211 cells by recruiting ESCRT components to the PM (Nabhan et al., 2012). Its absence suggests that it
212 does not mediate ESCRT-dependent MV release in the examined helminths and raises questions as
213 to whether ESCRT-dependent MV formation can occur in these parasites. Additionally, the
214 cytoskeletal regulator DIAPH3 is absent from all but the Clade IV nematodes, *Echinococcus*
215 *multilocularis* (Cestoda) and the blood flukes, suggesting that it is not involved in MV formation in
216 most helminths.

217 Whilst the loading of cargo into EVs can occur through direct interactions with the EV
218 biogenesis machinery, further proteins have been implicated in the active sorting of RNA into

219 exosomes (Supplementary Fig. S1C). These include argonaute 2 (Ago2) and major vault protein
220 (MVP), as well as NSEP1 (also called Y-box protein 1), hnRNPQ (also called SYNCRIP) and
221 hnRNPA2B1 which select RNA from the cytoplasm by recognising discrete sequence motifs (see
222 Supplementary Table S12 for references). As hEVs carry RNA species capable of interfering with
223 immune regulator gene expression in host cells (Wu et al., 2018), the loading of RNA into EVs
224 released by these pathogens is of particular interest. Whilst hnRNPA2B1, NSEP1 and Ago2 are
225 well-conserved in helminths (Table 1), orthologues of hnRNPQ are present exclusively in the
226 Nematoda. However, most of these proteins have not been detected in hEV proteomic data
227 (Supplementary Table S12), suggesting either that these proteins are present in low abundance
228 making them difficult to detect, they are only packaged into hEVs in specific biological contexts (as
229 is the case in mammalian cells, such as for Ago2; Weaver and Patton, 2020), or that other proteins
230 can act as RNA chaperones in helminths. Nematodes have a lineage-specific expansion of
231 argonaute proteins (WAGOs) and it was recently reported that a WAGO protein mediated the
232 export of small interfering RNAs in *H. bakeri* EVs in a similar manner to argonaute proteins in
233 mammalian cells (Chow et al., 2019). As this WAGO is conserved in Clade V nematodes (Chow et
234 al., 2019), it could compensate for the absence of other RNA loading machinery in nematode EVs
235 or indicate that specialised RNA secretion pathways exist in certain helminths.

236 Another exosomal RNA-loading protein, MVP, is conserved solely in platyhelminths (Table
237 1) and consistently found in platyhelminth EVs (Supplementary Table S12). Along with TEP1 and
238 PARP4, this protein forms the vault complex and ambiguity exists over whether MVP is loaded into
239 EVs or secreted as part of the vault complex and co-isolated during ultracentrifugation due to its
240 large size (Jeppesen et al., 2019). However, it has been shown that *E. multilocularis* secretes MVP
241 in a signal peptide-independent manner (Goto et al., 2013) and, given its role in exosomal RNA-
242 loading in mammalian systems (see Supplementary Table S12 for references), it is possible that
243 MVP carries out a similar role during platyhelminth EV production. Interestingly, when the current
244 search was expanded to include the other vault complex components, these were found to be absent
245 in nematodes and only TEP1 was conserved in platyhelminths (which has also been identified in
246 hEV proteomic data; Supplementary Table S12). TEP1 specifically binds vault RNA (vtRNA)
247 which is found enriched in mammalian EVs (Mateescu et al., 2017) and can downregulate target
248 gene expression in a manner similar to miRNA (Persson et al., 2009). It is unknown whether hEVs
249 are also enriched in vtRNA, but it is possible that TEP1 is also involved in shuttling non-coding
250 RNA into the EVs of some platyhelminth species. Indeed, the EV field is rapidly evolving and, with
251 new discoveries, the number of proteins implicated in EV biogenesis, and RNA loading, will
252 continue to expand beyond those listed in this study.

253 The helminth species analysed here were restricted to the genomes available at the time of
254 analysis. Consequently, this study involved genomes of varying qualities and the absence of some
255 orthologues (or the lack of domains/interacting regions) in some species may be due to incomplete
256 genome assembly. Nevertheless, it was found that conservation of EV biogenesis pathways in
257 helminths broadly follows an evolutionary route rather than being dependent on the environmental
258 niches of individual species. Furthermore, the presence of these pathways in species where EV
259 release has not yet been reported may indicate that they also secrete EVs. Whilst the conservation of
260 EV biogenesis machinery in helminths could be explained by their essential functions in other
261 cellular processes, such as that of ESCRT in cell division (Christ et al., 2017) and SMases in
262 membrane lesion removal (Andrews et al., 2014), these proteins are also crucial drivers of EV
263 biogenesis (see Supplementary Tables S7-S12 for references) and their presence in hEV proteomic
264 data (Fig. 2) supports their association with hEVs. Comparing the proteomes of platyhelminth and
265 nematode EVs, however, reveals a stark difference between the phyla: whilst 55 EV biogenesis
266 proteins were found in EVs released by platyhelminths, only 7 orthologues were present in
267 nematode EVs (Fig. 2; Supplementary Tables S7-S12). There may be both technical and biological
268 reasons for this difference. To date, 17 platyhelminth EV proteomic data sets have been published
269 as opposed to nine from nematodes, and most nematode EV studies have used isolation procedures
270 that focus on small EVs (Supplementary Tables S7-S12), perhaps leading to different protein
271 profiles. Alternatively, nematode EV biogenesis proteins may be retained by the vesicle-producing
272 cell, or other proteins detected in hEVs may provide an alternative mechanistic framework for EV
273 production in helminths and compensate for EV biogenesis pathway deficits identified in this study.
274 Determining the expression patterns of these proteins, in combination with functional studies, could
275 reveal which helminth proteins are the key drivers of EV biogenesis and identify targets to block
276 EV production for novel anti-parasitic therapies.

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360

361 **Legends to Figures**

362

363 **Fig. 1.** Proteins of the ESCRT-dependent exosome biogenesis pathway in mammalian cells. ‘*’
364 indicates proteins also implicated in microvesicle formation. The presence (coloured square) or
365 absence (white square) of an orthologue in the helminth genome is indicated. The colour of the
366 square represents the degree of conservation of the interacting regions relevant to extracellular
367 vesicle biogenesis (listed in Supplementary Table S13) in the predicted protein sequence of the
368 helminth orthologue. A blue square signifies that all of the identified interacting regions are
369 conserved; an orange square signifies that over half of the identified interacting regions are
370 conserved; a red square signifies that less than half of the identified interacting regions are
371 conserved. Supplementary Table S3 lists query protein identifiers. Supplementary Tables S7-S13
372 contain the references for the EV biogenesis proteins and interacting regions identified in Fig. 1,
373 Table 1 and Supplementary Tables S3-S6.

374

375 **Fig. 2.** Proteins found associated with platyhelminth (A) or nematode (B) extracellular vesicles
376 (EVs) by mass spectrometry that have been implicated in EV biogenesis in mammalian systems.
377 Proteins marked with an asterisk (*) have been implicated in both exosome and microvesicle (MV)
378 biogenesis. See Supplementary Tables S7-S12 for details of the EV biogenesis protein orthologues
379 found in helminth EVs listed by species, with corresponding references.

380

381 **Legends to Supplementary Figures**

382

383 **Supplementary Fig. S1.** Mechanisms of extracellular vesicle biogenesis and cargo loading (based
384 on studies in mammalian systems). (A) Intraluminal vesicle formation is driven by processes
385 dependent or independent of the endosomal sorting complex required for transport (ESCRT) which
386 generates multivesicular bodies (MVB). Trafficking of MVBs to the cell surface involves small Rab
387 GTPases, whilst SNARE proteins facilitate MVB fusion with the plasma membrane (PM) to release
388 exosomes. In contrast, microvesicles (MV) bud directly from the PM in a process driven by a
389 combination of cytoskeletal restructuring and PM modifications. (B) ESCRT proteins can drive
390 intraluminal vesicle (ILV) biogenesis in a canonical manner where the early ESCRT subunits
391 (ESCRT-0, -I, -II) sequester cargo and recruit ESCRT-III which forms coils and constricts the neck
392 of the budding ILV. Alternatively, the complex of syndecan and syntenin interact with ESCRT
393 components through the ESCRT accessory protein ALIX to regulate the biogenesis of syndecan-
394 syntenin-ALIX containing exosomes. (C) RNA species are packaged into budding intraluminal
395 vesicles by RNA binding proteins. Ago2, Argonaute 2; NSEP1, nuclease-sensitive element-binding

396 protein 1; MVP, major vault protein; A2B1, heterogenous nuclear ribonucleoprotein A2B1;
397 hnRNPQ, heterogenous nuclear ribonucleoprotein Q.

398

399 **Supplementary Fig. S2.** Cartoon representations of ESCRT pathway proteins showing that some
400 helminth species lack interacting regions found in their human counterparts. (A) *S. Stercoralis*
401 MVB12 is missing the UMA domain of human MVB12A/B and the SH3-binding motif of human
402 MVB12A which are involved in cargo recognition and interactions with other ESCRT proteins. (B)
403 Whilst *S. carpocapsae* ALIX retains the domain architecture of the human protein, it lacks the
404 TSG101-interacting motif PSAP. Domain architectures were generated using Interpro
405 (<https://www.ebi.ac.uk/interpro/>).

406

407 Table 1. Proteins involved in RNA sorting and transport in mammalian cells. ‘X’ indicates the
 408 presence of an orthologue in the helminth genome.
 409

		RNA sorting and transport				410
Nematoda						411
		hnRNP A2B1	hnRNP Q	NSEP1	Ago2	412
						413
Clade	Species					414
I/2A	<i>Romanomermis culicivorax</i>	X	X	X	X	415
I/2A	<i>Trichinella spiralis</i>	X	X	X	X	416
I/2A	<i>Trichuris trichiura</i>	X	X	X	X	417
III/8B	<i>Ascaris lumbricoides</i>	X	X	X	X	418
III/8B	<i>Enterobius vermicularis</i>	X	X	X	X	419
III/8B	<i>Dracunculus medinensis</i>	X	X	X	X	420
III/8B	<i>Brugia malayi</i>	X	X	X	X	421
III/8B	<i>Onchocerca volvulus</i>	X	X	X	X	422
IV/10A	<i>Steinernema carpocapsae</i>	X	X	X	X	423
IV/10B	<i>Strongyloides stercoralis</i>	X	X	X	X	424
IV/10B	<i>Bursaphelenchus xylophilus</i>	X	X	X	X	425
IV/12B	<i>Meloidogyne incognita</i>	X	X	X	X	426
V/9A	<i>Caenorhabditis elegans</i>	X	X	X	X	427
V/9B	<i>Heterorhabditis bacteriophora</i>	X				428
V/9B	<i>Necator americanus</i>	X	X	X	X	429
Platyhelminthes						430
Class	Species					431
Cestoda	<i>Echinococcus multilocularis</i>	X		X	X	432
Cestoda	<i>Taenia solium</i>	X		X	X	433
Trematoda	<i>Clonorchis sinensis</i>	X		X	X	434
Trematoda	<i>Opisthorchis viverrini</i>	X		X	X	435
Trematoda	<i>Echinostoma caproni</i>	X		X	X	436
Trematoda	<i>Fasciola hepatica</i>	X		X	X	437
Trematoda	<i>Schistosoma haematobium</i>	X		X	X	438
Trematoda	<i>Schistosoma mansoni</i>	X		X	X	439
Monogenea	<i>Protopolystoma xenopodis</i>	X		X	X	440
Rhabditophora	<i>Schmidtea mediterranea</i>	X		X	X	441
						442

444 **Highlights:**

445

446 • Canonical extracellular vesicle biogenesis pathways are conserved in helminths

447 • However, ESCRT proteins may operate in a non-canonical manner

448 • Helminths displayed phylum-level divergence of EV RNA-loading machinery

449

450

451 **Figure 1**

452

453

454

(A) Platyhelminth

(B) Nematode