

1 **Differential regulation of degradation and immune pathways underlies**
2 **adaptation of the ectosymbiotic nematode *Laxus oneistus* to oxic-anoxic**
3 **interfaces**

4

5 Running title: Transcriptomics and proteomics of an ectosymbiotic marine nematode

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21

22 **ABSTRACT**

23 Eukaryotes may experience oxygen deprivation under both physiological and
24 pathological conditions. Because oxygen shortage leads to a reduction in cellular energy
25 production, all eukaryotes studied so far conserve energy by suppressing their metabolism.
26 However, the molecular physiology of animals that naturally and repeatedly experience anoxia
27 is underexplored. One such animal is the marine nematode *Laxus oneistus*. It thrives,
28 invariably coated by its sulfur-oxidizing symbiont *Candidatus Thiosymbion oneisti*, in anoxic
29 sulfidic or hypoxic sand. Here, transcriptomics and proteomics showed that, whether in anoxia
30 or not, *L. oneistus* mostly expressed genes involved in ubiquitination, energy generation,
31 oxidative stress response, immune response, development, and translation. Importantly,
32 ubiquitination genes were also upregulated when the nematode was subjected to anoxic
33 sulfidic conditions, together with genes involved in autophagy, detoxification and ribosome
34 biogenesis. We hypothesize that these degradation pathways were induced to recycle
35 damaged cellular components (mitochondria) and misfolded proteins into nutrients.
36 Remarkably, when *L. oneistus* was subjected to anoxic sulfidic conditions, lectin and mucin
37 genes were also upregulated, potentially to promote the attachment of its thiotrophic symbiont.
38 Furthermore, the nematode appeared to survive oxygen deprivation by using an alternative
39 electron carrier (rhodoquinone) and acceptor (fumarate), to rewire the electron transfer chain.
40 On the other hand, under hypoxia, genes involved in costly processes (e.g., amino acid
41 biosynthesis, development, feeding, mating) were upregulated, together with the worm's Toll-
42 like innate immunity pathway and several immune effectors (e.g., Bacterial Permeability
43 Increasing proteins, fungicides).

44 In conclusion, we hypothesize that, in anoxic sulfidic sand, *L. oneistus* upregulates
45 degradation processes, rewires oxidative phosphorylation and by reinforces its coat of
46 bacterial sulfur-oxidizers. In upper sand layers, instead, it appears to produce broad-range
47 antimicrobials and to exploit oxygen for biosynthesis and development.

48 **INTRODUCTION**

49 Fluctuations that lead to a decrease in oxygen availability are common in nature
50 (Hermes-Lima and Zenteno-Savin, 2002). The physiological and behavioral response to
51 oxygen deprivation has been studied in animals that naturally experience oxygen deprivation,
52 such as frogs, goldfish, and turtles (Hochachka et al., 1996; 1997, 2001; Hermes-Lima and
53 Zenteno-Savin, 2002), as well as in invertebrate genetic models (Clegg 1997; Nystul et al.,
54 2003; Teodoro and O'Farrell, 2003; Haddad 2006). When oxygen deprived, these organisms
55 must face the challenge of a drastic drop in ATP (the energy-storing metabolite adenosine
56 triphosphate) production, which leads to the failure of energy-demanding processes that are
57 crucial for maintaining cellular homeostasis. Anoxia-tolerant organisms, however, are capable

58 to save energy by stopping energy-costly cellular functions (e.g., protein synthesis, ion
59 pumping, cell cycle progression), maintain stable and low permeability of membranes, and
60 produce ATP by anaerobic glycolysis (Hochachka et al., 1996; Teodoro and O'Farrell, 2003;
61 Liu & Simon, 2004; Liu et al., 2006; Galli et al., 2014).

62 When parasitic and free-living nematodes, including the model organism
63 *Caenorhabditis elegans*, are experimentally exposed to anoxia (<0.001 kPa O₂), the
64 intracellular ATP/ADP ratio drops dramatically and, within 10 h, they enter a state of reversible
65 metabolic arrest called *suspended animation*. Namely, they stop to eat, move, develop or lay
66 eggs, implying that oxygen deprivation affects their growth and behavior (Van Voorhies et al.,
67 2000; Padilla et al., 2002; Nystul and Roth, 2004; Powell-Coffmann 2010; Fawcett et al., 2015;
68 Kitazume et al., 2018). If these effects can be reversed upon oxygen reestablishment, the
69 latter can also provoke a massive and sudden production of reactive oxygen species (ROS)
70 that may overwhelm the organism's antioxidant defense, and cause its death (reviewed in
71 Hermes-Lima and Zenteno-Savin, 2002). Of note, an increase of mitochondrial ROS
72 production was also observed in worms under hypoxia, because of the inefficient transfer of
73 electrons to molecular oxygen (Nystul and Roth, 2004; Kim and Jin, 2015).

74 Because oxygen diffuses slowly through aqueous solutions, sharp concentration
75 gradients of this electron acceptor may occur in marine environments and wet soil (Denny et
76 al., 1993; Fawcett et al., 2015). It is at oxic-anoxic interfaces of marine sands that free-living
77 nematodes coated with sulfur-oxidizing Gammaproteobacteria (Stilbonematinae) abound (Ott
78 et al., 1989, 1991; Schiemer et al., 1990; Paredes et al., 2021). However, up to this study, the
79 molecular mechanisms allowing symbiotic nematodes to withstand anoxia, and the inherent
80 stress it is known to inflict upon metazoans, were unknown. Here, we incubated *Laxus*
81 *oneistus* (Ott et al., 1995) in conditions resembling those it encounters in its natural
82 environment (i.e. anoxic sulfidic or hypoxic), and applied comparative transcriptomics,
83 proteomics and lipidomics, to understand how it copes with oxygen deprivation. Contrarily to
84 our expectations, in anoxic sulfidic water *Laxus oneistus* did not appear to enter suspended
85 animation. However, it upregulated genes required for ribosome biogenesis and energy
86 generation, and for degradation pathways (e.g., ubiquitination-proteasome systems,
87 autophagy) likely involved in recycling damaged cellular components and misfolded proteins
88 into nutrients. Notably, under anoxic sulfidic conditions, it also upregulated putative symbiont-
89 binding molecules such as lectins. In the presence of oxygen, on the other hand, the worm
90 appeared to overexpress genes involved in energy-demanding processes (e.g., amino acid
91 synthesis, development, feeding, and mating) and upregulated the synthesis of broad-range
92 antimicrobials, likely via triggering the Toll/NF- κ B pathway.

93

94 **RESULTS AND DISCUSSION**

95 **The nematode *Laxus oneistus* did not enter suspended animation upon 24 h anoxia**

96 To survive anoxia, nematodes enter suspended animation to suppress metabolism and
97 conserve energy. The most notorious sign of suspended animation is the arrest of motility
98 (Nystul et al., 2003; Chan et al., 2010; Kitazume et al., 2018).

99 Surprisingly, although the whole population of four tested nematode species, including
100 *C. elegans*, was reported to be in suspended animation upon 10 h in anoxia (Kitazume et al.,
101 2018), *L. oneistus* kept moving not only after 24-h-long incubations, but also upon 6-day-long
102 incubations in anoxic seawater (three batches of 50 worms were incubated under each
103 condition). Additionally, the symbiotic nematodes appeared morphologically normal
104 ([Supplemental movies 1-4](#)).

105 The fact that we could not observed suspended animation, led us to hypothesize that *L.*
106 *oneistus* evolved different strategies to survive oxygen deprivation.

107

108 **Stable transcriptional profile under hypoxic or anoxic sulfidic conditions**

109 To understand the molecular mechanisms underlying *L. oneistus* response to oxygen,
110 we subjected it to various oxygen concentrations. Namely, nematode batches were incubated
111 under either normoxic (100% air saturation; O), hypoxic (30% air saturation; H) or anoxic (0%
112 air saturation; A) conditions for 24 h. Additionally, given that *L. oneistus* thrives in reduced
113 sand containing up to 25 μ M sulfide (Ott and Novak., 1989; Paredes et al., 2021), we also
114 incubated it in anoxic seawater supplemented with < 25 μ M sulfide (anoxic sulfidic condition;
115 AS).

116 While transcriptional differences of its symbiont (*Candidatus Thiosymbion oneisti*),
117 incubated under normoxic (O) and hypoxic (H) conditions were negligible (Paredes et al.,
118 2021), the expression profiles of nematode batches incubated under O conditions varied so
119 much that they did not cluster ([Figure S1](#)). Consequently, there was no detectable differential
120 expression between the transcriptomes of O nematodes and any of the other transcriptomes
121 (H, A or AS; [Figure S1B, C](#)). We attribute the erratic transcriptional response of *L. oneistus* to
122 normoxia to the fact that this concentration is not typically experienced by *L. oneistus* (Ott et
123 al., 1989; Paredes et al., 2021).

124 As for the expression profiles of nematodes subjected to the H, A or AS conditions,
125 replicates of each condition behaved more congruently ([Figure S1B](#)). While we did not find any
126 significant difference between the A and AS nematodes, only 0.05% of the genes (8 genes;
127 [Data S1](#)) were differentially expressed between the H and A nematodes and there was no
128 significant difference between the H and A proteomes (t-test, FDR, Benjamini-Hochberg
129 correction, $p < 0.05$; [Figure S2A, Data S1](#)). However, 4.8% of the expressed genes (787 out of
130 16,526) were differentially expressed between H and AS nematodes, with 434 upregulated
131 under AS and 353 genes upregulated under H conditions ([Figure S1C, Data S1](#)).

132 Collectively, our data suggests that *L. oneistus* may be ill-equipped to handle normoxic
133 sediment, but it maintains a largely stable physiological profile under both hypoxic and anoxic
134 sulfidic conditions. Before discussing the subset of biological processes differentially
135 upregulated in AS versus H nematodes and vice versa, we will present the physiological
136 processes the worm appears to mostly engage with, irrespectively of the environmental
137 conditions we experimentally subjected it to.

138

139 **Top-expressed transcripts under all tested conditions**

140 To gain insights on *L. oneistus* basal physiology, we treated all the 16 transcriptomes
141 as biological replicates (i.e., O, H, A and AS transcriptomes were pooled) and identified the
142 100 most abundant transcripts out of 16,526 based on functional categories extracted from the
143 UniProt database (2021) and comprehensive literature search (Figure 1, Data S2). Our manual
144 classification was supported by automatic eggNOG classification (Data S2). Similarly, the H
145 and A proteomes were pooled, and the 100 most abundant proteins out of 2,626 were
146 detected (Figure S2).

147 Based on median gene expression values of the top 100 expressed genes, we found
148 that some of the processes *L. oneistus* mostly engages with were ubiquitination (*ubq-1*,
149 Stringham et al., 1992), energy generation (globin *glb-1*-like (Geuens et al., 2010), cytochrome
150 c oxidase I subunit *ctc-1* (UniProtKB P24893), *nduo-4*-like (UniProtKB P24892), stress
151 response and detoxification (e.g., *hsp-1*, *hsp-90*, *hsp12.2*, and catalases *ctl-1* and *ctl-2*; Birnby
152 et al., 2000; Chávez et al., 2007), and immune defense (lysozyme-like proteins and *lec-3*)
153 (Figure 1, Data S2).

154 Lastly, 48 out of the top 100 most expressed genes, were also detected among the top
155 100 proteins (Figure 1, Figure S2, and Data S2, Supplemental material). Despite the modest
156 correlation between transcript and protein expression levels ($r = 0.4$) (Figure S3), there was an
157 overlap in the detected biological processes (e.g., energy generation, stress response or
158 detoxification categories, carbohydrate metabolism, cytoskeleton, locomotion, nervous system)
159 (Figure S2).

160 All in all, except for those encoding for immune effectors, top-transcribed *L. oneistus*
161 genes could not be ascribed to its symbiotic lifestyle. This differs to what observed for other
162 chemosynthetic hosts, such as giant tubeworms and clams. Indeed, it is perhaps because
163 these animals acquire their symbionts horizontally and feed on them as they are housed in
164 their cells (and not on their surface) that they were found to abundantly express genes
165 involved in symbiont acquisition, proliferation control and digestion (Sun et al., 2017, Hinzke et
166 al., 2019; Yuen et al., 2019). Notably, we did observe a partial overlap of the most expressed
167 gene categories (e.g., oxidative stress, energy generation, immune response), when *L.*
168 *oneistus* was compared to the marine gutless annelid *Olavius algarvensis*. We ascribe the

169 overlap to the fact that, albeit endosymbiotic, *O. algarvensis* also inhabits shallow water sand
170 (Figure S4, Supplemental material) and, as hypothesized for *L. oneistus*, it may also acquire its
171 symbionts vertically (Woyke et al., 2006; Dubilier et al., 2008; Wippler et al., 2016;
172 Zimmermann et al., 2016).

173 To conclude, although both symbiont- (Paredes et al., 2021) and host-transcriptomics
174 do not suggest a high degree of inter-partner metabolic dependence in the *L. oneistus*
175 ectosymbiosis, the nematode seems well-adapted to both anoxic sulfidic (AS) and hypoxic (H)
176 sand (Figure 2, Data S1). The transcriptional response of the worm to these two conditions is,
177 however, significant (Figure 2, Data S1), and it will be reported next.

178

179 **Genes upregulated in anoxic sulfidic (AS) nematodes**

180 **Chaperones and detoxification.** The expression of chaperone-encoding (e.g.,
181 *hsp12.2*, *grpE*, *dnaJ/dnj-2*, *pfd-1*, *pfd-6*; Naylor et al., 1996; Lundin et al., 2008; Bar-Lavan et
182 al., 2016), and ROS-detoxifying-related genes (e.g., superoxide dismutase *sod-2* and a
183 putative glutathione peroxidase, involved in the detoxification of superoxide dismutase and
184 hydrogen peroxide, respectively; Suzuki et al., 1996; Margis et al., 2008) were higher in AS
185 nematodes (Figures 2 and 3). Notably, transcripts encoding for the heme-binding cytochrome
186 P450 *cyp-13B1* were also more abundant in AS (Figure 3), perhaps to increase the worm's
187 capacity to cope with putative ROS formation (Oliveira et al., 2009). Indeed, as cells start being
188 oxygen-depleted, mitochondrial ROS accumulate because of the inefficient transfer of
189 electrons to molecular oxygen (Semenza, 1999; Nystul and Roth, 2004; Selivanov et al., 2009;
190 Kim and Jin, 2015). Alternatively, the upregulation of antioxidant-related genes in AS worms
191 could represent an anticipation response to an imminent reoxygenation. In animals alternating
192 between anoxic and oxygenated habitats, the re-exposure to oxygen can be very dangerous,
193 as it creates a sudden ROS overproduction that may overwhelm the organism's oxidative
194 defense mechanisms (Hermes-Lima and Zenteno-Savin, 2002; Hashimoto et al., 2004).
195 Although it has not been reported for nematodes, overexpression of ROS-counteracting genes
196 is consistent with what has been reported for vertebrates and marine gastropods which, just
197 like *L. oneistus*, alternate between oxygen-depletion and reoxygenation (Hermes-Lima and
198 Zenteno-Savin, 2002).

199 **Mitochondrial and cytoplasmic ribosome biogenesis.** In the cellular stress imposed
200 by oxygen deprivation, mitochondria are central to both death and survival (Borutaite et al.,
201 1995; Brookes et al., 2004; Brenner et al., 2012; Hawrysh et al., 2013; Galli et al., 2014). In
202 this scenario, calcium regulation, the scavenging of ROS or the suppression of their
203 production, and/or inhibition of the mitochondrial permeability transition pore (MPTP) opening,
204 might help to preserve mitochondrial function and integrity (Horwitz et al., 1994; Murphy et al.,
205 2008; Galli et al., 2014; Fanter et al., 2020). In addition, removal of specific mitochondrial

206 components (mitochondrial-associated protein degradation, MAD), might also arise to maintain
207 the overall mitochondrial homeostasis (Chatenay-Lapointe and Shadel, 2010; Heo et al.,
208 2010). Perhaps as a response to anoxia-induced stress (reviewed in Galli et al., 2014), a gene
209 involved in MAD (*vms-1*) (Chatenay-Lapointe and Shadel, 2010; Heo et al., 2010), was
210 upregulated in AS worms (Figure 4). More abundant in this condition were also transcripts
211 encoding for mitochondrial transmembrane transporters *tin-44*, *slc-25A26* and *C16C10.1*
212 (UniProtKB O02161, Q18934, Q09461), putatively transporting, peptide-containing proteins
213 from the inner membrane into the mitochondrial matrix, such as S-Adenosyl Methionine (Figure
214 6). Surprisingly, although the translation elongation factor *eef-1A.2* (Tullet, 2015) was
215 downregulated in AS worms, not only various mitochondrial ribosome structural components
216 (28S: *mrps*, 39S: *mrpl*; Kaushal et al., 2014), and mitochondrial translation-related genes (e.g.,
217 *C24D10.6* and *W03F8.3*; Sharika et al., 2018) were upregulated in AS nematodes, but also
218 several cytoplasmic ribosome biogenesis (40S: *rps*, 60S: *rpl*; Melnikov et al., 2012) and subunit
219 assembly genes (e.g., RRP7A-like, You et al., 2015) (Figure 4).

220 Taken together, the maintenance of mitochondrial homeostasis, an anticipatory
221 response to a potential upcoming ROS insult (see Chaperones and detoxification section)
222 and/or their involvement in extra-ribosomal functions (Chen et al., 2010; Savada et al., 2014;
223 Xu et al., 2016) might explain the upregulation of ribosomal biogenesis-related genes in AS
224 nematodes. Although upregulation of ribosomal proteins has also been observed in anoxic
225 gastropods (Larade et al., 2001), increased ribosomal biogenesis (which oftentimes directly
226 correlates with an increase of protein synthesis) is not expected in animals that must repress
227 their metabolism to cope with oxygen deprivation (Thomas et al., 2000; Hochachka and Lutz
228 2001; Shukla et al., 2012).

229 **Energy generation.** Equally surprising was the upregulation of all differentially
230 expressed genes related to energy generation in AS nematodes (Figure 4). Namely, besides
231 putative oxygen-binding globulin-like genes (e.g., *glb-1*, *glb-14*, Geuens et al., 2010), the
232 following were upregulated in AS nematodes: key structural genes (e.g., *atp-3*, *atp-5*, Xu et al.,
233 2018), assembly-related genes (H⁺-transport ATP synthase, Maglioni et al., 2016) of the
234 mitochondrial ATP synthase (complex V), genes related to complex I (*lpd-5*, *nuo-2*, McKay et
235 al., 2003; Rea et al., 2007), a subunit of the succinate dehydrogenase involved in complex II
236 (*mev-1*, Hartman et al., 2001), a mitochondrial cytochrome C oxidase subunit II assembly gene
237 related to complex IV (*sco-1*, Williams et al., 2005), and a mitochondrial gene (*coq-5*), involved
238 in the synthesis of either ubiquinone (Q, aerobic) or rholoquinone (RQ, anaerobic) electron
239 carriers (Buceta et al., 2019) (Figure 4). This suggests that, under anoxia, the electron transfer
240 chain (ETC) is rewired in such way that electrons still enter the ETC at complex I, but instead
241 of reaching complex III and IV they are transferred to RQ. This, in turn, shuttles the electrons to
242 succinate dehydrogenase. The latter enzyme uses fumarate as an alternative electron

243 acceptor, reducing it to succinate. This mechanism would maintain the flow of electrons
244 through the ETC, and, it would prevent mitochondrial ATP generation (complex V) from
245 shutting down (Buceta et al., 2019; Del Borrello et al., 2019).

246 In short, under AS, similarly to what has been observed in other free-living and parasitic
247 nematodes, complex I appears to be the sole proton pump in this truncated form of ETC
248 (Buceta et al., 2019; Del Borrello et al., 2019). In accordance with this hypothesis, tryptophan
249 (Trp) degradation-related genes (*acsd-1*, *acsd-2*) and the Trp RNA ligase (*wars-1*; Tsai et al.,
250 2017) that might be required to synthesize RQ (Buceta et al., 2019; Del Borrello et al., 2017;
251 Tan et al., 2020) were upregulated under AS. Intriguingly, upregulated was also an isocitrate
252 dehydrogenase gene (*idh-1*). This produces reducing equivalent (NADPH) carrying electrons
253 that may fuel complex I (Smolková et al., 2012; Martínez-Reyes et al., 2020), but it might also
254 add to the stimulation of the antioxidant capacity or to the maintenance of redox homeostasis
255 by regenerating reduced glutathione (Hermes-Lima and Zenteno-Savin, 2002; Penkov et al.,
256 2015; Yang et al., 2019).

257 If glycolysis is a key process for ATP generation in anoxia (Lutz et al., 1997; Semenza
258 et al., 2001; Hochachka et al., 2001; Huang et al., 2008; Larade et al., 2009) and if,
259 consistently, *hvk-2* was upregulated under this condition (Figure 6), based on the expression
260 levels of transcripts encoding for alpha-amylases (see Carbohydrate metabolism in Figure 6),
261 starch and/or glycogen (Jackson and McLaughlin, 2009) may be the prominent carbon sources
262 under anoxic sulfidic conditions.

263 **Ubiquitin-proteasome system and proteases.** Proteolysis supplies amino acids or
264 polypeptides to the cells, while impeding the accumulation of damaged or misfolded proteins.
265 The two main mechanisms of cellular proteolysis are the lysosome-mediated intracellular
266 protein degradation (autophagy) and the proteasome-mediated protein degradation (ubiquitin-
267 proteasome system, UPS). In the latter, ubiquitin-protein ligases covalently attach ubiquitin to
268 proteins, allowing their recognition and further degradation by the proteasome (Lodish et al.,
269 2008; Papaevgeniou and Chondrogianni, 2014).

270 As shown in Figure 1, transcripts encoding for polyubiquitin (*ubq-1*), had the highest
271 median gene expression across all transcriptomes. However, all ubiquitination-related genes
272 detected in the differential gene expression analysis between the AS and H conditions, were
273 upregulated in AS worms (Figure 2 and 3, Data S1). For example, *aos-1*, encoding for a
274 subunit of the ubiquitin-activating enzyme (E1) (Jones et al., 2001), two ubiquitin-protein
275 ligases (E3s without detected cullin domains; Papaevgeniou and Chondrogianni, 2014), and
276 kelch-like genes (e.g., *kel-8*-like and *kel-20*). The former are BTB-domain containing proteins
277 known to interact with E3 enzymes, with *kel-8* being involved in the degradation of glutamate
278 neuroreceptors (Schaefer and Rongo 2006; Stogios et al., 2005; Kim et al., 2018). Additional
279 ubiquitination-related genes upregulated in AS were *csn-2*, encoding for a component of the

280 COP9 signalosome complex (Pintard et al., 2003; Brockway et al., 2014), and proteasome
281 genes (*pas-2* and *pas-3*; Fraser et al., 2000; Blumenthal et al., 2002).

282 Among the proteases that were upregulated in AS worms, aspartyl proteases have
283 been involved in neurodegeneration (Syntichaki et al., 2002), whereas plasminogen and the
284 zinc matrix metalloproteinase ZMP-2 were both reported to mediate degradation of
285 extracellular matrix (ECM) (Vassalli et al., 1991; Altincicek et al., 2010; Fischer, et al., 2014)
286 (Figure 3). *C. elegans* ZMP-2 was also shown to prevent the accumulation of oxidized
287 lipoproteins (Fischer et al., 2014), and, therefore it may contribute to the enhanced antioxidant
288 response observed in this condition.

289 **Autophagy and amino acid degradation.** Besides acting coordinately to withstand
290 stress, autophagy cooperates with apoptotic UPS for the recovery and supply of nutrients
291 when these are scarce (Vabulas et al., 2005; Scott et al., 2004; Huber and Teis, 2016;
292 reviewed in Wang RC et al., 2010 and Russel et al., 2014). Transcripts of two autophagy-
293 related genes, *bec-1* (Liang et al., 1999) and the Ragulator complex protein LAMTOR4
294 (C7orf59-like) (Bar-Peled et al., 2012) were more abundant in AS nematodes (Figure 3). While
295 the former positively regulates autophagy (Liang et al., 1999; Meléndez et al., 2003), the latter
296 interacts with the mTOR Complex I (mTORC1), and tethers small GTPases (Rags and Rheb)
297 to the lysosomal surface (Bar-Peled et al., 2012). When amino acid levels are low, mTORC1 is
298 not translocated to the lysosomal surface (Wang et al., 2009; Bar-Peled et al., 2012), thereby
299 favoring catabolic processes such as autophagy (Thompson et al., 2005). We propose that
300 amino acid scarcity might result from the upregulation of genes involved in the degradation of
301 lysin, glycine, tyrosine, cysteine, leucine, isoleucine, valine or tryptophan (Figure 3, Data S1). This
302 would decrease mTORC1 activity and, in turn, stimulates nutrient recycling via autophagy in
303 AS worms.

304 Conversely, we hypothesize that in H worms, active mTORC1 interacts with the
305 ribosomal protein S6 kinase (S6K), encoded by the *rsks-1* gene which is also up in H worms
306 (Ladevaia et al., 2014) (Figure 3). This direct interaction, upon a cascade of phosphorylation
307 events, would stimulate translation, and ultimately cell growth and proliferation (Ma et al., 2009,
308 Howell et al., 2011, and Ladevaia et al., 2014).

309 All in all, although it is currently unclear whether increased autophagy is beneficial or
310 detrimental, under AS conditions, the upregulation of genes involved in self-digestion might
311 play a protective role and foster recovery from starvation (Thompson et al., 2005), pathogens
312 (Huber and Teis, 2016) or from neuronal and muscular degeneration induced by oxygen
313 deprivation (Murphy and Steenbergen 2008).

314 **Lectins and mucins.** Given that symbiont attachment may be mediated by Ca²⁺-
315 dependent lectins (Nussbaumer et al. 2004, Bulgheresi et al., 2006, 2011) and given that,
316 under anoxia, the symbiont appeared to proliferate more (Paredes et al., 2021), we expected

317 nematode lectins to be upregulated under this condition. Indeed, nine C-type lectin domain
318 (CTLD)-containing proteins were upregulated in AS *L. oneistus* adults and only two (*clec-78*
319 and *clec-78-like-2*) were upregulated in the presence of oxygen (Figure 4). In addition to
320 CTLD-containing proteins, mucins, a class of glycoproteins with more than 50% of its mass
321 attributable to O-glycans, were also upregulated in AS nematodes. Considering that mucin
322 glycans are used by vertebrate gut commensals for attachment, as well as a source of
323 nutrients (Koropatkin et al., 2012), it is conceivable that their upregulation in anoxia (Figure 4),
324 together with that of CTLD-containing proteins, would foster symbiont attachment.

325 We hypothesize that overexpression of two classes of putative symbiont-binding
326 molecules, lectins and mucins, under conditions favoring symbiont proliferation (i.e., AS
327 condition, Paredes et al., 2021) may mediate bacterial coat reinforcement.

328 **Apoptosis.** Mitochondria play an important role in apoptosis induction (Simon et al.,
329 2000; Martínez-Reyes et al., 2020). Indeed, MPTP opening due to ROS (or the severe ATP
330 decline imposed by the absence of oxygen) may cause cytochrome C release from
331 mitochondria and this, in turn, triggers caspase activation (Martinou et al., 2000; Simon et al.,
332 2000; Gogvadze et al., 2006; Galli et al., 2014). We observed that transcripts encoding for *sco-*
333 *1*, a gene needed for the synthesis and assembly of mitochondrial cytochrome C (Williams et
334 al., 2005) were more abundant in AS worms (Figure 4). Further, we observed upregulation of
335 Caspase-3 (*ced-3*) which belongs to a family of cysteine proteases involved in apoptosis
336 (Mangahas et al., 2005; Kaufmann et al., 2008) and which is activated upon mitochondrial
337 cytochrome C release into the cytosol (Liu et al., 1996; Tafani et al., 2000; Kaufmann et al.,
338 2008; Martínez-Reyes et al., 2020). Additional apoptosis-related genes that appeared to be
339 upregulated in AS worms were: *bec-1* (Figure 3), a gene that promotes autophagy and fine-
340 tunes the Ced-3-mediated apoptosis (Liang et al., 1999; Takacs-Vellai et al., 2005); *ttr-52*,
341 which mediates apoptotic cell recognition prior to engulfment (Wang, X. et al., 2010; Chen et
342 al., 2013); a BAG family molecular chaperone regulator 1 (BAG1-regulator); a cell-death-
343 related nuclease *crn-2* (Parrish et al., 2003; Samejima et al., 2005) and phagolysosome
344 forming *arl-8* (Sasaki et al., 2013), and a tyrosine kinase Abl-1, (*abl-1*) that modulates apoptotic
345 engulfment pathways (Hurwitz et al., 2009).

346 **Lipid catabolism.** Genes involved in lipid metabolism were similarly expressed
347 between the AS and H conditions (Figure 2, Data S1). In accordance, lipidomes of nematodes
348 incubated in the presence or absence of oxygen were not significantly different (Figure S5,
349 Supplemental material). However, in line with the overall upregulation of degradation
350 pathways, we observed upregulation of genes involved in FA beta-oxidation (*kat-1*;
351 Berdichevsky et al., 2010), in lipid digestion (the lipase *lipl-6*; UniProtKB E2S7J2), and lipid
352 degradation (a peripilin-2-like protein; Chughtai et al., 2015). Moreover, a gene that might be
353 involved in oxidative-stress tolerance (a stearic acid desaturase *fat-7* regulating the first step of

354 the fatty acid desaturation pathway (Horikawa et al., 2009) was also upregulated in AS worms.
355 Lipid degradation under anoxia might be a strategy to overcome starvation (Krivoruchko and
356 Storey, 2015).

357 Notably, we also observed an upregulation of two genes involved in
358 phosphatidylcholine (PC) synthesis (*pmt-1*, *pmt-2*, Brendza et al., 2007) (Figure 5). Intriguingly,
359 PC was more abundant in the anoxic symbiont (Paredes et al., 2021), although the latter
360 cannot synthesize it. Thus, their upregulation in AS worms suggests worm-symbiont lipid
361 transfer.

362 **GABA- and glutamate-mediated neurotransmission.** Upregulated genes related to
363 GABA synthesis were, *unc-25*, *unc-104* and *pdxk-1* (pyridoxal phosphate hexokinase)
364 (Thomas et al., 1990; McIntire et al., 1993; Jin et al., 1999; Gally et al., 2003; Nordquist et al.,
365 2018; Risley et al., 2016) (Figure 5, Data S1). Consistent with an expected increase in
366 glutamate requirement as a direct GABA precursor (Martin et al., 1993), we observed
367 downregulation of two glutamine synthetases and a delta-1-pyrroline-5-carboxylate synthase
368 (*gln-3* and *alh-13* respectively; van der Vos et al., 2012; Yen et al., 2021; Figure 6), known to
369 convert glutamate to glutamine or to proline, respectively. Furthermore, an *mgl-2* like gene
370 encoding for a glutamate receptor, which is activated in the presence of glutamate
371 (Tharmalingam et al., 2012), was up in AS worms. Note that, when oxygen is limited,
372 glutamate may act as a neurotoxic amino acid (Baker et al., 1991; Lutz et al., 2003a).
373 Therefore, increased GABA biosynthesis might, beneficially, prevent its accumulation (Milton et
374 al., 2002; Mathews et al., 2003).

375 GABA-mediated neurotransmission has been documented for facultative anaerobic
376 animals thriving in anoxic conditions (Lutz et al., 1997; Milton et al., 1998; Lutz et al., 2003a,
377 b). Due to its inhibitory nature, it contributes to avoid membrane depolymerization (Nilsson et
378 al., 1990; Milton et al., 1998). Moreover, given that it relaxes muscles, the increment of GABA
379 may impact the movement of the animal (McIntire et al., 1993; Schuske et al., 2004).
380 Therefore, upregulation of GABA-mediated neuronal activity might explain why anoxic *L.*
381 *oneistus* did not form tight worm clusters after 24h (Supplemental movie 3).

382 **Dopamine-mediated neurotransmission.** A gene encoding for the tyrosine
383 hydroxylase Cat-2 (*cat-2*), which is needed for dopamine biosynthesis (Sawin et al., 2000) and
384 two putative dopamine receptors (*protein-D2-like* and a G_PROTEIN_RECEP_F1_2 domain-
385 containing protein (*dop-5*); Sanyal et al., 2004) were upregulated in AS worms. Moreover, a
386 *dat-1*-like gene mediating dopamine reuptake into the presynaptic terminals was
387 downregulated (Gainetdinov et al., 2002; McDonald et al., 2006) in AS worms (Figure 5).

388 **Calcium-binding and -sensing proteins.** Finally, in AS worms several calcium-
389 binding or -sensing proteins (e.g., *ncs-2*, *cex-2*, and a calbindin-like (CALB1 homologue);
390 Soontornniyomkij et al., 2012; Hobert et al., 2018; Figure 5), as well as calcium transporters

391 (*cca-1*, Steger et al., 2005; Transport category, [Figure 6](#)) were upregulated. On the one hand,
392 we hypothesize their involvement in the inhibitory neural signaling described above (for
393 example, Ncs-2 mediates the cholinergic and GABAergic expression of *C. elegans* (Zhou et
394 al., 2017). On the other, they may protect cells against the stress inflicted by anoxia, which
395 involves calcium overload and consequent cellular acidification (Bickler et al., 1992; Dell'Anna
396 et al., 1996; Galli et al., 2014).

397

398 **Genes upregulated in hypoxic (H) nematodes**

399 **Innate immune pathways and effectors.** Animals recognize and respond to microbes
400 by means of immunoreceptors including Toll-like receptors, conserved from sponges to
401 humans (Akira et al., 2006). We identified almost all genes belonging to this pathway, including
402 the one encoding for the NF- κ B transcription factor. This came as a surprise given that, up to
403 now, the has not been identified in any other nematode NF- κ B (Pujol and Ewbank, submitted).
404 As surprising, was the fact that not only two Toll-like receptors (*tol-1* and *tol-1-like*), but also
405 genes encoding for antimicrobial proteins such as a peroxisome assembly factor involved in
406 defense against Gram- (*prx-11-like*, Wang, D. (2019), a putatively antifungal endochitinase
407 (Dravid et al., 2015) and Bactericidal Permeability Increasing proteins (BPIs) were also more
408 abundant in H worms. BPIs may bind LPS and perforate Gram- membranes and have shown
409 to play a symbiostatic role in other invertebrates (Bruno et al., 2019; Krasity et al., 2015; Chen
410 et al., 2017). However, it is unclear whether activation of the *L. oneistus* Toll pathway leads to
411 the nuclear NF- κ B switching on the expression of antimicrobial genes or whether, as shown in
412 *C. elegans*, the Toll pathway mediates behavioral avoidance of pathogens (Pradel et al., 2007;
413 Brandt et al., 2015).

414 Overall, the apparent oxygen stimulation of a central innate immunity pathway and,
415 directly or indirectly, of broad range anti-defense mechanisms could be adaptations to the fact
416 that in oxygenated environments (when crawling in superficial sand layers), *L. oneistus* is
417 exposed to predation from bigger animals, but also to pathogenic members of the
418 bacterioplankton. Overexpression of broad-range antimicrobials in response to oxygen might
419 therefore help *L. oneistus* to avoid colonization by potentially deleterious, fouling bacteria (e.g.,
420 *Vibrios*, *Roseobacters* and *Pseudoaltermonas/Alteromonadales*) when crawling close to the
421 water column (Dang and Lovell, 2016; M. Mussmann, personal communication).

422 **Development.** Although development-related genes were some of the most expressed
423 under all conditions ([Figure 1](#)), many were upregulated in H nematodes ([Figure 2](#) and [5](#)).
424 Among the development-related genes upregulated in H nematodes were those related to
425 molting (e.g., *nas-36*, *nas-38*, *chs-2*, *ptr-5*, *ptr-18*, *apl-1*, *myrf-1*; Suzuki et al., 2004; Zhang et
426 al., 2005; Zugasti et al., 2005; Hornsten et al., 2007; Russel et al., 2011), germ line
427 establishment (e.g., *ccm-3*, *rsks-1*; Pan et al., 2007; Pal et al., 2017),

428 oogenesis/spermatogenesis (*crt-1*, Park et al., 2001), embryonic development and yolk
429 production (*smp-1*, *cpna-1*, *plt-1*, *vit-6*, *crt-1*, *arrd-17*, *mlc-5*; Clark et al., 1997; Goedert et al.,
430 1996; Gatewood et al., 1997; Fuji et al., 2002; Gally et al., 2009; Zahreddine et al., 2010; Jee
431 et al., 2012; Warner et al., 2013; Fisher et al., 2014; Perez and Lehner, 2019), and/or larval
432 development (*nmy-1*, *ifb-1*; Ding et al., 2004; Osório et al., 2019), as well as male tip (Cdt1,
433 *plx-1*, *ver-3*; Nelson et al., 2011; Dalpé et al., 2004; Dalpe et al., 2013), vulva morphogenesis
434 (*hda-1*, *unc-62*), and a hermaphrodite-related gene (*hda-1*; Dufourcq et al., 2002; Choy et al.,
435 2007) (Figure 5). Moreover, transcripts encoding for a number of proteases shown to be
436 involved in *C. elegans* molting (e.g., *nas-38*, *nas-6*-like; Park et al., 2010), development (e.g.,
437 teneurin-a-like; Topf and Drabikowski, 2019), neuronal regrowth or locomotion (*tep-1*; Kim et
438 al., 2018) and pharyngeal pumping (e.g., neprilysin *nep-1*; Spanier et al., 2005) were also more
439 abundant in H worms. Remarkably, *vav-1*, which, besides being involved in male tip and vulva
440 morphogenesis (Nelson et al., 2011), may also regulate the concentration of intracellular
441 calcium (Norman et al., 2005), was one of the few development-related genes to be
442 downregulated in H nematodes (see previous section on Ca-binding proteins).

443 To sum up, and as expected, the host appears to exploit oxygen availability to
444 undertake energetically costly processes, such as development and molting (De Cuyper and
445 Vanfleteren 1982; Uppaluri and Brangwynne 2015).

446 **Carbohydrate metabolism.** If in AS nematodes, glycogen or starch appeared
447 prominent carbon sources, H worms seemed to exploit trehalose and cellulose instead.
448 Indeed, genes that degrade trehalose (*tre-1*, Pellerone et al., 2003) and cellulose (Ppa-*cel-2*,
449 Schuster et al., 2012) were upregulated in H worms, as well as a putative ADP-dependent
450 glucokinase (C50D2.7) involved in glycolysis (Yuan et al., 2012). The use of this pathway was
451 supported by the overexpression of four genes encoding for sugar transporters (Slc2-A1,
452 C35A11, K08F9.1, F53H8.3; Kitaoka et al., 2013; Bertoli et al., 2015), perhaps switched on by
453 active mTOR (see above) (Figure 6) (Howell et al., 2011).

454 Additionally, *L. oneistus* appeared to exploit oxygen to synthesize complex
455 polysaccharides, such as heparan sulfate (*hst-1*-like; Miyagawa et al. 1988; Bhattacharya et
456 al., 2009) and glycan (Gcnt3-like) (Figure 6), as an ortholog of the N-deacetylase/N-
457 sulfotransferase *hst-1*, related to heparin biosynthesis was also upregulated (Bhattacharya et
458 al., 2009).

459 Although glycolysis seems to generate ATP in both AS and H worms, it is not clear why
460 the latter would prefer to respire cellulose or trehalose instead of starch. Given its role as a
461 membrane stabilizer, we speculate that AS worms might prioritize the storage of trehalose over
462 its degradation to preserve membrane integrity (Figure 6) (Crowe et al 1987; Carpenter et al.,
463 1988; Clegg et al., 1997; Chen et al., 2002; Haddad 2006). Of note, based on its genome draft,
464 the symbiont may synthesize and transport trehalose, but it may not use it (Paredes et al.,

465 2021). Therefore, we hypothesize symbiont-to-host transfer of trehalose under hypoxia.
466 Consistently, the symbiont's trehalose synthesis-related gene (*otsB*; Paredes et al., 2021), and
467 the host trehalase (*tre-1*; Figure 6) were both upregulated under hypoxia and metabolomics
468 could detect trehalose in both partners (Table S1). Metabolomics also detected sucrose in both
469 the holobiont and the symbiont fraction (Table S1). Given that, based on transcriptomics and
470 proteomics, the nematode can utilize sucrose but cannot synthesize it (Data S1), whereas the
471 symbiont can (Paredes et al., 2021), as in the case for trehalose, we hypothesize symbiont-to-
472 host sucrose transfer.

473 **Acetylcholine-mediated neurotransmission.** Instead of upregulating genes involved
474 in inhibitory (GABA and dopamine-mediated) neurotransmission, hypoxic worms appeared to
475 use excitatory acetylcholine-mediated neurotransmission as indicated by the upregulation of
476 *molo-1*, *acr-20*, *cup-4*, *lev-9*, and sphingosine kinase *sphk-1* that promotes its release (Mongan
477 et al., 2002; Patton et al., 2005; Gendrel et al., 2009; Boulin et al., 2012; Chan et al., 2012)
478 (Figure 5). On the one hand, acetylcholine-mediated neurotransmission might promote ROS
479 detoxification in H worms (Sun et al., 2014). On the other hand, its downregulation in AS
480 worms may beneficially decrease calcium influx (Hochachka and Lutz, 2001).

481 **Feeding, mating, mechanosensory behavior and axon guidance and**
482 **fasciculation.** Transcripts related to the neuronal regulation of energy-demanding activities
483 such as feeding, mating, motion, as well as nervous system development were more abundant
484 in H nematodes (Figure 5, and Data S1). More precisely, upregulated genes were involved in
485 pharyngeal pumping (*nep-1*, *lat-2*; Spanier et al., 2005; Guest et al., 2007), male mating
486 behavior and touch (*pdf-1*, *tbb-4*, *ebax-1*, Hurd et al., 2010; Wang, Z. et al., 2013), axon
487 guidance and fasciculation (*spon-1*, *igcm-1*, *ebax-1*, *tep-1*; Kim et al., 2018; Woo et al., 2008;
488 Schwarz et al., 2009; Wang, Z et al., 2013), mechanosensory behavior (e.g., *mec-12*, *delm-2*;
489 Gu et al., 1996; Han et al., 2013). Additionally, we also observed the upregulation of a gene
490 encoding for a glutamate receptor (*glr-7*) possibly involved in feeding facilitation (Li et al.,
491 2012).

492 **Amino acid biosynthesis.** Transcripts of genes involved in the synthesis of glutamine
493 and proline (*gln-3* and *alh-13*, respectively), aspartate (L-asparaginases; Tsuji et al., 1999) and
494 S-adenosyl-L-methionine (SAM) (*sams-4*; Chen et al., 2020) were all upregulated in H worms
495 (Figure 6), as well as one encoding for the ornithine decarboxylase *odc-1* which is involved in
496 biosynthesis of the polyamine putrescine, and is essential for cell proliferation and tissue growth
497 (Russell et al., 1968; Heby, 1981). Moreover, polyamines, with their high charge-to-mass ratio
498 may protect against superoxide radicals, which, as mentioned, harm cell membranes and
499 organelles, oxidize proteins, and damage DNA (Gilad et al., 1991; Longo et al., 1993).

500 **Lipid biosynthesis.** Genes upregulated in H worms mediate the biosynthesis of long
501 chain fatty acids (*acs-3*, *acs-14*, *elo-3* but not *acs-5*; Yuan et al., 2012; Ward et al., 2014;

502 Wang et al., 2021), sphingolipids (a sphingosine kinase-1 (*sphk-1*) and *egl-8*, which controls
503 egg laying and pharyngeal pumping in *C. elegans* (Bastiani et al., 2003). Notably, sphingolipids
504 may be anti-apoptotic (Taha et al., 2006) or result in acetylcholine release (Chan et al., 2012).

505 On the other hand, ceramides, which have antiproliferative properties and who may
506 mediate resistance to severe oxygen deprivation (Deng et al., 2008; Menuz, et al. 2009),
507 appeared to be mainly synthesized in AS worms, as indicated by the upregulation of genes
508 involved in ceramide biosynthesis (*asm-3*, *ttm-5*; Watts et al., 2017) (Figure 6).

509 **Transport.** As anticipated in the introduction, anoxia-tolerant animals switch off ATP-
510 demanding processes such as ion pumping (Lutz et al., 1996; Galli et al., 2014). Indeed,
511 transcripts encoding for proteins involved in cation channel activity (*gtl-2*, voltage gated H
512 channel 1; Teramoto et al., 2010), sodium transport (*delm-2*-like; Han et al., 2013), chloride
513 transport (*anoh-1*, *best-13*, *best-14*; Tsunenari et al., 2013; Wang, Y. et al., 2013; Goh et al.,
514 2018), ABC transport (*wht-2*, *pgp-2*, *slcr-46.3*, *F23F12.3*, *hmit-1.3*; Currie et al., 2007;
515 Schroeder et al., 2007; Kage-Nakadai et al., 2011) and organic transport (*F47E1.2*, *oct-2*; Pao
516 et al., 1998) were all more abundant in H than AS worms (Figure 6).

517 **Sulfur metabolism.** The *mpst-7* gene which is involved in organismal response to
518 selenium and it is switched on in hypoxic *C. elegans* (Romanelli-Credrez et al., 2020) was
519 upregulated in H nematodes (Figure 6). Given that the latter is thought to catalyze the
520 conversion of sulfite and glutathione persulfide (GSSH) to thiosulfate and glutathione (GSH)
521 (Filipovic et al., 2018), hypoxia-experiencing *L. oneistus* might express this enzyme to
522 recharge the cells with GSH and hence, help to cope with oxidative stress (Hayes and
523 McLellan, 1999; Mytilineou et al., 2002; Diaz-Vivancos et al., 2015). Also more abundant in H
524 worms were transcripts encoding for the sulfatases 2 (*sul-2*) (Morimoto-Tomita et al., 2002)
525 and a PAPS-producing *pps-1* (3'-phospho-adenosine-5'-phosphosulfate (PAPS) considered
526 the universal sulfur donor; Bhattacharya et al., 2009), as well as for the chaperones *pdi-6* and
527 protein-disulfide-isomerase-A5-like which require oxygen to mediate correct disulfide bond
528 formation in protein folding (Teodoro and O'Farrell, 2003; Rose et al., 2017; Livshits et al.,
529 2017) (Figure 6).

530 Conversely, a putative sulfide-producing enzyme (*mpst-1*) who protects *C. elegans*
531 from mitochondrial damage (Qabazard et al., 2014; Ng et al., 2019; Kimura, 2020) was
532 upregulated in AS nematodes. Notably, under AS, *L. oneistus* might detoxify sulfide by
533 producing glutathione and taurine (Rose et al., 2017), as a persulfide dioxygenase (*ethe-1*)
534 and a cysteine dioxygenase (*cdo-1*) which catalyzes taurine synthesis via cysteine degradation
535 were upregulated. Sulfide detoxification via taurine accumulation is a common strategy in
536 chemosynthetic animals (reviewed in Cavanaugh et al., 2006).

537 All in all, *L. oneistus* appeared to limit excess accumulation of free sulfide in anoxia and
538 to free sulfate when oxygen was available.

539

540 **Conclusions**

541 Overall and irrespectively of the conditions it was subjected to, *L. oneistus* mostly
542 expressed genes involved in degradation, energy generation, stress response and immune
543 defense. Astonishingly, *L. oneistus* did not enter suspended animation when subjected to
544 anoxic sulfidic conditions for days. We hypothesize that in the absence of oxygen, ATP
545 production is supported by trehalose and cellulose catabolism, and by rewiring the ETC in
546 such way as to use rodoquinone (RQ) as electron carrier, and fumarate as electron acceptor.
547 Moreover, the nematode activates several degradation pathways (e.g., ubiquitin-proteasome
548 system (UPS), autophagy, and apoptosis) to gain nutrients from anoxia- or ROS-damaged
549 proteins and mitochondria. Further, AS worms also upregulated genes encoding for ribosomal
550 proteins and putative symbiont-binding proteins (lectins). Finally, as proposed for other anoxic-
551 tolerant animals, the worm seems to upregulate its antioxidant capacity in anticipation of
552 reoxygenation. When in hypoxic conditions (Figure 7, left), instead, we speculate that the worm
553 uses starch for energy generation to engage in costly developmental processes such as
554 molting, feeding, and mating, likely relying on excitatory neurotransmitters (e.g., acetylcholine),
555 and it upregulates the Toll immune pathway and, directly or indirectly, the synthesis of broad
556 range antimicrobials (e.g., fungicides, bactericidal permeability increasing proteins).

557 When looking at the *Laxus-Thiosymbion* symbiosis in light of what was recently
558 published (Paredes et al., 2021), we could identify two signs of inter-partner metabolic
559 dependence: in anoxia worms might transfer lipids to their symbionts, and in hypoxia the
560 symbionts might transfer trehalose to their hosts.

561 Furthermore, we may conclude that, wherever in the sand the consortium is, one of the
562 two partners is bound to be stressed: in anoxia, the symbiont appear to proliferate more, while
563 its animal host engages in degradation of damaged proteins and mitochondria and in
564 detoxification. In the presence of oxygen, the situation is inverted: the symbiont seems
565 massively stressed, while the host can afford energy costly biosynthetic processes to develop
566 and reproduce (Figure 7). It is therefore fascinating that, in spite of the dramatically different
567 needs a bacterium and animal must have, the *Laxus-Thiosymbion* symbiosis evolved.

568

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582

583 **MATERIALS AND METHODS**

584 **Sample collection**

585 *Laxus oneistus* individuals were collected on multiple field trips (2016-2019) at approximately 1
586 m depth from sand bars off the Smithsonian Field Station, Carrie Bow Cay in Belize
587 (16°48'11.01"N, 88°4'54.42"W). The collection of the nematodes, the incubations set up for
588 RNA sequencing, lipidomics, proteomics and metabolomics, as well as the RNA extraction,
589 and library preparation are described in Paredes et al., 2021. Importantly, the nematodes had
590 a bright white appearance and replicate incubations were started simultaneously. Note that the
591 [Supplemental material](#) describes changes in the lipidomics and proteomics pipelines, as well
592 as the metabolomics, and sequencing data of *Olavius algarvensis*.

593 **Host transcriptome de novo assembly**

594 In preparation for the assembly, reads from each sample were first mapped to the symbiont as
595 described before (Paredes et al., 2021), and remaining rRNA reads from all domains of life
596 were removed from unmapped reads using sortmerna v2.1 in combination with the
597 SSURef_NR99_119_SILVA_14_07_14 and LSURef_119_SILVA_15_07_14 databases.
598 Further, exact duplicate reads were removed using PRINSEQ lite's derep option. Read files
599 free of symbiont reads, rRNA reads and exact duplicates were used as input for transcriptome
600 sub-assemblies via Trinity v2.6.6 with the strand-specific option (--SS_lib_type F) (Grabherr et
601 al., 2011). Two sub-assemblies differing in the number and type of input read files were
602 performed: (1) 9 input read files including biological triplicates from 3 incubation conditions (O,
603 H, A) and (2) 4 input read files including a single replicate from 4 incubation conditions (O, H, A
604 and hyper-O). Hyper-O refers to an incubation in which air was pumped directly into the
605 exetainers for the entire incubation period to supersaturate the seawater (300 %O₂). However,
606 as this incubation condition yielded an incongruous transcriptional response by the symbiont
607 (data not shown), these read data were only used to extend the host transcriptome's coding
608 repertoire. The qualities of both sub-assemblies were assessed as described below.

609 We then performed an intra-assembly clustering step as described in (Cerveau and
610 Jackson, 2016), during which identical transcripts were removed from the sub-assemblies
611 using CD-HIT-EST (Fu et al., 2012). To further reduce redundant transcripts, only the longest
612 isoform for each 'gene' identified by Trinity was kept using Trinity's

613 `get_longest_isoform_seq_per_trinity_gene.pl` utility. The remaining transcripts of each sub-
614 assembly were then concatenated to produce a merged transcriptome assembly. The final
615 assembly was created by applying another sequence clustering using CD-HIT-EST to avoid
616 inter-assembly redundancy. Here, the identity parameter of 80% (`-c 0.8`) combined with a
617 minimal coverage ratio of the shorter sequence of 80% (`-aS 0.8`) and minimal coverage ratio of
618 the longest sequence of 0.005% (`-aL 0.005`) yielded the best-performing assembly in terms of
619 number of transcripts (162,455) and contiguity (N50 value of 770) (data not shown).
620 Assembly completeness was assessed by estimating completeness via BUSCO nematode
621 single-copy orthologs (Simão et al., 2015). Importantly, the merged assembly yielded a higher
622 BUSCO-based completeness compared with the two sub-assemblies; 79.2% of the BUSCO
623 nematode single-copy orthologs were found to be present and complete in the final assembly
624 (636 single-copy/142 duplicated), whereas assembly (1) scored 77.8% (233 single-copy/531
625 duplicated) and assembly (2) was 76.2% complete (314 single-copy/434 duplicated). Further,
626 assembled transcripts were filtered based on taxonomic classification. Transcripts were
627 matched against the RefSeq protein database using `blastx` (E value $1E-3$), and the output was
628 then used as input for taxonomic assignment via MEGAN v5 (Huson et al., 2007). Only
629 transcripts classified as belonging to 'Eukarya' were kept (MEGAN parameters: Min Score: 50,
630 Max Expected: $1E-2$, Top Percent: 2), which reduced the number of putative *L. oneistus*
631 transcripts to 30,562. Assembled transcripts were also functionally annotated using Trinotate
632 (Bryant et al., 2017). Briefly, predicted protein coding regions were extracted using
633 TransDecoder (<https://github.com/TransDecoder>), both transcripts and predicted protein
634 sequences were searched for protein homology via `blastx` and `blastp`, respectively, and
635 predicted protein sequences were annotated for protein domains (`hmmscan`), signal peptides
636 (`signalP`) and transmembrane domains (THMMM). 85,859 transcripts exhibited at least one
637 functional annotation. Finally, only taxonomy-filtered transcripts with at least one functional
638 annotation were kept, thereby further reducing the number of putative host transcripts to
639 27,984, with 22,072 thereof predicted to contain protein coding regions. BUSCO-based
640 completeness for this filtered host transcriptome assembly was 78.8% (635 single-copy/139
641 duplicated).

642 **Gene expression analysis**

643 Raw sequencing reads quality assessment and preprocessing of data was followed as
644 described in Paredes et al., 2021. Trimmed reads were mapped to the de novo transcriptome
645 assembly and transcript abundance was estimated using RSEM v1.3.1 (Li and Dewey 2011) in
646 combination with `bowtie` with default settings except for the application of strandedness (`--`
647 `strandedness forward`). Read counts per transcript were used for differential expression
648 analysis, and TPM (transcripts per kilobase million) values were transformed to \log_2 TPMs as
649 described in Paredes et al. 2021.

650 Gene and differential expression analyses were conducted using the R software
651 environment and the Bioconductor package edgeR v3.28.1 (Gentleman et al., 2004; Robinson
652 et al., 2010; R core Team, 2013), and as shown in Paredes et al., 2021. Here, we only
653 describe the modifications that were made to the pipeline. Genes were considered expressed
654 if at least ten reads in at least three replicates of one of the four conditions could be assigned.
655 Excluding the replicates of the oxic condition, we found that 74.9% of all predicted nematode
656 protein-encoding genes to be expressed (16,526 genes out of 22,072). Log₂TPM were used to
657 assess sample similarities via multidimensional scaling based on Euclidean distances (R Stats
658 package) (R core Team, 2013) (Figure S1B), and the average of replicate log₂TPM values
659 per expressed gene and condition was used to estimate expression strength. Median gene
660 expression of entire metabolic processes and pathways per condition was determined from
661 average log₂TPM values.

662 Expression of genes was considered significantly different if their expression changed
663 1.5-fold between two treatments with a false-discovery rate (FDR) ≤ 0.05 (Rapaport et al.,
664 2013). Throughout the paper, all genes meeting these thresholds are either termed
665 differentially expressed or up- or downregulated. For the differential expression analyses
666 between the AS, H and A conditions see Data S1. Heatmaps show mean-centered log₂TPM
667 expression values to highlight gene expression change.

668 All predicted *L. oneistus* proteins were automatically annotated using eggNOG-mapper
669 v2 (Cantalapiedra et al., 2021) against eggNOG 5.0 (Huerta-Cepas et al., 2019) using
670 diamond v2.0.4 (Buchfink et al., 2021). All genes that are shown and involved in a particular
671 process were manually curated by blasting them against both the NCBI BLASTP nr database
672 (Altschul et al., 1990) and the WormBase (Harris et al., 2020;
673 https://wormbase.org/tools/blast_blat).

674

675 **Data availability.** This Transcriptome Shotgun Assembly project has been deposited at
676 DDBJ/EMBL/GenBank under the accession GJNO00000000. The version described in this
677 paper is the first version, GJNO01000000. RNA-Seq data are available at the Gene
678 Expression Omnibus (GEO) database and are accessible through accession number
679 GSE188619.

680

681 REFERENCES

- 682 1. Hermes-Lima, M., & Zenteno-Savín, T. (2002). Animal response to drastic changes in
683 oxygen availability and physiological oxidative stress. *Comparative Biochemistry and*
684 *Physiology Part C: Toxicology & Pharmacology*, 133(4), 537-556.

- 685 2. Hochachka, P. W., Buck, L. T., Doll, C. J., & Land, S. C. (1996). Unifying theory of
686 hypoxia tolerance: molecular/metabolic defense and rescue mechanisms for surviving
687 oxygen lack. *Proceedings of the National Academy of Sciences*, 93(18), 9493-9498.
- 688 3. Hochachka, P. W., Land, S. C., & Buck, L. T. (1997). Oxygen sensing and signal
689 transduction in metabolic defense against hypoxia: lessons from vertebrate facultative
690 anaerobes. *Comparative Biochemistry and Physiology Part A: Physiology*, 118(1), 23-
691 29.
- 692 4. Hochachka, P. W., & Lutz, P. L. (2001). Mechanism, origin, and evolution of anoxia
693 tolerance in animals. *Comparative Biochemistry and Physiology Part B: Biochemistry
694 and Molecular Biology*, 130(4), 435-459.
- 695 5. Clegg, J. (1997). Embryos of *Artemia franciscana* survive four years of continuous
696 anoxia: the case for complete metabolic rate depression. *The Journal of Experimental
697 Biology*, 200(3), 467-475.
- 698 6. Nystul, T. G., Goldmark, J. P., Padilla, P. A., & Roth, M. B. (2003). Suspended
699 animation in *C. elegans* requires the spindle checkpoint. *Science*, 302(5647), 1038-
700 1041.
- 701 7. Teodoro, R. O., and O'Farrell, P. H. (2003). Nitric oxide-induced suspended animation
702 promotes survival during hypoxia. *The EMBO Journal*, 22(3), 580-587.
- 703 8. Haddad, G. G. (2006). Tolerance to low O₂: lessons from invertebrate genetic models.
704 *Experimental physiology*, 91(2), 277-282.
- 705 9. Liu, L., & Simon, M. C. (2004). Regulation of transcription and translation by hypoxia.
706 *Cancer biology & therapy*, 3(6), 492-497.
- 707 10. Liu, L., Cash, T. P., Jones, R. G., Keith, B., Thompson, C. B., & Simon, M. C. (2006).
708 Hypoxia-induced energy stress regulates mRNA translation and cell growth. *Molecular
709 cell*, 21(4), 521-531.
- 710 11. Galli, G. L., & Richards, J. G. (2014). Mitochondria from anoxia-tolerant animals reveal
711 common strategies to survive without oxygen. *Journal of Comparative Physiology
712 B*, 184(3), 285-302.
- 713 12. Van Voorhies, W. A., & Ward, S. A. M. U. E. L. (2000). Broad oxygen tolerance in the
714 nematode *Caenorhabditis elegans*. *Journal of Experimental Biology*, 203(16), 2467-
715 2478.
- 716 13. Padilla, P. A., Nystul, T. G., Zager, R. A., Johnson, A. C., & Roth, M. B. (2002).
717 Dephosphorylation of cell cycle-regulated proteins correlates with anoxia-induced
718 suspended animation in *Caenorhabditis elegans*. *Molecular biology of the cell*, 13(5),
719 1473-1483.

- 720 14. Nystul, T. G., & Roth, M. B. (2004). Carbon monoxide-induced suspended animation
721 protects against hypoxic damage in *Caenorhabditis elegans*. Proceedings of the
722 National Academy of Sciences, 101(24), 9133-9136.
- 723 15. Powell-Coffman, J. A. (2010). Hypoxia signaling and resistance in *C. elegans*. Trends
724 in Endocrinology & Metabolism, 21(7), 435-440.
- 725 16. Fawcett, E. M., Hoyt, J. M., Johnson, J. K., & Miller, D. L. (2015). Hypoxia disrupts
726 proteostasis in *Caenorhabditis elegans*. Aging Cell, 14(1), 92-101.
- 727 17. Kitazume, H., Dayi, M., Tanaka, R., & Kikuchi, T. (2018). Assessment of the behaviour
728 and survival of nematodes under low oxygen concentrations. PloS one, 13(5),
729 e0197122.
- 730 18. Kim, K. W., & Jin, Y. (2015). Neuronal responses to stress and injury in *C. elegans*.
731 FEBS letters, 589(14), 1644-1652.
- 732 19. Denny, M. (1993). Air and Water: the Biology and Physics of Life's Media. Princeton,
733 NJ: Princeton University Press. 341pp.
- 734 20. Ott, J. A., & Novak, R. (1989). Living at an interface: Meiofauna at the oxygen/sulfide
735 boundary of marine sediments.
- 736 21. Ott, J. A., Novak, R., Schiemer, F., Hentschel, U., Nebelsick, M., & Polz, M. (1991).
737 Tackling the sulfide gradient: a novel strategy involving marine nematodes and
738 chemoautotrophic ectosymbionts. Marine Ecology, 12(3), 261-279.
- 739 22. Schiemer, F., Novak, R., & Ott, J. (1990). Metabolic studies on thiobiotic free-living
740 nematodes and their symbiotic microorganisms. Marine Biology, 106(1), 129-137.
- 741 23. Paredes, G. F., Viehboeck, T., Lee, R., Palatinszky, M., Mausz, M. A., Reipert, S., ... &
742 König, L. (2021). Anaerobic sulfur oxidation underlies adaptation of a chemosynthetic
743 symbiont to oxic-anoxic interfaces. mSystems, 6(3), e01186-20.
- 744 24. Ott, J. A., Bauer-Nebelsick, M., & Novotny, V. (1995). The genus *Laxus* Cobb, 1984
745 (Stilbonematinae: Nematoda): description of two new species with ectosymbiotic
746 chemoautotrophic bacteria. Proceedings of the Biological Society of Washington,
747 108(3), 508-527.
- 748 25. Chan, K., Goldmark, J. P., & Roth, M. B. (2010). Suspended animation extends survival
749 limits of *Caenorhabditis elegans* and *Saccharomyces cerevisiae* at low temperature.
750 Molecular biology of the cell, 21(13), 2161-2171.
- 751 26. UniProt: the universal protein knowledgebase in 2021. *Nucleic Acids Research*, 2021,
752 49. Jg., Nr. D1, S. D480-D489.
- 753 27. Stringham, E. G., Jones, D., & Candido, E. P. M. (1992). Expression of the
754 polyubiquitin-encoding gene (*ubq-1*) in transgenic *Caenorhabditis elegans*. Gene,
755 113(2), 165-173.

- 756 28. Geuens, E., Hoogewijs, D., Nardini, M., Vinck, E., Pesce, A., Kiger, L., ... & Dewilde, S.
757 (2010). Globin-like proteins in *Caenorhabditis elegans*: in vivo localization, ligand
758 binding and structural properties. *BMC biochemistry*, 11(1), 1-15.
- 759 29. Birnby, D. A., Link, E. M., Vowels, J. J., Tian, H., Colacurcio, P. L., & Thomas, J. H.
760 (2000). A transmembrane guanylyl cyclase (DAF-11) and Hsp90 (DAF-21) regulate a
761 common set of chemosensory behaviors in *Caenorhabditis elegans*. *Genetics*, 155(1),
762 85-104.
- 763 30. Chávez, V., Mohri-Shiomi, A., Maadani, A., Vega, L. A., & Garsin, D. A. (2007).
764 Oxidative stress enzymes are required for DAF-16-mediated immunity due to
765 generation of reactive oxygen species by *Caenorhabditis elegans*. *Genetics*, 176(3),
766 1567-1577.
- 767 31. Sun, J., Zhang, Y., Xu, T., Zhang, Y., Mu, H., Zhang, Y., ... & Qian, P. Y. (2017).
768 Adaptation to deep-sea chemosynthetic environments as revealed by mussel genomes.
769 *Nature Ecology & Evolution*, 1(5), 1-7.
- 770 32. Hinzke, T., Kleiner, M., Breusing, C., Felbeck, H., Häsler, R., Sievert, S. M., ... &
771 Markert, S. (2019). Host-microbe interactions in the chemosynthetic *Riftia pachyptila*
772 symbiosis. *Mbio*, 10(6), e02243-19.
- 773 33. Yuen, B., Polzin, J., & Petersen, J. M. (2019). Organ transcriptomes of the lucinid clam
774 *Loripes orbiculatus* (Poli, 1791) provide insights into their specialized roles in the
775 biology of a chemosymbiotic bivalve. *BMC genomics*, 20(1), 1-14.
- 776 34. Woyke, T., Teeling, H., Ivanova, N. N., Huntemann, M., Richter, M., Gloeckner, F. O.,
777 ... & Dubilier, N. (2006). Symbiosis insights through metagenomic analysis of a
778 microbial consortium. *Nature*, 443(7114), 950-955.
- 779 35. Dubilier, N., Bergin, C., & Lott, C. (2008). Symbiotic diversity in marine animals: the art
780 of harnessing chemosynthesis. *Nature Reviews Microbiology*, 6(10), 725-740.
- 781 36. Wippler, J., Kleiner, M., Lott, C., Gruhl, A., Abraham, P. E., Giannone, R. J., ... &
782 Dubilier, N. (2016). Transcriptomic and proteomic insights into innate immunity and
783 adaptations to a symbiotic lifestyle in the gutless marine worm *Olavius algarvensis*.
784 *BMC genomics*, 17(1), 1-19.
- 785 37. Zimmermann, J., Wentrup, C., Sadowski, M., Blazejak, A., Gruber-Vodicka, H. R.,
786 Kleiner, M., ... & Dubilier, N. (2016). Closely coupled evolutionary history of ecto-and
787 endosymbionts from two distantly related animal phyla. *Molecular ecology*, 25(13),
788 3203-3223.
- 789 38. Naylor, D. J., Hoogenraad, N. J., & Høj, P. B. (1996). Isolation and characterisation of a
790 cDNA encoding rat mitochondrial GrpE, a stress-inducible nucleotide-exchange factor
791 of ubiquitous appearance in mammalian organs. *FEBS letters*, 396(2-3), 181-188.

- 792 39. Lundin, V. F., Srayko, M., Hyman, A. A., & Leroux, M. R. (2008). Efficient chaperone-
793 mediated tubulin biogenesis is essential for cell division and cell migration in *C.*
794 *elegans*. *Developmental biology*, 313(1), 320-334.
- 795 40. Bar-Lavan, Y., Shemesh, N., Dror, S., Ofir, R., Yeger-Lotem, E., & Ben-Zvi, A. (2016).
796 A differentiation transcription factor establishes muscle-specific proteostasis in
797 *Caenorhabditis elegans*. *PLoS genetics*, 12(12), e1006531.
- 798 41. Suzuki, N., Inokuma, K., Yasuda, K., & Ishii, N. (1996). Cloning, sequencing and
799 mapping of a manganese superoxide dismutase gene of the nematode *Caenorhabditis*
800 *elegans*. *DNA research*, 3(3), 171-174.
- 801 42. Margis, R., Dunand, C., Teixeira, F. K., & Margis-Pinheiro, M. (2008). Glutathione
802 peroxidase family—an evolutionary overview. *The FEBS journal*, 275(15), 3959-3970.
- 803 43. Oliveira, R. P., Abate, J. P., Dilks, K., Landis, J., Ashraf, J., Murphy, C. T., & Blackwell,
804 T. K. (2009). Condition-adapted stress and longevity gene regulation by *Caenorhabditis*
805 *elegans* SKN-1/Nrf. *Aging cell*, 8(5), 524-541.
- 806 44. Selivanov, V. A., Votyakova, T. V., Zeak, J. A., Trucco, M., Roca, J., & Cascante, M.
807 (2009). Bistability of mitochondrial respiration underlies paradoxical reactive oxygen
808 species generation induced by anoxia. *PLoS computational biology*, 5(12), e1000619.
- 809 45. Semenza, G. L. (1999). Perspectives on oxygen sensing. *Cell*, 98(3), 281-284.
- 810 46. Hashimoto, T., Yonetani, M., & Nakamura, H. (2004). Selective brain hypothermia
811 protects against hypoxic-ischemic injury in newborn rats by reducing hydroxyl radical
812 production. *Kobe Journal of Medical Sciences*, 49(3/4), 83-92.
- 813 47. Borutaite, V., Mildaziene, V., Brown, G. C., & Brand, M. D. (1995). Control and kinetic
814 analysis of ischemia-damaged heart mitochondria: which parts of the oxidative
815 phosphorylation system are affected by ischemia?. *Biochimica et Biophysica Acta*
816 *(BBA)-Molecular Basis of Disease*, 1272(3), 154-158.
- 817 48. Brookes, P. S., Yoon, Y., Robotham, J. L., Anders, M. W., & Sheu, S. S. (2004).
818 Calcium, ATP, and ROS: a mitochondrial love-hate triangle. *American Journal of*
819 *Physiology-Cell Physiology*, 287(4), C817-C833.
- 820 49. Brenner, C., & Moulin, M. (2012). Physiological roles of the permeability transition
821 pore. *Circulation research*, 111(9), 1237-1247.
- 822 50. Hawrysh, P. J., & Buck, L. T. (2013). Anoxia-mediated calcium release through the
823 mitochondrial permeability transition pore silences NMDA receptor currents in turtle
824 neurons. *Journal of Experimental Biology*, 216(23), 4375-4387.
- 825 51. Horwitz, L. D., Fennessey, P. V., Shikes, R. H., & Kong, Y. (1994). Marked reduction in
826 myocardial infarct size due to prolonged infusion of an antioxidant during
827 reperfusion. *Circulation*, 89(4), 1792-1801.

- 828 52. Murphy, E., & Steenbergen, C. (2008). Mechanisms underlying acute protection from
829 cardiac ischemia-reperfusion injury. *Physiological reviews*, 88(2), 581-609.
- 830 53. Fanter, C. E., Lin, Z., Keenan, S. W., Janzen, F. J., Mitchell, T. S., & Warren, D. E.
831 (2020). Development-specific transcriptomic profiling suggests new mechanisms for
832 anoxic survival in the ventricle of overwintering turtles. *Journal of Experimental*
833 *Biology*, 223(4), jeb213918.
- 834 54. Chatenay-Lapointe, M., & Shadel, G. S. (2010). Stressed-out mitochondria get MAD.
835 *Cell metabolism*, 12(6), 559-560.
- 836 55. Heo, J. M., Livnat-Levanon, N., Taylor, E. B., Jones, K. T., Dephoure, N., Ring, J., ... &
837 Rutter, J. (2010). A stress-responsive system for mitochondrial protein degradation.
838 *Molecular cell*, 40(3), 465-480.
- 839 56. Tullet, J. M. (2015). DAF-16 target identification in *C. elegans*: past, present and
840 future. *Biogerontology*, 16(2), 221-234.
- 841 57. Kaushal, P. S., Sharma, M. R., Booth, T. M., Haque, E. M., Tung, C. S., Sanbonmatsu,
842 K. Y., ... & Agrawal, R. K. (2014). Cryo-EM structure of the small subunit of the
843 mammalian mitochondrial ribosome. *Proceedings of the National Academy of*
844 *Sciences*, 111(20), 7284-7289.
- 845 58. Sharika, R., Subbaiah, P., & Balamurugan, K. (2018). Studies on reproductive stress
846 caused by candidate Gram positive and Gram negative bacteria using model organism,
847 *Caenorhabditis elegans*. *Gene*, 649, 113-126.
- 848 59. Melnikov S, Ben-Shem A, Garreau de Loubresse N, Jenner L, Yusupova G, Yusupov
849 M. One core, two shells: bacterial and eukaryotic ribosomes. *Nat Struct Mol*
850 *Biol*. 2012;19(6):560–567
- 851 60. You, K. T., Park, J., & Kim, V. N. (2015). Role of the small subunit processome in the
852 maintenance of pluripotent stem cells. *Genes & development*, 29(19), 2004-2009.
- 853 61. Chen, J., & Kastan, M. B. (2010). 5'-3'-UTR interactions regulate p53 mRNA translation
854 and provide a target for modulating p53 induction after DNA damage. *Genes &*
855 *development*, 24(19), 2146-2156.
- 856 62. Savada, R. P., & Bonham-Smith, P. C. (2014). Differential transcript accumulation and
857 subcellular localization of *Arabidopsis* ribosomal proteins. *Plant Science*, 223, 134-145.
- 858 63. Xu, X., Xiong, X., & Sun, Y. (2016). The role of ribosomal proteins in the regulation of
859 cell proliferation, tumorigenesis, and genomic integrity. *Science China Life*
860 *Sciences*, 59(7), 656-672.
- 861 64. Larade, K., Nimigan, A., & Storey, K. B. (2001). Transcription pattern of ribosomal
862 protein L26 during anoxia exposure in *Littorina littorea*. *Journal of Experimental*
863 *Zoology*, 290(7), 759-768.

- 864 65. Thomas, G. (2000). An encore for ribosome biogenesis in the control of cell
865 proliferation. *Nature cell biology*, 2(5), E71-E72.
- 866 66. Shukla, S. K., & Kumar, V. (2012). Hepatitis B virus X protein and c-Myc cooperate in
867 the upregulation of ribosome biogenesis and in cellular transformation. *The FEBS*
868 *journal*, 279(20), 3859-3871.
- 869 67. Xu, C., Hwang, W., Jeong, D. E., Ryu, Y., Ha, C. M., Lee, S. J. V., ... & He, Z. M.
870 (2018). Genetic inhibition of an ATP synthase subunit extends lifespan in *C. elegans*.
871 *Scientific reports*, 8(1), 1-14.
- 872 68. Maglioni, S., & Ventura, N. (2016). *C. elegans* as a model organism for human
873 mitochondrial associated disorders. *Mitochondrion*, 30, 117-125.
- 874 69. McKay, R. M., McKay, J. P., Avery, L., & Graff, J. M. (2003). *C. elegans*: a model for
875 exploring the genetics of fat storage. *Developmental cell*, 4(1), 131-142.
- 876 70. Rea, S. L., Ventura, N., & Johnson, T. E. (2007). Relationship between mitochondrial
877 electron transport chain dysfunction, development, and life extension in *Caenorhabditis*
878 *elegans*. *PLoS biology*, 5(10), e259.
- 879 71. Hartman, P. S., Ishii, N., Kayser, E. B., Morgan, P. G., & Sedensky, M. M. (2001).
880 Mitochondrial mutations differentially affect aging, mutability and anesthetic sensitivity
881 in *Caenorhabditis elegans*. *Mechanisms of ageing and development*, 122(11), 1187-
882 1201.
- 883 72. Williams, J. C., Sue, C., Banting, G. S., Yang, H., Glerum, D. M., Hendrickson, W. A., &
884 Schon, E. A. (2005). Crystal structure of human SCO1: implications for redox signaling
885 by a mitochondrial cytochrome c oxidase "assembly" protein. *Journal of Biological*
886 *Chemistry*, 280(15), 15202-15211.
- 887 73. Buceta, P. M. R., Romanelli-Cedrez, L., Babcock, S. J., Xun, H., VonPaige, M. L.,
888 Higley, T. W., ... & Salinas, G. (2019). The kynurenine pathway is essential for
889 rholoquinone biosynthesis in *Caenorhabditis elegans*. *Journal of Biological*
890 *Chemistry*, 294(28), 11047-11053.
- 891 74. Del Borrello, S., Lautens, M., Dolan, K., Tan, J. H., Davie, T., Schertzberg, M. R., ... &
892 Fraser, A. G. (2019). Rholoquinone biosynthesis in *C. elegans* requires precursors
893 generated by the kynurenine pathway. *Elife*, 8, e48165.
- 894 75. Tsai, P. C., Soong, B. W., Mademan, I., Huang, Y. H., Liu, C. R., Hsiao, C. T., ... & Lee,
895 Y. C. (2017). A recurrent WARS mutation is a novel cause of autosomal dominant distal
896 hereditary motor neuropathy. *Brain*, 140(5), 1252-1266.
- 897 76. Tan, J. H., Lautens, M., Romanelli-Cedrez, L., Wang, J., Schertzberg, M. R., Reinl, S.
898 R., ... & Salinas, G. (2020). Alternative splicing of *coq-2* controls the levels of
899 rholoquinone in animals. *Elife*, 9, e56376.

- 900 77. Smolková, K., & Ježek, P. (2012). The role of mitochondrial NADPH-dependent
901 isocitrate dehydrogenase in cancer cells. *International journal of cell biology*, 2012.
- 902 78. Martínez-Reyes, I., & Chandel, N. S. (2020). Mitochondrial TCA cycle metabolites
903 control physiology and disease. *Nature communications*, 11(1), 1-11.
- 904 79. Penkov, S., Kaptan, D., Erkut, C., Sarov, M., Mende, F., & Kurzchalia, T. V. (2015).
905 Integration of carbohydrate metabolism and redox state controls dauer larva formation
906 in *Caenorhabditis elegans*. *Nature communications*, 6(1), 1-10.
- 907 80. Yang, H. C., Yu, H., Liu, Y. C., Chen, T. L., Stern, A., Lo, S. J., & Chiu, D. T. Y. (2019).
908 IDH-1 deficiency induces growth defects and metabolic alterations in GSPD-1-deficient
909 *Caenorhabditis elegans*. *Journal of Molecular Medicine*, 97(3), 385-396.
- 910 81. Lutz, P. L., & Nilsson, G. E. (1997). Contrasting strategies for anoxic brain survival--
911 glycolysis up or down. *The Journal of experimental biology*, 200(2), 411-419.
- 912 82. Semenza, G. L. (2001). HIF-1, O₂, and the 3 PHDs: how animal cells signal hypoxia to
913 the nucleus. *Cell*, 107(1), 1-3.
- 914 83. Huang, S., Colmer, T. D., & Millar, A. H. (2008). Does anoxia tolerance involve altering
915 the energy currency towards P_{Pi}? *Trends in plant science*, 13(5), 221-227.
- 916 84. Larade, K., & Storey, K. B. (2009). Living without oxygen: anoxia-responsive gene
917 expression and regulation. *Current Genomics*, 10(2), 76-85.
- 918 85. Jackson, A. D., & McLaughlin, J. (2009). Digestion and absorption. *Surgery*
919 (*oxford*), 27(6), 231-236.
- 920 86. Lodish, H., Berk, A., Kaiser, C. A., Kaiser, C., Krieger, M., Scott, M. P., ... & Matsudaira,
921 P. (2008). *Molecular cell biology*. Macmillan.
- 922 87. Papaevgeniou, N., & Chondrogianni, N. (2014). The ubiquitin proteasome system in
923 *Caenorhabditis elegans* and its regulation. *Redox biology*, 2, 333-347.
- 924 88. Jones, D., Crowe, E., Stevens, T. A., & Candido, E. P. M. (2001). Functional and
925 phylogenetic analysis of the ubiquitylation system in *Caenorhabditis elegans*: ubiquitin-
926 conjugating enzymes, ubiquitin-activating enzymes, and ubiquitin-like
927 proteins. *Genome biology*, 3(1), 1-15.
- 928 89. Schaefer, H., & Rongo, C. (2006). KEL-8 is a substrate receptor for CUL3-dependent
929 ubiquitin ligase that regulates synaptic glutamate receptor turnover. *Molecular biology*
930 *of the cell*, 17(3), 1250-1260.
- 931 90. Stogios, P. J., Downs, G. S., Jauhal, J. J., Nandra, S. K., & Privé, G. G. (2005).
932 Sequence and structural analysis of BTB domain proteins. *Genome biology*, 6(10),
- 933 91. Kim, K. W., Tang, N. H., Piggott, C. A., Andrusiak, M. G., Park, S., Zhu, M., ... & Jin, Y.
934 (2018). Expanded genetic screening in *Caenorhabditis elegans* identifies new
935 regulators and an inhibitory role for NAD⁺ in axon regeneration. *Elife*, 7, e39756.

- 936 92. Pintard, L., Kurz, T., Glaser, S., Willis, J. H., Peter, M., & Bowerman, B. (2003).
937 Neddylolation and deneddylolation of CUL-3 is required to target MEI-1/Katanin for
938 degradation at the meiosis-to-mitosis transition in *C. elegans*. *Current Biology*, 13(11),
939 911-921.
- 940 93. Brockway, H., Balukoff, N., Dean, M., Alleva, B., & Smolikove, S. (2014). The
941 CSN/COP9 signalosome regulates synaptonemal complex assembly during meiotic
942 prophase I of *Caenorhabditis elegans*. *PLoS genetics*, 10(11), e1004757.
- 943 94. Fraser, A. G., Kamath, R. S., Zipperlen, P., Martinez-Campos, M., Sohrmann, M., &
944 Ahringer, J. (2000). Functional genomic analysis of *C. elegans* chromosome I by
945 systematic RNA interference. *Nature*, 408(6810), 325-330.
- 946 95. Blumenthal, T., Evans, D., Link, C. D., Guffanti, A., Lawson, D., Thierry-Mieg, J., ... &
947 Kim, S. K. (2002). A global analysis of *Caenorhabditis elegans*
948 operons. *Nature*, 417(6891), 851-854.
- 949 96. Syntichaki, P., Xu, K., Driscoll, M., & Tavernarakis, N. (2002). Specific aspartyl and
950 calpain proteases are required for neurodegeneration in *C. elegans*. *Nature*, 419(6910),
951 939-944.
- 952 97. Vassalli, J. D., Sappino, A. P., & Belin, D. (1991). The plasminogen activator/plasmin
953 system. *The Journal of clinical investigation*, 88(4), 1067-1072.
- 954 98. Altincicek, B., Fischer, M., Fischer, M., Lüersen, K., Boll, M., Wenzel, U., & Vilcinskas,
955 A. (2010). Role of matrix metalloproteinase ZMP-2 in pathogen resistance and
956 development in *Caenorhabditis elegans*. *Developmental & Comparative Immunology*,
957 34(11), 1160-1169.
- 958 99. Fischer, M., Fitzenberger, E., Kull, R., Boll, M., & Wenzel, U. (2014). The zinc matrix
959 metalloproteinase ZMP-2 increases survival of *Caenorhabditis elegans* through
960 interference with lipoprotein absorption. *Genes & nutrition*, 9(4), 414.
- 961 100. Vabulas, R. M., & Hartl, F. U. (2005). Protein synthesis upon acute nutrient
962 restriction relies on proteasome function. *Science*, 310(5756), 1960-1963.
- 963 101. Scott, R. C., Schuldiner, O., & Neufeld, T. P. (2004). Role and regulation of
964 starvation-induced autophagy in the *Drosophila* fat body. *Developmental cell*, 7(2), 167-
965 178.
- 966 102. Huber, L. A., & Teis, D. (2016). Lysosomal signaling in control of degradation
967 pathways. *Current opinion in cell biology*, 39, 8-14.
- 968 103. Wang, R. C., & Levine, B. (2010). Autophagy in cellular growth control. *FEBS*
969 *letters*, 584(7), 1417-1426.
- 970 104. Russell, R. C., Yuan, H. X., & Guan, K. L. (2014). Autophagy regulation by
971 nutrient signaling. *Cell research*, 24(1), 42-57.

- 972 105. Liang, X. H., Jackson, S., Seaman, M., Brown, K., Kempkes, B., Hibshoosh, H.,
973 & Levine, B. (1999). Induction of autophagy and inhibition of tumorigenesis by beclin
974 1. *Nature*, 402(6762), 672-676.
- 975 106. Bar-Peled, L., Schweitzer, L. D., Zoncu, R., & Sabatini, D. M. (2012). Ragulator
976 is a GEF for the rag GTPases that signal amino acid levels to mTORC1. *Cell*, 150(6),
977 1196-1208.
- 978 107. Meléndez, A., Tallóczy, Z., Seaman, M., Eskelinen, E. L., Hall, D. H., & Levine,
979 B. (2003). Autophagy genes are essential for dauer development and life-span
980 extension in *C. elegans*. *Science*, 301(5638), 1387-1391.
- 981 108. Wang, X., & Proud, C. G. (2009). Nutrient control of TORC1, a cell-cycle
982 regulator. *Trends in cell biology*, 19(6), 260-267.
- 983 109. Thompson, A. R., & Vierstra, R. D. (2005). Autophagic recycling: lessons from
984 yeast help define the process in plants. *Current opinion in plant biology*, 8(2), 165-173.
- 985 110. Ladevaia, V., Liu, R., & Proud, C. G. (2014, December). mTORC1 signaling
986 controls multiple steps in ribosome biogenesis. In *Seminars in cell & developmental*
987 *biology* (Vol. 36, pp. 113-120). Academic Press.
- 988 111. Ma, X. M., & Blenis, J. (2009). Molecular mechanisms of mTOR-mediated
989 translational control. *Nature reviews Molecular cell biology*, 10(5), 307-318.
- 990 112. Howell, J. J., & Manning, B. D. (2011). mTOR couples cellular nutrient sensing
991 to organismal metabolic homeostasis. *Trends in Endocrinology & Metabolism*, 22(3),
992 94-102.
- 993 113. Nussbaumer, A.D., Bright, M., Baranyi, C., Beisser, C.J., and Ott, J.A. (2004)
994 Attachment mechanism in a highly specific association between ectosymbiotic bacteria
995 and marine nematodes. *Aquat Microb Ecol* 34:239–246
- 996 114. Bulgheresi S, Schabussova I, Chen T, Mullin NP, Maizels RM, Ott JA. A new C-
997 type lectin similar to the human immunoreceptor DC-SIGN mediates symbiont
998 acquisition by a marine nematode. *Appl Environ Microbiol*. 2006;72:2950–2956
- 999 115. Bulgheresi S, Gruber-Vodicka HR, Heindl NR, Dirks U, Kostadinova M,
1000 Breiteneder H, Ott JA. Sequence variability of the pattern recognition receptor Mermaid
1001 mediates specificity of marine nematode symbioses. *ISME J*. 2011;5:986–998
- 1002 116. Koropatkin, N. M., Cameron, E. A., & Martens, E. C. (2012). How glycan
1003 metabolism shapes the human gut microbiota. *Nature Reviews Microbiology*, 10(5),
1004 323-335.
- 1005 117. Simon, H. U., Haj-Yehia, A., & Levi-Schaffer, F. (2000). Role of reactive oxygen
1006 species (ROS) in apoptosis induction. *Apoptosis*, 5(5), 415-418.
- 1007 118. Martinou, J. C., Desagher, S., & Antonsson, B. (2000). Cytochrome c release
1008 from mitochondria: all or nothing. *Nature cell biology*, 2(3), E41-E43.

- 1009 119. Gogvadze, V., Orrenius, S., & Zhivotovsky, B. (2006). Multiple pathways of
1010 cytochrome c release from mitochondria in apoptosis. *Biochimica et Biophysica Acta*
1011 *(BBA)-Bioenergetics*, 1757(5-6), 639-647.
- 1012 120. Mangahas, P. M., & Zhou, Z. (2005, April). Clearance of apoptotic cells in
1013 *Caenorhabditis elegans*. In *Seminars in cell & developmental biology* (Vol. 16, No. 2,
1014 pp. 295-306). Academic Press.
- 1015 121. Kaufmann, S. H., Lee, S. H., Meng, X. W., Loegering, D. A., Kottke, T. J.,
1016 Henzing, A. J., ... & Earnshaw, W. C. (2008). Apoptosis-associated caspase activation
1017 assays. *Methods*, 44(3), 262-272.
- 1018 122. Liu, X., Kim, C. N., Yang, J., Jemmerson, R., & Wang, X. (1996). Induction of
1019 apoptotic program in cell-free extracts: requirement for dATP and cytochrome
1020 c. *Cell*, 86(1), 147-157.
- 1021 123. Tafani, M., Schneider, T. G., Pastorino, J. G., & Farber, J. L. (2000).
1022 Cytochrome c-dependent activation of caspase-3 by tumor necrosis factor requires
1023 induction of the mitochondrial permeability transition. *The American journal of*
1024 *pathology*, 156(6), 2111-2121.
- 1025 124. Takacs-Vellai, K., Vellai, T., Puoti, A., Passannante, M., Wicky, C., Streit, A., ...
1026 & Müller, F. (2005). Inactivation of the autophagy gene bec-1 triggers apoptotic cell
1027 death in *C. elegans*. *Current biology*, 15(16), 1513-1517.
- 1028 125. Wang, X., Li, W., Zhao, D., Liu, B., Shi, Y., Chen, B., ... & Xue, D. (2010).
1029 *Caenorhabditis elegans* transthyretin-like protein TTR-52 mediates recognition of
1030 apoptotic cells by the CED-1 phagocyte receptor. *Nature cell biology*, 12(7), 655-664.
- 1031 126. Chen, Y. Z., Mapes, J., Lee, E. S., Skeen-Gaar, R. R., & Xue, D. (2013).
1032 Caspase-mediated activation of *Caenorhabditis elegans* CED-8 promotes apoptosis
1033 and phosphatidylserine externalization. *Nature communications*, 4(1), 1-9.
- 1034 127. Parrish, J. Z., & Xue, D. (2003). Functional genomic analysis of apoptotic DNA
1035 degradation in *C. elegans*. *Molecular cell*, 11(4), 987-996.
- 1036 128. Samejima, K., & Earnshaw, W. C. (2005). Trashing the genome: the role of
1037 nucleases during apoptosis. *Nature reviews Molecular cell biology*, 6(9), 677-688.
- 1038 129. Sasaki, A., Nakae, I., Nagasawa, M., Hashimoto, K., Abe, F., Saito, K., ... &
1039 Kontani, K. (2013). Arl8/ARL-8 functions in apoptotic cell removal by mediating
1040 phagolysosome formation in *Caenorhabditis elegans*. *Molecular biology of the*
1041 *cell*, 24(10), 1584-1592.
- 1042 130. Hurwitz, M. E., Vanderzalm, P. J., Bloom, L., Goldman, J., Garriga, G., &
1043 Horvitz, H. R. (2009). Abl kinase inhibits the engulfment of apoptotic cells in
1044 *Caenorhabditis elegans*. *PLoS biology*, 7(4), e1000099.

- 1045 131. Berdichevsky, A., Nedelcu, S., Boulias, K., Bishop, N. A., Guarente, L., &
1046 Horvitz, H. R. (2010). 3-Ketoacyl thiolase delays aging of *Caenorhabditis elegans* and
1047 is required for lifespan extension mediated by sir-2.1. *Proceedings of the National*
1048 *Academy of Sciences*, 107(44), 18927-18932.
- 1049 132. Chughtai, A. A., Kaššák, F., Kostrouchová, M., Novotný, J. P., Krause, M. W.,
1050 Saudek, V., ... & Kostrouchová, M. (2015). Perilipin-related protein regulates lipid
1051 metabolism in *C. elegans*. *PeerJ*, 3, e1213.
- 1052 133. Horikawa, M., & Sakamoto, K. (2009). Fatty-acid metabolism is involved in
1053 stress-resistance mechanisms of *Caenorhabditis elegans*. *Biochemical and biophysical*
1054 *research communications*, 390(4), 1402-1407.
- 1055 134. Krivoruchko, A., & Storey, K. B. (2015). Turtle anoxia tolerance: biochemistry
1056 and gene regulation. *Biochimica et Biophysica Acta (BBA)-General Subjects*, 1850(6),
1057 1188-1196.
- 1058 135. Brendza, K. M., Haakenson, W., Cahoon, R. E., Hicks, L. M., Palavalli, L. H.,
1059 Chiapelli, B. J., ... & Jez, J. M. (2007). Phosphoethanolamine N-methyltransferase
1060 (PMT-1) catalyses the first reaction of a new pathway for phosphocholine biosynthesis
1061 in *Caenorhabditis elegans*. *Biochemical Journal*, 404(3), 439-448.
- 1062 136. Thomas, J. H. (1990). Genetic analysis of defecation in *Caenorhabditis*
1063 *elegans*. *Genetics*, 124(4), 855-872.
- 1064 137. McIntire, S. L., Jorgensen, E., Kaplan, J., & Horvitz, H. R. (1993). The
1065 GABAergic nervous system of *Caenorhabditis elegans*. *Nature*, 364(6435), 337-341.
- 1066 138. Jin, Y., Jorgensen, E., Hartwig, E., & Horvitz, H. R. (1999). The *Caenorhabditis*
1067 *elegans* gene *unc-25* encodes glutamic acid decarboxylase and is required for synaptic
1068 transmission but not synaptic development. *Journal of Neuroscience*, 19(2), 539-548.
- 1069 139. Gally, C., & Bessereau, J. L. (2003). GABA is dispensable for the formation of
1070 junctional GABA receptor clusters in *Caenorhabditis elegans*. *Journal of*
1071 *Neuroscience*, 23(7), 2591-2599.
- 1072 140. Nordquist, S. K., Smith, S. R., & Pierce, J. T. (2018). Systematic functional
1073 characterization of human 21st chromosome orthologs in *Caenorhabditis elegans*. *G3:*
1074 *Genes, Genomes, Genetics*, 8(3), 967-979.
- 1075 141. Risley, M. G., Kelly, S. P., Jia, K., Grill, B., & Dawson-Scully, K. (2016).
1076 Modulating behavior in *C. elegans* using electroshock and antiepileptic drugs. *PLoS*
1077 *One*, 11(9), e0163786.
- 1078 142. Martin, D. L., & Rimvall, K. (1993). Regulation of γ -aminobutyric acid synthesis
1079 in the brain. *Journal of neurochemistry*, 60(2), 395-407.
- 1080 143. van der Vos KE, Coffey PJ. Glutamine metabolism links growth factor signaling
1081 to the regulation of autophagy. *Autophagy*. 2012;8:1862–1864

- 1082 144. Yen, C. A., & Curran, S. P. (2021). Incomplete proline catabolism drives
1083 premature sperm aging. *Aging cell*, 20(2), e13308.
- 1084 145. Tharmalingam, S., Burns, A. R., Roy, P. J., & Hampson, D. R. (2012).
1085 Orthosteric and allosteric drug binding sites in the *Caenorhabditis elegans mgl-2*
1086 metabotropic glutamate receptor. *Neuropharmacology*, 63(4), 667-674.
- 1087 146. Baker, A. J., Zornow, M. H., Scheller, M. S., Yaksh, T. L., Skilling, S. R.,
1088 Smullin, D. H., ... & Kuczenski, R. (1991). Changes in extracellular concentrations of
1089 glutamate, aspartate, glycine, dopamine, serotonin, and dopamine metabolites after
1090 transient global ischemia in the rabbit brain. *Journal of neurochemistry*, 57(4), 1370-
1091 1379.
- 1092 147. Lutz, P. L., Nilsson, G. E., & Prentice, H. M. (2003a). The brain without oxygen:
1093 causes of failure-physiological and molecular mechanisms for survival. Springer
1094 Science & Business Media.
- 1095 148. Milton, S. L., Thompson, J. W., & Lutz, P. L. (2002). Mechanisms for
1096 maintaining extracellular glutamate levels in the anoxic turtle striatum. *American*
1097 *Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 282(5),
1098 R1317-R1323.
- 1099 149. Mathews, G. C., & Diamond, J. S. (2003). Neuronal glutamate uptake
1100 contributes to GABA synthesis and inhibitory synaptic strength. *Journal of*
1101 *Neuroscience*, 23(6), 2040-2048.
- 1102 150. Milton, S. L., & Lutz, P. L. (1998). Low extracellular dopamine levels are
1103 maintained in the anoxic turtle (*Trachemys scripta*) striatum. *Journal of Cerebral Blood*
1104 *Flow & Metabolism*, 18(7), 803-807.
- 1105 151. Lutz, P. L., Prentice, H. M., & Milton, S. L. (2003b). Is turtle longevity linked to
1106 enhanced mechanisms for surviving brain anoxia and reoxygenation?. *Experimental*
1107 *Gerontology*, 38(7), 797-800.
- 1108 152. Nilsson, G. E. (1990). Long-term anoxia in crucian carp: changes in the levels of
1109 amino acid and monoamine neurotransmitters in the brain, catecholamines in
1110 chromaffin tissue, and liver glycogen. *Journal of Experimental Biology*, 150(1), 295-320.
- 1111 153. Schuske, K., Beg, A. A., & Jorgensen, E. M. (2004). The GABA nervous system
1112 in *C. elegans*. *Trends in neurosciences*, 27(7), 407-414.
- 1113 154. Sawin, E. R., Ranganathan, R., & Horvitz, H. R. (2000). *C. elegans* locomotory
1114 rate is modulated by the environment through a dopaminergic pathway and by
1115 experience through a serotonergic pathway. *Neuron*, 26(3), 619-631.
- 1116 155. Sanyal, S., Wintle, R. F., Kindt, K. S., Nuttley, W. M., Arvan, R., Fitzmaurice, P.,
1117 ... & Van Tol, H. H. (2004). Dopamine modulates the plasticity of mechanosensory
1118 responses in *Caenorhabditis elegans*. *The EMBO journal*, 23(2), 473-482.

- 1119 156. Gainetdinov, R. R., Sotnikova, T. D., & Caron, M. G. (2002). Monoamine
1120 transporter pharmacology and mutant mice. *Trends in pharmacological sciences*, 23(8),
1121 367-373.
- 1122 157. McDonald, P. W., Jessen, T., Field, J. R., & Blakely, R. D. (2006). Dopamine
1123 signaling architecture in *Caenorhabditis elegans*. *Cellular and molecular neurobiology*,
1124 26(4), 591-616.
- 1125 158. Soontornniyomkij, V., Risbrough, V. B., Young, J. W., Soontornniyomkij, B.,
1126 Jeste, D. V., & Achim, C. L. (2012). Hippocampal calbindin-1 immunoreactivity correlate
1127 of recognition memory performance in aged mice. *Neuroscience letters*, 516(1), 161-
1128 165.
- 1129 159. Hobert, O. (2018). The neuronal genome of *Caenorhabditis elegans*.
1130 WormBook: The Online Review of *C. elegans* Biology
- 1131 160. Steger, K. A., Shtonda, B. B., Thacker, C., Snutch, T. P., & Avery, L. (2005).
1132 The *C. elegans* T-type calcium channel CCA-1 boosts neuromuscular transmission.
1133 *Journal of Experimental Biology*, 208(11), 2191-2203.
- 1134 161. Zhou, K., Cherra III, S. J., Goncharov, A., & Jin, Y. (2017). Asynchronous
1135 cholinergic drive correlates with excitation-inhibition imbalance via a neuronal Ca²⁺
1136 sensor protein. *Cell reports*, 19(6), 1117-1129.
- 1137 162. Bickler, P. E. (1992). Cerebral anoxia tolerance in turtles: regulation of
1138 intracellular calcium and pH. *American Journal of Physiology-Regulatory, Integrative
1139 and Comparative Physiology*, 263(6), R1298-R1302.
- 1140 163. Dell'Anna, E., Geloso, M. C., Magarelli, M., & Molinari, M. (1996). Development
1141 of GABA and calcium binding proteins immunoreactivity in the rat hippocampus
1142 following neonatal anoxia. *Neuroscience letters*, 211(2), 93-96.
- 1143 164. Akira, S., Uematsu, S., & Takeuchi, O. (2006). Pathogen recognition and innate
1144 immunity. *Cell*, 124(4), 783-801.
- 1145 165. Ott et al., 2021. BOOK CHAPTER
- 1146 166. Wang, D. (2019). Epidermal Barrier for Nematodes Against Toxicity of
1147 Environmental Toxicants or Stresses. In *Target Organ Toxicology in Caenorhabditis
1148 elegans* (pp. 97-122). Springer, Singapore.
- 1149 167. Dravid, P., Kaushal, D. C., Saxena, J. K., & Kaushal, N. A. (2015). Isolation and
1150 characterization of endochitinase and exochitinase of *Setaria cervi*. *Parasitology
1151 international*, 64(6), 579-586.
- 1152 168. Krasity, B. C., Troll, J. V., Lehnert, E. M., Hackett, K. T., Dillard, J. P., Apicella,
1153 M. A., ... & McFall-Ngai, M. J. (2015). Structural and functional features of a
1154 developmentally regulated lipopolysaccharide-binding protein. *MBio*, 6(5), e01193-15.

- 1155 169. Chen, F., Krasity, B. C., Peyer, S. M., Koehler, S., Ruby, E. G., Zhang, X., &
1156 McFall-Ngai, M. J. (2017). MBio.
- 1157 170. Pradel, E., Zhang, Y., Pujol, N., Matsuyama, T., Bargmann, C. I., & Ewbank, J.
1158 J. (2007). Detection and avoidance of a natural product from the pathogenic bacterium
1159 *Serratia marcescens* by *Caenorhabditis elegans*. Proceedings of the National Academy
1160 of Sciences, 104(7), 2295-2300.
- 1161 171. Brandt, J. P., & Ringstad, N. (2015). Toll-like receptor signaling promotes
1162 development and function of sensory neurons required for a *C. elegans* pathogen-
1163 avoidance behavior. Current Biology, 25(17), 2228-2237.
- 1164 172. Dang, H., & Lovell, C. R. (2016). Microbial surface colonization and biofilm
1165 development in marine environments. Microbiology and molecular biology reviews,
1166 80(1), 91-138.
- 1167 173. Suzuki, M., Sagoh, N., Iwasaki, H., Inoue, H., & Takahashi, K. (2004).
1168 Metalloproteases with EGF, CUB, and thrombospondin-1 domains function in molting of
1169 *Caenorhabditis elegans*.
- 1170 174. Zhang, Y., Foster, J. M., Nelson, L. S., Ma, D., & Carlow, C. K. (2005). The
1171 chitin synthase genes *chs-1* and *chs-2* are essential for *C. elegans* development and
1172 responsible for chitin deposition in the eggshell and pharynx, respectively.
1173 Developmental biology, 285(2), 330-339.
- 1174 175. Zugasti, O., Rajan, J., & Kuwabara, P. E. (2005). The function and expansion of
1175 the Patched-and Hedgehog-related homologs in *C. elegans*. Genome research, 15(10),
1176 1402-1410.
- 1177 176. Hornsten, A., Lieberthal, J., Fadia, S., Malins, R., Ha, L., Xu, X., ... & Li, C.
1178 (2007). APL-1, a *Caenorhabditis elegans* protein related to the human β -amyloid
1179 precursor protein, is essential for viability. Proceedings of the National Academy of
1180 Sciences, 104(6), 1971-1976.
- 1181 177. Russel, S., Frand, A. R., & Ruvkun, G. (2011). Regulation of the *C. elegans*
1182 molt by *pqn-47*. Developmental biology, 360(2), 297-309.
- 1183 178. Pan, K. Z., Palter, J. E., Rogers, A. N., Olsen, A., Chen, D., Lithgow, G. J., &
1184 Kapahi, P. (2007). Inhibition of mRNA translation extends lifespan in *Caenorhabditis*
1185 *elegans*. Aging cell, 6(1), 111-119.
- 1186 179. Pal, S., Lant, B., Yu, B., Tian, R., Tong, J., Krieger, J. R., ... & Derry, W. B.
1187 (2017). CCM-3 promotes *C. elegans* germline development by regulating vesicle
1188 trafficking cytokinesis and polarity. Current Biology, 27(6), 868-876.
- 1189 180. Park, B. J., Lee, D. G., Yu, J. R., Jung, S. K., Choi, K., Lee, J., ... & Ahnn, J.
1190 (2001). Calreticulin, a calcium-binding molecular chaperone, is required for stress

- 1191 response and fertility in *Caenorhabditis elegans*. *Molecular Biology of the Cell*, 12(9),
1192 2835-2845.
- 1193 181. Clark, S. G., Shurland, D. L., Meyerowitz, E. M., Bargmann, C. I., & Van Der
1194 Blik, A. M. (1997). A dynamin GTPase mutation causes a rapid and reversible
1195 temperature-inducible locomotion defect in *C. elegans*. *Proceedings of the National*
1196 *Academy of Sciences*, 94(19), 10438-10443.
- 1197 182. Goedert, M., Baur, C. P., Ahringer, J., Jakes, R., Hasegawa, M., Spillantini, M.
1198 G., ... & Hill, F. (1996). PTL-1, a microtubule-associated protein with tau-like repeats
1199 from the nematode *Caenorhabditis elegans*. *Journal of cell science*, 109(11), 2661-
1200 2672.
- 1201 183. Gatewood, B. K., & Bucher, E. A. (1997). The mup-4 locus in *Caenorhabditis*
1202 *elegans* is essential for hypodermal integrity, organismal morphogenesis and
1203 embryonic body wall muscle position. *Genetics*, 146(1), 165-183.
- 1204 184. Fujii, T., Nakao, F., Shibata, Y., Shioi, G., Kodama, E., Fujisawa, H., & Takagi,
1205 S. (2002). *Caenorhabditis elegans* PlexinA, PLX-1, interacts with transmembrane
1206 semaphorins and regulates epidermal morphogenesis.
- 1207 185. Gally, C., Wissler, F., Zahreddine, H., Quintin, S., Landmann, F., & Labouesse,
1208 M. (2009). Myosin II regulation during *C. elegans* embryonic elongation: LET-
1209 502/ROCK, MRCK-1 and PAK-1, three kinases with different
1210 roles. *Development*, 136(18), 3109-3119.
- 1211 186. Zahreddine, H., Zhang, H., Diogon, M., Nagamatsu, Y., & Labouesse, M.
1212 (2010). CRT-1/calreticulin and the E3 ligase EEL-1/HUWE1 control hemidesmosome
1213 maturation in *C. elegans* development. *Current Biology*, 20(4), 322-327.
- 1214 187. Jee, C., Choi, T. W., Kalichamy, K., Yee, J. Z., Song, H. O., Ji, Y. J., ... & Lee,
1215 S. K. (2012). CNP-1 (ARRD-17), a novel substrate of calcineurin, is critical for
1216 modulation of egg-laying and locomotion in response to food and lysine sensation in
1217 *Caenorhabditis elegans*. *Journal of molecular biology*, 417(3), 165-178.
- 1218 188. Warner, A., Xiong, G., Qadota, H., Rogalski, T., Vogl, A. W., Moerman, D. G., &
1219 Benian, G. M. (2013). CPNA-1, a copine domain protein, is located at integrin adhesion
1220 sites and is required for myofilament stability in *Caenorhabditis elegans*. *Molecular*
1221 *biology of the cell*, 24(5), 601-616.
- 1222 189. Perez, M. F., & Lehner, B. (2019). Vitellogenins-yolk gene function and
1223 regulation in *Caenorhabditis elegans*. *Frontiers in physiology*, 10, 1067.
- 1224 190. Ding, M., Woo, W. M., & Chisholm, A. D. (2004). The cytoskeleton and
1225 epidermal morphogenesis in *C. elegans*. *Experimental cell research*, 301(1), 84-90.

- 1226 191. Osório, D. S., Chan, F. Y., Saramago, J., Leite, J., Silva, A. M., Sobral, A. F., ...
1227 & Carvalho, A. X. (2019). Crosslinking activity of non-muscle myosin II is not sufficient
1228 for embryonic cytokinesis in *C. elegans*. *Development*, 146(21), dev179150.
- 1229 192. Nelson, M. D., Zhou, E., Kiontke, K., Fradin, H., Maldonado, G., Martin, D., ... &
1230 Fitch, D. H. (2011). A bow-tie genetic architecture for morphogenesis suggested by a
1231 genome-wide RNAi screen in *Caenorhabditis elegans*. *PLoS genetics*, 7(3), e1002010.
- 1232 193. Dalpé, G., Zhang, L. W., Zheng, H., & Culotti, J. G. (2004). Conversion of cell
1233 movement responses to Semaphorin-1 and Plexin-1 from attraction to repulsion by
1234 lowered levels of specific RAC GTPases in *C. elegans*.
- 1235 194. Dalpe, G., Tarsitano, M., Persico, M. G., Zheng, H., & Culotti, J. (2013). *C.*
1236 *elegans* PVF-1 inhibits permissive UNC-40 signalling through CED-10 GTPase to
1237 position the male ray 1 sensillum. *Development*, 140(19), 4020-4030.
- 1238 195. Dufourcq, P., Victor, M., Gay, F., Calvo, D., Hodgkin, J., & Shi, Y. (2002).
1239 Functional requirement for histone deacetylase 1 in *Caenorhabditis elegans*
1240 gonadogenesis. *Molecular and cellular biology*, 22(9), 3024-3034.
- 1241 196. Choy, S. W., Wong, Y. M., Ho, S., & Chow, K. L. (2007). *C. elegans* SIN-3 and
1242 its associated HDAC corepressor complex act as mediators of male sensory ray
1243 development. *Biochemical and biophysical research communications*, 358(3), 802-807.
- 1244 197. Park, J. O., Pan, J., Möhrle, F., Schupp, M. O., Johnsen, R., Baillie, D. L., ... &
1245 Hutter, H. (2010). Characterization of the astacin family of metalloproteases in *C.*
1246 *elegans*. *BMC developmental biology*, 10(1), 1-13.
- 1247 198. Topf, U., & Drabikowski, K. (2019). Ancient function of teneurins in tissue
1248 organization and neuronal guidance in the nematode *Caenorhabditis elegans*. *Frontiers*
1249 *in neuroscience*, 13, 205.
- 1250 199. Spanier, B., Stürzenbaum, S. R., Holden-Dye, L. M., & Baumeister, R. (2005).
1251 *Caenorhabditis elegans* neprilysin NEP-1: an effector of locomotion and pharyngeal
1252 pumping. *Journal of molecular biology*, 352(2), 429-437.
- 1253 200. Norman, K. R., Fazzio, R. T., Mellem, J. E., Espelt, M. V., Strange, K., Beckerle,
1254 M. C., & Maricq, A. V. (2005). The Rho/Rac-family guanine nucleotide exchange factor
1255 VAV-1 regulates rhythmic behaviors in *C. elegans*. *Cell*, 123(1), 119-132.
- 1256 201. De Cuyper, C., & Vanfleteren, J. R. (1982). Oxygen consumption during
1257 development and aging of the nematode *Caenorhabditis elegans*. *Comparative*
1258 *Biochemistry and Physiology Part A: Physiology*, 73(2), 283-289.
- 1259 202. Uppaluri, S., & Brangwynne, C. P. (2015). A size threshold governs
1260 *Caenorhabditis elegans* developmental progression. *Proceedings of the Royal Society*
1261 *B: Biological Sciences*, 282(1813), 20151283.

- 1262 203. Pellerone, F. I., Archer, S. K., Behm, C. A., Grant, W. N., Lacey, M. J., &
1263 Somerville, A. C. (2003). Trehalose metabolism genes in *Caenorhabditis elegans* and
1264 filarial nematodes. *International journal for parasitology*, 33(11), 1195-1206.
- 1265 204. Schuster, L. N., & Sommer, R. J. (2012). Expressional and functional variation
1266 of horizontally acquired cellulases in the nematode *Pristionchus pacificus*. *Gene*,
1267 506(2), 274-282.
- 1268 205. Yuan, Y., Kadiyala, C. S., Ching, T. T., Hakimi, P., Saha, S., Xu, H., ... & Feng,
1269 Z. (2012). Enhanced energy metabolism contributes to the extended life span of
1270 calorie-restricted *Caenorhabditis elegans*. *Journal of Biological Chemistry*, 287(37),
1271 31414-31426.
- 1272 206. Kitaoka, S., Morielli, A. D., & Zhao, F. Q. (2013). FGT-1 is a mammalian
1273 GLUT2-like facilitative glucose transporter in *Caenorhabditis elegans* whose
1274 malfunction induces fat accumulation in intestinal cells. *PLoS One*, 8(6), e68475.
- 1275 207. Bertoli, S., Neri, I. G., Trentani, C., Ferraris, C., De Amicis, R., Battezzati, A., ...
1276 & Tagliabue, A. (2015). Short-term effects of ketogenic diet on anthropometric
1277 parameters, body fat distribution, and inflammatory cytokine production in GLUT1
1278 deficiency syndrome. *Nutrition*, 31(7-8), 981-987.
- 1279 208. Miyagawa, K., Sakamoto, H., Yoshida, T., Yamashita, Y., Mitsui, Y., Furusawa,
1280 M.... & Terada, M. (1988). hst-1 transforming protein: expression in silkworm cells and
1281 characterization as a novel heparin-binding growth factor. *Oncogene*, 3(4), 383-389.
- 1282 209. Bhattacharya, R., Townley, R. A., Berry, K. L., & Bülow, H. E. (2009). The
1283 PAPS transporter PST-1 is required for heparan sulfation and is essential for viability
1284 and neural development in *C. elegans*. *Journal of cell science*, 122(24), 4492-4504.
- 1285 210. Crowe, J. H., Crowe, L. M., Carpenter, J. F., & Wistrom, C. A. (1987).
1286 Stabilization of dry phospholipid bilayers and proteins by sugars. *Biochemical Journal*,
1287 242(1), 1.
- 1288 211. Carpenter, J. F., & Crowe, J. H. (1988). Modes of stabilization of a protein by
1289 organic solutes during desiccation. *Cryobiology*, 25(5), 459-470.
- 1290 212. Chen, Q., Ma, E., Behar, K. L., Xu, T., & Haddad, G. G. (2002). Role of
1291 trehalose phosphate synthase in anoxia tolerance and development in *Drosophila*
1292 *melanogaster*. *Journal of Biological Chemistry*, 277(5), 3274-3279.
- 1293 213. Mongan, N. P., Jones, A. K., Smith, G. R., Sansom, M. S., & Sattelle, D. B.
1294 (2002). Novel α 7-like nicotinic acetylcholine receptor subunits in the nematode
1295 *Caenorhabditis elegans*. *Protein Science*, 11(5), 1162-1171.
- 1296 214. Patton, A., Knuth, S., Schaheen, B., Dang, H., Greenwald, I., & Fares, H.
1297 (2005). Endocytosis function of a ligand-gated ion channel homolog in *Caenorhabditis*
1298 *elegans*. *Current biology*, 15(11), 1045-1050.

- 1299 215. Gendrel, M., Rapti, G., Richmond, J. E., & Bessereau, J. L. (2009). A secreted
1300 complement-control-related protein ensures acetylcholine receptor clustering. *Nature*,
1301 461(7266), 992-996.
- 1302 216. Boulin, T., Rapti, G., Briseño-Roa, L., Stigloher, C., Richmond, J. E., Paoletti,
1303 P., & Bessereau, J. L. (2012). Positive modulation of a Cys-loop acetylcholine receptor
1304 by an auxiliary transmembrane subunit. *Nature neuroscience*, 15(10), 1374-1381.
- 1305 217. Chan, J. P., Hu, Z., & Sieburth, D. (2012). Recruitment of sphingosine kinase to
1306 presynaptic terminals by a conserved muscarinic signaling pathway promotes
1307 neurotransmitter release. *Genes & development*, 26(10), 1070-1085.
- 1308 218. Sun, L., Zang, W. J., Wang, H., Zhao, M., Yu, X. J., He, X., ... & Zhou, J. (2014).
1309 Acetylcholine promotes ROS detoxification against hypoxia/reoxygenation-induced
1310 oxidative stress through FoxO3a/PGC-1 α dependent superoxide dismutase. *Cellular*
1311 *Physiology and Biochemistry*, 34(5), 1614-1625.
- 1312 219. Guest, M., Bull, K., Walker, R. J., Amliwala, K., O'Connor, V., Harder, A., ... &
1313 Hopper, N. A. (2007). The calcium-activated potassium channel, SLO-1, is required for
1314 the action of the novel cyclo-octadepsipeptide anthelmintic, emodepside, in
1315 *Caenorhabditis elegans*. *International journal for parasitology*, 37(14), 1577-1588.
- 1316 220. Hurd, D. D., Miller, R. M., Núñez, L., & Portman, D. S. (2010). Specific α -and β -
1317 tubulin isotypes optimize the functions of sensory cilia in *Caenorhabditis elegans*.
1318 *Genetics*, 185(3), 883-896.
- 1319 221. Wang, Z., Hou, Y., Guo, X., van der Voet, M., Boxem, M., Dixon, J. E., ... & Jin,
1320 Y. (2013). The EBAX-type Cullin-RING E3 ligase and Hsp90 guard the protein quality
1321 of the SAX-3/Robo receptor in developing neurons. *Neuron*, 79(5), 903-916.
- 1322 222. Woo, W. M., Berry, E. C., Hudson, M. L., Swale, R. E., Goncharov, A., &
1323 Chisholm, A. D. (2008). The *C. elegans* F-spondin family protein SPON-1 maintains cell
1324 adhesion in neural and non-neural tissues.
- 1325 223. Schwarz, V., Pan, J., Voltmer-Irsch, S., & Hutter, H. (2009). IgCAMs
1326 redundantly control axon navigation in *Caenorhabditis elegans*. *Neural development*,
1327 4(1), 1-15.
- 1328 224. Gu, G. U. O. Q. I. A. N. G., Caldwell, G. A., & Chalfie, M. (1996). Genetic
1329 interactions affecting touch sensitivity in *Caenorhabditis elegans*. *Proceedings of the*
1330 *National Academy of Sciences*, 93(13), 6577-6582.
- 1331 225. Han, L., Wang, Y., Sangaletti, R., D'Urso, G., Lu, Y., Shaham, S., & Bianchi, L.
1332 (2013). Two novel DEG/ENaC channel subunits expressed in glia are needed for nose-
1333 touch sensitivity in *Caenorhabditis elegans*. *Journal of Neuroscience*, 33(3), 936-949.

- 1334 226. Li, Z., Li, Y., Yi, Y., Huang, W., Yang, S., Niu, W., ... & Xu, T. (2012). Dissecting
1335 a central flip-flop circuit that integrates contradictory sensory cues in *C. elegans* feeding
1336 regulation. *Nature communications*, 3(1), 1-8.
- 1337 227. Tsuji, N., Morales, T. H., Ozols, V. V., Carmody, A. B., & Chandrashekar, R.
1338 (1999). Identification of an asparagine amidohydrolase from the filarial parasite
1339 *Dirofilaria immitis*. *International journal for parasitology*, 29(9), 1451-1455.
- 1340 228. Chen, C. C., Lim, C. Y., Lee, P. J., Hsu, A. L., & Ching, T. T. (2020). S-adenosyl
1341 methionine synthetase SAMS-5 mediates dietary restriction-induced longevity in
1342 *Caenorhabditis elegans*. *PloS one*, 15(11), e0241455.
- 1343 229. Russell, D., & Snyder, S. H. (1968). Amine synthesis in rapidly growing tissues:
1344 ornithine decarboxylase activity in regenerating rat liver, chick embryo, and various
1345 tumors. *Proceedings of the National Academy of Sciences of the United States of*
1346 *America*, 60(4), 1420.
- 1347 230. Heby, O. (1981). Role of polyamines in the control of cell proliferation and
1348 differentiation. *Differentiation*, 19(1-3), 1-20.
- 1349 231. Gilad, G. M., & Gilad, V. H. (1991). Polyamines can protect against ischemia-
1350 induced nerve cell death in gerbil forebrain. *Experimental neurology*, 111(3), 349-355.
- 1351 232. Longo, L. D., Packianathan, S., McQueary, J. A., Stagg, R. B., Byus, C. V., &
1352 Cain, C. D. (1993). Acute hypoxia increases ornithine decarboxylase activity and
1353 polyamine concentrations in fetal rat brain. *Proceedings of the National Academy of*
1354 *Sciences*, 90(2), 692-696.
- 1355 233. Ward, J. D., Mullaney, B., Schiller, B. J., He, L. D., Petnic, S. E., Couillault, C.,
1356 ... & Yamamoto, K. R. (2014). Defects in the *C. elegans* acyl-CoA synthase, *acs-3*, and
1357 nuclear hormone receptor, *nhr-25*, cause sensitivity to distinct, but overlapping
1358 stresses. *PloS one*, 9(3), e92552.
- 1359 234. Wang, F., Dai, Y., Zhu, X., Chen, Q., Zhu, H., Zhou, B., ... & Pang, S. (2021).
1360 Saturated very long chain fatty acid configures glycosphingolipid for lysosome
1361 homeostasis in long-lived *C. elegans*. *Nature Communications*, 12(1), 1-14.
- 1362 235. Bastiani, C. A., Gharib, S., Simon, M. I., & Sternberg, P. W. (2003).
1363 *Caenorhabditis elegans* *Gaq* regulates egg-laying behavior via a PLC β -independent
1364 and serotonin-dependent signaling pathway and likely functions both in the nervous
1365 system and in muscle. *Genetics*, 165(4), 1805-1822.
- 1366 236. Taha, T. A., Kitatani, K., El-Alwani, M., Bielawski, J., Hannun, Y. A., & Obeid, L.
1367 M. (2006). Loss of sphingosine kinase-1 activates the intrinsic pathway of programmed
1368 cell death: modulation of sphingolipid levels and the induction of apoptosis. *The FASEB*
1369 *journal*, 20(3), 482-484.

- 1370 237. Deng, X., Yin, X., Allan, R., Lu, D. D., Maurer, C. W., Haimovitz-Friedman, A., ...
1371 & Kolesnick, R. (2008). Ceramide biogenesis is required for radiation-induced
1372 apoptosis in the germ line of *C. elegans*. *Science*, 322(5898), 110-115.
- 1373 238. Menuz, V., Howell, K. S., Gentina, S., Epstein, S., Riezman, I., Fornallaz-
1374 Mulhauser, M., ... & Martinou, J. C. (2009). Protection of *C. elegans* from anoxia by
1375 HYL-2 ceramide synthase. *Science*, 324(5925), 381-384.
- 1376 239. Watts, J. L., & Ristow, M. (2017). Lipid and carbohydrate metabolism in
1377 *Caenorhabditis elegans*. *Genetics*, 207(2), 413-446.
- 1378 240. Lutz, P. L., Nilsson, G. E., & Pérez-Pinzón, M. A. (1996). Anoxia tolerant
1379 animals from a neurobiological perspective. *Comparative Biochemistry and Physiology*
1380 *Part B: Biochemistry and Molecular Biology*, 113(1), 3-13.
- 1381 241. Teramoto, T., Sternick, L. A., Kage-Nakadai, E., Sajjadi, S., Siembida, J.,
1382 Mitani, S., ... & Lambie, E. J. (2010). Magnesium excretion in *C. elegans* requires the
1383 activity of the GTL-2 TRPM channel. *PloS one*, 5(3), e9589.
- 1384 242. Tsunenari, T., Sun, H., Williams, J., Cahill, H., Smallwood, P., Yau, K. W., &
1385 Nathans, J. (2003). Structure-function analysis of the bestrophin family of anion
1386 channels. *Journal of Biological Chemistry*, 278(42), 41114-41125.
- 1387 243. Wang, Y., Alam, T., Hill-Harfe, K., Lopez, A. J., Leung, C. K., Iribarne, D., ... &
1388 Choe, K. P. (2013). Phylogenetic, expression, and functional analyses of anoctamin
1389 homologs in *Caenorhabditis elegans*. *American Journal of Physiology-Regulatory,*
1390 *Integrative and Comparative Physiology*, 305(11), R1376-R1389.
- 1391 244. Goh, K. Y., & Inoue, T. (2018). A large transcribed enhancer region regulates *C.*
1392 *elegans* *bed-3* and the development of egg laying muscles. *Biochimica et Biophysica*
1393 *Acta (BBA)-Gene Regulatory Mechanisms*, 1861(5), 519-533.
- 1394 245. Currie, E., King, B., Lawrenson, A. L., Schroeder, L. K., Kershner, A. M., &
1395 Hermann, G. J. (2007). Role of the *Caenorhabditis elegans* multidrug resistance gene,
1396 *mrp-4*, in gut granule differentiation. *Genetics*, 177(3), 1569-1582.
- 1397 246. Schroeder, L. K., Kremer, S., Kramer, M. J., Currie, E., Kwan, E., Watts, J. L., ...
1398 & Hermann, G. J. (2007). Function of the *Caenorhabditis elegans* ABC transporter
1399 PGP-2 in the biogenesis of a lysosome-related fat storage organelle. *Molecular biology*
1400 *of the cell*, 18(3), 995-1008.
- 1401 247. Kage-Nakadai, E., Uehara, T., & Mitani, S. (2011). H⁺/myo-inositol transporter
1402 genes, *hmit-1.1* and *hmit-1.2*, have roles in the osmoprotective response in
1403 *Caenorhabditis elegans*. *Biochemical and biophysical research communications*,
1404 410(3), 471-477.
- 1405 248. Pao, S. S., Paulsen, I. T., & Saier Jr, M. H. (1998). Major facilitator superfamily.
1406 *Microbiology and molecular biology reviews*, 62(1), 1-34.

- 1407 249. Romanelli-Credrez, L., Doitsidou, M., Alkema, M. J., & Salinas, G. (2020). HIF-1
1408 Has a Central Role in *Caenorhabditis elegans* Organismal Response to
1409 Selenium. *Frontiers in genetics*, 11, 63.
- 1410 250. Filipovic, M. R., Zivanovic, J., Alvarez, B., & Banerjee, R. (2018). Chemical
1411 biology of H₂S signaling through persulfidation. *Chemical reviews*, 118(3), 1253-1337.
- 1412 251. Hayes, J. D., & McLellan, L. I. (1999). Glutathione and glutathione-dependent
1413 enzymes represent a coordinately regulated defense against oxidative stress. *Free
1414 radical research*, 31(4), 273-300.
- 1415 252. Mytilineou, C., Kramer, B. C., & Yabut, J. A. (2002). Glutathione depletion and
1416 oxidative stress. *Parkinsonism & related disorders*, 8(6), 385-387.
- 1417 253. Diaz-Vivancos, P., de Simone, A., Kiddle, G., & Foyer, C. H. (2015).
1418 Glutathione—linking cell proliferation to oxidative stress. *Free Radical Biology and
1419 Medicine*, 89, 1154-1164.
- 1420 254. Morimoto-Tomita, M., Uchimura, K., Werb, Z., Hemmerich, S., & Rosen, S. D.
1421 (2002). Cloning and characterization of two extracellular heparin-degrading
1422 endosulfatases in mice and humans. *Journal of Biological Chemistry*, 277(51), 49175-
1423 49185.
- 1424 255. Rose, P., Moore, P. K., & Zhu, Y. Z. (2017). H₂S biosynthesis and catabolism:
1425 new insights from molecular studies. *Cellular and Molecular Life Sciences*, 74(8), 1391-
1426 1412.
- 1427 256. Livshits, L., Chatterjee, A. K., Karbian, N., Abergel, R., Abergel, Z., & Gross, E.
1428 (2017). Mechanisms of defense against products of cysteine catabolism in the
1429 nematode *Caenorhabditis elegans*. *Free Radical Biology and Medicine*, 104, 346-359.
- 1430 257. Qabazard, B., Li, L., Gruber, J., Peh, M. T., Ng, L. F., Kumar, S. D., ... & Moore,
1431 P. K. (2014). Hydrogen sulfide is an endogenous regulator of aging in *Caenorhabditis
1432 elegans*. *Antioxidants & redox signaling*, 20(16), 2621-2630.
- 1433 258. Ng, L. F., Ng, L. T., van Breugel, M., Halliwell, B., & Gruber, J. (2019).
1434 Mitochondrial DNA damage does not determine *C. elegans* lifespan. *Frontiers in
1435 genetics*, 10, 311.
- 1436 259. Kimura, H. (2020). Hydrogen sulfide signaling in the central nervous system-
1437 Comparison with nitric oxide. Authorea Preprints.
- 1438 260. Cavanaugh, C. M., McKiness, Z. P., Newton, I. L., & Stewart, F. J. (2006).
1439 Marine chemosynthetic symbioses. *The prokaryotes*, 1, 475-507.
- 1440 261. Grabherr, M.G., Haas, B.J., Yassour, M., Levin, J.Z., Thompson, D.A., Amit, I.,
1441 et al. (2011) Full-length transcriptome assembly from RNA-Seq data without a
1442 reference genome. *Nat Biotechnol* 29: 644–52

- 1443 262. Cerveau, N., and Jackson, D.J. (2016) Combining independent de novo
1444 assemblies optimizes the coding transcriptome for nonconventional model eukaryotic
1445 organisms. *BMC Bioinformatics* 17: 525
- 1446 263. Fu, L., Niu, B., Zhu, Z., Wu, S., and Li, W. (2012) CD-HIT: Accelerated for
1447 clustering the next-generation sequencing data. *Bioinformatics* 28: 3150–3152
- 1448 264. Simão, F.A., Waterhouse, R.M., Ioannidis, P., Kriventseva, E. V., and Zdobnov,
1449 E.M. (2015) BUSCO: assessing genome assembly and annotation completeness with
1450 single-copy orthologs. *Bioinformatics* 31: 3210–3212
- 1451 265. Huson, D.H., Auch, A.F., Qi, J., and Schuster, S.C. (2007) MEGAN analysis of
1452 metagenomic data. *Genome Res* 17: 377–386.
- 1453 266. Bryant, D.M., Johnson, K., DiTommaso, T., Tickle, T., Couger, M.B., Payzin-
1454 Dogru, D., et al. (2017) A Tissue-Mapped Axolotl De Novo Transcriptome Enables
1455 Identification of Limb Regeneration Factors. *Cell Rep* 18: 762–776
- 1456 267. Li, B., & Dewey, C. N. (2011). RSEM: accurate transcript quantification from
1457 RNA-Seq data with or without a reference genome. *BMC bioinformatics*, 12(1), 1-16.
- 1458 268. Gentleman, R. C., Carey, V. J., Bates, D. M., Bolstad, B., Dettling, M., Dudoit,
1459 S., ... & Zhang, J. (2004). Bioconductor: open software development for computational
1460 biology and bioinformatics. *Genome biology*, 5(10), 1-16.
- 1461 269. Robinson, M. D., McCarthy, D. J., & Smyth, G. K. (2010). edgeR: a
1462 Bioconductor package for differential expression analysis of digital gene expression
1463 data. *Bioinformatics*, 26(1), 139-140.
- 1464 270. Team, R. C. (2013). R: a language and environment for statistical computing. R
1465 Foundation for Statistical Computing, Vienna, Austria.
- 1466 271. Rapaport, F., Khanin, R., Liang, Y., Pirun, M., Krek, A., Zumbo, P., ... & Betel,
1467 D. (2013). Comprehensive evaluation of differential gene expression analysis methods
1468 for RNA-seq data. *Genome biology*, 14(9), 1-13.
- 1469 272. Cantalapiedra, C. P., Hernández-Plaza, A., Letunic, I., Bork, P., & Huerta-
1470 Cepas, J. (2021). eggNOG-mapper v2: Functional Annotation, Orthology Assignments,
1471 and Domain Prediction at the Metagenomic Scale. *Molecular Biology and Evolution*.
1472 msab293.
- 1473 273. Huerta-Cepas, J., Szklarczyk, D., Heller, D., Hernández-Plaza, A., Forslund, S.
1474 K., Cook, H., ... & Bork, P. (2019). eggNOG 5.0: a hierarchical, functionally and
1475 phylogenetically annotated orthology resource based on 5090 organisms and 2502
1476 viruses. *Nucleic acids research*, 47(D1), D309-D314.
- 1477 274. Buchfink, B., Reuter, K., & Drost, H. G. (2021). Sensitive protein alignments at
1478 tree-of-life scale using DIAMOND. *Nature methods*, 18(4), 366-368.

- 1479 275. Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic
1480 local alignment search tool. *Journal of molecular biology*, 215(3), 403-410.
- 1481 276. Harris, T. W., Arnaboldi, V., Cain, S., Chan, J., Chen, W. J., Cho, J., ... &
1482 Sternberg, P. W. (2020). WormBase: a modern model organism information resource.
1483 Nucleic Acids Research, 48(D1), D762-D767.

1484 **Figure legends**

1485

1486 **Figure 1. Relative transcript abundance and expression levels of the top 100 expressed**
1487 **genes of *L. oneistus* across all conditions.** (A) Relative transcript abundance (%) of the top
1488 100 expressed genes with a manually curated functional category. The top 100 expressed
1489 genes were collected by averaging the expression values (\log_2 TPM) across all replicates of all
1490 incubations ([Figure S1A](#), [Data S1](#), and [S2](#)). Functional classifications were extracted from the
1491 curated database UniProt and from comprehensive literature search focused mainly on *C.*
1492 *elegans*, and confirmed with the automatic annotated eggNOG classification ([Data S1](#)). (B)
1493 Median gene expression levels of selected *L. oneistus* manually annotated functional
1494 categories of the top 100 expressed genes. Metabolic processes include both differentially and
1495 constitutively expressed genes. Each dot represents the average \log_2 TPM value per gene
1496 across all replicates of all incubations. All gene names (or locus tags for unidentified gene
1497 names) are listed in [Data S2](#).

1498 **Figure 2. Median gene expression levels of selected *L. oneistus* metabolic processes**
1499 **among the differentially expressed genes between the hypoxic (H) and anoxic sulfidic**
1500 **(AS) conditions after 24 h.** Individual processes among the differentially expressed genes are
1501 ordered according to their difference in median expression between the AS and H incubations.
1502 Namely, detoxification (far left) had the largest difference in median expression in the AS
1503 condition, whereas immune response (far right) had the largest median expression difference
1504 in the H condition. The absolute number of genes are indicated at the top of each process.
1505 Metabolic processes were manually assigned and confirmed with the automatic annotated
1506 eggNOG classification. For specific gene assignments see [Data S1](#). Some genes are present
1507 in more than one functional category and processes comprising only one gene are not
1508 displayed in the figure but listed in [Data S1](#).

1509 **Figure 3. Genes involved in detoxification, ubiquitin-proteasome, autophagy, apoptosis,**
1510 **and amino acids degradation were predominantly expressed in AS worms.** Heatmap
1511 displaying genes upregulated in AS (anoxic sulfidic) relative to H (hypoxic) worms after 24 h-
1512 long incubations under one of the two conditions (1.5-fold change, $FDR \leq 0.05$). Expression
1513 levels are displayed as mean-centered \log_2 TPM value (transcripts per kilobase million). Genes
1514 are ordered by function in their respective metabolic pathways. For each process, the minority
1515 of genes that were upregulated in H worms is shown in [Data S1](#). Red denotes upregulation,
1516 and blue downregulation. Prot. protein, COP9: Constitutive photomorphogenesis 9. Dcp:
1517 domain-containing proteins. Put. glut. peroxid.: putative glutamate peroxidase. Put. sarc. oxid.:
1518 putative sarcosine oxidase.

1519 **Figure 4. Genes involved in translation and energy generation and genes encoding for**
1520 **C-type lectins and mucins were predominantly expressed in AS worms.** Heatmap

1521 displaying genes upregulated in AS (anoxic sulfidic) relative to H (hypoxic) worms, upon 24 h-
1522 long incubations under one of the two conditions (1.5-fold change, $FDR \leq 0.05$). Expression
1523 levels are displayed as mean-centered \log_2 TPM values (transcripts per kilobase million).
1524 Genes are ordered by function in their respective metabolic pathways. For each process, the
1525 minority of genes that were upregulated in H worms is shown in [Data S1](#). Red denotes
1526 upregulation, and blue downregulation. Fp: family-containing protein. Cytoch. C ox. su. II.:
1527 cytochrome c oxidase subunit II. Ubiqu./rhodoq biosynth.: Ubiquinone or rholoquinone
1528 biosynthesis.

1529 **Figure 5. Genes involved in immune response, development and nervous system were**
1530 **predominantly expressed in hypoxic (H) worms.** Heatmap displaying genes upregulated in
1531 H relative to AS worms, upon 24 h-long incubations under one of the two conditions (1.5-fold
1532 change, $FDR \leq 0.05$). Expression levels are displayed as mean-centered \log_2 TPM value
1533 (transcripts per kilobase million). Genes are ordered by function in their respective metabolic
1534 pathways. For each process, the minority of genes that were upregulated in AS worms is
1535 shown in [Data S1](#). Red denotes upregulation and blue downregulation. MN: mechanosensory
1536 neurons. Embr. body wall muscle posit.: Embryonic body wall muscle positioning. Put.:
1537 putative.

1538 **Figure 6. Genes involved in carbohydrate, lipid- and sulfur-metabolism, amino acids**
1539 **biosynthesis, and transport were predominant expressed in hypoxic (H) worms.**
1540 Heatmap displaying genes upregulated in H relative to AS worms, upon 24 h-long incubations
1541 under one of the two conditions (1.5-fold change, $FDR \leq 0.05$). Expression levels are displayed
1542 as mean-centered \log_2 TPM values (transcripts per kilobase million). Genes are ordered by
1543 function in their respective metabolic pathways. For each process, the minority of genes that
1544 were upregulated in AS worms is shown in [Data S1](#). Red denotes upregulation, and blue
1545 downregulation. FA: fatty acids. PC: phosphatidylcholine. PL: phospholipids. Metab:
1546 metabolism. Synth: synthesis. Assim: assimilation. Oxid: oxidation. Transp: transporters.

1547 **Figure 7. Schematic representation of *Laxus oneistus* physiology in anoxic and hypoxic**
1548 **sand.** In anoxic sulfidic sand (left) *L. oneistus* does not enter suspended animation. Instead, it
1549 upregulates the expression of genes mediating inhibitory neurotransmission, involved in
1550 symbiosis establishment (e.g., lectins, mucins) and in ribosome biogenesis. Metabolism may
1551 be supported by the degradation of starch and by rewiring the electron transfer chain:
1552 rholoquinone (RQ) is used as electron carrier and fumarate as electron acceptor. Moreover,
1553 the worm activates degradation pathways (e.g., ubiquitin-proteasome system (UPS),
1554 autophagy, and apoptosis) and may anticipate reoxygenation by upregulating superoxide
1555 dismutase (SOD) and glutathione peroxidase (GP).

1556 In hypoxic sand (right), instead, *L. oneistus* appears to use trehalose and cellulose for
1557 energy generation, while engaging in costly processes such as development, molting, feeding,

1558 and mating. Genes involved in excitatory neurotransmission are also upregulated, together
1559 with Toll receptors and immune effectors (e.g., fungicides, bactericidal permeability increasing
1560 proteins).

1561

1562 SUPPLEMENTAL MATERIAL LEGENDS

1563

1564 **Figure S1. Experimental conditions, sample similarity and differential expression.** (A)
1565 Experimental setup was previously described (Paredes et al. 2021). Briefly, nematodes were
1566 subjected to different oxygen concentrations for 24 h: anoxic with sulfide (AS: 0mM O₂, 25mM
1567 sodium sulfide added), anoxic without sulfide (A, 0mM O₂), hypoxic (H, 60mM O₂ after 24 h),
1568 and oxic (O, 100mM O₂ after 24 h). The box around the anoxic incubation vials illustrates that
1569 these incubations were carried out in a polyethylene glove bag. (B) Similarity between
1570 transcriptome samples based on Euclidean distances between expression values (log₂TPM),
1571 and visualized by means of multidimensional scaling (C) Differential gene expression (DE)
1572 analysis between incubations showed that the number of DE genes was low (maximum value
1573 was 4.8% of all expressed genes for the H vs AS conditions). Genes were considered
1574 differentially expressed if their expression changed 1.5-fold with a false-discovery rate (FDR) of
1575 ≤ 0.05.

1576 **Figure S2. Statistical analysis, relative transcript abundance and expression levels of**
1577 **the top 100 detected proteins of *L. oneistus* across all conditions.** (A) Relative protein
1578 abundance (%) of the top 100 detected proteins present in a particular manually curated
1579 functional category. The top 100 proteins were collected by averaging the expression values
1580 across all replicates of all incubations ([Figure S1A](#), [Data S2](#)). Functional classifications were
1581 extracted from the curated database UniProt and from comprehensive literature search
1582 focused mainly on *C. elegans*, and confirmed with the automatic annotated eggNOG
1583 classification ([Data S1](#)). (B) Median gene expression levels of selected *L. oneistus* manually
1584 annotated functional categories of the top 100 expressed proteins. Each dot represents the
1585 average %cOrgNSAF per protein across all replicates of all incubations. Notice that some
1586 categories were created with genes of overlapping functions (e.g.,
1587 cytoskeleton/locomotion/nervous system). All protein names (or locus tags for unidentified
1588 protein names) are listed in [Data S2](#).

1589 **Figure S3. Transcriptomics vs proteomics comparison.** Pearson correlation between all
1590 transcripts and proteins ([Data S1](#)) automatically classified based on their functional category.
1591 The Pearson correlation between all expressed transcripts and all detected proteins (r = 0.4)
1592 was found to be low ([Figure S3](#)).

1593 **Figure S4. Relative transcript abundance and expression levels of the top 100**
1594 **expressed genes of *O. algarvensis* across all conditions.** (A) Relative transcript

1595 abundance (%) of the top 100 expressed genes with a manually curated functional category.
1596 The top 100 expressed genes were collected by averaging the expression values (\log_2 TPM)
1597 across all replicates of all incubations (see [Supplemental material](#)). Functional classifications
1598 were extracted from the curated database UniProt and from comprehensive literature search
1599 focused mainly on *C. elegans*). (B) Median gene expression levels of selected *O. algarvensis*
1600 manually annotated functional categories of the top 100 expressed genes. Metabolic
1601 processes include both differentially and constitutively expressed genes. Each dot represents
1602 the average \log_2 TPM value per gene across all replicates of all incubations.

1603 **Figure S5. *L. oneistus* lipid composition in anoxic and oxic conditions after 24 h.** Major
1604 lipid classes and their abundance relative to all lipids detected showed no statistical difference
1605 between both conditions. For details on methodology see [Supplemental material](#).

1606 **Table S1.** Metabolites detected in at least two biological replicates of either the holobiont
1607 fraction (*Laxus oneistus* and its ectosymbiont) or in the symbiont fraction (see [Supplemental](#)
1608 [material](#)). RT: retention time. Area: area of a peak from a specific compound detected in the
1609 GC-MS chromatograms. Grey boxes: no metabolites detected. Blank boxes: Unknown
1610 metabolites that are either below the detected threshold (< 700) or might be products of
1611 derivatization reagents. Note that cholestane and ribitol were used as internal standards.

1612 **Data S1.** Ca. T. oneisti genes, functional annotations, transcript and protein expression.

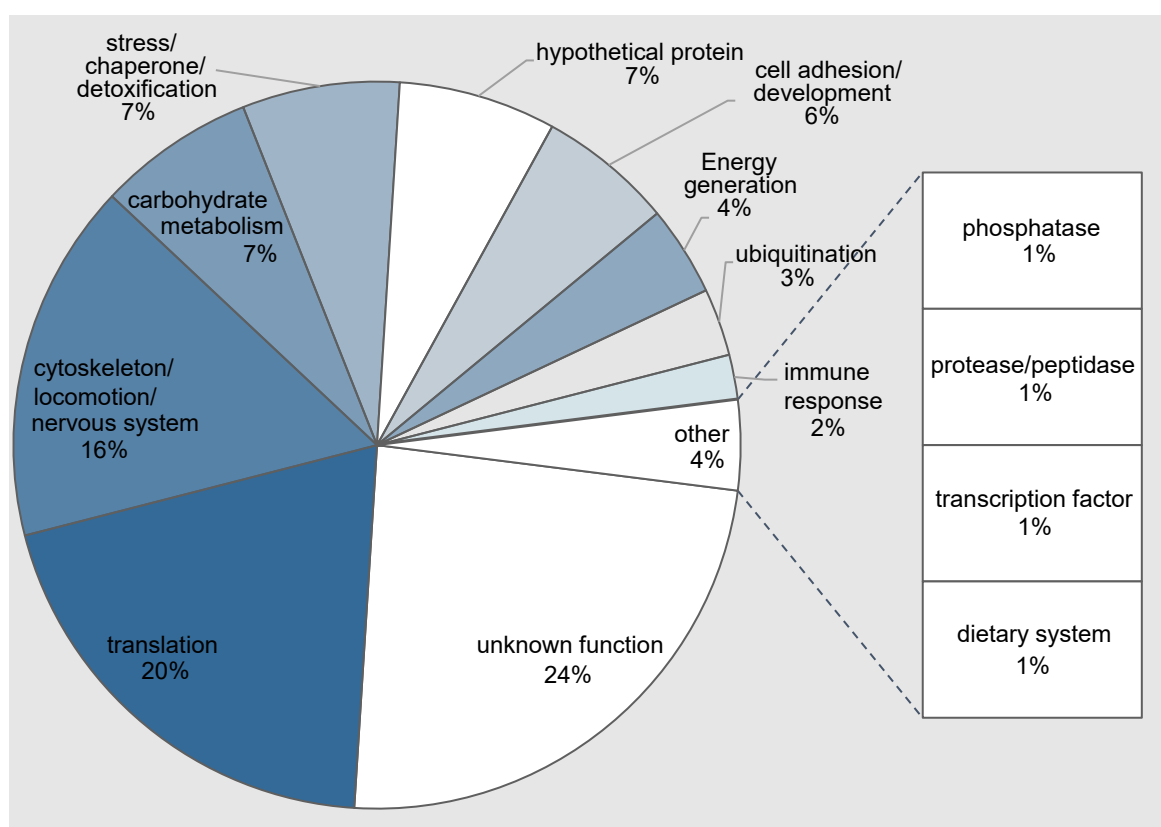
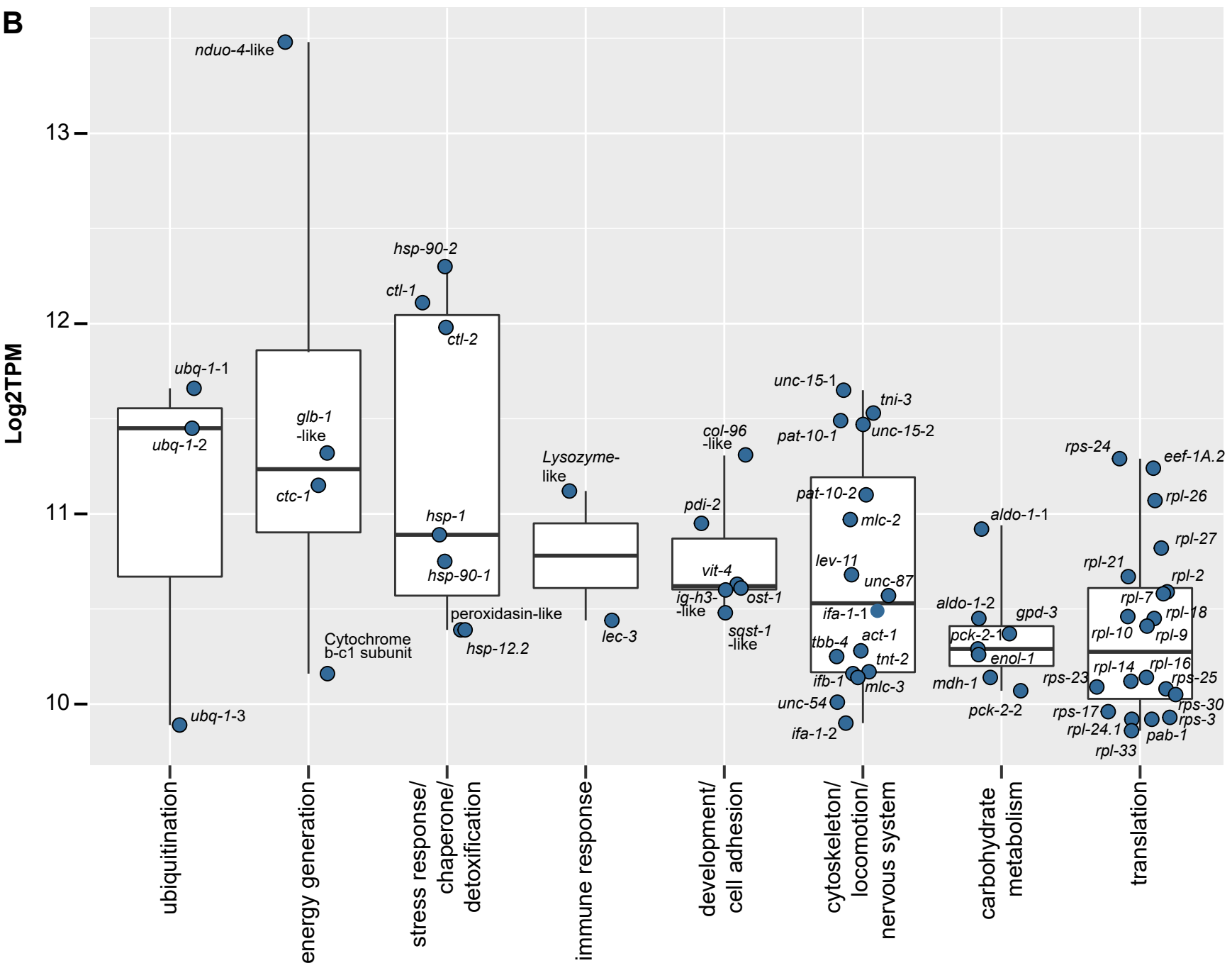
1613 **Data S2.** Top 100 expressed genes (RNA-Seq) and detected proteins (proteomics data).

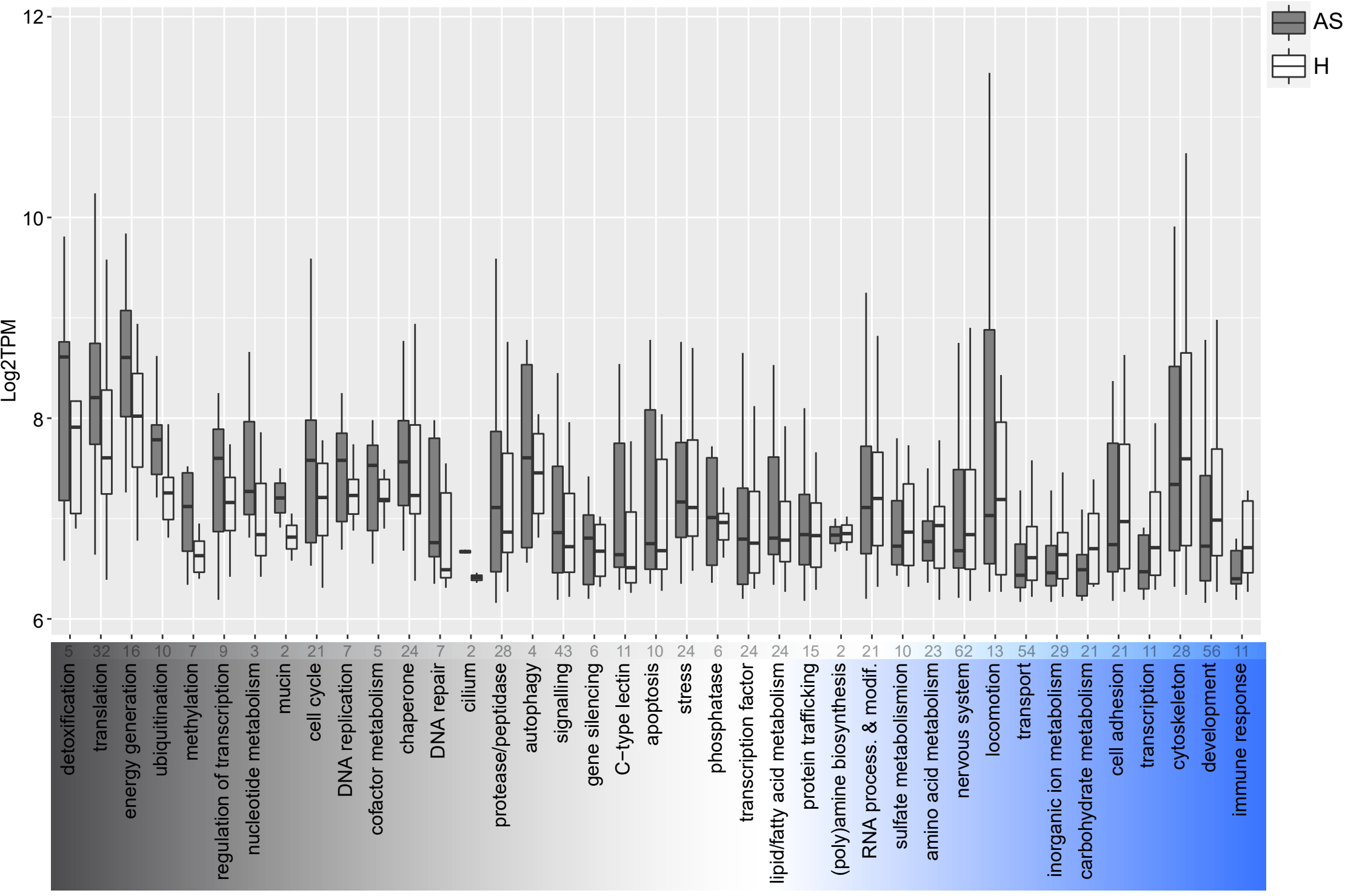
1614 **Supplemental video 1.** A batch of 50 *Laxus oneistus* after 6 days in anoxic seawater.

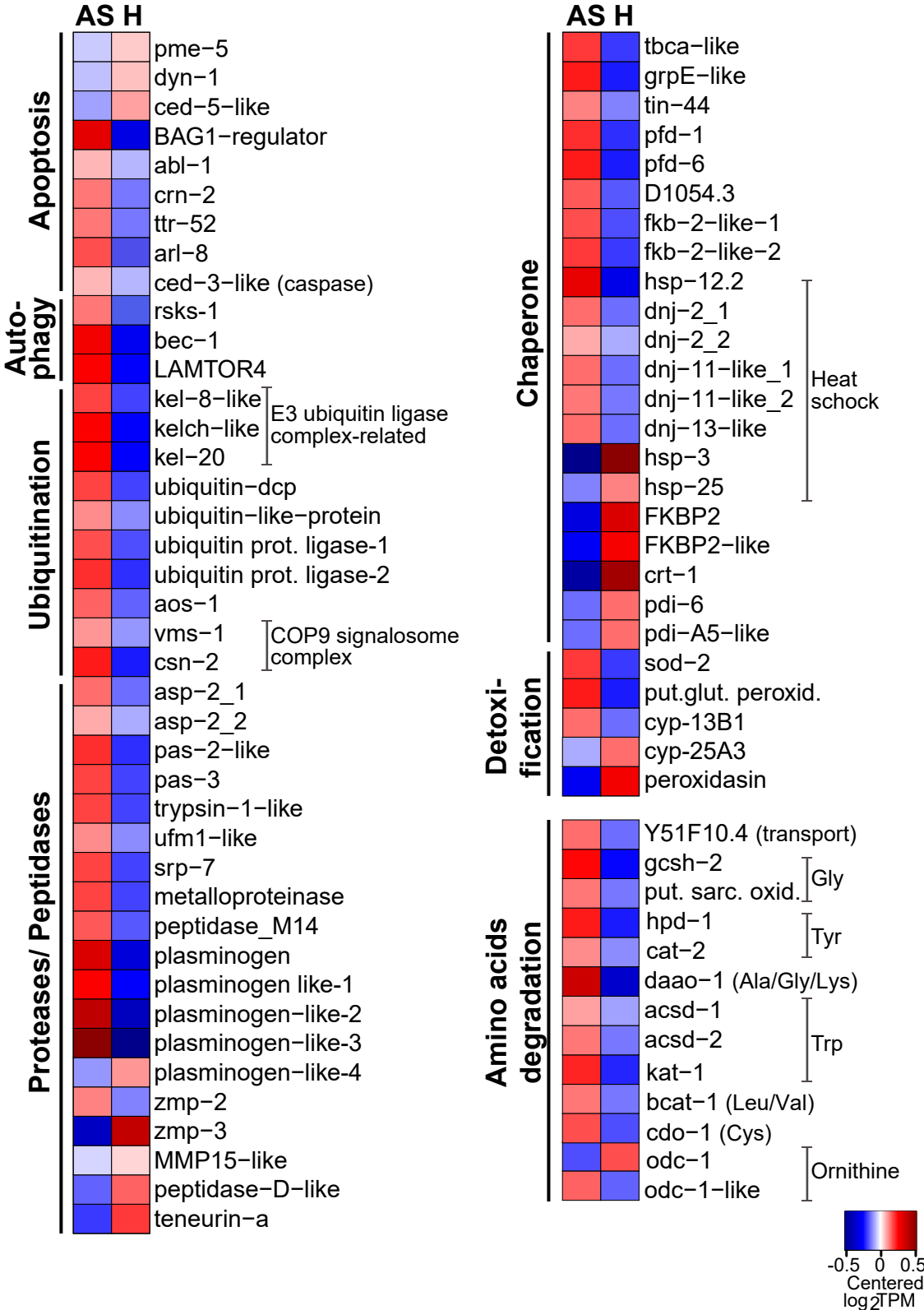
1615 **Supplemental video 2.** A batch of 50 *Laxus oneistus* at the beginning (T0) of the incubations.

1616 **Supplemental video 3.** A batch of 50 *Laxus oneistus* after 1 day (T24 h) in anoxic sulfidic
1617 seawater (0 % air saturation, 25 μ M H₂S).

1618 **Supplemental video 4.** A batch of 50 *Laxus oneistus* after 1 day (T24 h) in oxic seawater (87
1619 % air saturation, 0 μ M H₂S).

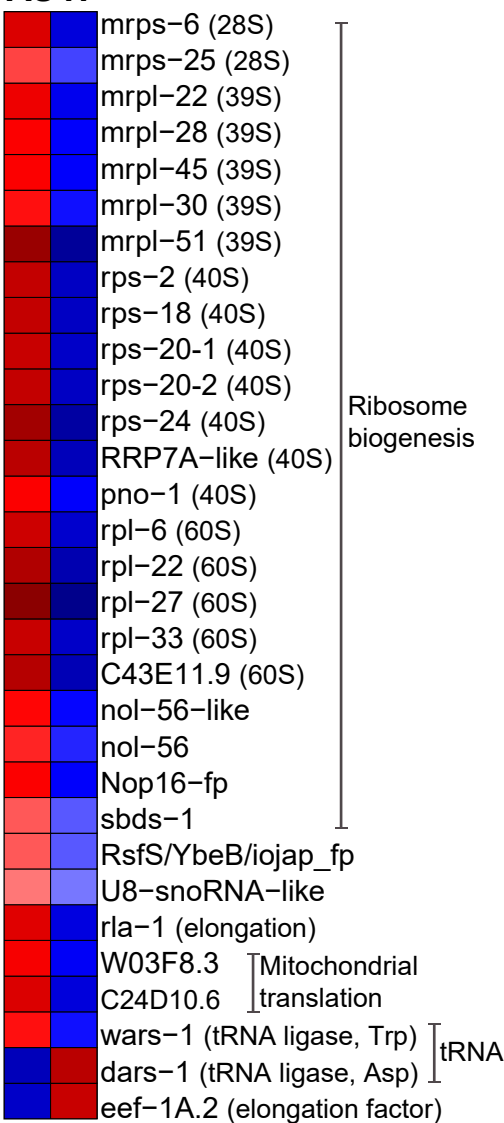
A**B**





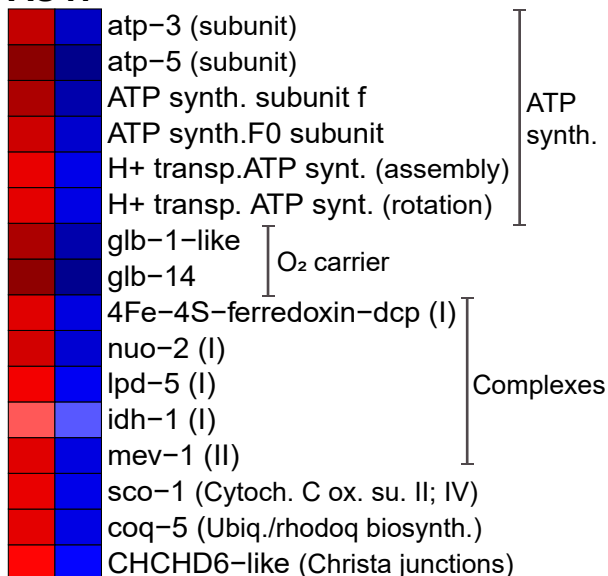
Translation

AS H



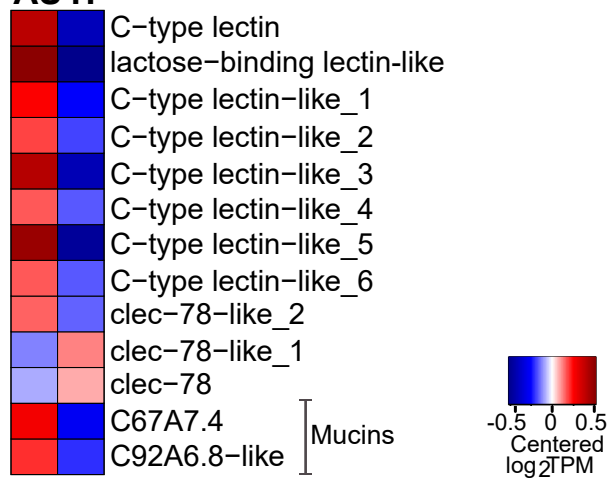
Energy generation

AS H



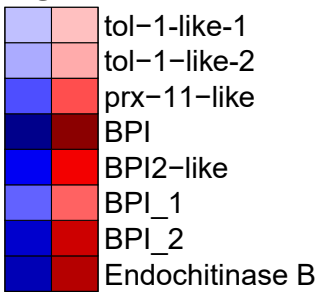
C-type lectins/mucins

AS H



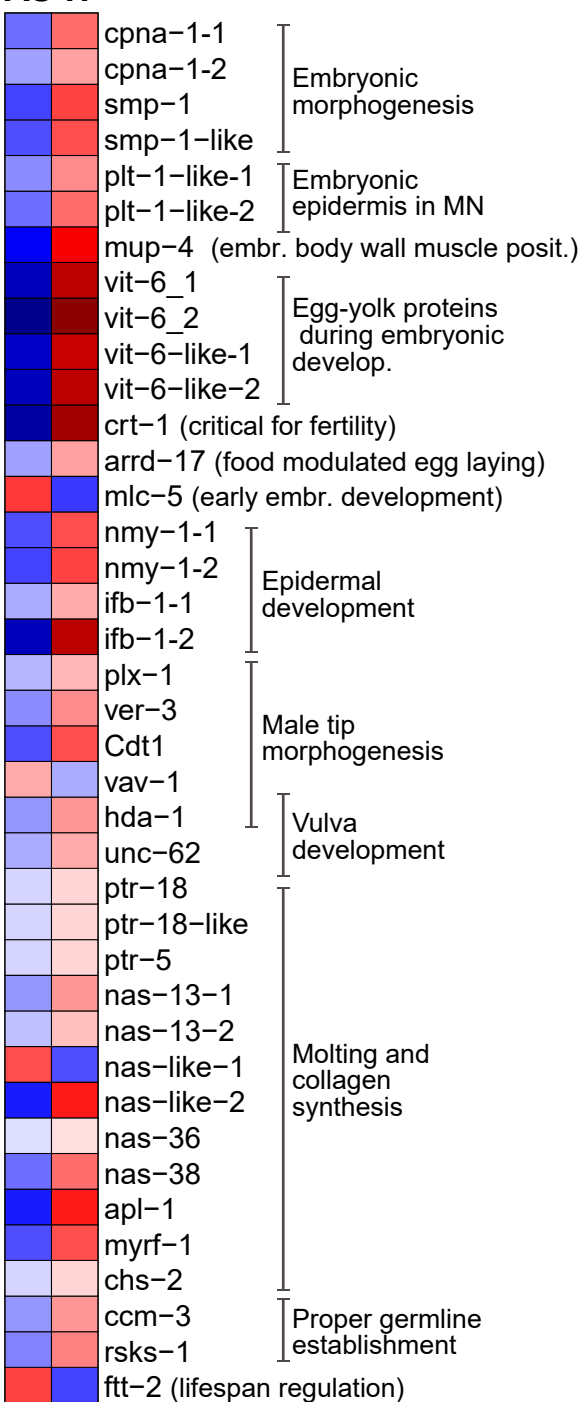
Immune system

AS H



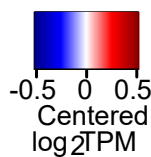
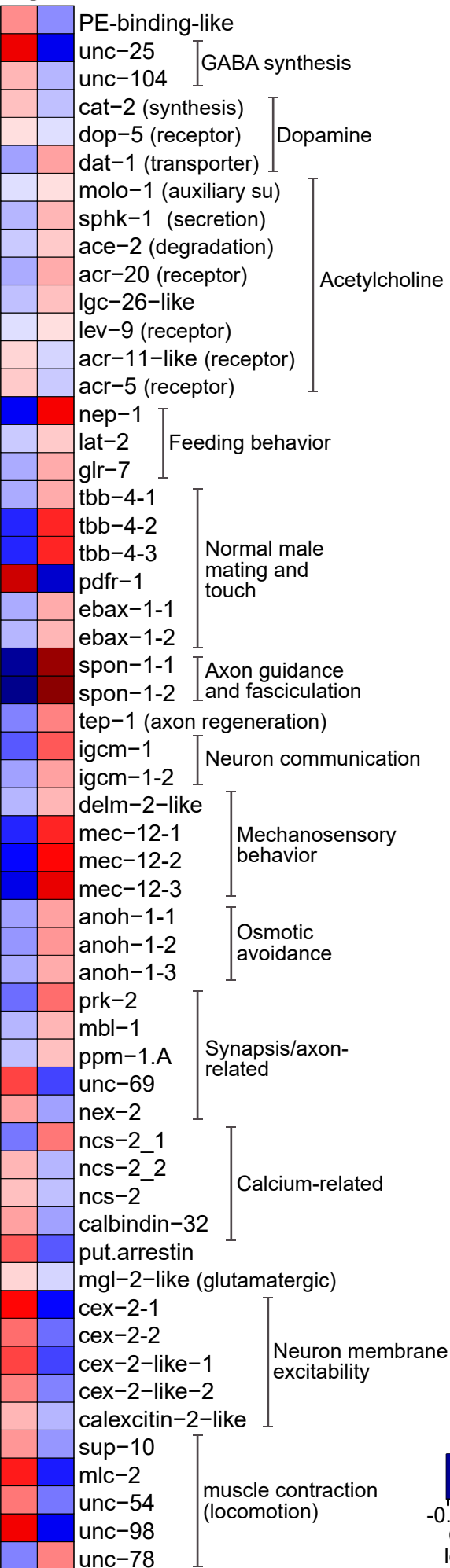
Development

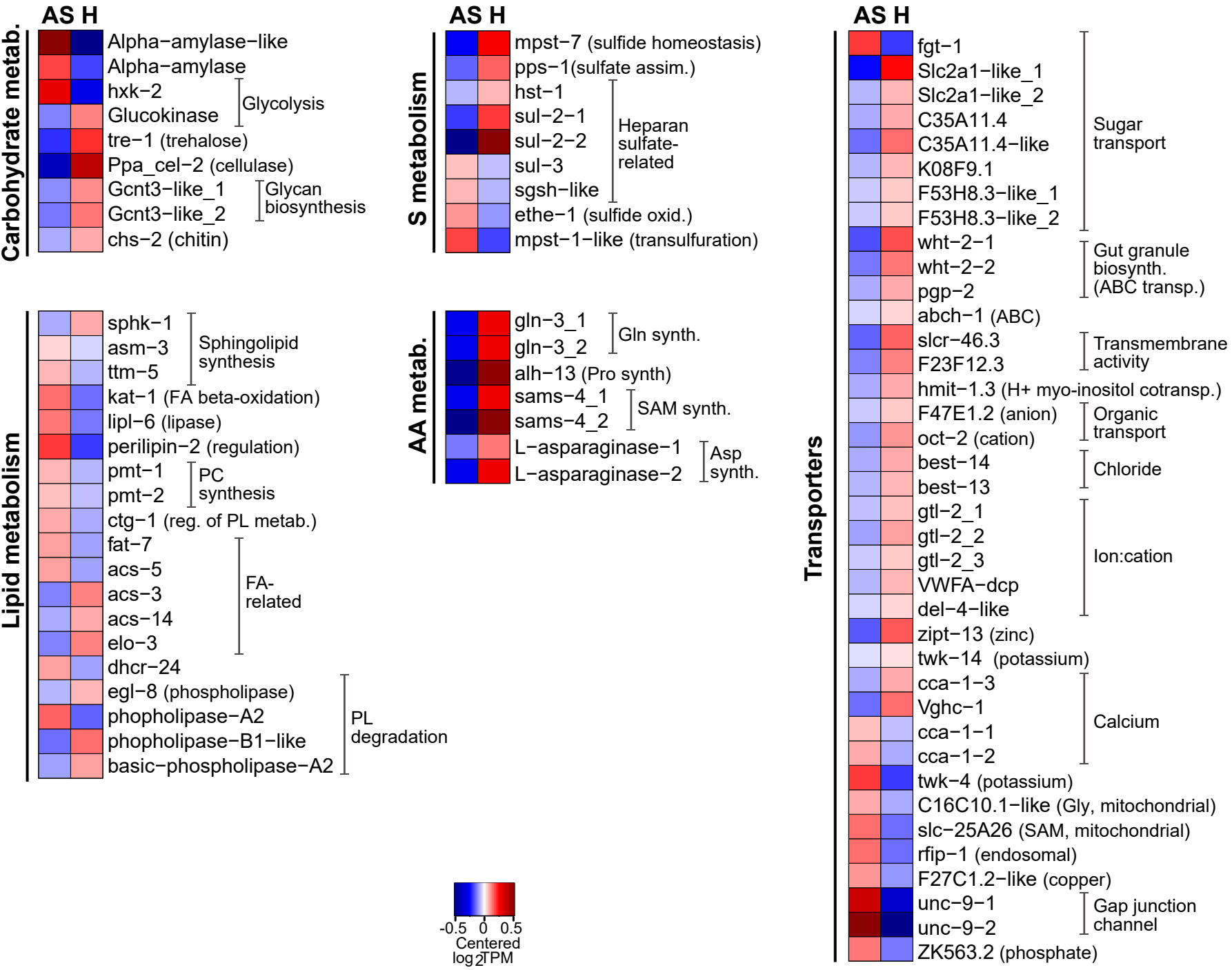
AS H



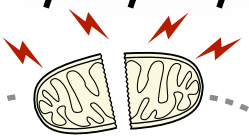
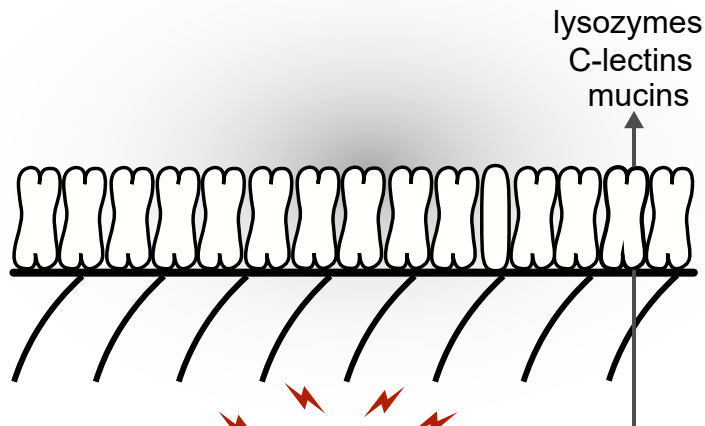
Nervous system

AS H





ANOXIC-SULFIDIC

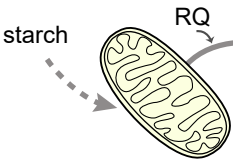


Degradation

UPS
apoptosis
autophagy
proteolysis
aa and lipid catabolism

**Anticipation
of oxidative
stress**

chaperones
anti-ROS (e.g. SOD, GP)
ceramides



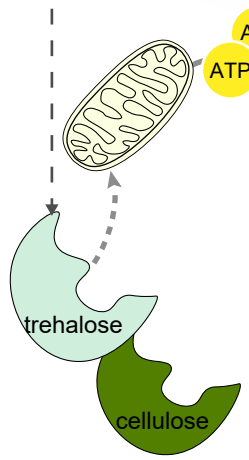
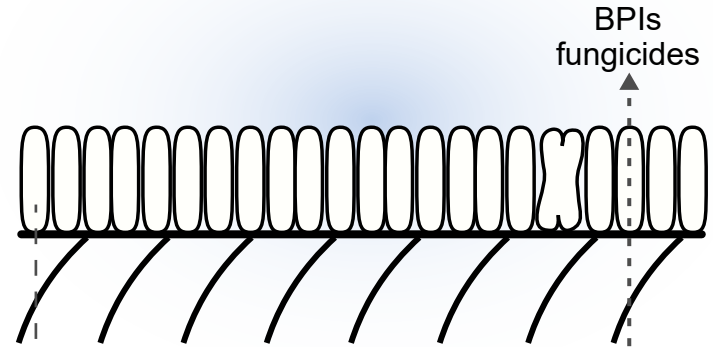
ATP

ribosome
biogenesis

inhibitory
neurotransmission



lysozymes
C-lectins
mucins



ATP
ATP
ATP

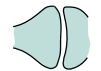
Growth

molting
mating
development

Biosynthesis

long chain FAs
aa
heparan sulfate
glycan

excitatory
neurotransmission



Toll/ Nf-kB
pathway
activation

HYPoxic

BPIs
fungicides