Title: Interplay between mitochondria and diet mediates pathogen and stress resistance in *C. elegans*

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Summary:

Diet is a crucial determinant of organismal biology. Here we demonstrate the dramatic impact of a subtle shift in diet on the ability of *Caenorhabditis elegans* to survive pathogenic or abiotic stress. Interestingly, this shift occurs independently of canonical host defense pathways, arising instead from improvements in mitochondrial health. Using a variety of assays, we reveal that the most common *C. elegans* food source (*E. coli* OP50) results in a vitamin B12 deficiency that compromises mitochondrial homeostasis. Increasing B12 supply by feeding on *E. coli* HT115 or by supplementing bacterial media with methylcobalamin restored mitochondrial function, even if the bacteria were dead. B12 supplementation also efficiently increased host health without adversely affecting lifespan. Our study forges a molecular link between a dietary deficiency (nutrition/microbiota) and a physiological consequence (host sensitivity), using the host-microbiota-diet framework. The ubiquity of B12 deficiency (~10-40% of US adults) highlights the importance of our findings.

Keywords: *C. elegans*; *E coli* HT115, OP50, BW25113; mitochondrial function; vitamin B12; mitochondrial homeostasis; pathogen sensitivity

26 **Introduction**

27 The diet and microbiota of an organism define its biology as much as its genome. Unfortunately,
28 an avalanche of descriptive studies on model organism microbiomes have yielded very little
29 mechanistic understanding of the relationships that define the host-microbiota-nutrition axis. One
30 explanation for this is that the interplay amongst the three participants is incredibly complex and
31 surprisingly dynamic. For example, the host and microbiota actively shape and, are in turn shaped
32 by, their nutrition. Host genetics and environment determine initial susceptibility to microbial
33 colonization, which will go on to influence all aspects of health. Establishing a mechanistic
34 understanding of these interrelationships is crucial to the life history of an organism.

35 *Caenorhabditis elegans* offers a tantalizing system for simplifying these studies without
36 sacrificing the ability to make discoveries that will be a useful starting point for more advanced
37 organisms. At its most reduced form, the host-microbiota-nutrition axis of *C. elegans* can be
38 collapsed to a binary system comprised of only two species, both of which are genetically tractable.
39 As *C. elegans* is a bacteriovore, a single bacterial species can form the diet and the intestinal flora
40 (due to incomplete disruption and digestion of bacterial food, which then colonizes the intestine).
41 Generation of gnotobiotic worms is also simple and inexpensive.

42 Even this binary system has yielded significant insight regarding interactions between the host
43 and its bacterial counterpart. For example, studies have shown that not only the relative
44 biomacromolecular content of their diet (i.e., lipids, carbohydrates, etc.) but also bacterial
45 metabolites informed the host's biology, including folate (Virk et al., 2012), nitric oxide (Gusarov et
46 al., 2013), and tryptophan (Anyanful et al., 2005; Gracida and Eckmann, 2013). Indeed, even the
47 rate of bacterial respiration affected the metabolic state of the host (Saiki et al., 2008).

48 In this report, we show that differences in bacterial strains drive increased host sensitivity to a
49 variety of stresses, including exposure to *P. aeruginosa*, oxidative stress, or hyperthermia. Through
50 a panel of orthogonal assays, we demonstrated that a diet of *E. coli* strain OP50 causes a chronic

51 vitamin B12 deficiency that perturbs mitochondrial health and function. B12 supplementation,
52 even in the absence of living bacteria, increased resistance without shortening lifespan. Our
53 findings provide a mechanistic explanation for the link between diet, cellular homeostasis, and
54 organismal health.
55
56 **Results**
57 *A diet of E. coli strain HT115 confers resistance to a variety of stresses in C. elegans*
58 While characterizing a novel *C. elegans-P. aeruginosa* Liquid Killing assay (Conery et al., 2014;
59 Kirienko et al., 2013), we made the unexpected observation that worms reared on *E. coli* HT115
60 exhibited increased resistance to *P. aeruginosa* compared to worms reared on *E. coli* OP50 (**Fig 1A,**
61 **B**). This was true even if the bacteria lacked the L4440 RNAi vector. Pathogenesis in this assay
62 requires the siderophore pyoverdine, which compromises host metabolism by removing ferric iron
63 (Kirienko et al., 2015). Since exogenous iron strongly limits host killing (Kirienko et al., 2013), we
64 tested whether worms reared on HT115 contained more iron, indirectly increasing their resistance
65 to pyoverdine. However, neither a fluorometric iron (III) assay nor mass spectrometric
66 measurement of total iron showed significant diet-dependent differences in host iron concentration
67 (**Fig S1A, B**).
68 Substitution of *E. coli* OP50 with a variety of other bacterial foods (or UV- or heat-killed OP50)
69 increases *C. elegans'* lifespan (Gomez et al., 2012; Kim, 2013; Win et al., 2013; Yu et al., 2015). These
70 observations are typically interpreted to mean that OP50 is weakly pathogenic to *C. elegans*
71 (Garigan et al., 2002; Garsin et al., 2001). On this basis, we surveyed basal gene expression levels for
72 downstream effectors of a variety of *C. elegans* immune pathways, including PMK-1/p38, ZIP-
73 2/bZIP, DAF-16/FOXO, FSHR-1/FSH, and SKN-1/Nrf2 (Estes et al., 2010; Garsin et al., 2003; Kim et
74 al., 2004; Papp et al., 2012; Powell et al., 2009; Troemel et al., 2006). Worms reared on OP50 and

75 HT115 had indistinguishable basal levels of expression of downstream innate immune effectors
76 associated with these pathways (**Fig S2**).
77 Using either the conventional HT115 RNAi strain or an RNAi-competent OP50 derivative (Xiao
78 et al., 2015), we knocked down key defense pathways and assayed sensitivity to *P. aeruginosa*
79 exposure. Although some knockdowns affected the timing of death (e.g. *daf-2(RNAi)* prolonged
80 survival, *daf-16(RNAi)* hastened death), worms fed HT115 survived longer in each case (**Fig S3**).
81 These data indicate that weak OP50 pathogenicity (if it does exist) is unlikely to underlie the
82 difference in sensitivity to *P. aeruginosa*.
83 Many defense pathways confer resistance to both abiotic and pathogenic factors. Therefore, we
84 tested whether HT115 increased resistance to additional pathogens and abiotic stresses. As
85 anticipated, survival was significantly increased when HT115-fed worms were infected with
86 *Enterococcus faecalis* or *E. faecium* or were subjected to a heat shock or exposed juglone-induced
87 oxidative stress or to the iron-scavenging xenobiotic 1,10-phenanthroline (**Fig 1C-G**). Increased
88 resistance to pathogens and abiotic stresses is commonly associated with longer lifespan but, to our
89 surprise, feeding HT115 did not extend lifespan (**Fig 1H**).
90
91 *Transcriptome profiling implicates mitochondrial defects in diet-induced stress sensitivity*
92 To gain an unbiased snapshot of gene expression in worms fed OP50 or HT115, we performed
93 transcriptome profiling. Interestingly, the number of differentially-regulated genes was very small,
94 with only 35 genes upregulated between 2- and 8-fold in HT115 and 22 genes upregulated between
95 2- and 20-fold in OP50 (**Table S1**). Strikingly, of the latter 22 genes, 12 encoded proteins localized
96 to the mitochondria (**Table 1**). This enrichment was highly significant (p=2.2*10^{-16}), particularly
97 given that the fraction of nuclear genes that encode mitochondrial proteins in *C. elegans* and
98 humans is ~6% and 7% respectively (Li et al., 2009; Prokisch et al., 2006). This enrichment was
99 specific to genes upregulated by an OP50 diet; amongst genes expressed at higher levels in HT115-

100 fed worms, only two mitochondrial genes (*acox-2* and T22B7.7) were observed (2/35=5.7%,
101 p=0.9896). These data argue that feeding with OP50 increases expression of mitochondrial genes.
102 Two genes in particular caught our attention: *hsp-60*, which encodes a mitochondrial chaperone
103 expressed constitutively at low levels but induced upon mitochondrial damage (Benedetti et al.,
104 2006; Haynes et al., 2007; Yoneda et al., 2004), and *acdh-1*, which encodes a short-chain acyl-CoA
105 dehydrogenase that has been used as an indicator of branched chain amino acids and/or propionyl-
106 CoA, a mitotoxic byproduct of their metabolism (Schwab et al., 2006; Watson et al., 2014).
107
108 *A diet of HT115 improves the vitamin B12 deficiency caused by OP50*
109 Consistent with our microarray results, we observed increased *acdh-1::*GFP expression in OP50-
110 fed worms, whether measured by conventional imaging (**Fig 2A,B**) or by COPAS flow vermimetry
111 (**Fig 2C**). Vitamin B12 is an essential micronutrient for most organisms, including *C. elegans* and *E.*
112 *coli* and is required for efficient metabolism of branched chain amino acids and propionyl-CoA
113 (Watson et al., 2014; Watson et al., 2015). Our data, and those of the Walhout lab, demonstrate that
114 an OP50 diet induces a B12 deficiency that triggers compensatory increases in expression of *acdh-*
115 *1*::GFP and other mitochondria-related genes (Watson et al., 2016).
116 We attempted to use mass spectrometry to measure S-adenosylcobalamin in worms fed either
117 HT115 or OP50 (or in the bacteria themselves) to obtain quantitative measurements of vitamin
118 B12. Unfortunately, B12 levels for all samples measured were below the detection threshold, even
119 when samples were concentrated (*data not shown*). To circumvent this limitation we used sodium
120 propionate sensitivity as a functional readout of B12 sufficiency. Consistent with other data, a diet
121 of HT115 significantly increased resistance to propionate, indicating that it improves the B12
122 shortage (**Fig 2D**).
123
124 *Vitamin B12 deficiency disrupts mitochondrial homeostasis*

125 We hypothesized that the dietary B12 deficiency causes buildup of propionyl-CoA, which would
126 lead to mitochondrial damage. Therefore, we assayed mitochondrial homeostasis in *C. elegans* fed
127 either OP50 or HT115. Under normal conditions, mitochondrial quality control involves constant
128 fission and fusion events that serve to pool healthy, functional mitochondrial content, while
129 damaged material is sequestered for autophagic recycling (Liesa and Shirihai, 2013). Compared to
130 mammals, *C. elegans* mitochondria generally exhibit greater interconnectivity and longer tubular
131 architecture (e.g., disrupting *C. elegans* mitochondrial genes almost always increases fragmentation
132 (Ichishita et al., 2008)). We used a *C. elegans* strain expressing a mitochondrially-targeted GFP
133 (Benedetti et al., 2006) to assay mitochondrial health after OP50 or HT115 consumption. Worms
134 fed HT115 demonstrated increased connectivity and less clumping (i.e., punctae) than OP50 (**Fig**
135 **2E**), indicating improved mitochondrial health.
136
137 *Vitamin B12 supplementation improves mitochondrial health*
138 To determine whether we could remedy the B12 limitation, we spiked OP50 growth medium
139 with exogenous methylcobalamin to a final concentration of 0.2 μg/mL. Supplementation
140 dramatically decreased *acdh-1::GFP* fluorescence (**Fig 2A-C**), increased resistance to propionate
141 (**Fig 2D**), and improved mitochondrial network architecture (**Fig 2E**). Combined, these data
142 suggested that methylcobalamin supplementation significantly improved mitochondrial health.
143 We assayed other metrics of mitochondrial health, including mitochondrial count (**Fig 2F**),
144 membrane potential (**Fig 2G**), production of reactive oxygen species (ROS, **Fig 2H**), and ATP (**Fig**
145 **2I**) to verify this conclusion. Interestingly, methylcobalamin supplementation decreased the
146 number of mitochondria while mitochondrial membrane potential remained steady, indicating that
147 the average membrane potential of each mitochondrion was slightly increased by treatment.
148 Methylcobalamin also significantly decreased ROS production, while ATP production decreased

149 only slightly. The most parsimonious explanation for these observations is that a dietary B12
150 deficiency significantly compromises mitochondrial health and efficiency.
151
152 *Vitamin B12 deficiency in OP50 diet drives sensitivity to stress*
153 We predicted that the mild mitochondrial dysfunction exhibited by OP50-fed worms may drive
154 their increased sensitivity to stress. We reared worms on OP50 or OP50 supplemented with
155 methylcobalamin (OP50/B12), and tested their resistance to *P. aeruginosa*. We observed a dramatic
156 difference in host survival: virtually all OP50-fed worms were dead by the time OP50/B12-fed
157 worms showed ~10% death (**Fig 3A, B**). Bacterial metabolism of B12 was superfluous for this
158 effect; *C. elegans* fed heat-killed *E. coli* spotted onto plates containing methylcobalamin-
159 supplemented media also exhibited virtually no *acdh-1*::GFP expression and increased survival
160 when exposed to *P. aeruginosa* (**Figs S4A, 3C**). Cobalamin supplementation also substantially
161 improved survival after infection with *E. faecalis* or *E. faecium* (**Fig 3D, E**). Next, we examined
162 whether cobalamin was beneficial during exposure to more generic stressors, like hyperthermia.
163 Supplementation again significantly increased *C. elegans* survival (**Fig 3F**).
164 A growing body of evidence suggests that decreasing mitochondrial activity, either by
165 genetically compromising oxidative phosphorylation or by caloric restriction, extends lifespan (Luz
166 et al., 2016; Yang and Hekimi, 2010). Since B12 supplementation improves mitochondrial function,
167 we wanted to know whether it may be beneficial during adverse conditions, but would also
168 ultimately shorten lifespan. No significant changes in lifespan were seen in worms receiving a diet
169 supplemented with methylcobalamin (**Fig 3G**).
170 All of the experiments described above were performed in a *glp-4(bn2ts)* background, which
171 fails to generate a germline and is sterile at non-permissive temperatures. Sterility is necessary to
172 avoid non-specific death in the Liquid-Killing assay. To rule out the possibility that the observed

173 phenotypes were a result of the *glp-4 (bn2)* background, we tested wild-type N2 worms. In every
174 case tested, wild-type worms recapitulated our findings from *glp-4(bn2)* mutants (**Fig 3H-J**).
175
176 *The E. coli TonB transporter mediates vitamin B12 internalization and subsequent host health*
177 Since *E. coli* is unable to synthesize vitamin B12, it must be imported from the extracellular
178 milieu. Efficient import has been reported to require TonB activity (Bassford et al., 1976; Kadner,
179 1990), so we evaluated *acdh-1*::GFP fluorescence in worms fed *E. coli* with deletions of the *tonB*
180 gene (Baba et al., 2006). While *acdh-1*::GFP fluorescence in worms reared on the parental strain (*E.*
181 *coli* BW25113) was similar to OP50 (**Fig S4B**), *tonB* deletion slightly, but reproducibly, increased
182 GFP level (**Fig S4C**, $p<0.01$), consistent with a previous report (Watson et al., 2014).
183 Correspondingly, *tonB* deletion also increased sensitivity to *P. aeruginosa* exposure (**Fig S4D**). As
184 with OP50, supplementation of wild-type BW25113 with methylcobalamin completely abolished
185 *acdh-1*::GFP fluorescence (**Fig S4E**). In contrast, *tonB* mutants showed only partial attenuation of
186 GFP expression after methylcobalamin supplementation, indicating that the bacteria were unable to
187 efficiently internalize the vitamin (**Fig S4F, G**). One possible explanation for the attenuation in GFP
188 fluorescence that we observed in these mutants is that *E. coli* has a salvage pathway to acquire
189 precursors for B12 biosynthesis (Fang et al., 2017). Our observation that *tonB* mutation increases
190 host sensitivity to stress still further (**Fig S4D**) indicates a role for TonB activity in the health of the
191 host.
192
193 **Discussion**
194 Recently, Leulier and colleagues defined the "nutrient-microbiota-host axis" to incorporate all
195 of these determinants into a single conceptual framework (Leulier et al., 2017). Our results leverage
196 this idea to connect diverse phenomena into an articulated whole. For example, our data indicate
197 that a dietary deficiency in vitamin B12 deficiency causes mitochondrial defects, likely through the

198 buildup of propionyl-CoA (a known mitochondrial toxin). We also demonstrated that this damage
199 significantly increased sensitivity to a variety of stresses. Recently, Lin and Wang demonstrated
200 that methionine supplementation alters the metabolism of the food-microbiota of *C. elegans*,
201 directly influencing host mitochondrial health (Lin and Wang, 2017). Interestingly, methionine
202 supplementation did not influence host survival after exposure to *P. aeruginosa* in Liquid Killing
203 conditions (56.5% *vs* 54.2%, p=0.363).

204 Our findings provide a mechanistic explanation for clinical observations that patients with
205 methylmalonic or propionic acidemias (either of which can arise from vitamin B12 deficiency)
206 exhibit symptoms that are strikingly similar to those with congenital mitochondrial defects
207 (including poor growth, muscle weakness, and disorders of the liver, kidney, and gastrointestinal
208 and respiratory tracts). It also explains why mice with inactive methylmalonyl-CoA mutase (one of
209 two B12-dependent enzymes) exhibit reduced respiratory chain activity (Chandler et al., 2009).
210 Vitamin B12 is clearly important for normal cellular stress resistance and healthy aging. Despite
211 this, estimates of deficiency range between 10-40% of the population, with increased prevalence in
212 the elderly (Spence, 2016).

213 Previous studies from the Walhout lab showed that some diets replete in B12 (including
214 *Comamonas aquaticus* and *E. coli* HT115) were associated with shorter lifespans, although the
215 difference is not directly due to B12 (MacNeil et al., 2013). We also saw a small, but statistically
216 significant, decrease in maximum lifespan when *C. elegans* was reared on *E. coli* HT115.
217 Intriguingly, methylcobalamin did not affect the lifespan of worms when it was added to an OP50
218 diet (**Fig 3E**), suggesting that the improvement in mitochondrial health provided by B12
219 supplementation can be unlinked from lifespan shortening. Currently, we hypothesize that some
220 other aspect of *Comamonas* and HT115 diets, probably a common metabolite, was responsible for
221 this effect.

222 Interestingly, even though we started to query the host-microbiota-diet axis with a minimal,
223 binary system (*C. elegans* and *E. coli*), a number of unexpected complexities have arisen. For
224 example, subtle differences in the biology of *C. elegans* reared on OP50 or HT115 have generally
225 been attributed to the differences in their origins (i.e., OP50 was derived from an *E. coli* B strain
226 while HT115 came from *E. coli* K12). However, BW25113 (which also originated from K12) showed
227 *acdh-1*::GFP fluorescence similar to that of OP50. Heat-killed OP50 showed *acdh-1*::GFP
228 fluorescence similar to that of HT115-fed *C. elegans*. These data indicate that *acdh-1*::GFP
229 expression and mitochondrial health poorly correlate with strain origin and are likely to be
230 dynamic, illustrating the subtle nuances that lie along the nutrient/microbiota axis and the
231 dramatic impacts they have on host phenotype.

232 We also showed that this framework can help identify an important mechanism used by *C.*
233 *elegans*, and presumably more complex metazoans, to fine-tune their cell metabolism to resist
234 adverse environmental factors (including pathogens, heat, and toxins), even without activating
235 canonical host defense pathways. Interestingly, the sensitivity to stress caused by B12 deficiency
236 seems to correlate with the degree of mitochondrial involvement in the stress-induced pathology.
237 For example, we observed that methylcobalamin supplementation decreased *acdh-1* expression by
238 10-fold or more while proportional increases were seen in resistance to propionate or *P. aeruginosa*
239 in Liquid Killing. This result corroborates our recent findings that place mitochondrial homeostasis
240 at the heart of this pathogenesis model (Tjahjono and Kirienko, 2017). Although the effect was
241 smaller, benefits from B12 supplementation were still observed when stresses were more
242 pleiotropic (such as heat shock or oxidative stress, where intracellular contents are more uniformly
243 damaged). Taken together, our findings emphasize the importance of using a bottom-up approach
244 to effectively understand the mechanisms that connect nutrition, metabolic activity, and the host's
245 microbiota to its health.
246

247 **Experimental Procedures**

248 Additional detailed methods are presented in the Supplemental Experimental Procedures available
249 online.

250

251 *C. elegans pathogenesis and stress assays*

252 Liquid- and Slow-Killing assays were performed as described elsewhere (Kirienko et al., 2014).
253 Killing assays using *Enterococcus faecalis* strain OG1RF and *E. faecium* strain E007 assays were
254 performed similarly, except that *P. aeruginosa* strain PA14 was substituted with the relevant
255 pathogen, 10% brain-heart infusion medium was substituted for 20% slow-kill media, and host
256 death was measured after 4 days of infection. Propionate toxicity was measured as described
257 (Watson et al., 2014). For oxidative and iron removal stresses, NGM agar plates were supplemented
258 with 120 μM of juglone (Kirienko and Fay, 2010) or 100 μM of phenanthroline, respectively. Heat
259 shock experiments were carried out at 30 ˚C, lifespan was measured at 25 ˚C. Wild-type *C. elegans*
260 were rendered sterile by *cdc-25.1(RNAi)* when appropriate. For agar-based assays, three plates with
261 50 worms/plate/strain/biological replicate were used. For liquid-based experiments, 10 wells with
262 20 worms/well/strain/biological replicate were used. At least three biological replicates were
263 performed for each experiment. Statistical significance was calculated based on log-rank test,
264 except Student's t-test was used for Liquid Killing and propionate toxicity.

265

266 **Author Contributions**

267 AVR, RL, and NVK conceived of and performed experiments. AVR and NK analyzed data and wrote
268 the manuscript. NK edited the manuscript and supervised the work.

269

270 **Acknowledgements**

271 We wish to thank Alex Kang and Dr. Chris Pennington for technical assistance with ICP-MS. *E. coli*
272 OP50(xu363) was provided by Dr Eyleen O'Rourke, *E. faecalis* and *E. faecium* strains were the kind
273 gift of Dr. Danielle Garsin; some strains were provided by the CGC, which is funded by NIH Office of
274 Research Infrastructure Programs (P40 OD010440). This study was supported by the Cancer
275 Prevention and Research Institute of Texas (CPRIT) RR150044 and National Institutes of Health
276 K22 AI110552 awarded to NVK.
277

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409

410 **Figure Legends**

411 **Fig 1. HT115 increases *C. elegans'* resistance to stresses.** (**A**) Representative brightfield (top)
412 and fluorescent (bottom) images of *C. elegans* exposed to *P. aeruginosa* after feeding on OP50 or
413 HT115. Sytox Orange, a cell-impermeant, fluorescent dye was used to mark dead worms. (**B**) Time
414 course of quantification of staining for conditions in (**A**). (**C-G**) Survival of OP50- and HT115-fed
415 worms after infection with *E. faecalis* or *E. faecium*, or exposure to thermal or oxidative stressors, or
416 acute iron removal. (**H**) Lifespan of *C. elegans* fed with OP50 or HT115. *p*-value for (**B-G**) <0.01, (**H**)
417 – n.s. See also Figures S1-3.

418

419 **Fig 2. HT115 or methylcobalamin supplementation improve mitochondrial health.** (**A-C**)
420 Visualization and quantification of *acdh-1*::GFP reporter fluorescence in worms fed various diets, as
421 indicated. (**D-E**) Effect of the diet on propionate toxicity (**D**) and connectivity of mitochondrial
422 network (**E**). (**F-I**) Impact of methylcobalamin (B12) supplementation on mitochondrial count (**F**),
423 membrane potential (**G**), and production of ROS (**H**) or ATP (**I**). * - $p<0.01$, # - $p<0.05$

424

425 **Fig 3. Methylcobalamin supplementation increases resistance to stress.** (**A-B**) Representative
426 images and quantification of survival of *C. elegans* exposed to *P. aeruginosa* after feeding on OP50
427 with or without methylcobalamin (B12) supplementation. Fraction of surviving worms here and
428 elsewhere was inferred based on staining with Sytox Orange, a cell impermeant dye. (**C**) Relative
429 survival of *C. elegans*, grown on NGM or NGM/methylcobalamin plates with heat-killed OP50 as the
430 food source, after exposure to *P. aeruginosa*. (**D-G**) Effect of methylcobalamin-supplemented OP50
431 feeding on *C. elegans* resistance to infection with *E. faecalis*, *E. faecium,* heat shock, or lifespan. (**H-J**)
432 Survival of wild-type worms fed OP50 with or without methylcobalamin supplementation after
433 exposure to propionate (**H**), *P. aeruginosa* (**I**), or hyperthermia (**J**). * - $p<0.01$ (**B-E, H, I**). See also
434 Figure S4.

435 **Table 1. Mitochondrial genes upregulated by feeding on *E. coli* OP50**

Gene	Name	Evidence
C05C10.3		Ortholog of human mitochondrial CoA transferase (NP_000427.1); in *C. elegans* mitochondrial proteome (Li et al., 2009)
C55B7.4	*acdh-1*	Ortholog to human mitochondrial short chain specific acyl-CoA dehydrogenase (NP_001600.1); responds to propionate level in *C. elegans* mitochondria (MacNeil et al., 2013); in *C. elegans* mitochondrial proteome (Li et al., 2009)
F09E10.3	*dhs-25*	Ortholog of mitochondrial dehydrogenase (NP_055049.1) (Venkatesan et al., 2014); in *C. elegans* mitochondrial proteome (Li et al., 2009)
F09F7.4	*hach-1*	Ortholog of human mitochondrial CoA hydrolase (NP_055177.2), in *C. elegans* mitochondrial proteome (Li et al., 2009)
F22B8.7		Ortholog of human mitochondrial amidoxime reducing component (NP_073583.3), in *C. elegans* mitochondrial proteome (Li et al., 2009)
F28F8.2	*acs-2*	Ortholog of human mitochondrial acyl-CoA synthetase (NP_079425.3), localized to mitochondria (Van Gilst et al., 2005); in *C. elegans* mitochondrial proteome (Li et al., 2009)
F32D8.12		Ortholog of human mitochondrial lactate dehydrogenase (NP_705690.2); in *C. elegans* mitochondrial proteome (Li et al., 2009)
F37B4.7	*folt-2*	In *C. elegans* mitochondrial proteome (Li et al., 2009)
F44G3.2	*argk-1*	Ortholog of human mitochondrial creatine kinase (NP_001814)
F54D5.12		Ortholog of human mitochondrial hydroxyglutarate dehydrogenase (NP_689996.4), in *C. elegans* mitochondrial proteome (Li et al., 2009)
Y22D7AL.5	*hsp-60*	Mitochondrial heat shock protein (Yoneda et al., 2004); in *C. elegans* mitochondrial proteome (Li et al., 2009)
Y38F1A.6	*hphd-1*	Ortholog of human mitochondrial hydroxyacid-oxoacid transhydrogenase (NP_653251.2), in *C. elegans* mitochondrial proteome (Li et al., 2009)

436

437 See also Table S1.

438

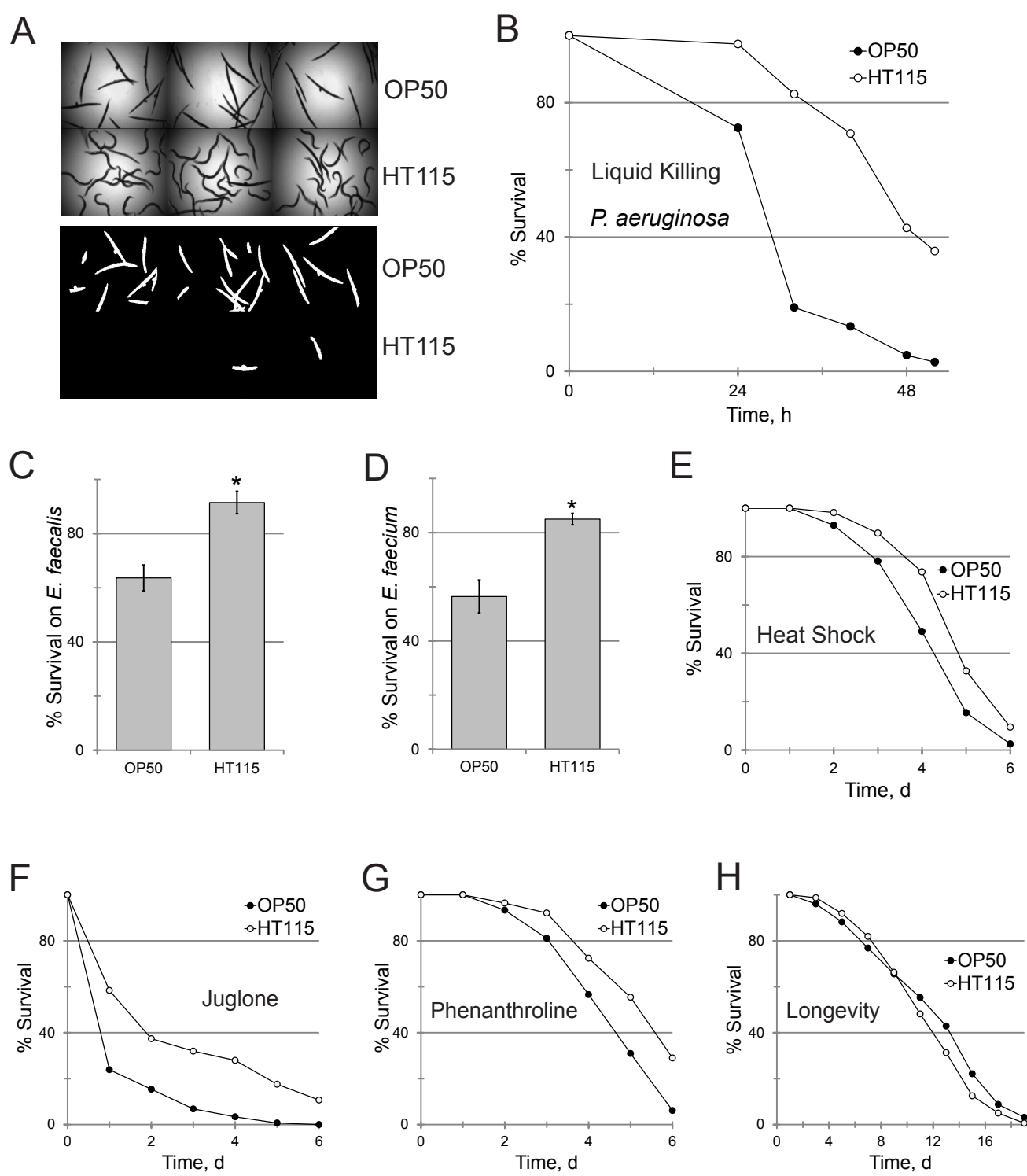
A
OP50
HT115
OP50
HT115
B
OP50
HT115
Liquid Killing
P. aeruginosa
% Survival
Time, h
C
% Survival on E. faecalis
OP50
HT115
D
% Survival on E. faecium
OP50
HT115
E
OP50
HT115
Heat Shock
% Survival
Time, d
F
OP50
HT115
Juglone
% Survival
Time, d
G
OP50
HT115
Phenanthroline
% Survival
Time, d
H
OP50
HT115
Longevity
% Survival
Time, d

Figure 1

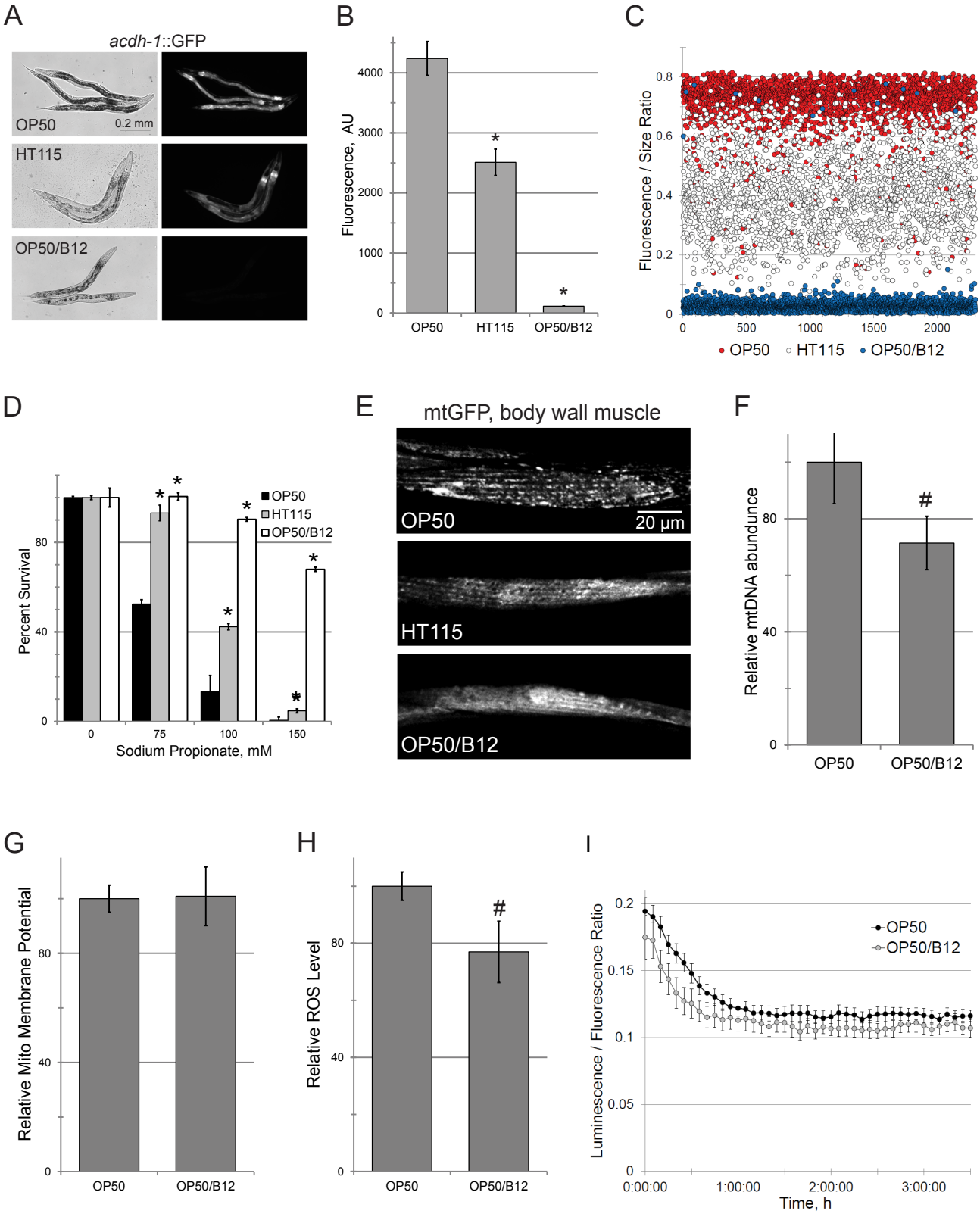
A
acdh-1::GFP
OP50
0.2 mm
HT115
OP50/B12
B
Fluorescence, AU
4000
3000
2000
1000
0
OP50
HT115
OP50/B12
C
Fluorescence / Size Ratio
0.8
0.6
0.4
0.2
0
0
500
1000
1500
2000
OP50
HT115
OP50/B12
D
Percent Survival
80
40
0
OP50
HT115
OP50/B12
0
75
100
150
Sodium Propionate, mM
E
mtGFP, body wall muscle
OP50
20 µm
HT115
OP50/B12
F
Relative mtDNA abundunce
80
40
0
#
OP50
OP50/B12
G
Relative Mito Membrane Potential
80
40
0
OP50
OP50/B12
H
Relative ROS Level
80
40
0
#
OP50
OP50/B12
I
Luminescence / Fluorescence Ratio
0.2
0.15
0.1
0.05
0
OP50
OP50/B12
0:00:00
1:00:00
2:00:00
3:00:00
Time, h

Figure 2

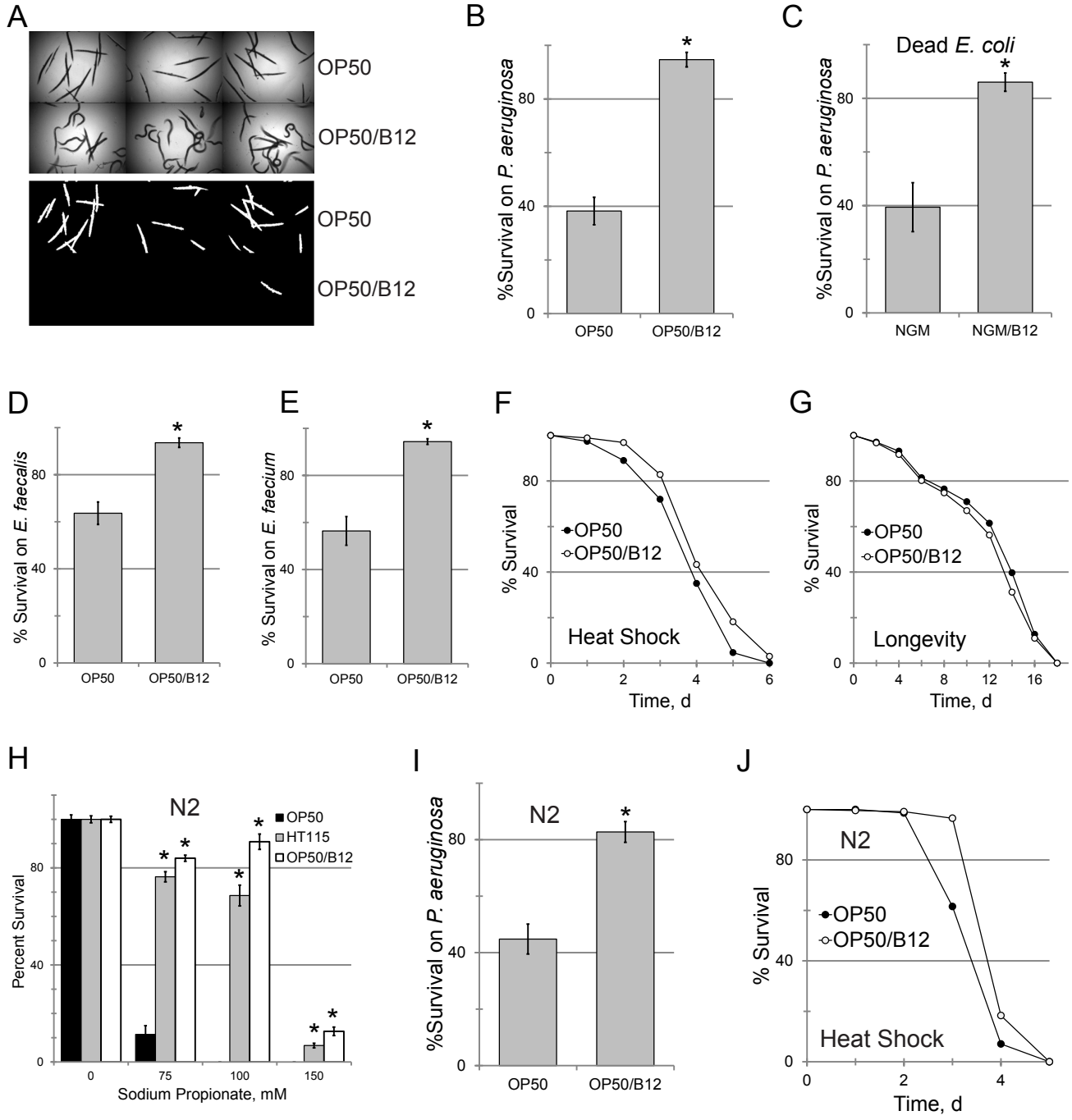
A
OP50
OP50/B12
OP50
OP50/B12
B
%Survival on P. aeruginosa
0
40
80
*
OP50
OP50/B12
C
Dead E. coli
%Survival on P. aeruginosa
0
40
80
*
NGM
NGM/B12
D
% Survival on E. faecalis
0
40
80
*
OP50
OP50/B12
E
% Survival on E. faecium
0
40
80
*
OP50
OP50/B12
F
% Survival
0
40
80
OP50
OP50/B12
Heat Shock
0
2
4
6
Time, d
G
% Survival
0
40
80
OP50
OP50/B12
Longevity
0
4
8
12
16
Time, d
H
N2
OP50
HT115
OP50/B12
Percent Survival
0
40
80
*
0
75
100
150
Sodium Propionate, mM
I
N2
%Survival on P. aeruginosa
0
40
80
*
OP50
OP50/B12
J
N2
% Survival
0
40
80
OP50
OP50/B12
Heat Shock
0
2
4
Time, d

Figure 3