

1 **Title:** Interplay between Mitochondria and Diet Mediates Pathogen and Stress Resistance in *C.*

2 *elegans*

3

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9

10 **Abstract:**

11 Diet is a crucial determinant of organismal biology. Here we demonstrate the dramatic impact of a

12 subtle shift in diet on the ability of *Caenorhabditis elegans* to survive pathogenic or abiotic stress.

13 Interestingly, this shift occurs independently of canonical host defense pathways, arising instead

14 from improvements in mitochondrial health. Using a variety of assays, we reveal that the most

15 common *C. elegans* food source (*E. coli* OP50) results in a vitamin B12 deficiency that compromises

16 mitochondrial homeostasis. Increasing B12 supply by feeding on *E. coli* HT115 or by supplementing

17 bacterial media with methylcobalamin restored mitochondrial function, even if the bacteria were

18 dead. B12 supplementation also efficiently increased host health without adversely affecting

19 lifespan. Our study forges a molecular link between a dietary deficiency (nutrition/microbiota) and

20 a physiological consequence (host sensitivity), using the host-microbiota-diet framework. The

21 ubiquity of B12 deficiency (~10-40% of US adults) highlights the importance of our findings.

22

23

24 **Introduction**

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

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

40 Even this binary system has yielded significant insight regarding interactions between the host
41 and its bacterial counterpart. For example, studies have shown that not only the relative
42 biomacromolecular content of their diet (i.e., lipids, carbohydrates, etc.) but also bacterial
43 metabolites informed the host's biology, including folate ¹, nitric oxide ², and tryptophan ^{3,4}. Indeed,
44 even the rate of bacterial respiration affected the metabolic state of the host ⁵.

45 In this report, we show that differences in bacterial strains drive increased host sensitivity to a
46 variety of stresses, including exposure to *P. aeruginosa*, oxidative stress, or hyperthermia. Through
47 a panel of orthogonal assays, we demonstrated that a diet of *E. coli* strain OP50 causes a chronic
48 vitamin B12 deficiency that perturbs mitochondrial health and function. B12 supplementation,

49 even in the absence of living bacteria, increased resistance without shortening lifespan. Our
50 findings provide a mechanistic explanation for the link between diet, cellular homeostasis, and
51 organismal health.

52

53 **Results**

54 *A diet of E. coli strain HT115 confers resistance to a variety of stresses in C. elegans*

55 While characterizing a novel *C. elegans*-*P. aeruginosa* Liquid Killing assay ^{6,7}, we made the
56 unexpected observation that worms reared on *E. coli* HT115 exhibited increased resistance to *P.*
57 *aeruginosa* compared to worms reared on *E. coli* OP50 (**Fig 1A, B**). This was true even if bacteria
58 lacked the L4440 RNAi vector. Pathogenesis in this assay requires the siderophore pyoverdine,
59 which compromises host metabolism by removing ferric iron ⁸. Since exogenous iron strongly limits
60 host killing ⁷, we tested whether worms reared on HT115 contained more iron, indirectly
61 increasing their resistance to pyoverdine. However, neither a fluorometric iron (III) assay nor mass
62 spectrometric measurement of total iron showed significant diet-dependent differences in host iron
63 concentration (**Fig S1A, B**).

64 Substitution of *E. coli* OP50 with a variety of other bacterial foods (or UV- or heat-killed OP50)
65 increases *C. elegans*' lifespan ⁹⁻¹². These observations are typically interpreted to mean that OP50 is
66 weakly pathogenic to *C. elegans* ^{13,14}. On this basis, we surveyed basal gene expression levels for
67 downstream effectors of a variety of *C. elegans* immune pathways, including PMK-1/p38, ZIP-
68 2/bZIP, DAF-16/FOXO, FSHR-1/FSH, and SKN-1/Nrf2 ¹⁵⁻²⁰. Worms reared on OP50 and HT115 had
69 indistinguishable basal levels of expression of downstream innate immune effectors associated
70 with these pathways (**Fig S2**).

71 Using either the conventional HT115 RNAi strain or an RNAi-competent OP50 derivative ²¹, we
72 knocked down key defense pathways and assayed sensitivity to *P. aeruginosa* exposure. Although
73 some knockdowns affected the timing of death (e.g. *daf-2(RNAi)* prolonged survival, *daf-16(RNAi)*

74 hastened death), worms fed HT115 survived longer in each case (**Fig S3**). These data indicate that
75 weak OP50 pathogenicity (if it does exist) is unlikely to underlie the difference in sensitivity to *P.*
76 *aeruginosa*.

77 Many defense pathways confer resistance to both abiotic and pathogenic factors. Therefore, we
78 tested whether HT115 increased resistance to abiotic stresses. As anticipated, survival was
79 significantly increased when HT115-fed worms were exposed to the iron-scavenging xenobiotic
80 1,10-phenanthroline, heat shock, or juglone-induced oxidative stress (**Fig 1C-E**). Increased
81 resistance to pathogens and abiotic stresses is commonly associated with longer lifespan but, to our
82 surprise, feeding HT115 did not extend lifespan (**Fig 1F**).

83

84 *Transcriptome profiling implicates mitochondrial defects in diet-induced stress sensitivity*

85 To gain an unbiased snapshot of gene expression in worms fed OP50 or HT115, we performed
86 transcriptome profiling. Interestingly, the number of differentially-regulated genes was very small,
87 with only 35 genes upregulated between 2- and 8-fold in HT115 and 22 genes upregulated between
88 2- and 20-fold in OP50 (**Table S1**). Strikingly, of the latter 22 genes, 12 encoded proteins localized
89 to the mitochondria (**Table 1**). This enrichment was highly significant ($p=2.2*10^{-16}$), particularly
90 given that the fraction of nuclear genes that encode mitochondrial proteins in *C. elegans* and
91 humans is ~6% and 7% respectively^{22,23}. This enrichment was specific to genes upregulated by an
92 OP50 diet; amongst genes expressed at higher levels in HT115-fed worms, only two mitochondrial
93 genes (*acox-2* and T22B7.7) were observed (2/35=5.7%, $p=0.9896$). These data argue that feeding
94 with OP50 increases expression of mitochondrial genes. Two genes in particular caught our
95 attention: *hsp-60* encodes a mitochondrial chaperone that is constitutively expressed at low levels
96 and is induced when worms are exposed to mitochondria-damaging treatments²⁴⁻²⁶. *acdh-1*
97 encodes a short-chain acyl-CoA dehydrogenase that has been used as an indicator of branched
98 chain amino acids and/or propionyl-CoA, a mitotoxic byproduct of their metabolism²⁷.

99

100 *A diet of HT115 improves the vitamin B12 deficiency caused by OP50*

101 Consistent with our microarray results, we observed increased *acdH-1::GFP* expression in OP50-
102 fed worms, whether measured by conventional imaging (**Fig 2A,B**) or by COPAS flow vermimetry
103 (**Fig 2C**). Efficient metabolism of branched chain amino acids and propionyl-CoA requires vitamin
104 B12 ^{28,29}, an essential micronutrient for most organisms, including *C. elegans* and *E. coli*. Based on
105 these results, we hypothesized that a diet of OP50 causes a B12 deficiency that triggers
106 compensatory increases in expression of *acdH-1::GFP* and other mitochondria-related genes.

107 We attempted to use mass spectrometry to measure S-adenosylcobalamin in worms fed either
108 HT115 or OP50 (or in the bacteria themselves) to obtain quantitative measurements of vitamin
109 B12. Unfortunately, B12 levels for all samples measured were below the detection threshold, even
110 when samples were concentrated (*data not shown*). To circumvent this limitation we used sodium
111 propionate sensitivity as a functional readout of B12 sufficiency. Consistent with other data, a diet
112 of HT115 significantly increased resistance to propionate, indicating that it improves the B12
113 shortage (**Fig 2D**).

114

115 *Vitamin B12 deficiency disrupts mitochondrial homeostasis*

116 We hypothesized that the dietary B12 deficiency causes buildup of propionyl-CoA, which would
117 lead to mitochondrial damage. Therefore, we assayed mitochondrial homeostasis in *C. elegans* fed
118 either OP50 or HT115. Under normal conditions, mitochondrial quality control involves constant
119 fission and fusion events that serve to pool healthy, functional mitochondrial content, while
120 damaged material is sequestered for autophagic recycling ³⁰. Compared to mammals, *C. elegans*
121 mitochondria generally exhibit greater interconnectivity and longer tubular architecture (e.g.,
122 disrupting *C. elegans* mitochondrial genes almost always increases fragmentation ³¹). We used a *C.*
123 *elegans* strain expressing a mitochondrially-targeted GFP ²⁶ to assay mitochondrial health after

124 OP50 or HT115 consumption. Worms fed HT115 demonstrated increased connectivity and less
125 clumping (i.e., punctae) than OP50 (**Fig 2E**), indicating improved mitochondrial health.

126

127 *Vitamin B12 supplementation improves mitochondrial health*

128 To determine whether we could remedy the B12 limitation, we spiked OP50 growth medium
129 with exogenous methylcobalamin to a final concentration of 0.2 $\mu\text{g}/\text{mL}$. Supplementation
130 dramatically decreased *acd-1::GFP* fluorescence (**Fig 2A-C**), increased resistance to propionate
131 (**Fig 2D**), and improved mitochondrial network architecture (**Fig 2E**). Combined, these data
132 suggested that methylcobalamin supplementation significantly improved mitochondrial health.

133 We assayed other metrics of mitochondrial health, including mitochondrial count (**Fig 2F**),
134 membrane potential (**Fig 2G**), production of reactive oxygen species (ROS, **Fig 2H**), and ATP (**Fig**
135 **2I**) to verify this conclusion. Interestingly, methylcobalamin supplementation decreased the
136 number of mitochondria while mitochondrial membrane potential remained steady, indicating that
137 the average membrane potential of each mitochondrion was slightly increased by treatment.
138 Methylcobalamin also significantly decreased ROS production, while ATP production decreased
139 only slightly. The most parsimonious explanation for these observations is that a dietary B12
140 deficiency significantly compromises mitochondrial health and efficiency.

141

142 *Vitamin B12 deficiency in OP50 diet drives sensitivity to stress*

143 We predicted that the mild mitochondrial dysfunction exhibited by OP50-fed worms may drive
144 their increased sensitivity to stress. We reared worms on OP50 or OP50 supplemented with
145 methylcobalamin (OP50/B12), and tested their resistance to *P. aeruginosa*. We observed a dramatic
146 difference in host survival: virtually all OP50-fed worms were dead by the time OP50/B12-fed
147 worms showed $\sim 10\%$ death (**Fig 3A, B**). Bacterial metabolism of B12 was superfluous for this
148 effect. *C. elegans* fed heat-killed *E. coli* spotted onto plates containing methylcobalamin-

149 supplemented media also exhibited virtually no *acdH-1::GFP* expression and increased survival
150 when exposed to *P. aeruginosa* (**Figs S4A, 3C**). Next, we examined whether cobalamin was
151 beneficial during exposure to more generic stressors, like hyperthermia. Supplementation again
152 significantly increased *C. elegans* survival (**Fig 3D**).

153 A growing body of evidence suggests that decreasing mitochondrial activity, either by
154 genetically compromising oxidative phosphorylation or by caloric restriction, extends lifespan^{32,33}.
155 Since B12 supplementation improves mitochondrial function, we wanted to know whether it may
156 be beneficial during adverse conditions, but would also ultimately shorten lifespan. No significant
157 changes in lifespan were seen in worms receiving a diet supplemented with methylcobalamin (**Fig**
158 **3E**).

159 All of the experiments described above were performed in a *glp-4(bn2ts)* background, which
160 fails to generate a germline and is sterile at non-permissive temperatures. Sterility is necessary to
161 avoid non-specific death in the Liquid-Killing assay. To rule out the possibility that the observed
162 phenotypes were a result of the *glp-4 (bn2)* background, we tested wild-type N2 worms. In every
163 case tested, wild-type worms recapitulated our findings from *glp-4(bn2)* mutants (**Fig 3F-H**).

164

165 *The E. coli TonB transporter mediates vitamin B12 internalization and subsequent host health*

166 Since *E. coli* is unable to synthesize vitamin B12, it must be imported from the extracellular
167 milieu. As efficient import has been reported to require TonB activity^{34,35}, we evaluated *acdH-*
168 *1::GFP* fluorescence in worms fed *tonB* deletion mutants³⁶. While *acdH-1::GFP* fluorescence in
169 worms reared on the parental strain (*E. coli* BW25113) was similar to OP50 (**Fig S4B**), *tonB*
170 deletion slightly, but reproducibly, increased GFP level (**Fig S4C**, $p < 0.01$). Correspondingly, *tonB*
171 deletion also increased sensitivity to *P. aeruginosa* exposure (**Fig S4D**). As with OP50,
172 supplementation of wild-type BW25113 with methylcobalamin completely abolished *acdH-1::GFP*
173 fluorescence (**Fig S4E**). In contrast, *tonB* mutants showed only partial attenuation of GFP

174 expression after methylcobalamin supplementation, indicating that the bacteria were unable to
175 efficiently internalize the vitamin (**Fig S4F, G**). One possible explanation for the attenuation in GFP
176 fluorescence that we observed in these mutants is that *E. coli* has a salvage pathway to acquire
177 precursors for B12 biosynthesis³⁷. Our observation that *tonB* mutation increases host sensitivity to
178 stress still further (**Fig S4D**) indicates a role for TonB activity in the health of the host.

179

180 **Discussion**

181 Recently, Leulier and colleagues defined the “nutrient-microbiota-host axis” to incorporate all
182 of these determinants into a single conceptual framework³⁸. Our results leverage this idea to
183 connect diverse phenomena into an articulated whole. For example, our data indicate that a dietary
184 deficiency in vitamin B12 deficiency causes mitochondrial defects, likely through the buildup of
185 propionyl-CoA (a known mitochondrial toxin). We also demonstrated that this damage significantly
186 increased sensitivity to a variety of stresses. Our findings provide a mechanistic explanation for
187 clinical observations that patients with methylmalonic or propionic acidemias (either of which can
188 arise from vitamin B12 deficiency) exhibit symptoms that are strikingly similar to those with
189 congenital mitochondrial defects (including poor growth, muscle weakness, and disorders of the
190 liver, kidney, and gastrointestinal and respiratory tracts). It also explains why mice with inactive
191 methylmalonyl-CoA mutase (one of two B12-dependent enzymes) exhibit reduced respiratory
192 chain activity³⁹. Vitamin B12 is clearly important for normal cellular stress resistance and healthy
193 aging. Despite this, estimates of deficiency range between 10-40% of the population, with increased
194 prevalence in the elderly⁴⁰.

195 Previous studies from the Walhout lab have linked B12 replete diets (including *Comamonas*
196 *aquaticus* and *E. coli* HT115) to shortened lifespan⁴¹. We also saw a small, but statistically
197 significant, decrease in maximum lifespan when *C. elegans* was reared on *E. coli* HT115.
198 Intriguingly, methylcobalamin did not affect the lifespan of worms when it was added to an OP50

199 diet (**Fig 3E**), suggesting that the improvement in mitochondrial health provided by B12
200 supplementation can be unlinked from lifespan shortening. Currently, we hypothesize that some
201 other aspect of *Comamonas* and HT115 diets, probably a common metabolite, was responsible for
202 this effect.

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

213 We also showed that this framework can help identify an important mechanism used by *C.*
214 *elegans*, and presumably more complex metazoans, to fine-tune their cell metabolism to resist
215 adverse environmental factors (including pathogens, heat, and toxins), even without activating
216 canonical host defense pathways. Interestingly, the sensitivity to stress caused by B12 deficiency
217 seems to correlate with the degree of mitochondrial involvement in the stress-induced pathology.
218 For example, we observed that methylcobalamin supplementation decreased *acdH-1* expression by
219 10-fold or more while proportional increases were seen in resistance to propionate or *P. aeruginosa*
220 in Liquid Killing. This result corroborates our recent findings that place mitochondrial homeostasis
221 at the heart of this pathogenesis model (Tjahjono E, Kirienko N, *personal communication*). Although
222 the effect was smaller, benefits from B12 supplementation were still observed when stresses were
223 more pleiotropic (such as heat shock or oxidative stress, where intracellular contents are more

224 uniformly damaged). Taken together, our findings emphasize the importance of using a bottom-up
225 approach to effectively understand the mechanisms that connect nutrition, metabolic activity, and
226 the host's microbiota to its health.

227

228 **Methods**

229 *C. elegans* strains

230 All *C. elegans* strains were maintained on nematode growth medium (NGM) seeded with
231 *Escherichia coli* strain OP50, HT115 or BW25113 (see below) with or without supplementation
232 with methylcobalamin at a final concentration of 0.2 mg/L. Worms were reared and passaged at
233 15°C ⁴² unless otherwise noted. For RNAi-mediated gene knockdown, plasmids from the Ahringer
234 library ⁴³ were either used in the HT115(DE3) strain supplied or purified and transformed into an
235 RNAi-competent strain of OP50 (xu363) ²¹. All plasmids were sequence verified.

236 *C. elegans* strains used in this study included N2 Bristol (wild-type), SS104 [*glp-4(bn2)*] ⁴⁴,
237 NVK44 [*glp-4(bn2); zcls14{myo-3::GFP(mt)}*], PE327 (ATP reporter): *glp-4(bn2); feIs5* [sur-
238 5p::luciferase::GFP + rol-6(su1006)] ⁴⁵, and VL749 [wwIs24{*acdh-1p::GFP + unc-119(+)*}] ⁴¹,

239

240 *Bacterial strains*

241 Bacterial strains used in this study were *E. coli* OP50 and OP50(xu363), *E. coli* HT115(DE3); *E.*
242 *coli* BW25113, BW25113 *tonB* #1 and BW25113 *tonB* #2 were obtained from Keio Knockout
243 Collection (GE Dharmacon). For pathogenesis assays, *P. aeruginosa* PA14 was used ⁴⁶.

244

245 *C. elegans* pathogenesis and stress assays

246 Liquid- and Slow-Killing assays were performed as described elsewhere ⁴⁷. Propionate toxicity
247 was measured as described ²⁸. For oxidative and iron removal stresses, NGM agar plates were
248 supplemented with 120 μM of juglone ⁴⁸ or 100 μM of phenanthroline, respectively. Heat shock

249 experiments were carried out at 30 °C, lifespan was measured at 25 °C. Wild-type *C. elegans* were
250 rendered sterile by *cdc-25.1(RNAi)* when appropriate. For agar-based assays, three plates with 50
251 worms/plate/strain/biological replicate were used. For liquid-based experiments, 10 wells with 20
252 worms/well/strain/biological replicate were used. At least three biological replicates were
253 performed for each experiment. Statistical significance was calculated based on log-rank test,
254 except Student's t-test was used for Liquid Killing and propionate toxicity.

255

256 *Detection of total iron via ICP-MS*

257 For ICP-MS experiments, worms were prepared as for the measurement of internalized
258 pyoverdine. In brief, approximately 24,000 *C. elegans* L1 larvae per sample were raised on NGM
259 plates seeded with *E. coli* strains OP50 or HT115. When they reached young adulthood, they were
260 transferred to 15 mL conicals, washed four times, and then collected with uniform volumes.
261 Samples were frozen, thawed, and disrupted via sonication using a 2 min cycle on a Branson 250
262 Sonifier set to 70% output, 10% duty cycle. After lysis, samples were transferred to glass
263 scintillation vials and water was evaporated by heating at 100°C on a heating block. Upon the loss
264 of all visible moisture, samples were digested in 4 mL of concentrated, trace-metals grade nitric
265 acid (GFS Chemicals) overnight at 98 °C. For ICP analysis, samples were resuspended in 1 mL
266 concentrated nitric acid, diluted 1/40 into 2% HNO₃ (v:v), 2% ethanol (v:v), and spiked with
267 yttrium/indium as internal standards. For iron quantitation, a seven point calibration curve from 3
268 ug/L to 200 ug/L was prepared in 2% HNO₃ (v:v), 2% ethanol (v:v) and spiked with
269 yttrium/indium internal standards. Linear correlation coefficients for the iron calibration curve
270 were at least 0.9998.

271 Quantitation of iron was carried out by inductively-coupled plasma mass spectrometry (ICP-
272 MS) analysis, using a Perkin Elmer Nexion 300X ICP-MS system, operated in the kinetic energy
273 discrimination (KED) mode. Iron was monitored at m/z 57. Yttrium and indium were used as

274 internal standards and were monitored at m/z 89 and m/z 115, respectively. Quadrupole scanning
275 parameters were set to 20 scans per read, 1 read per replicate, and 3 replicates per sample. The
276 instrument was operated in the peak hopping mode with a dwell time of 50 ms. For KED mode, the
277 cell gas pressure was set at 5, the RPa and RPq were set at 0 and 0.25 respectively. The detector
278 was operated in dual mode. Plasma and auxiliary gas flows were set at 16 and 1.2 L/min
279 respectively. Nebulizer gas flow was tuned at the time of analysis and typically ranged between
280 0.99 and 1.02 L/min. Plasma power was 1600 W. The peristaltic pump was operated at 20 rpm for
281 analysis and 40 rpm for flushing. Read delay, flush, and wash times were 60, 90 and 120 seconds
282 respectively.

283

284 *Detection of host ferric iron*

285 Fluorometric determination of ferric iron was performed as follows. First, lysates from young
286 adult *C. elegans*, fed on OP50 or HT115 were prepared as described above. Next, a standard solution
287 of pyoverdine, with fluorescence within linear range of detection by spectrometry (15,000 – 20,000
288 AU) was prepared. This material was mixed (1:1, v/v) with water, ferric iron, or lysates from *C.*
289 *elegans*, and incubated for 10 min. Pyoverdine fluorescence was measured using excitation 405 nm,
290 emission 460 nm. As pyoverdine has a 1:1 stoichiometric relationship with iron, the difference
291 between initial and final fluorescence is equivalent to the available ferric iron remaining within *C.*
292 *elegans*.

293

294 *Microarray and Quantitative Real-Time PCR (qPCR)*

295 For RNA collection, *glp-4(bn2)* worms were grown on appropriate plates until reaching young
296 adult stage. RNA was purified and hybridized to Affymetrix GeneChips for *C. elegans* at the Partners
297 Center for Personalized Genetic Medicine, Boston, MA, according to manufacturer's protocols.
298 Three biological replicates were tested for each condition. Gene expression was analyzed using

299 GCRMA (<http://www.bioconductor.org>). Differentially upregulated genes were determined on the
300 basis of fold change (>2) and the value of modified Wilcoxon rank test >1.5. Wilcoxon coefficient
301 was determined for each probeset as the smallest expression value in the condition with higher
302 average divided by the highest expression value in the condition with lower average. Microarray
303 data were deposited in GEO database and are available using following link:
304 <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?token=kjibcyuydrstvuj&acc=GSE97678>

305 cDNA synthesis was performed according to manufacturers' protocols (Ambion). qPCR was
306 performed using SYBR Green iQ mix (Bio-Rad). Fold changes were calculated with a $\Delta\Delta C_t$ method.
307 Primer sequences are available upon request.

308

309 *Imaging*

310 For visualization of *myo-3::GFP(mt)* and *acdh-1::GFP* on slides, young adults were grown on
311 appropriate food. Images were acquired and fluorescence was quantified using a Zeiss Axio Imager
312 M2 upright microscope with a Zeiss AxioCam 506 Mono camera and Zen 2 pro (Zeiss) software.
313 Fluorescence was quantified for at least 50 worms per condition per biological replicate. All the
314 fluorescent images were taken under identical exposure conditions. At least three biological
315 replicates were performed. Statistical significance was determined by Student's t-test.

316 For determination of ATP concentrations, approximately 200 PE327 worms were grown until
317 young adults on OP50 or HT115. The worms were transferred to 96-well, white clear bottom plates
318 and washed in S-basal five times. 150 μ Luminescence buffer (0.14M K₂PO₄, 0.03M sodium citrate,
319 1% DMSO, 1mM luciferin) was added to each well in the plate (Luz et al., 2016). Bioluminescence
320 and GFP fluorescence was measured kinetically every 5 minutes for 4h using Cytation5 plate reader
321 / imager (BioTek). Luminescence values were normalized to GFP fluorescence.

322

323 *Flow vermimetry*

324 Flow cytometry for measurement of *acdH-1::GFP*, MitoTracker Red CMXRos (Invitrogen), or
325 dihydroethidium (Fisher) was performed using COPAS FlowSort as described previously⁸. For each
326 reporter, all the measurements were taken under same settings. At least 2,000
327 worms/condition/biological replicate were used; at least three biological replicates were
328 performed. Statistical significance was determined by Student's t-test.

329

330 *Statistical analysis.*

331 For all of the experiments described in the paper at least 3 biological replicates were
332 performed. For Figures 1B-F and 3D, E, and H, statistical significance was determined using a log-
333 rank test (<http://bioinf.wehi.edu.au/software/russell/logrank/>). For Figures 2B, D, F, G, and H and
334 3B, C, F, and G, statistical significance was determined using two-tailed Student's t-test. * - $p < 0.01$, #
335 - $p < 0.05$. For determining enrichment of mitochondrial genes amongst the total pool of genes
336 upregulated in OP50, a p -value was calculated using hypergeometric probability distribution. For
337 Supplemental Figures S1-3 and S4 C, D, statistical significance was determined using Student's t-
338 test. * - $p < 0.01$. All error bars represent SEM.

339

340

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482

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489

490 **Author Contributions**

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

493

494 **Competing financial interests**

495 The authors declare no competing financial interests.

496

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499

500 **Figure Legends**

501 **Fig 1. HT115 increases *C. elegans*' resistance to stresses.** (A) Representative brightfield (top)
502 and fluorescent (bottom) images of *C. elegans* exposed to *P. aeruginosa* after feeding on OP50 or
503 HT115. Sytox Orange, a cell-impermeant, fluorescent dye was used to mark dead worms. (B) Time
504 course of quantification of staining for conditions in (A). (C-E) Survival of OP50- and HT115-fed
505 worms after exposure to thermal and oxidative stressors, and acute iron removal. (F) Lifespan of *C.*
506 *elegans* fed with OP50 or HT115. *p*-value for (B-E) <0.01, (F) - n.s.

507
508 **Fig 2. HT115 or methylcobalamin supplementation improve mitochondrial health.** (A-C)
509 Visualization and quantification of *acdH-1::GFP* reporter fluorescence in worms fed various diets, as
510 indicated. (D-E) Effect of the diet on propionate toxicity (D) and connectivity of mitochondrial
511 network (E). (F-I) Impact of methylcobalamin (B12) supplementation on mitochondrial count (F),
512 membrane potential (G), and ROS (H) or ATP (I) production. * - *p*<0.01, # - *p*<0.05

513
514 **Fig 3. Methylcobalamin supplementation increases resistance to stress.** (A-B) Representative
515 images and quantification of *C. elegans* exposed to *P. aeruginosa* after feeding on OP50 with or
516 without methylcobalamin (B12) supplementation. Fraction of dead worms here and elsewhere (1
517 corresponds to 100% dead) was inferred based on staining with Sytox Orange, a cell impermeant
518 dye. (C) Relative death of *C. elegans*, grown on NGM or NGM/methylcobalamin plates with heat-
519 killed OP50 as food source, after exposure to *P. aeruginosa*. (D-E) Effect of methylcobalamin-
520 supplemented OP50 feeding on *C. elegans* resistance to heat shock (*p*<0.01) or lifespan (*p*>0.05).
521 (F-H) Survival of wild-type worms fed OP50 with or without methylcobalamin supplementation
522 after exposure to *P. aeruginosa* (F), propionate (G), or hyperthermia (H). * - *p*<0.01 (B, C, F, G), for
523 (D, H), *p*<0.01.

524 **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 <i>C. elegans</i> mitochondrial proteome ²²
C55B7.4	<i>acdh-1</i>	Ortholog to human mitochondrial short chain specific acyl-CoA dehydrogenase (NP_001600.1); responds to propionate level in <i>C. elegans</i> mitochondria ⁴¹ ; in <i>C. elegans</i> mitochondrial proteome ²²
F09E10.3	<i>dhs-25</i>	Ortholog of mitochondrial dehydrogenase (NP_055049.1) ⁴⁹ ; in <i>C. elegans</i> mitochondrial proteome ²²
F09F7.4	<i>hach-1</i>	Ortholog of human mitochondrial CoA hydrolase (NP_055177.2), in <i>C. elegans</i> mitochondrial proteome ²²
F22B8.7		Ortholog of human mitochondrial amidoxime reducing component (NP_073583.3), in <i>C. elegans</i> mitochondrial proteome ²²
F28F8.2	<i>acs-2</i>	Ortholog of human mitochondrial acyl-CoA synthetase (NP_079425.3), localized to mitochondria ⁵⁰ ; in <i>C. elegans</i> mitochondrial proteome ²²
F32D8.12		Ortholog of human mitochondrial lactate dehydrogenase (NP_705690.2); in <i>C. elegans</i> mitochondrial proteome ²²
F37B4.7	<i>fol-2</i>	In <i>C. elegans</i> mitochondrial proteome ²²
F44G3.2	<i>argk-1</i>	Ortholog of human mitochondrial creatine kinase (NP_001814)
F54D5.12		Ortholog of human mitochondrial hydroxyglutarate dehydrogenase (NP_689996.4), in <i>C. elegans</i> mitochondrial proteome ²²
Y22D7AL.5	<i>hsp-60</i>	Mitochondrial heat shock protein ²⁴ ; in <i>C. elegans</i> mitochondrial proteome ²²
Y38F1A.6	<i>hphd-1</i>	Ortholog of human mitochondrial hydroxyacid-oxoacid transhydrogenase (NP_653251.2), in <i>C. elegans</i> mitochondrial proteome ²²

525

526

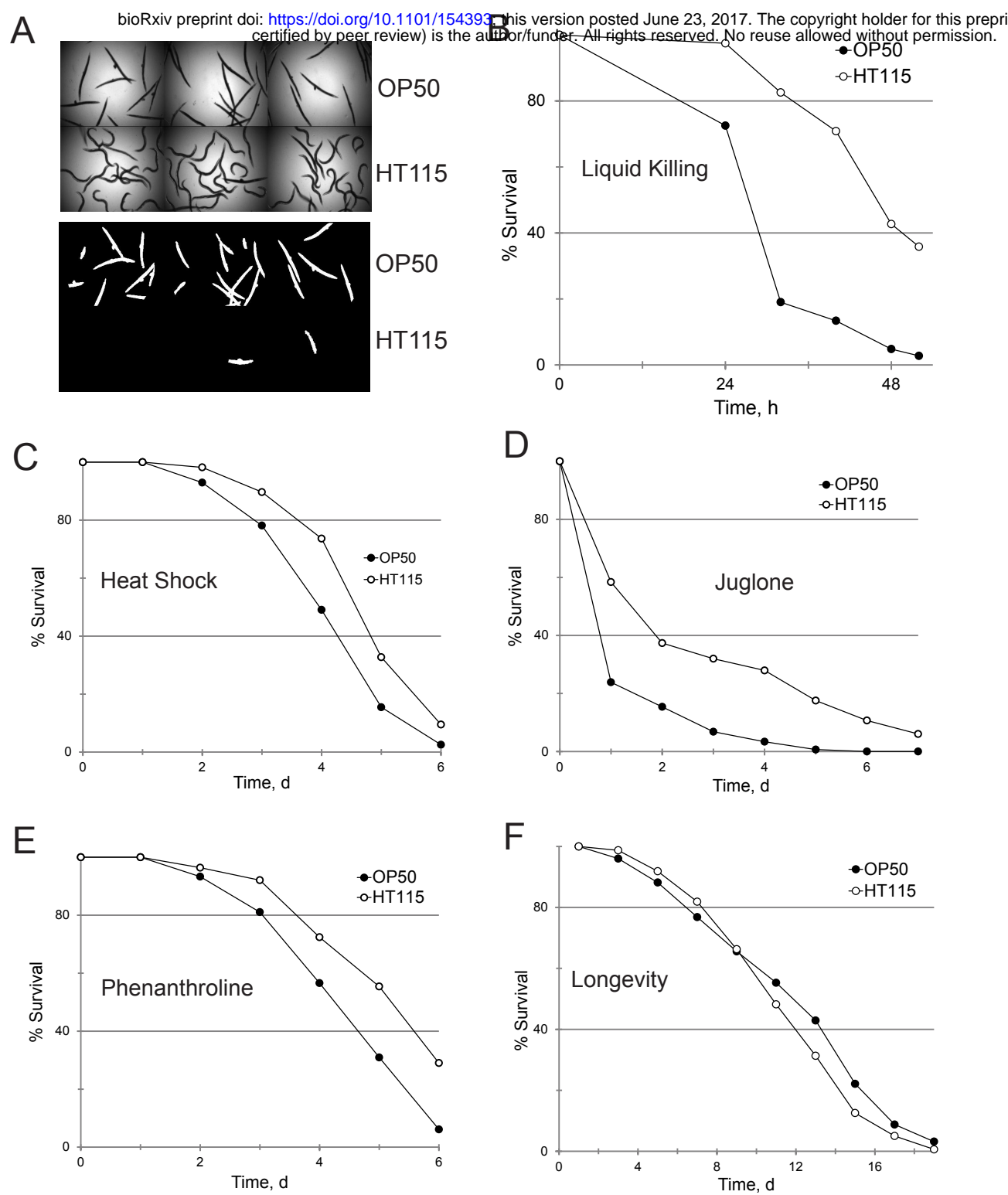


Figure 1

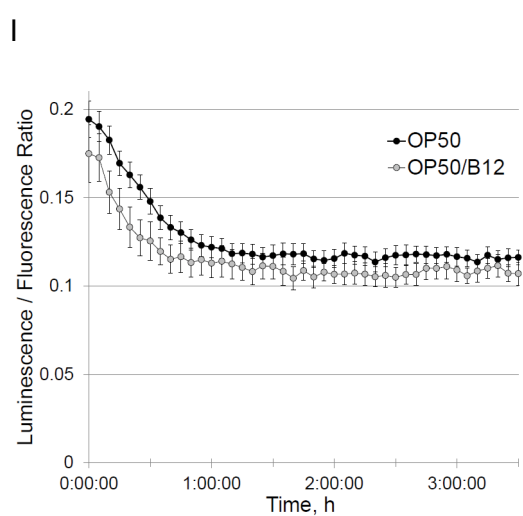
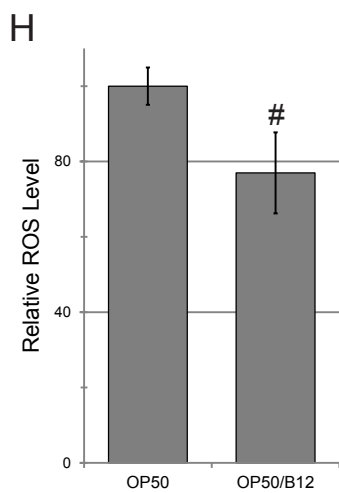
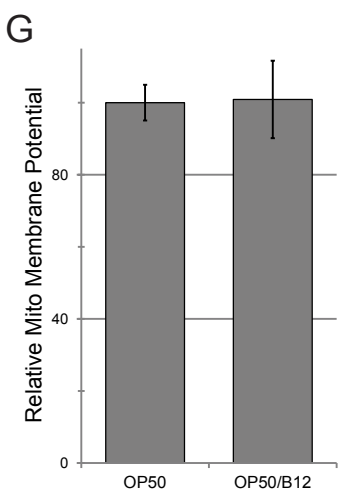
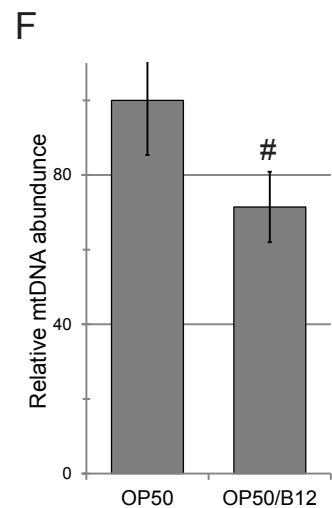
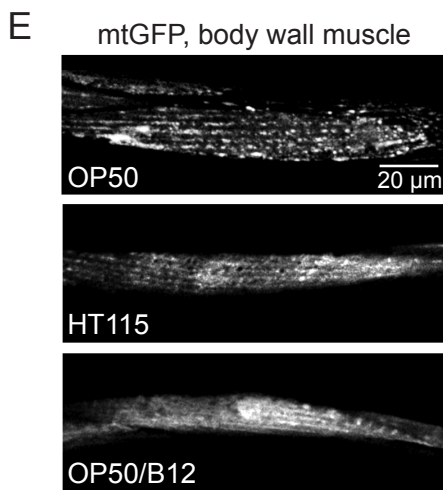
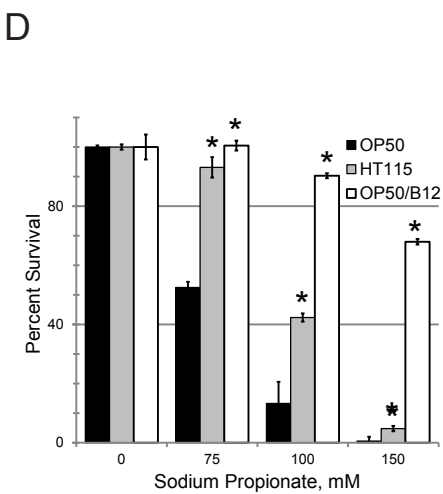
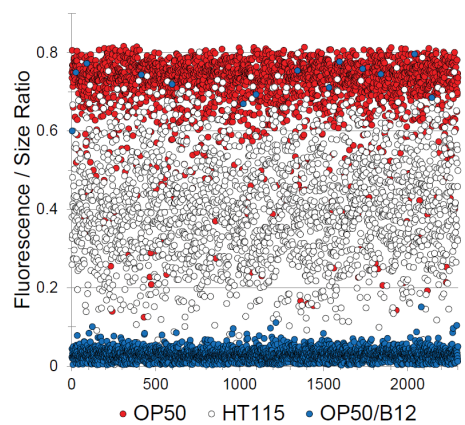
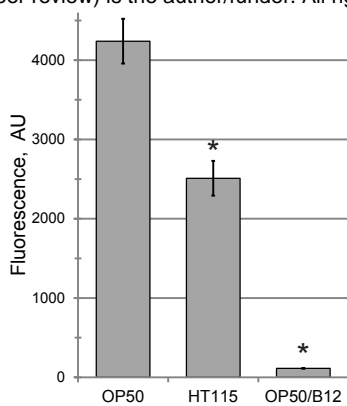
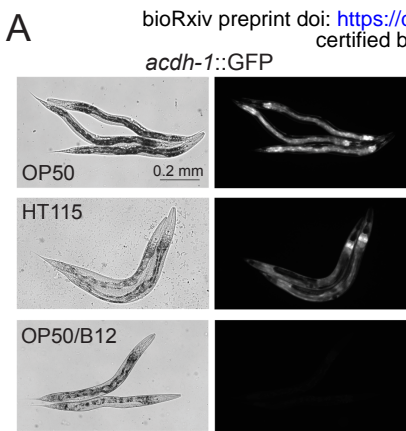


Figure 2

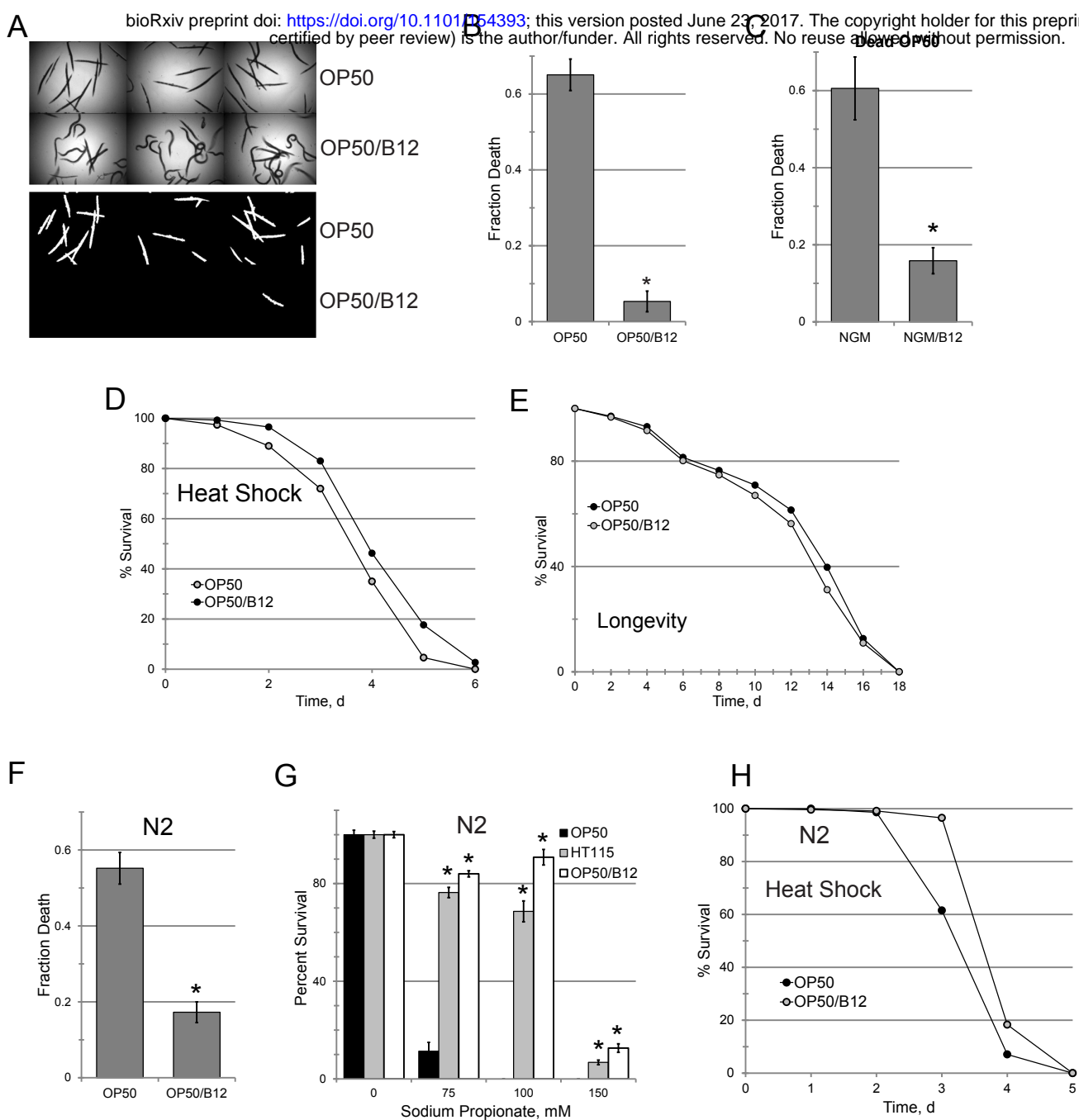


Figure 3