C. elegans transgenerationally adapts to infection by increasing the expression of cysteine synthases in progeny

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Abstract

Parental exposure to pathogens can prime offspring immunity in diverse organisms. The mechanisms by which parental exposure to pathogens can prime offspring immunity remain largely unknown. Here we report that the soil bacteria *Pseudomonas vranovensis* is a natural pathogen of the nematode *Caenorhabditis elegans* and that parental exposure of animals to *P. vranovensis* promotes offspring resistance to infection. This adaptation can be transmitted transgenerationally such that infection of adults can enhance the immunity of their descendants four generations later. We find that parental infection by *P. vranovensis* results in increased expression of the cysteine synthases CYSL-1 and CYSL-2 and the regulator of hypoxia inducible factor RHY-1 in progeny, that the expression of these three genes in offspring is required for adaptation to *P. vranovensis,* and that the expression of these genes is regulated by the WD40 repeat protein WDR-23.

87 **Introduction**

88

89 Intergenerational and transgenerational responses to environmental stress have been reported
90 in evolutionarily diverse organisms[1–7]. These studies investigated a variety of types of
91 stresses ranging from osmotic stress[1], to mitochondrial stress[5], to pathogen infection[8]. In each
92 case, parental exposure to stress appeared to prime offspring to respond to a similar stress.
93 For example, parental exposure of the nematode *Caenorhabditis elegans* to the opportunistic
94 pathogen *Pseudomonas aeruginosa* was reported to alter offspring behaviour in a way that
95 promotes offspring avoidance of *P. aeruginosa* and enhances offspring survival[8]. Despite this
96 apparent adaptation, parental exposure of *C. elegans* to *P. aeruginosa* also has been reported
97 to have deleterious effects; parental exposure to *P. aeruginosa* results in offspring that are
98 more susceptible to osmotic stress[1]. Taken together, these observations suggest that parents
99 might prime their offspring to adapt to stresses they have faced at the expense of offspring's
100 ability to respond to other stresses. However, whether these observations represent rare
101 examples and potential laboratory artifacts or if such programmed predictive adaptive
102 responses might be ubiquitous throughout evolution remains unclear.

103

104 Among the diverse stresses an organism might encounter, pathogens such as viruses, some
105 bacteria and eukaryotes are among the most ubiquitous stresses found in nature[9]. To adapt to
106 the constant threat of pathogens, many organisms have evolved mechanisms by which
107 parental exposure to pathogens can prime offspring immunity[6,10–15]. For example, in
108 mammals, a mother can transfer specific antibodies to her offspring via milk to prime
109 offspring immunity[15]. Similar observations of parents priming offspring immunity in
110 response to pathogens have been reported in both plants[14] and invertebrates[10], even though

111 these organisms lack antibodies. These findings suggest that multiple independent
112 mechanisms have evolved for parents to prime offspring immunity.
113
114 The mechanisms by which parental exposure to pathogens might program predictive adaptive
115 responses in offspring in organisms that lack antibodies remain largely unknown. Here we
116 report that *C. elegans* can transgenerationaly adapt to a natural pathogen, *Pseudomonas*
117 *vranovensis*. In this case, we find that parental exposure to *P. vranovensis* results in an
118 increase in the expression of cysteine synthases in offspring which promotes resistance to *P.*
119 *vranovensis*. This mechanism is distinct from any previously proposed pathway[8].
120 Collectively, these observations describe a new mechanism by which parents can prime
121 offspring immunity in response to a bacterial infection.
122
123 **Results**
124
125 One of the major obstacles in determining the molecular mechanisms underlying heritable
126 adaptations to pathogen infection is the lack of robust models that can be easily analyzed in
127 the laboratory. To address this obstacle, we sought to develop a model of a heritable
128 adaptation to bacterial infection in the nematode *C. elegans* by testing whether *C. elegans*
129 could heritably adapt to potential bacterial pathogens from its natural environment. Previous
130 sampling of bacterial species from the natural environments of *C. elegans* identified 49 as yet
131 undescribed bacterial isolates that induce the expression of the immune response genes *irg-1*
132 or *irg-5*[16]. We tested whether parental exposure to these potentially pathogenic bacteria could
133 enhance offspring survival in response to future exposure to the same bacteria. We found that
134 parental exposure to two of the isolates, BIGb446 and BIGb468, significantly enhanced
135 offspring survival in response to future exposure to the same bacteria (Fig. 1a). Specifically,

136 approximately 95% of newly hatched larvae from parents fed a standard laboratory diet of *E.*
137 *coli* HB101 died within 24 hours of hatching on BIGb446 or BIGb468 (Fig. 1a). By contrast,
138 45% of embryos from adults briefly exposed to BIGb446 or BIGb468 were still alive at 24
139 hours (Fig. 1a) and a majority of these larvae survived through adulthood (Fig. 1b-c). In
140 addition, we found that adults were more resistant to BIGb446 than larval animals
141 (Supplementary Fig. 1) and that UV-killed bacteria did not cause lethality (Fig. 1d). We
142 conclude that parental exposure to bacterial isolates BIGb446 and BIGb468 enhances
143 offspring survival in response to future exposure to these bacteria.
144
145 To determine the species identity of bacterial isolates BIGb446 and BIGb468 we performed
146 long read whole genome sequencing and assembled the genomes of these bacteria. We found
147 that the genomes of both BIGb446 and BIGb468 were approximately 5.9 Mb (Supplementary
148 File 1 and 2) and were 99.38% identical across the entire genome (Supplementary Table 1
149 and Supplementary Fig. 2). We concluded that BIGb446 and BIGb468 are isolates of the
150 same species. We compared the 16S rRNA sequence of BIGb446 to known bacterial
151 genomes using BLAST. We found that the 16s rRNA sequence from BIGb446 is 99.93%
152 identical to *Pseudomonas vranovensis* strain 15D11. Previous studies of *Pseudomonas*
153 phylogeny have used the DNA sequences of *gyrB*, *rpoB*, and *recA* to differentiate species of
154 *Pseudomonas,* with similarity above 97% set as the accepted species threshold[17,18]. We found
155 that the sequence of *gyrB* was 98.05% identical, the sequence of *rpoB* was 99.44% identical,
156 and the sequence of *recA* was 98.66% identical to the sequences of homologous genes in *P.*
157 *vranovensis* strain 15D11. No other species of *Pseudomonas* was greater than 97% identical
158 to *Pseudomonas sp.* BIGb446 at any of these three genes. Furthermore, we compared the
159 average nucleotide identity (ANI) of our assembly of *Pseudomonas sp.* BIGb446 with the
160 genome of *P. vranovensis* strain 15D11 using OrthoANIu[19]. We found these two genomes

161 had an average nucleotide identity of 97.33%. We conclude that *Pseudomonas sp.* BIGb446
162 and *Pseudomonas sp.* BIGb468 are isolates of *Pseudomonas vranovensis*, a Gram-negative
163 soil bacteria[20].
164
165 In some cases, the effects of parental stress on offspring have been reported to be
166 intergenerational and only last a single generation[1]. In other cases, the effects of parental
167 stress on offspring have been reported to persist transgenerationally and thus affect
168 descendants several generations later[5,8,21]. To test whether exposure to BIGb446 had any
169 transgenerational effects on immunity we fed adult animals BIGb446 for 24 hours and
170 assayed the response of progeny two generations later. We found that a single exposure of
171 adult animals to BIGb446 enhanced their offspring's survival (one generation later) but did
172 not enhance the survival of their descendants two generations later (Fig. 2a), indicating that
173 the effects of parental exposure to BIGb446 only last a single generation under these
174 conditions. Some studies of the effects of parental environment on offspring have found that
175 consecutive exposure of multiple generations of animals to the same stress can enhance
176 adaptation to stress in descendants[5]. We therefore tested whether the exposure of five
177 consecutive generations (P0, F1, F2, F3, and F4) of animals to BIGb446 could enhance
178 progeny (F5) survival in response to BIGb446 but found that this was not the case (Fig. 2a).
179 Finally, we tested whether the exposure of four consecutive generations of animals to
180 BIGb446 could enhance survival in progeny for more than a single generation. Unlike a
181 single generation of exposure to BIGb446, we found that the enhanced survival of
182 descendants of animals exposed to BIGb446 for four consecutive generations persisted for
183 two generations (Fig. 2a). Furthermore, we found that three consecutive generations of
184 exposure to BIGb446 was required for resistance to BIGb446 to persist transgenerationally
185 (Fig. 2b). We conclude that under conditions of multiple consecutive generations of animals

186 being exposed to BIGb446 the effects of exposure to BIGb446 can be transmitted
187 transgenerationally.
188
189 Multiple signaling pathways and epigenetic modifiers have been proposed to mediate
190 heritable and transgenerational responses to environmental stress. We tested whether
191 mutations in any factors previously reported to be involved heritable or transgenerational
192 responses to stress were required for *C. elegans* heritable adaptation to BIGb446 including
193 small RNA mediated pathways (*hrde-1*[22], *prg-1*[8]), H3K9 methylation (*set-32*[23] and *met-2*[4]),
194 H3K4 methylations (*set-2, wdr-5.1, spr-5*)[5,21], DNA adenosine methylation (*damt-1*)[5], and the
195 RAS/ERK signaling pathway (*lin-45*)[1]. We found that none of these factors were required for
196 *C. elegans* adaptation to BIGb446 (Fig. 2c) These data suggest that adaptation to *P.*
197 *vranovensis* involves an as-yet-unknown mechanism of inheritance.
198
199 Bacterial isolates BIGb446 and BIGb468 were originally reported to promote the expression
200 of immune response gene *irg-5*[16]. The expression of *irg-5* in response to pathogen infection is
201 controlled by the p38-like MAP kinase PMK-1[24]. We tested whether PMK-1 is required for
202 resistance to BIGb446 by placing wild-type and *pmk-1* mutant adults on plates seeded with
203 BIGb446. We found that 100% of *pmk-1* mutants were dead after 48 hours while more than
204 40% of wild-type animals remained alive (Supplementary Fig. 1). These results indicate that
205 PMK-1 promotes resistance to BIGb446. We tested whether PMK-1 was required for
206 heritable adaptation to BIGb446 by exposing wild-type and *pmk-1* mutants to BIGb446 for
207 24 hours and assaying the survival of their offspring in response to repeated exposure to
208 BIGb446. We found that PMK-1 is not required for the heritable adaptation to BIGb446 (Fig.
209 2d).
210

211 *C. elegans'* heritable adaptation to *P. vranovensis* might be a general response to pathogen
212 infection or specific to *P. vranovensis.* To test whether parental infection by different
213 bacterial pathogens could protect offspring from BIGb446 we exposed adult animals to two
214 additional bacterial pathogens of *C. elegans*, *Pseudomonas aeruginosa* PA14[25] and
215 *Photorhabdus luminescens* Hb[26], and assayed the response of their offspring to BIGb446.
216 We found that parental infection by these two pathogens did not enhance offspring survival
217 in response to BIGb446 (Fig. 2e). These results indicate that adaptation to *P. vranovensis* is
218 not a generic response to pathogenic bacteria.
219
220 Previous studies found that *C. elegans* can heritably adapt to osmotic stress and starvation by
221 regulating gene expression and metabolism in offspring[1,3]. To identify how parental infection
222 by BIGb446 affects gene expression and metabolism in offspring we profiled gene
223 expression by RNA-seq and metabolite levels by LC/MS. Using RNA-seq we identified
224 1,153 genes that exhibit increased expression greater than 2-fold and 491 genes that
225 decreased expression greater than 2-fold in embryos from parents fed BIGb446 when
226 compared to embryos from parents fed HB101 (Fig. 3a and Supplementary Table 2). We also
227 quantified gene expression in young adults exposed to BIGb446. We found that 1,275 genes
228 exhibited a greater than 2-fold change in expression when compared to young adults fed
229 HB101 (Supplementary Table 2). Of these 1,275 genes, 398 were also observed to change in
230 embryos (Fig. 3b). We conclude that infection by *P. vranovensis* BIGb446 results in similar,
231 but distinct, effects on adult and embryonic gene expression. Using LC/MS we profiled 92
232 lipid metabolites, including those previously observed to heritably change in abundance in
233 response to osmotic stress[27], and found that 6 metabolites exhibit modest changes in
234 abundance in embryos from parents fed BIGb446 when compared to embryos from parents
235 fed HB101 (Fig. 3c and Supplementary Table 3). We conclude that parental exposure to the

236 pathogenic diet of *P. vranovensis* BIGb446 alters gene expression and metabolite abundance
237 in embryos.
238
239 We previously found that parental infection by *P. aeruginosa* PA14 and *P. luminescens* Hb
240 did not protect offspring from BIGb446 (Fig. 2e). We hypothesized that different parental
241 infections might result in different gene expression and metabolite changes in embryos and
242 that the specific changes caused by parental infection by BIGb446 are required for offspring
243 adaptation to BIGb446. To compare how parental infection by different bacterial pathogens
244 affects offspring gene expression and metabolism we exposed young adults to *P. aeruginosa*
245 PA14 or *P. luminescens* Hb for 24 hours and collected embryos from these animals to profile
246 gene expression by RNA-seq and metabolites by LC/MS. We found that only 41 genes
247 exhibited a greater than 2-fold change in expression in embryos from parents exposed to
248 PA14 when compared to embryos from parents exposed to HB101 and of these genes 29 also
249 exhibited altered gene expression in embryos from parents exposed to BIGb446 (Fig. 3d-e,
250 Supplementary Table 4). Separately, we found that only 26 genes exhibited a greater than 2-
251 fold change in expression in embryos from parents exposed to *P. luminescens Hb* when
252 compared to parents fed HB101 (Fig. 3d-e and Supplementary Table 4). Of these genes, only
253 5 also exhibited altered expression in embryos from parents exposed to BIGb446 (Fig. 3d).
254 Collectively, these results indicate that parental infection by *P. aeruginosa* and *P.*
255 *luminescens* had only a limited effect on offspring gene expression when compared to
256 parental infection by *P. vranovensis*. Only a single lipid metabolite measured exhibited a
257 statistically significant change in abundance in embryos from parents fed *P. aeruginosa* and
258 *P. luminescens* (Supplementary Table 3). We conclude that parental exposure to different
259 pathogenic diets has distinct effects on offspring gene expression.
260

261 We hypothesized that the specific changes in gene expression in embryos from parents
262 exposed to *P. vranovensis* BIGb446 mediate the observed adaptation to this pathogen.
263 Among the genes that we found to specifically increase in expression in response to BIGb446
264 was the glutathione-s-transferase *gst-31* (among the top 10 most upregulated genes (Table
265 S2)) and we confirmed that *C. elegans'* exposure to BIGb446 resulted in increased
266 expression of *gst-31* in both adults and embryos using a GFP reporter (Fig. 4a-b). To identify
267 factors that regulate animals' gene expression in response to BIGb446 we performed a
268 chemical mutagenesis screen for mutants that exhibit increased expression of *gst-31* even
269 when fed a normal laboratory diet of *E. coli* HB101. We identified four mutants from this
270 screen and all four contain mutations in the conserved WD40 repeat protein *wdr-23* (Fig. 4c).
271 Consistent with our findings, multiple previous studies have identified that *wdr-23* mutants
272 exhibit increased expression of glutathione-s-transferases, including *gst-31*[28–30]. We conclude
273 that WDR-23 negatively regulates the expression of *gst-31.*
274
275 We tested whether the loss of WDR-23 affected *C. elegans* adaptation to *P. vranovensis* and
276 found that multiple independent *wdr-23* mutants were unable to heritably adapt to BIGb446
277 (Fig. 4d). These results indicate that loss of WDR-23 prevents animals from being able to
278 adapt to BIGb446. WDR-23 is a negative regulator of the Nrf2-like transcription factor SKN-
279 1 and the loss of WDR-23 results in SKN-1 activation[31]. Activation of SKN-1 in turn
280 promotes the expression of oxidative stress response genes[30] and mutations in *skn-1* suppress
281 many of the defects observed in *wdr-23* mutants[31]. We exposed wild-type, *skn-1* partial loss-
282 of-function, and *wdr-23;skn-1* double mutant adults to BIGb446 and assayed the response of
283 their offspring to BIGb446. We found that partial loss-of-function mutations in *skn-1* (null
284 mutants are embryonic lethal) also reduced animals' ability to adapt to BIGb446 and that this
285 mutation in *skn-1* did not suppress the adaptation defect of *wdr-23* mutants (Fig. 4d).

286 Collectively, these results indicate that mutations that both increase and decrease the activity
287 of SKN-1 disrupt animals' ability to adapt to *P. vranovensis.* We propose that proper
288 signaling via WDR-23 and SKN-1 is critical for *C. elegans* to adapt to *P. vranovensis.*
289
290 We previously observed that parental exposure to BIGb446 modifies gene expression in
291 offspring. We hypothesized that parental infection by BIGb446 might regulate the expression
292 of pathogen or stress response genes in offspring via WDR-23 and SKN-1. To test this
293 hypothesis, we first performed RNA-seq of wild-type and *wdr-23* mutant embryos. We
294 identified that 1,634 genes exhibited a greater than 2-fold change in expression in *wdr-23*
295 mutants (Fig. 4e and Supplementary Table 5). Of these genes, we found that 634 also exhibit
296 a greater than 2-fold change in expression in embryos from parents exposed to BIGb446 (Fig.
297 4e and Supplementary Table 5). Despite regulating an overlapping set of genes, we found
298 that parental exposure to BIGb446 and the loss of WDR-23 resulted in distinct gene
299 expression patterns (Fig. 4f). These data indicate that exposure to *P. vranovensis* and
300 signaling via WDR-23 regulate the expression of a significantly overlapping set of target
301 genes and are consistent with our hypothesis that parental exposure to BIGb446 modifies
302 gene expression in offspring in part via WDR-23.
303
304 94% of genes that exhibit a greater than 2-fold change in expression in response to both *P.*
305 *vranovensis* and loss of WDR-23 exhibit an increase in mRNA expression (Supplementary
306 Tables 2 and 5). Among these genes we identified the cysteine synthases CYSL-1 and CYSL-
307 2 (Fig. 5a and Supplementary Tables 2 and 5), which were previously reported to be involved
308 in breaking down bacterial toxins produced by *P. aeruginosa*[32]. We confirmed that exposure
309 of parents to BIGb446 increases CYSL-2 expression in embryos using a GFP reporter (Fig.
310 5b)[33]. We hypothesized that increased expression of *cysl-1* and *cysl-2* in offspring promotes

311 adaptation to *P. vranovensis*. We assayed seven independent alleles of *cysl-1* and two
312 independent mutants lacking *cysl-2* and found that these mutants were unable to adapt to
313 BIGb446 (Fig. 5c-d and Supplementary Fig. 3). In addition, we found that these defects could
314 be rescued by expressing a wild-type copy of *cysl-2* (Fig. 5d). We conclude that CYSL-1 and
315 CYSL-2 are required for adaptation to BIGb446.
316
317 CYSL-1 and CYSL-2 were previously found to function in a signaling pathway with the
318 regulator of hypoxia factor, RHY-1, the EGLN1 homolog EGL-9, and the hypoxia inducible
319 factor HIF-1 to regulate animals' response to hypoxia [33]. We found that one of these genes,
320 *rhy-1*, also exhibited increased expression in both animals exposed to BIGb446 and in *wdr-*
321 *23* mutants (Fig. 6a). We tested whether mutants lacking RHY-1, EGL-9, and HIF-1 also
322 exhibited altered adaptation to BIGb446. We found that three independent mutations in *rhy-1*
323 resulted in animals that did not adapt to BIGb446 (Fig. 6b), similar to *cysl-1* and *cysl-2*
324 mutants. This defect was rescued by expressing a wild-type copy of *rhy-1* (Fig. 6b). By
325 contrast, we found that the loss of *hif-1* and *egl-9* did not affect adaptation to BIGb446 (Fig.
326 6c). These results indicate that RHY-1, CYSL-1, and CYSL-2 function separately from EGL-
327 9 and HIF-1 to promote animals' adaptation to *P. vranovensis.*
328
329 **Discussion**
330
331 Collectively, our results demonstrate that *P. vranovensis* is a pathogen of *C. elegans* and that
332 parental exposure of *C. elegans* to *P. vranovensis* can protect offspring from future infection
333 by increasing the expression of the cysteine synthases *cysl-1* and *cysl-2* and the regulator of
334 hypoxia inducible factor *rhy-1* in progeny. The ability of *P. vranovensis* to kill *C. elegans*
335 larvae within 24 hours and the observation that *C. elegans* activates genes associated with

336 oxidative stress in response to *P. vranovensis* suggests that *P. vranovensis* produces a toxic
337 molecule(s) that is lethal to *C. elegans*. The cysteine synthases CYSL-1 and CYSL-2 have
338 been reported to break down toxic molecules such as hydrogen cyanide and hydrogen sulfide
339 [32]. Based on these observations, we propose that *C. elegans* can transgenerationally adapt to
340 infection *by P. vranovensis* by increasing the expression of CYSL-1 and CYSL-2 in offspring
341 which protects animals by breaking down bacterial toxins. Thus, we demonstrate a new
342 transgenerationally adaptive mechanism for *C. elegans* to fight a natural pathogen.
343
344 The expression of *cysl-1*, *cysl-2* and *rhy-1* is regulated by signaling via WDR-23, however,
345 we found that mutations that both increase and decrease SKN-1 activity disrupt adaptation to
346 *P. vranovensis.* One possible explanation for these results is that specific signaling via WDR-
347 23 and SKN-1, likely in combination with signaling via other pathogen response pathways, is
348 required to elicit a distinct response to *P. vranovensis* and that completely abolishing
349 signalling via this pathway using genetic mutants results in an inappropriate response to *P.*
350 *vranovensis* and animals that are unable to adapt. Such a model is similar to other core
351 signaling pathways that regulate animals' response to multiple different stresses, such as the
352 insulin-like signaling pathway in *C. elegans* that functions in combination with signaling via
353 SSU-1 and NHR-1 to specify *C. elegans* distinct responses to both starvation and osmotic
354 stress[27]. Alternatively, it is possible that WDR-23 and SKN-1 regulate the expression of
355 CYSL-1, CYSL-2, and RHY-1 in parallel to infection by *P. vraonvensis.* In this case,
356 disrupted signaling via WDR-23 and SKN-1 might result in animals that are simply more
357 susceptible to infection and thus unable to adapt. Interestingly, loss of RHY-1 alone also
358 modifies CYSL-1 activity and results in significantly increased expression of CYSL-2[33]. It is
359 possible that RHY-1, WDR-23, and SKN-1 all function by regulating the activity of CYSL-1

360 and CYSL-2 and that the failure of *rhy-1, wdr-23,* and *skn-1* mutants to adapt to *P.*
361 *vraonvensis* is due to abnormal regulation of these two cysteine synthases.
362
363 A single generation of exposure to *P. vranovensis* only resulted in an intergenerational
364 adaptation to infection (Figure 2A). By contrast, we found that multiple successive
365 generations of exposure to *P. vranovensis* could result in transgenerational adaptation to
366 infection that persisted for two generations, however, the second generation exhibited a
367 significantly less robust adaptation than the offspring of infected animals (Figure 2A). This
368 transgenerational effect required 3 consecutive generations of exposure to *P. vranovensis*
369 (Figure 2B). These observations are consistent with an as yet unknown factor potentially
370 accumulating in germ cells and embryos and that is diluted out after two generations of
371 removal from the pathogen as a food source. Such a factor could be an epigenetic mark, a
372 signalling molecule, the altered abundance of a metabolite, or the presence of a bacterial
373 toxin. The identification of the molecular identity of this factor will be crucial to determining
374 the nature of this transgenerational response.
375
376 Recent studies of *C. elegans* response to the human opportunistic pathogen *P. aeruginosa*
377 have also described a transgenerational response to this pathogenic species of *Pseudomonas*[8].
378 In this paradigm researchers found that exposure of a single generation of *C. elegans* to *P.*
379 *aeruginosa* resulted in a heritable change in avoidance behaviour that persisted for four
380 generations and was dependent on the PIWI Argonaute homolog PRG-1[8]. By contrast, we
381 found that exposure of a single generation of *C. elegans* to *P. vranovensis* did not result in a
382 transgenerational adaptation to infection, but rather the heritable increase in immunity only
383 lasted one generation (Fig. 2a-b). Transgenerational effects of *P. vranovensis* infection were
384 only observed after several consecutive generations of exposure to *P. vranovensis* (Fig. 2b).

385 In addition, we found that *C. elegans* heritable adaptation to *P. vranovensis* did not require
386 PRG-1 (Fig. 2c). The explanation for why *C. elegans* elicits different responses to *P.*
387 *aeruginosa* when compared to *P. vranovensis* remains unclear. It is possible that *C. elegans*
388 evolved distinct responses to *P. vranovensis* when compared to *P. aeruginosa*. Alternatively,
389 studies of *C. elegans'* heritable responses to *P. aeruginosa* focused on changes in avoidance
390 behaviour while our studies of *C. elegans* heritable responses to *P. vranovensis* focused on
391 changes in survival under conditions where animals were unable to avoid the pathogen. It is
392 possible that heritable changes in behaviour in response to bacterial infection in *C. elegans*
393 are mediated by a small RNA pathway and PRG-1, while changes in immunity and the
394 expression of cysteine synthases are controlled by a mechanism that is PRG-1 independent.
395 Future studies will be important in differentiating between these possibilities and determining
396 how parental infection can heritably prime offspring immunity, if these mechanisms are
397 evolutionarily conserved, and how much they contribute to organisms' responses to
398 pathogens.
399
400 Finally, studies of *C. elegans* interaction with the opportunistic pathogen *P. aeruginosa*
401 indicate that parental exposure to *P. aeruginosa* can have both adaptive[8] and deleterious[1]
402 consequences for offspring. We suspect that similar trade-offs, where adaptation to one stress
403 comes at the cost of fitness in a different environment, are also likely to be the case for *C.*
404 *elegans* adaptation to *P. vranovensis.* Future studies will likely be critical in determining
405 what the costs of intergenerational and transgenerational responses to stress are, and we
406 propose that such studies might be able to identify links between different types of stress
407 responses. For example, previous studies have observed that *C. elegans* transcriptional
408 response to osmotic stress and pathogen infection appear to be related[34]. These findings, in
409 combination with observations that parental infection by *P. aeruginosa* results in offspring

410 that are more susceptible to osmotic stress[1], suggest that adapting to pathogen infection might
411 generally disrupt animals' ability to respond to osmotic stress and explain why animals are
412 not normally resistant to this pathogen.

413

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423

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425 and E.A.M analysed the data. N.O.B., C. R., A.D., J.P., A.K., and B.J. performed the
426 experiments. N.O.B. wrote the manuscript.

427

428 **Declarations of Interest:** The authors declare that they have no competing interests.

429

430 **Data and materials availability:** RNA-seq data that support the findings of this study have
431 been deposited at the European Nucleotide Archive (ENA) under the accession code
432 PRJEB32993. The raw data for assembling the genomes of *P. vranovensis* isolate BIGb446 is
433 available at the ENA under the accession code ERS3670403 and BIGb468 is available under
434 the accession code ERS3670404. The raw data related to metabolomics is available on Dryad

using doi:10.5061/dryad.8t6q5f5. Raw data supporting all figures is provided in Supplementary Table 6.

483
484 Figure 1. *C. elegans* heritably adapts to infection by *Pseudomonas vranovensis.* (a) Percent of
485 wild-type animals surviving on plates seeded with either *E. coli* HB101 or bacterial isolates
486 BIGb446 and BIGb468 after 24 hrs. Error bars, s.d. n = 3 experiments of >100 animals. (b)
487 Percent of wild-type animals surviving on plates seeded with bacterial isolate BIGb446. Error
488 bars, s.d. n = 3 experiments of 500 animals. (c) Images of wild-type animals surviving after
489 120 hrs of feeding on bacterial isolate BIGb446. 1000 animals were used at t = 0 in each
490 condition and surviving animals were resuspended in 20 µl M9 and imaged. Scale bars 1 mm.
491 (d) Percent of wild-type animals surviving on *E. coli* HB101 or bacterial isolate BIGb446
492 after 24 hrs. Error bars, s.d. n = 3 experiments of >100 animals. * = $p < 0.05$, ** = $p < 0.01$,
493 *** = $p < 0.001$, **** $p < 0.0001$.
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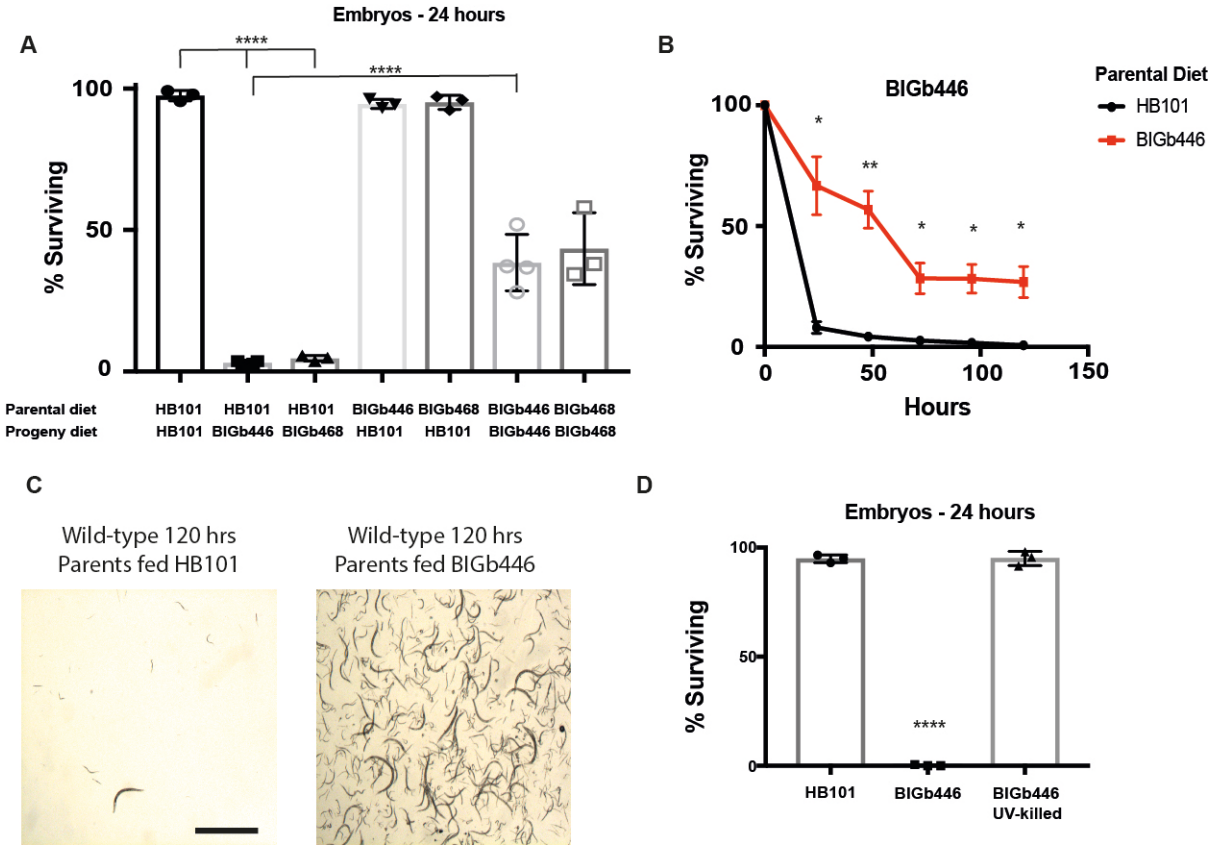
A
Embryos - 24 hours
****
****
100
50
0
% Surviving
Parental diet
Progeny diet
HB101 HB101
HB101 BIGb446
HB101 BIGb468
BIGb446 HB101
BIGb468 HB101
BIGb446 BIGb446
BIGb468 BIGb468
B
BIGb446
Parental Diet
HB101
BIGb446
100
50
0
% Surviving
*
**
*
*
*
0
50
100
150
Hours
C
Wild-type 120 hrs
Parents fed HB101
Wild-type 120 hrs
Parents fed BIGb446
D
Embryos - 24 hours
100
50
0
% Surviving
****
HB101
BIGb446
BIGb446
UV-killed

515 Figure 2. *C. elegans* adaptation to *P. vranovensis* can be inherited transgenerationally. (a)
516 Percent of wild-type animals surviving on plates seeded with bacterial isolate BIGb446 after
517 24 hrs. Error bars s.d. n = 3 experiments of > 100 animals. (b) Percent of wild-type animals
518 surviving on plates seeded with bacterial isolate BIGb446 after 24 hrs. Error bars s.d. n = 3
519 experiments of 500 animals. Wild-type animals carried the integrated transgene *nIs470.* (c)
520 Percent of wild-type, *set-32(ok1457), prg-1(n4357), hrde-1(tm1200), damt-1(gk961032),*
521 *wdr-5.1(ok1417), set-2(ok952), spr-5(by134), lin-45(n2018),* and *met-2(n4256)* mutants
522 surviving on plates seeded with bacterial isolates BIGb446 after 24 hrs. Error bars, s.d. n = 3
523 experiments of >100 animals. (d) Percent of wild-type and *pmk-1(km25)* mutants surviving
524 on plates seeded with bacterial isolates BIGb446 after 24 hrs. Error bars, s.d. n = 3
525 experiments of >100 animals. (e) Percent of wild-type animals surviving on plates seeded
526 with bacterial isolate BIGb446 after 24 hrs. Error bars s.d. n = 3 experiments of 500 animals.
527 *** = $p < 0.001$, **** $p < 0.0001$.
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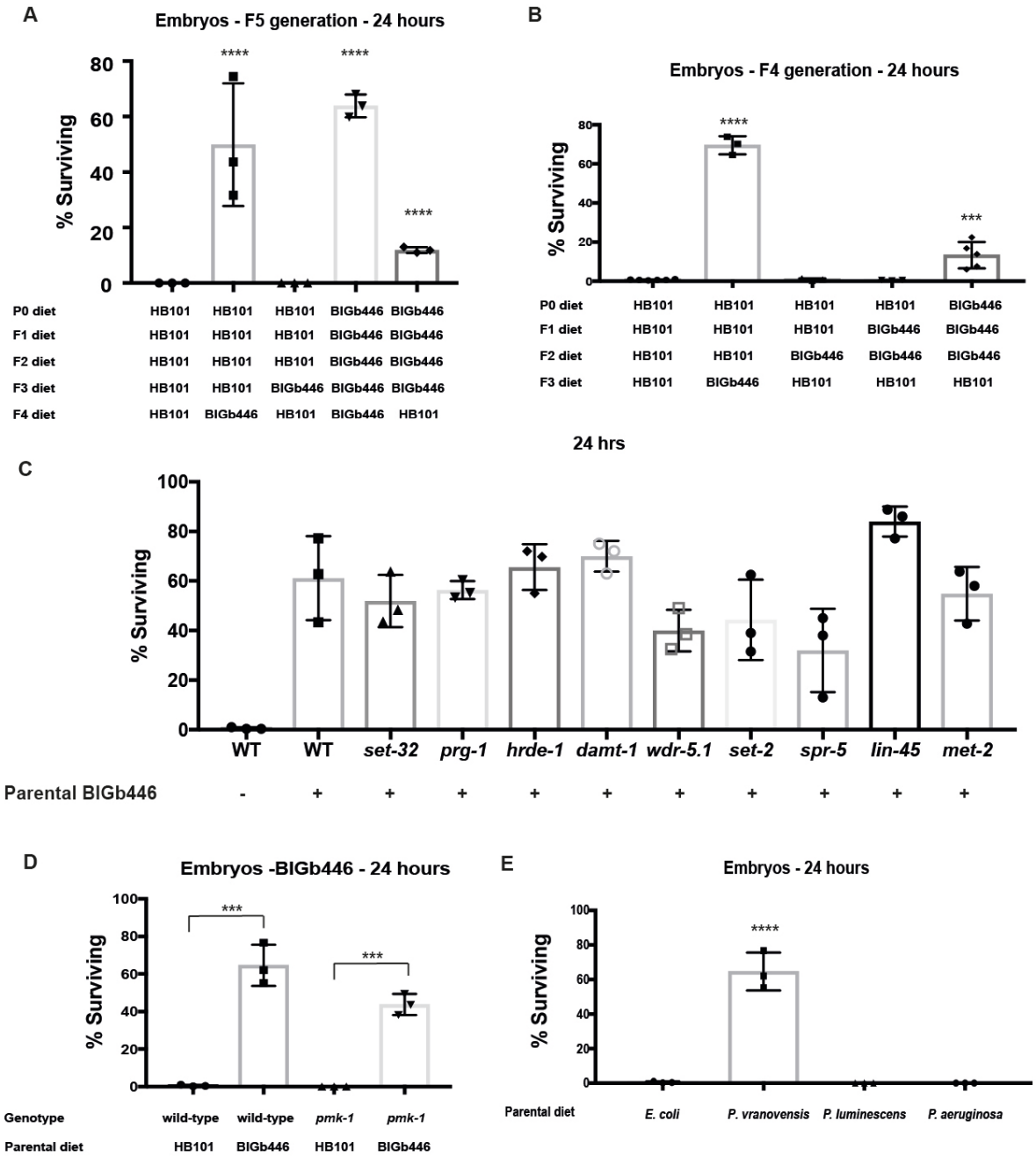

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540 Figure 3. Parental infection by *P. vranovensis* alters gene expression and metabolism in
541 offspring. (a) Gene expression changes in embryos from parents fed *P. vranovensis* BIGb446
542 when compared to embryos from parents fed *E. coli* HB101. Values represent averages from
543 three replicates. (b) Venn diagram of genes exhibiting a greater than 2-fold change in RNA
544 expression in adults fed *P. vranovensis* BIGb446 and embryos from adults fed *P. vranovensis*
545 BIGb446. *p*-value represents normal approximation to the hypergeometric probability (See
546 Statistics and Reproducibility) (c) μM abundance of lipid metabolites exhibiting a greater
547 than 2-fold change in abundance in embryos from parents fed *P. vranovensis* BIGb446 when
548 compared to embryos from parents fed *E. coli* HB101. Error bars, s.d. n = 3 replicates. (d)
549 Venn diagram of genes exhibiting a greater than 2-fold change in RNA expression in
550 embryos from parents fed *P. vranovensis* BIGb446, *P. aeruginosa* PA14, and *P. luminescens*
551 Hb. *p*-value represents normal approximation to the hypergeometric probability (See
552 Statistics and Reproducibility) (e) Principal component analysis (PCA) plot of mRNA
553 expression data from RNA-seq of embryos from parents fed *E. coli* HB101, *P. vranovensis*
554 BIGb446 and BIGb468, *P. aeruginosa* PA14, or *P. luminescens* Hb.
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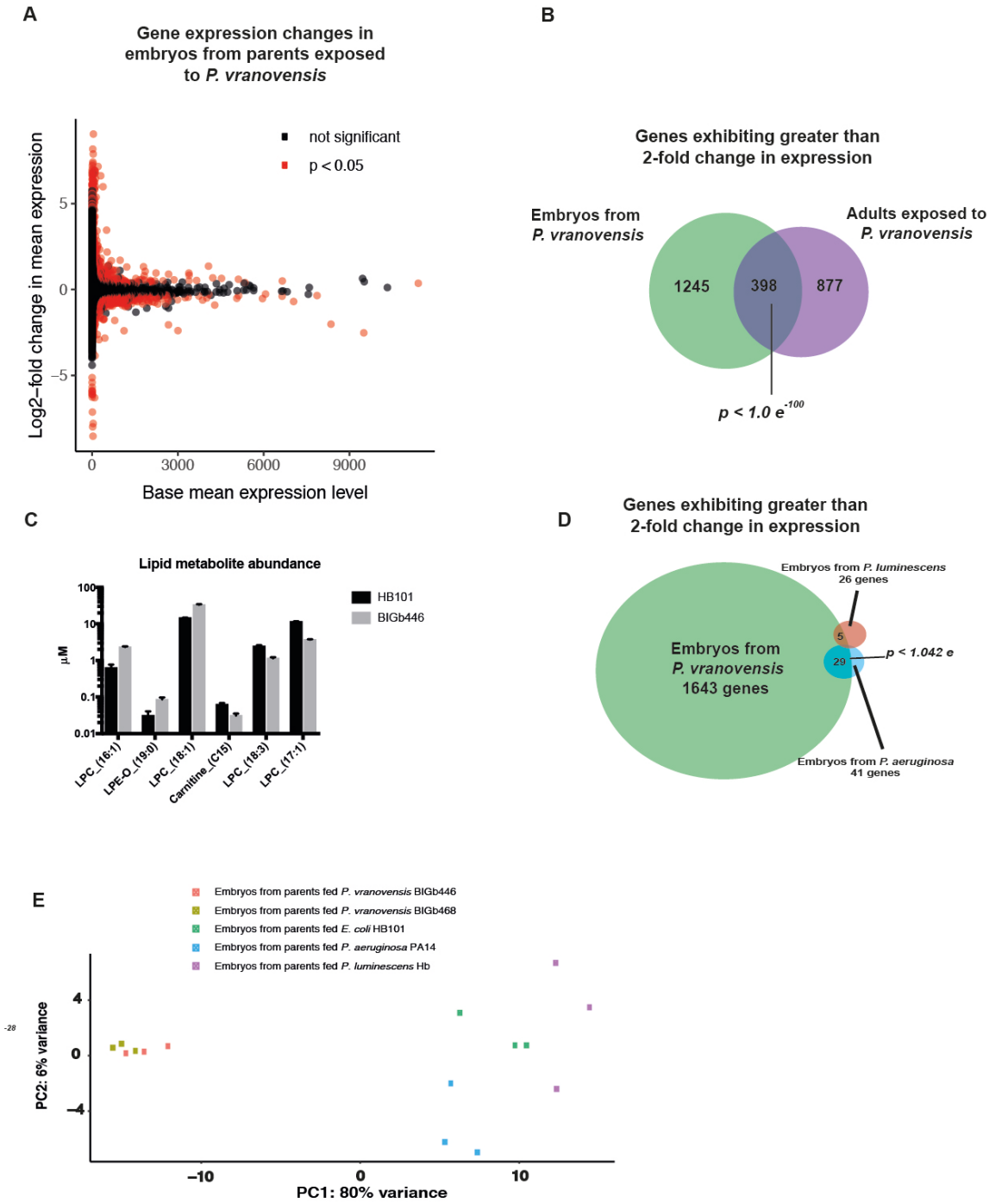
A
Gene expression changes in embryos from parents exposed to P. vranovensis
not significant
p < 0.05
Log2−fold change in mean expression
Base mean expression level
B
Genes exhibiting greater than 2-fold change in expression
Embryos from P. vranovensis
Adults exposed to P. vranovensis
1245
398
877
p < 1.0 e-100
C
Lipid metabolite abundance
HB101
BIGb446
µM
LPC_(16:1)
LPE-O_(19:0)
LPC_(18:1)
Carnitine_(C15)
LPC_(18:3)
LPC_(17:1)
D
Genes exhibiting greater than 2-fold change in expression
Embryos from P. luminescens 26 genes
Embryos from P. vranovensis 1643 genes
5
29
p < 1.042 e
Embryos from P. aeruginosa 41 genes
E
Embryos from parents fed P. vranovensis BIGb446
Embryos from parents fed P. vranovensis BIGb468
Embryos from parents fed E. coli HB101
Embryos from parents fed P. aeruginosa PA14
Embryos from parents fed P. luminescens Hb
PC2: 6% variance
PC1: 80% variance

567 Figure 4. Signaling via WDR-23 regulates *C. elegans* response to *P. vranovensis.* (a)
568 Representative images of *gst-31::GFP* expression in wild-type embryos from parents fed
569 either *E. coli* HB101 or *P. vranovensis* BIGb446. Scale bars 10 μm. (b) Representative
570 images of *gst-31::GFP* expression in wild-type adults fed either *E. coli* HB101 or *P.*
571 *vranovensis* BIGb446 for 24 hrs. Scale bars 100 μm. (c) Schematic of *wdr-23* mutations that
572 cause increased *gst-31::GFP* expression when animals are fed *E. coli* HB101. Scale bar, 100
573 bp. (d) Percent of wild-type, *wdr-23(mac51, tm1817),* and *skn-1(zj15)* mutants surviving on
574 plates seeded with bacterial isolates BIGb446 after 24 hrs. Error bars, s.d. n = 3 experiments
575 of >100 animals. **** $p < 0.0001$. (e) Venn diagram of genes exhibiting a greater than 2-fold
576 change in RNA expression in embryos from parents fed *P. vranovensis* BIGb446 and *wdr-*
577 *23(bur9)* mutant embryos from parents fed *E. coli* HB101. *p*-value represents normal
578 approximation to the hypergeometric probability (See Statistics and Reproducibility). (f)
579 Principal component analysis (PCA) plot of mRNA expression data from RNA-seq of wild-
580 type and *wdr-23(bur9)* mutant embryos from parents fed *E. coli* HB101, *P. vranovensis*
581 BIGb446, *P. aeruginosa* PA14, or *P. luminescens* Hb.
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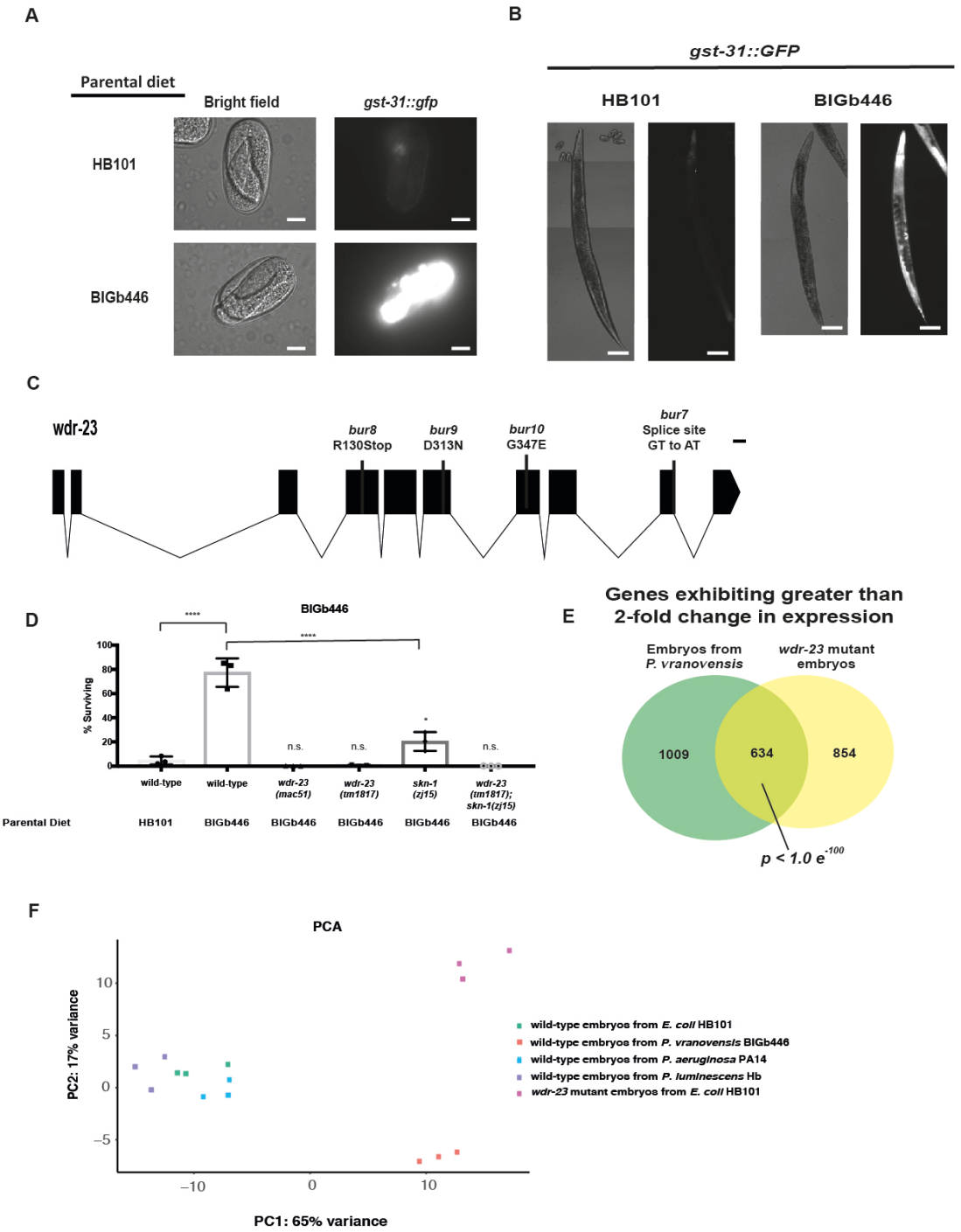

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596 Figure 5. CYSL-1 and CYSL-2 are required for *C. elegans* to adapt to *P. vranovensis.* (a)
597 Transcripts per million (TPM) of *cysl-2* in wild-type and *wdr-23(bur9)* mutant embryos from
598 parents fed *E. coli* HB101 or *P. vraonvensis* BIGb446. Error bars, s.d. n = 3 replicates. Data
599 is the same as found in Table S2 and S5. (b) Representative images of *cysl-2::GFP* in
600 embryos from parents fed *E. coli* HB101 or *P. vraonvensis* BIGb446. Scale bars 100 μm. (c)
601 Percent of wild-type and *cysl-1(ok762)* mutants surviving on plates seeded with bacterial
602 isolates BIGb446 after 24 hrs. Error bars, s.d. n = 3 experiments of >100 animals. (d) Percent
603 of wild-type and *cysl-2(ok3516)* mutants surviving on plates seeded with bacterial isolates
604 BIGb446 after 24 hrs. Error bars, s.d. n = 3 experiments of >100 animals. *** = $p < 0.001$,
605 **** $p < 0.0001$.

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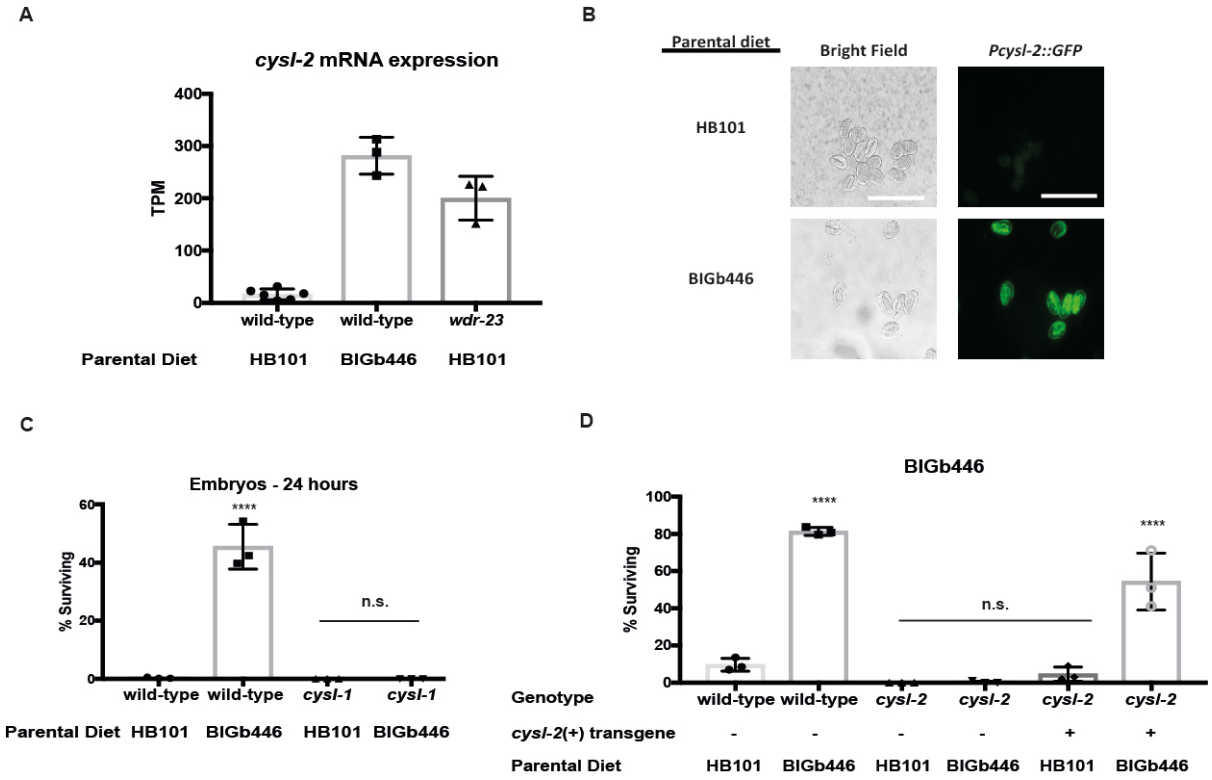
A
cysl-2 mRNA expression
TPM
400
300
200
100
0
wild-type
wild-type
wdr-23
Parental Diet
HB101
BIGb446
HB101
B
Parental diet
Bright Field
Pcysl-2::GFP
HB101
BIGb446
C
Embryos - 24 hours
% Surviving
60
40
20
0
****
n.s.
wild-type
wild-type
cysl-1
cysl-1
Parental Diet
HB101
BIGb446
HB101
BIGb446
D
BIGb446
% Surviving
100
80
60
40
20
0
****
n.s.
****
Genotype
wild-type
wild-type
cysl-2
cysl-2
cysl-2
cysl-2
cysl-2(+) transgene
-
-
-
-
+
+
Parental Diet
HB101
BIGb446
HB101
BIGb446
HB101
BIGb446

629

630 Figure 6. RHY-1 is required for *C. elegans* adaptation to *P. vranovensis*. (a) Transcripts per
631 million (TPM) of *rhy-1* in wild-type and *wdr-23(bur9)* mutant embryos from parents fed *E.*
632 *coli* HB101 or *P. vraonvensis* BIGb446. Error bars, s.d. n = 3 replicates. Data is the same as
633 found in Table S2 and S5. (b) Percent of wild-type and *rhy-1* mutants surviving on plates
634 seeded with bacterial isolates BIGb446 after 24 hrs. Error bars, s.d. n = 3 experiments of
635 >100 animals. (c) Percent of wild-type, *egl-9(n586), hif-1(ia4),* and *rhy-1(n5500)* mutants
636 surviving on plates seeded with bacterial isolates BIGb446 after 24 hrs. Error bars, s.d. n = 3
637 experiments of >100 animals. The wild-type data in panels B and C and Figure 5C are
638 identical as these experiments were run together. *** = $p < 0.001$, **** $p < 0.0001$.

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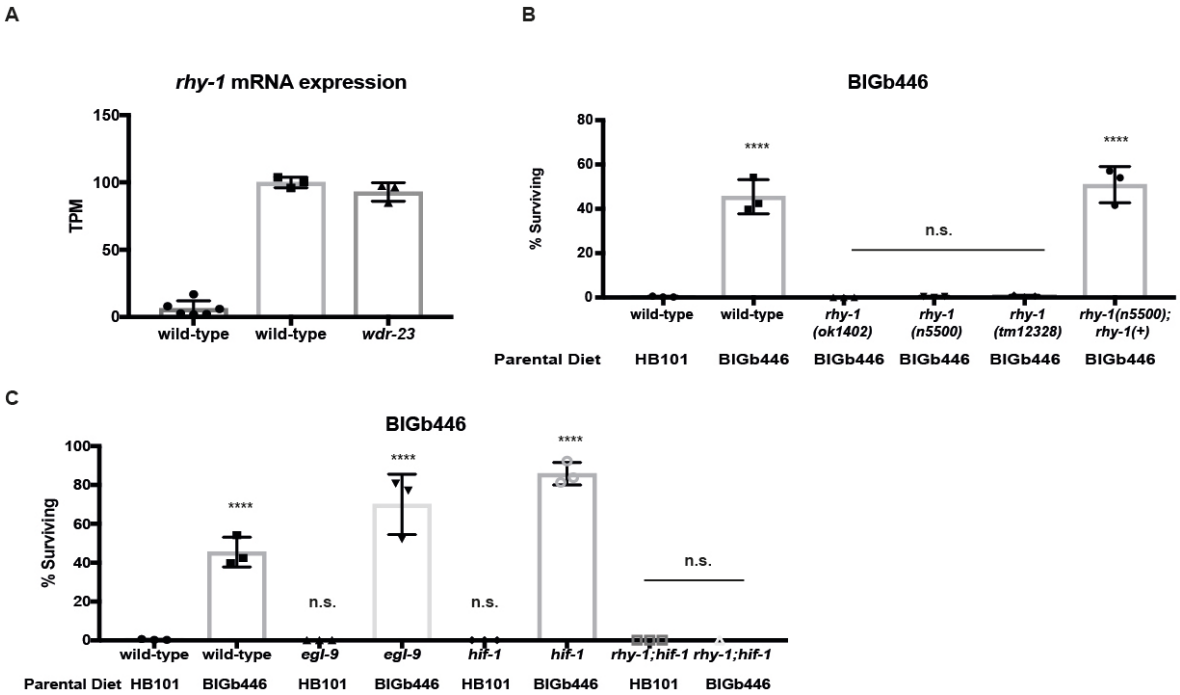

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664 **Methods**
665
666
667 *Strains.* *C. elegans* strains were cultured as described[35] and maintained at 20 °C unless noted
668 otherwise. The Bristol strain N2 was the wild-type strain.
669 **LGI:** *set-32(ok1457), prg-1(n4357), spr-5(by134), wdr-23(bur7, bur8, bur9, bur10, tm1817,*
670 *mac51)*
671 **LGII:** *cysl-2(ok3516, syb1431), damt-1(gk961032), rhy-1(n5500, ok1402, tm12398))*
672 **LGIII:** *wdr-5.1(ok1417), hrde-1(tm1200), set-2(ok952), met-2(n4256)*
673 **LGIV:** *pmk-1(km25), skn-1(zj15), pgl-1(bn102), lin-45(n2018), nIs470[cysl-2::Venus; myo-*
674 *2::RFP]*
675 **LGV:** *egl-9(n586), hif-1(ia4)*
676 **LGX:** *cysl-1(ok762, mr23, mr25, mr26, mr29, mr39, mr40)*
677 **Unknown linkage:** *burIs2[gst-31::gfp]*
678 **Extrachromosomal arrays:** *nEx1763[rhy-1(+); myo-2::RFP], burEx1 [cysl-2(+); myo-*
679 *3::RFP]*
680
681 *Sequencing of* Pseudomonas vranovensis *BIGb446 and BIGb468*
682 Genomic DNA was prepped using a Gentra Puregene kit (QIAGEN). DNA was sheared to 10
683 kb using gTUBE (Covaris). Sheared DNA was barcoded and multiplexed for PacBio
684 sequencing using Template Prep Kit 1.0-SPv3 and Barcoded Adapter Kit 8A (PacBio).
685 Genomic DNA was sequenced using the PacBio Sequel system using version 3.0 sequencing
686 reagents (PacBio) and 1M v3 SMRT cell.
687
688 *Genome assembly of BIGb446 and BIGb468*
689 The genomes of BIGb446 and BIGb46 were assembled using HGAP4 from SMRT Link
690 version 5.1.0.26412 with estimated genome sizes of 5 MB.

691
692 *Assays of adaptation to* Pseudomonas vranovensis *BIGb446 and BIGb468*

693 *P. vranovensis* BIGb446 and BIGb468 was cultured in LB at 37 °C overnight. 1 mL of
694 overnight culture was seeded onto 50 mm NGM agar plates and dried in a laminar flow hood
695 (bacterial lawns completely covered the plate such that animals could not avoid the
696 pathogen). All plates seeded with BIGb446 or BIGb468 were used the same day they were
697 seeded. Young adult animals were placed onto 50 mm NGM agar plates seeded with 1 mL
698 either *E. coli* HB101 or *P. vranovensis* BIGb446 or BIGb468 for 24 hours at room
699 temperature. Embryos from these animals were collected and placed onto fresh NGM agar
700 plates seeded with BIGb446 or BIGb468. Percent surviving were counted after 24 hours at
701 room temperature unless otherwise noted.

702

703 *Transgenerational adaptation to* P. vranovensis

704 *P. vranovensis* BIGb446 was cultured in LB at 37 °C overnight. 1 mL of overnight culture
705 was seeded onto 50 mm NGM agar plates and dried in a laminar flow hood (bacterial lawns
706 completely covered the plate). All plates seeded with BIGb446 were used the same day they
707 were seeded. All animals in each generation were grown from embryos to young adults on
708 NGM agar plates seeded with *E. coli* HB101. Young adults were then moved to fresh plates
709 seeded with BIGb446 at room temperature for 24 hours. 24 hours of feeding on BIGb446 was
710 counted as one generation of exposure to a BIGb446 diet. Embryos from parents fed
711 BIGb446 were collected and placed onto plates seeded with *E. coli* HB101 until they were
712 young adults and then moved to fresh plates seeded with BIGb446 for additional generations.

713

714 *Imaging of wild-type animals surviving after* P. vranovensis *exposure*

715 1,000 embryos were placed onto NGM agar plates seeded with *P. vranovensis* BIGb446 at t =
716 0 in each condition and surviving animals at 120 hours were washed off of plates and
717 resuspended in 20 µl M9 and imaged.
718
719 *Assays of C. elegans response to* P. aeruginosa *and* P. luminescens
720 Young adult animals were placed onto slow-killing assay (*P. aeruginosa*) plates or NGM
721 agar (*P. luminescens*) plates seeded with either *P. aeruginosa* PA14 or *P. luminescens* Hb for
722 24 hours at room temperature. Embryos from these animals were collected and snap frozen in
723 liquid nitrogen for RNA sequencing and metabolomics analysis or placed onto fresh NGM
724 agar plates seeded with BIGb446. Percent of animals placed on BIGb446 surviving were
725 counted after 24 hours at room temperature.
726
727 *Assay of adult survival*
728 Greater than 100 young adult animals were placed onto placed onto NGM agar plates seeded
729 with *P. vranovensis* BIGb446 from overnight cultures in LB. Percent of animals surviving
730 was counted at 24 hour intervals. Animals were scored as alive if they were mobile and dead
731 if they were immobile and did not respond to touch.
732
733 *RNA-seq*
734 Young adult animals were placed onto NGM agar plates seeded with *E. coli* HB101, *P.*
735 *vranovensis* BIGb446 or BIGb468, or *P. luminescens* Hb or slow-killing assay plates seeded
736 with *P. aeruginosa* PA14 for 24 hours at room temperature. Adult animals or embryos
737 collected from adult animals after 24 hours were snap frozen in liquid nitrogen. Samples were
738 lysed using a BeadBug microtube homogenizer (Sigma) and 0.5 mm Zirconium beads
739 (Sigma). RNA was extracted using a RNeasy Plus Mini kit (Qiagen). mRNA was enriched

740 using an NEBNext rRNA Depletion kit (NEB). Libraries for sequencing were prepped using
741 an NEBNext Ultra II Library prep kit for Illumina (NEB) and loaded for paired-end
742 sequencing using the Illumina HiSeq 1500.
743
744 *Mutagenesis screening*
745 Approximately 20,000 L4 stage animals expressing *gst-31::GFP* (*burIs2*) were incubated
746 with 20 µL of EMS in 4 mL of M9 for 4 h at 20 °C, and approximately 200,000 F3 generation
747 embryos were placed onto NGM agar Petri plates seeded with *E. coli* HB101 and screened
748 for mutants that expressed *gst-31::GFP* even in the absence of pathogen infection.
749
750 gst-31::GFP *imaging*
751 To image adults and embryos, young adult animals expressing *burIs2* were placed onto NGM
752 agar plates seeded with BIGb446 at room temperature for 24 hours. Adults and embryos were
753 collected and immediately imaged using a Zeiss AXIO imager A1 microscope and a
754 Hamamatsu ORCA-ER camera.
755
756 cysl-2::GFP *imaging*
757 To image adults and embryos, young adult animals expressing *nIs470* were placed onto
758 NGM agar plates seeded with BIGb446 at room temperature for 24 hours. Embryos were
759 collected and immediately imaged using a Zeiss AXIO imager A1 microscope and a
760 Hamamatsu ORCA-ER camera.
761
762 *UV-killing bacteria*

763 1 mL of overnight culture of BIGb446 was seeded onto 50 mm NGM agar plates and dried in
764 the laminar flow hood. Seeded plates were then exposed to 20 μW/cm^2 for 1 hour. Complete
765 killing of bacteria was confirmed by testing inoculations of bacteria in LB overnight.
766
767 cysl-2 *cloning and rescue*
768 Genomic *cysl-2* DNA was amplified from wild-type animals using the primers
769 ACGATTGGGTTGGCTGTAAG and GGTCGTACGTGTTCGTTGTG. Extrachromosomal
770 arrays were generated by injecting the corresponding PCR fragment and co-injection marker
771 into the gonad of one-day old adults at the specified concentrations. *nobEx1* was generated by
772 injecting *cysl-2* genomic DNA at 20 ng/μl and *myo-3::RFP* was injected at 10 ng/μl. Final
773 injection DNA concentration was brought up to 150 ng/μl using DNA ladder (1kb
774 HyperLadder – Bioline).
775
776 *Generation of* cysl-2 *CRISPR alleles*
777 *syb1431* was generated by SunyBiotech. *syb1431* contains a 50 bp frameshift deletion in the
778 first exon with the following flanking sequences
779 ACCGGTGGTGAGCTCATCGGAAACACCCCA and
780 GGTAGAGTACATGAACCCTGCCTGCTC.
781
782 *LC/MS lipid profiling*
783 *C. elegans* was prepared for LC-MS lipidomics and acyl-carnitine analysis as previously
784 described [36] with minor modifications. Briefly, ~40 μL of concentrated embryos were re-
785 suspended in 100 μL of water, then 0.4 mL of chloroform was added to each sample followed
786 by 0.2 mL of methanol containing the stable isotope labelled acyl-carnitine internal standards
787 (Butyryl-L-carnitine$_{-d7}$ at 5 μM and Hexadecanoyl-L-carnitine$_{-d3}$ at 5 μM). The samples were

788 then homogenised by vortexing then transferred into a 2 mL Eppendorf screw-cap tube. The
789 original container was washed out with 0.5 mL of chloroform: methanol (2: 1, respectively)
790 and added to the appropriate 2 mL Eppendorf screw-cap tube. This was followed by the
791 addition of 150 µL of the following stable isotope labelled internal standards (approximately
792 10 to 50 µM in methanol): Ceramide_C16$_{d31}$, LPC_(C14:0$_{d42}$), PC_(C16:0$_{d31}$ / C18:1),
793 PE_(C16:0$_{d31}$ / C18:1), PG_(C16:0$_{d31}$ / C18:1), PI_(C16:0$_{d31}$ / C18:1), PS_(C16:0$_{d62}$),
794 SM_(C16:0$_{d31}$), TG_(45:0$_{d29}$) and TG_(48:0$_{d31}$). Then, 400 µL of sterile water was added. The
795 samples were vortexed for 1 min, and then centrifuged at ~20,000 rpm for 5 minutes.
796
797 For the intact lipid sample preparation, 0.3 mL of the organic layer (the lower chloroform layer)
798 was collected into a 2 mL amber glass vial (Agilent Technologies, Santa Clara California,
799 USA) and dried down to dryness in an Eppendorf Concentrator Plus system (Eppendorf,
800 Stevenage, UK) run for 60 minutes at 45 °C. The dried lipid samples were then reconstituted
801 with 100 µL of 2:1:1 solution of propan-2-ol, acetonitrile and water, respectively, and then
802 vortexed thoroughly. The lipid samples were then transferred into a 300 µL low-volume vial
803 insert inside a 2 mL amber glass auto-sample vial ready for liquid chromatography separation
804 with mass spectrometry detection (LC-MS) of intact lipid species.
805
806 For the acyl-carnitine sample preparation, 0.2 mL of the organic layer (the lower chloroform
807 layer) and 0.2 mL of the aqueous layer (the top water layer) were mixed into a 2 mL amber
808 glass vial and dried down to dryness. The dried acyl-carnitine samples were then reconstituted
809 with 100 µL of water and acetonitrile (4: 1, respectively) and thoroughly vortexed. The acyl-
810 carnitine samples were then transferred into a 300 µL low-volume vial insert inside a 2 mL
811 amber glass auto-sample vial ready for liquid chromatography separation with mass
812 spectrometry detection (LC-MS) of the acyl-carnitine species.

813

814 Full chromatographic separation of intact lipids [37] was achieved using Shimadzu HPLC System
815 (Shimadzu UK Limited, Milton Keynes, United Kingdom) with the injection of 10 µL onto a
816 Waters Acquity UPLC® CSH C18 column; 1.7 µm, I.D. 2.1 mm X 50 mm, maintained at 55
817 °C. Mobile phase A was 6:4, acetonitrile and water with 10 mM ammonium formate. Mobile
818 phase B was 9:1, propan-2-ol and acetonitrile with 10 mM ammonium formate. The flow was
819 maintained at 500 µL per minute through the following gradient: 0.00 minutes_40% mobile
820 phase B; 0.40 minutes_43% mobile phase B; 0.45 minutes_50% mobile phase B; 2.40
821 minutes_54% mobile phase B; 2.45 minutes_70% mobile phase B; 7.00 minutes_99% mobile
822 phase B; 8.00 minutes_99% mobile phase B; 8.3 minutes_40% mobile phase B; 10
823 minutes_40% mobile phase B; 10.00 minutes_40% mobile phase B. The sample injection
824 needle was washed using 9:1, 2-propan-2-ol and acetonitrile with 0.1 % formic acid. The mass
825 spectrometer used was the Thermo Scientific Exactive Orbitrap with a heated electrospray
826 ionisation source (Thermo Fisher Scientific, Hemel Hempstead, UK). The mass spectrometer
827 was calibrated immediately before sample analysis using positive and negative ionisation
828 calibration solution (recommended by Thermo Scientific). Additionally, the heated
829 electrospray ionisation source was optimised at 50:50 mobile phase A to mobile phase B for
830 spray stability (capillary temperature; 380 °C, source heater temperature; 420 °C, sheath gas
831 flow; 60 (arbitrary), auxiliary gas flow; 20 (arbitrary), sweep gas; 5 (arbitrary), source voltage;
832 3.5 kV. The mass spectrometer resolution was set to 25,000 with a full-scan range of m/z 100
833 to 1,800 Da, with continuous switching between positive and negative mode. Lipid
834 quantification was achieved using the area under the curve (AUC) of the corresponding high
835 resolution extracted ion chromatogram (with a window of ± 8 ppm) at the indicative retention
836 time. The lipid analyte AUC relative to the associated internal standard AUC for that lipid class
837 was used to semi-quantify and correct for any extraction/instrument variation.

838

839 Acyl-carnitine chromatographic separation was achieved using an ACE Excel 2 C18-PFP (150
840 mm, I.D. 2.1 mm, 2 µm) LC-column with a Shimadzu UPLC system. The column was
841 maintained at 55 ºC with a flow rate of 0.5 mL/min. A binary mobile phase system was used
842 with mobile phase A; water (with 0.1% formic acid), and mobile phase B; acetonitrile (with
843 0.1% formic acid). The gradient profile was as follows; at 0 minutes_0% mobile phase B, at
844 0.5 minutes_100% mobile phase B, at 5.5 minutes_100% mobile phase B, at 5.51 minutes_0%
845 mobiles phase B, at 7 minutes_0% mobile phase B. Mass spectrometry detection was
846 performed on a Thermo Exactive orbitrap mass spectrometer operating in positive ion mode.
847 Heated electrospray source was used; the sheath gas was set to 40 (arbitrary units), the aux gas
848 set to 15 (arbitrary units) and the capillary temperature set to 250°C. The instrument was
849 operated in full scan mode from m/z 75–1000 Da. Acyl-carnitine quantification was achieved
850 using the area under the curve (AUC) of the corresponding high resolution extracted ion
851 chromatogram (with a window of ± 8 ppm) at the indicative retention time. The acyl-carnitine
852 analyte AUC relative to the associated internal standard AUC was used to semi-quantify and
853 correct for any extraction/instrument variation. All lipid values were normalized to total lipid.
854

855 *RNA-seq and principal component analysis*

856 Cutadapt version 1.18 was used to remove adapter sequences
857 (AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCG
858 ATC for HS678 and AGATCGGAAGAGCACACGTCTGAACTCCAGTCA (forward) and
859 AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT (reverse) for HS755. Cutadapt was
860 also used to trim the 3' ends of reads when the phred quality value was below 20. Reads
861 shorter than 40 bp were discarded. The genome sequence in FASTA format and annotation
862 file in GFF3 format of *C. elegans* were downloaded from Ensembl release 96. The genome

863 sequence was index with the annotation with hisat2 version 2.1.0. hisat2 was used for
864 aligning reads to the reference genome and the maximum number of alignments to be
865 reported per read was set to 5000.Featurecounts from the conda subread package version
866 1.6.3 was used to count the number of reads per gene. The Ensembl release 96 annotation file
867 in GTF format for *C. elegans* was used. Fragments were counted when they overlapped an
868 exon by at least 1 nucleotide and fragments are reported at the gene level. The option for the
869 stranded protocol was turned off. Only read pairs that had both ends aligned were counted.
870 Given our average fragment length of 300 bp, a distance of between 50 and 600 nucleotides
871 was tolerated for read pairs. Read pairs that had their two ends mapping to different
872 chromosomes or mapping to the same chromosome but on different strands were not counted.
873 Multi-mapping reads were not counted.The raw counts table was imported into R 3.5.1 for
874 differential expression analysis with DESeq2 version 1.22.1 Normalisation was carried out
875 within each contrast. The PCA plots were produced with DESeq2 function plotPCA() after
876 variance stabilising transformation of the data. A snakemake workflow (CITE snakemake [38])
877 was created for the RNA-seq analysis and can it be found at
878 https://github.com/cristianriccio/celegans-pathogen-adaptation.
879
880 *Genomic DNA alignment*
881 MUMmer (*Version 3.0, default parameters*) and MUMmer-plot[39] were used to visualize
882 global alignments of BIGb446 and BIGb468 whole genome assemblies. Further to this
883 NUCmer [39] and the associated dnadiff script was used to produce statistics on the alignment
884 of the two bacterial genomes.
885
886 *Statistics and reproducibility*

887 ANOVA analysis with post hoc p-value calculations was used for Fig. 1a, 1d, 2a, 2b, 2c, 2d,
888 2e, 4d, 5c, 5d, 6b, 6c, and Supplementary Fig. 3. Two-tail t-tests were used for Fig. 1b and
889 Supplementary Fig. 1a. For Fig. 3b, 3d, and 4e the *p*-value was calculated using a normal
890 approximation to the hypergeometric probability, as in
891 http://nemates.org/MA/progs/representation.stats.html. For lipidomics data (Fig. 3c and
892 Supplementary Table 3) p-values were calculated using two-tail t-tests and statistical
893 significance was determined using Bonferonni correction for multiple hypotheses. * = $p <$
894 0.05, ** = $p < 0.01$, *** = $p < 0.001$, **** $p < 0.0001$. No statistical method was used to
895 predetermine sample size. Sample sizes were chosen based on similar studies in the relevant
896 literature. The experiments were not randomized. The investigators were not blinded to
897 allocation during experiments and outcome assessment.
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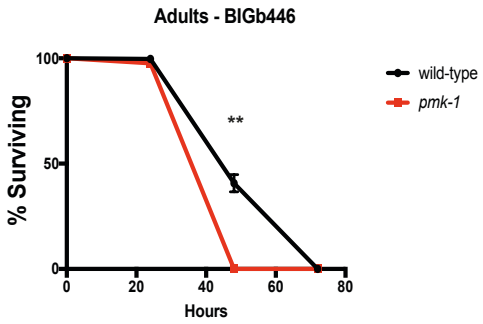
Supplementary Figure 1. Genes associated with transgenerational inheritance and pathogen infection in *C. elegans* are not required for heritable adaptation to *P. vranovensis* BIGb446. Percent of wild-type and *pmk-1(km25)* mutant adults surviving on NGM plates seeded with P. vranovenis BIGb446. Error bars, s.d. n = 3 replicates of 100 animals. ** = $p < 0.01$



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958 Supplementary Figure 2. BIGb446 and BIGb468 are isolates of a single species. MUMmer

959 plot of the alignments of contigs of BIGb446 and BIGb468 assembled genomes.

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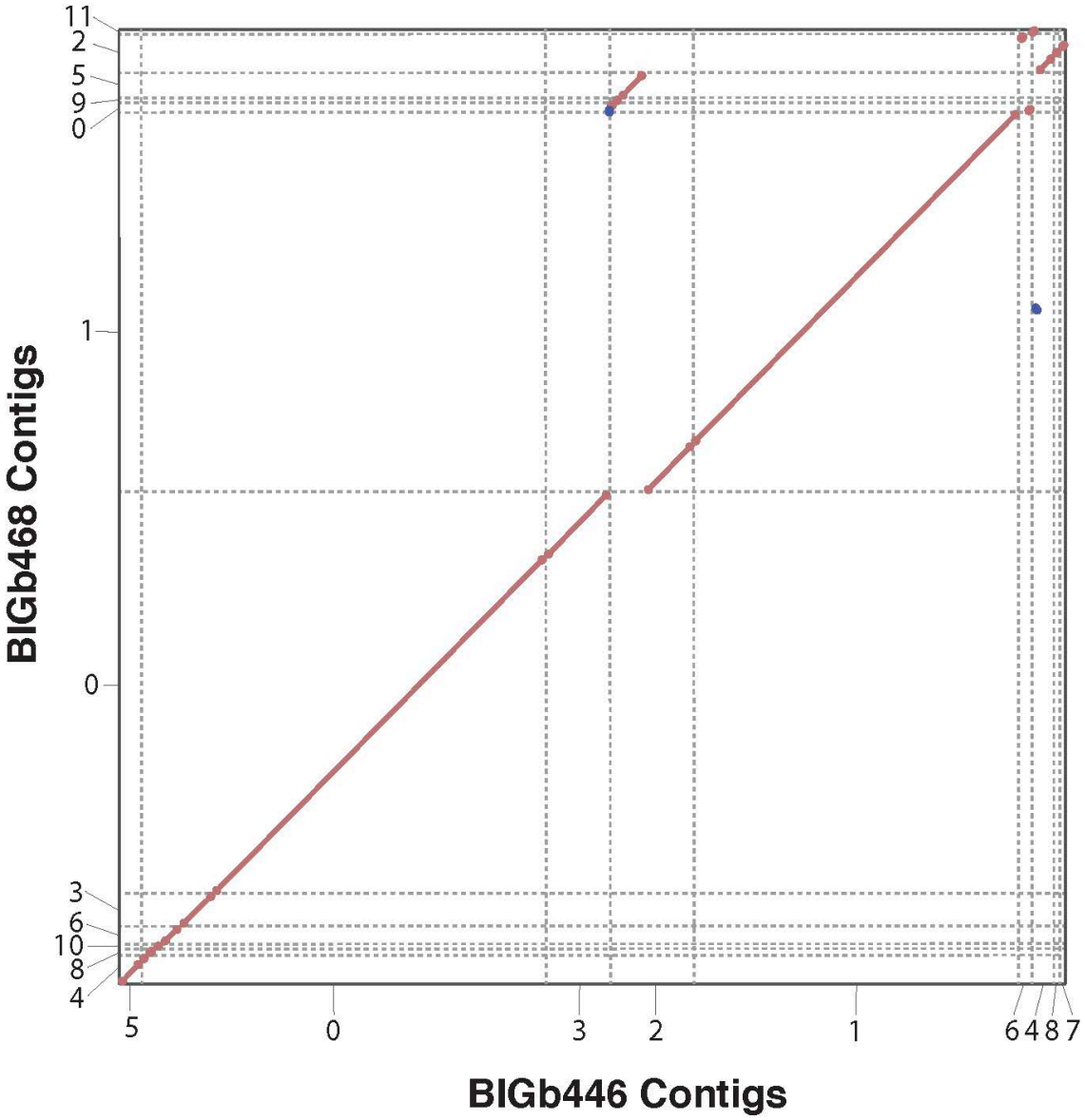
BIGb468 Contigs
BIGb446 Contigs
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1005 Supplementary Figure 3. CYSL-1 and CYSL-2 are required for adaptation to *P. vranovensis*

1006 BIGb446. Percent of wild-type and *cysl-1(mr23, mr25, mr26, mr29, mr39, mr40)* and *cysl-*

1007 *2(syb1431)* mutants surviving on plates seeded with bacterial isolates BIGb446 after 24 hrs.

1008 Error bars, s.d. n = 3 experiments of >100 animals. **** $p < 0.0001$.

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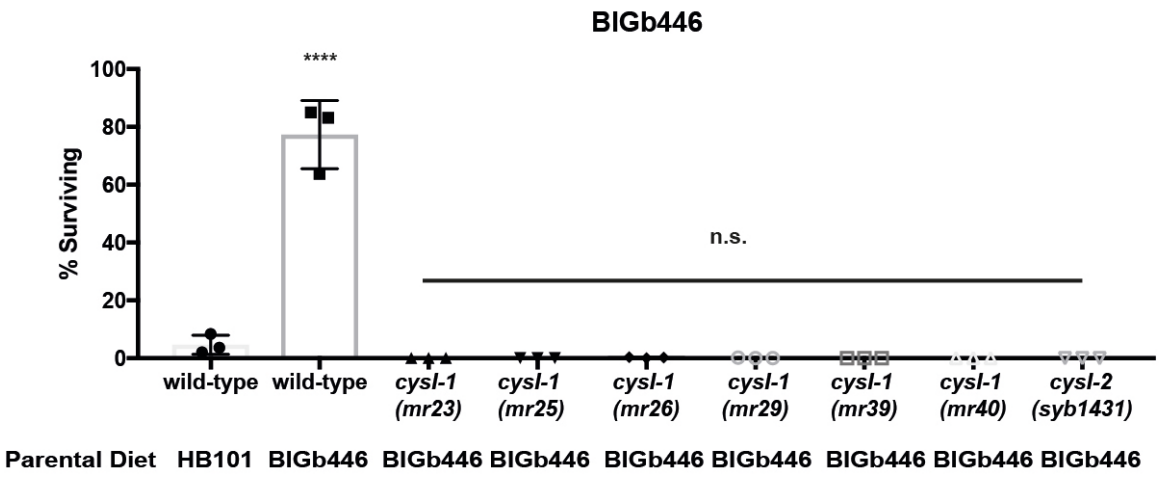
BIGb446
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40
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% Surviving
n.s.
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wild-type
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cysl-1 (mr25)
cysl-1 (mr26)
cysl-1 (mr29)
cysl-1 (mr39)
cysl-1 (mr40)
cysl-2 (syb1431)
Parental Diet
HB101
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1044 Supplementary File 1. Genome assembly of *P. vranovensis* BIGb446. FASTA format file of
1045 *P. vranovensis* BIGb446 genomic DNA.
1046
1047 Supplementary File 2. Genome assembly of *P. vranovensis* BIGb468. FASTA format file of
1048 *P. vranovensis* BIGb468 genomic DNA.
1049
1050 Supplementary Table 1. Comparison of genomic DNA sequences of BIGb446 and BIGb468
1051 to *P. vranovensis.*
1052
1053 Supplementary Table 2. DEseq2 and TPM analysis of mRNA expression in wild-type
1054 embryos from parents fed *E. coli* HB101 or *P. vranovensis* BIGb446.
1055
1056 Supplementary Table 3. Profile of lipid metabolite abundances in wild-type embryos from
1057 parents fed *E. coli* HB101 or *P. vranovensis* BIGb446.
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1059 Supplementary Table 4. DEseq2 analysis of mRNA expression in wild-type embryos from
1060 parents fed *E. coli* HB101, *P. aeruginosa* PA14, or *P. luminescens* Hb.
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1062 Supplementary Table 5. DEseq2 analysis of mRNA expression in wild-type and *wdr-*
1063 *23(bur9)* mutant embryos from parents fed *E. coli* HB101. Input for wild-type embryos fed *E.*
1064 *coli* HB101 are the same as Supplementary Table 4 and these samples were analysed at the
1065 same time.
1066
1067 Supplementary Table 6. Statistics Source Data
1068

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