The Elongator Complex is Required to Maintain Locomotor Healthspan in *Caenorhabditis elegans*

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Abstract: An inherited mutation is not always immediately toxic. Some mutations cause symptoms during youth, while other mutations cause symptoms during adulthood. Mutant animals that show delayed onset of disease symptoms may provide insights into mechanisms that maintain functional capacities during adulthood. Here, we take advantage of the relatively short lifespan of the nematode *Caenorhabditis elegans* and develop a novel screening procedure to collect mutants with locomotor deficits that become apparent in adulthood. After ethyl methanesulfonate mutagenesis, we isolated five *C. elegans* mutant strains that progressively lose adult locomotor activity. In one of the mutant strains, a nonsense mutation in Elongator Complex Protein Component 2 (*elpc-2)* causes a progressive decline in locomotor function. Other *C. elegans elpc* mutants were also unable to maintain locomotor function during adulthood, indicating that the Elongator complex plays a critical role in maintaining locomotor healthspan in *C. elegans*.

Significance Statement

Two people with the same lifespan do not necessarily have the same healthspan. One person may retain locomotor and cognitive functions until the end of life, while another person may lose them during adulthood. Currently, the network of genes that regulate healthspan is largely unknown. Unbiased searches for genes that work to maintain functional capacities during adulthood may uncover key regulators of healthspan. Here we isolated five *Caenorhabditis elegans* mutant strains that progressively lose their locomotor function in adulthood. Mutant analysis suggests that the Elongator complex regulates locomotor healthspan. Mutants and mutations identified in the present screen may provide insights into mechanisms of age-related locomotor impairment and may provide clues for improving healthspan.

Introduction

Inherited mutations can exhibit delayed onset of toxic effects. In theory, three types of mutations can do so: (1) Gain-of-function mutation that causes a slow, cumulative form of toxicity; (2) Gain-of-function mutation in which toxicity is triggered by changes related to aging or adulthood; and (3) Loss-of-function mutation of a gene that is not required for development, but is required for maintenance of functions during adulthood. These types of mutations can be underlying causes of familial cases of adult-onset disease and of more prevalent age-related impairments.

Mutations that cause functional impairment in adulthood take a long time to study in animals with long lifespans. *Caenorhabditis elegans* is a short-lived model organism that has been used to identify key genes involved in complex biological processes. A forward genetic screen for long-lived mutants led to identification of the insulin-signaling pathway as a regulator of lifespan (1–3). Downregulation of the insulin-signaling pathway also prolongs healthspan, or the organism's ability to maintain its functional capacity during adulthood (4–6).

However, recent studies have suggested that the genetic bases of lifespan and healthspan may not completely overlap (7, 8). From a candidate-based genetic screen, Iwasa *et al*. found that activation of the epidermal growth factor signaling pathway prolongs adult swimming ability in *C. elegans* without large effects on lifespan (8). More examples of genetic pathways that work to maintain healthspan may be discovered by carrying out unbiased searches for mutant animals that show progressive declines in functional capacity.

Locomotor ability can serve as an indicator of animal healthspan across species such as worms, flies, mice, and humans (6, 9–11). A forward genetic screen using *C. elegans* has previously been employed to identify genes that affect locomotor function during development (12). However, unbiased screens that focus on locomotor deficits occurring later in life have not been carried out, in part due to the difficulty in

70 distinguishing whether symptoms observed during adulthood were already present during
71 development.
72 In the present study, we established the "Edge Assay" to measure locomotor
73 ability of hundreds of adult worms at once. Using the Edge Assay, we developed a
74 screening procedure to remove mutant worms with strong developmental locomotor
75 defects on the first day of adulthood, and then isolated mutant worms that progressively
76 lose their locomotor function on the third or fifth days of adulthood. After ethyl
77 methanesulfonate-mutagenesis, we isolated five mutant strains that progressively lose
78 their ability to complete the Edge Assay. In one mutant strain, we found that a mutation
79 in the *elpc-2* gene causes progressive loss of locomotor function. *elpc-2* works with
80 other Elongator complex genes, *elpc-1* and *elpc-3*, to maintain adult locomotor function
81 in *C. elegans*. Along with Elongator complex mutants, isolated mutants from our screen
82 can be used as tools to explore mechanisms that lead to a delayed onset of disease
83 symptoms in *C. elegans*.
84
85 **Results**
86 **The "Edge Assay" can test locomotor function of hundreds of worms**
87 Our forward genetic screen isolates mutant worms that progressively lose
88 locomotor function. We established the Edge Assay to measure locomotor activity of
89 hundreds of worms at once. The Edge Assay is carried out on a 9-cm agar plate with *E.*
90 *coli* bacterial feed spread only on the outer edge of the plate. Up to a few hundred adult
91 worms are placed on the center of the plate where there is no food (Fig. 1A; Fig. S1).
92 Motile worms reach the *E. coli* on the edge of the plate, while worms with defects in
93 locomotion or chemotaxis remain in the center of the plate.
94 On the first day of adulthood, 91.3% of wild-type worms reached the edge in 15
95 min and 99.6% reached the edge in 60 min (Fig. 1B; Fig. S1). *C. elegans* mutant strains
96 that are defective in the function of neurons (*unc-13(e51)*, *unc-43(e408)*) (13, 14) or
97 muscles (*unc-54(e190)*) (15) could not reach the edge in 15 min on the first day of
98 adulthood (Fig. 1C). After 60 min, 26% of *unc-54(e190)* mutants, 6.4% of *unc-43(e408)*
99 mutants, and 0% of *unc-13(e51)* mutants reached the edge (Fig. 1C). Therefore, carrying
100 out the Edge Assay for 15 min on the first day of adulthood can separate wild-type worms
101 from worms with strong developmental locomotor defects.
102 On average, over 90% of wild-type worms could complete the Edge Assay in 60
103 min during the first five days of adulthood (Fig. 1B). A *C. elegans* model of amyotrophic
104 lateral sclerosis (Hsa-sod-1(127X)) (16) showed a significant reduction in Edge Assay
105 completion rate compared to wild-type worms on the fifth day of adulthood (Fig. 1D).
106 Therefore, carrying out the Edge Assay for 60 min on the fifth day of adulthood can
107 separate wild-type worms from worms that progressively lose their locomotor activity.
108

109 **Isolation of mutants that progressively lose locomotor activity during adulthood**

110 We mutagenized wild-type N2 worms using ethyl methanesulfonate, and
111 screened 3352 F2 offspring from 500 F1 worms (1000 genomes) (Table S1). We carried
112 out the Edge Assay for the mutagenized F2 offspring on the first day of adulthood (Fig.
113 2A). To remove worms with developmental defects, worms that could not complete the
114 Edge Assay in 15 min were aspirated away (Fig. 2A). Only worms that completed the
115 Edge Assay on the first day of adulthood were kept for further screening. On the third and
116 fifth days of adulthood, we tested the worms again with the Edge Assay and collected
117 slow or uncoordinated mutants that remained near the center of the Edge Assay plate after
118 60 min (Fig. 2A). By removing worms with strong developmental defects on the first day
119 of adulthood, we were able to isolate worms that progressively lost locomotor function
120 during adulthood. We isolated 22 viable mutants, and created individual strains from
121 those mutants (Table S1). Five of those mutant strains reproducibly showed progressive
122 deficits in completing the Edge Assay during adulthood (Fig. 2B).

123 To determine whether isolated mutant strains have deficits in locomotor function
124 and not sensory function or search behavior, we measured locomotor function of worms
125 on an agar plate without food. We recorded one-minute videos of 15 worms freely moving
126 on a plate, and measured the maximum velocities and total travel distances for each worm.
127 For each strain, we recorded three plates of 15 worms on the first, third, and fifth days of
128 adulthood. All isolated mutant strains showed significantly greater reductions in
129 maximum velocity and travel distance from the first to fifth days of adulthood compared
130 to wild type except for *ix240* worms (Fig. 3A, B, D; Fig. S2A–D; Fig. S3A–D). In the
131 *ix240* worms, progressive deficits other than locomotor function, such as sensory function
132 or search behavior, may cause the reduction in Edge Assay completion rate.

133

134 ***ix241* and *ix243* mutant strains show progressive decline in locomotor function**

135 *ix241* and *ix243* worms were backcrossed with the parental N2 strain to reduce
136 the number of mutation sites that do not affect locomotor function. After each backcross,
137 we checked for individual lines that still showed a progressive decline in locomotor
138 function. We measured the maximum velocity and travel distance of individual worms
139 on an agar plate without food on the first, third, and fifth days of adulthood. *ix241* and
140 *ix243* worms still showed significant reductions in both maximum velocity and travel
141 distance after the fourth backcross (Fig. 3A, B, D; Fig. S3A–D).

142 To check whether *ix241* and *ix243* worms were simply aging faster than wild-
143 type worms, we measured lifespans of the two strains. The lifespan of *ix241* worms was
144 not significantly shortened compared to that of wild type (Fig. 3E; Table S2). The median
145 lifespan of the *ix243* worms was shortened by two days (Fig. 3C; Table S2). To compare
146 relative reductions in lifespan and locomotor healthspan, we measured the maximum
147 velocities of wild type and *ix243* worms for 10 days (Fig. S4A). We quantified the percent
148 decrease in lifespan by comparing the areas under the survival curves of wild type and

149 *ix243* worms (Fig. S4B). We quantified the percent decrease in locomotor healthspan by
150 comparing the areas under the decline in maximum velocity curves of wild type and *ix243*
151 worms (Fig. S4C). For *ix243* worms, there is an average 11.5% reduction in lifespan,
152 while there is a significantly greater 18.5% reduction in locomotor healthspan (Fig. S4D).
153 *ix243* worms take a 13.9% longer time to reach adulthood (Table S3). The
154 developmental delay was taken into account for locomotor and lifespan measurements by
155 allowing *ix243* worms an extra 10 h to develop, and starting locomotor and lifespan
156 measurements from the first day of adulthood. *ix243* worms show a 17.7% decrease in
157 maximum locomotor activity on the first day of adulthood compared to wild-type worms
158 (Fig. 3B). The deficit in locomotor capacity compared to wild-type worms increases to
159 54.8% on the fifth day of adulthood (Fig. 3B). These results suggest that the *ix243* mutant
160 allele has modest negative effects on development and lifespan, with relatively stronger
161 negative effects on locomotor healthspan.
162 *ix241* worms take 4.0% longer to reach adulthood (Table S3) and show an 18.1%
163 decrease in maximum locomotor activity on the first day of adulthood compared to wild-
164 type worms (Fig. 3D). The deficit in locomotor function compared to wild-type worms
165 increases to 43.0% on the fifth day of adulthood (Fig. 3D). The *ix241* mutant allele has
166 no negative effect on lifespan, a modest negative effect on development, and a relatively
167 stronger negative effect on locomotor healthspan.
168
169 **Nonsense mutation in *elpc-2* causes progressive loss of adult locomotor function in**
170 ***ix243* worms**
171 We used whole genome sequencing and a modified version of the sibling
172 subtraction method to identify the causative mutation site in the *ix243* strain (Fig. S5)
173 (17). Mutations were evenly induced on all chromosomes in the *ix243* mutant strain
174 before backcrossing (Fig. 4A). Many mutations remained on Chromosome III after
175 comparing mutations in backcrossed strains that show a progressive loss of adult
176 locomotor function and subtracting mutations in backcrossed strains that do not show
177 progressive loss of adult locomotor function (Fig. 4B; Table S4). A nonsense mutation
178 from TGG to TAG within the protein coding region of *elpc-2* was predicted to disrupt
179 protein function (Fig. 4C; Table S4). Presence of the *elpc-2* mutation site was confirmed
180 by Sanger sequencing (Fig. 4D).
181 To test whether loss of *elpc*-2 causes a progressive decline in locomotor
182 function, we injected a genomic fragment of *elpc-2* including 2090-base pairs (bp)
183 upstream of the start codon and 851-bp downstream of the stop codon in the *ix243*
184 mutant strain. The wild-type *elpc-2* fragment rescued the progressive loss of adult
185 locomotor function (Fig. 4E, F; Fig. S6A, B). These results suggest that *elpc-2* is
186 required for maintenance of adult locomotor function in *C. elegans*. The *ix243* mutant

187 strain is the first reported mutant of the *elpc-2* gene in *C. elegans*.
188
189 **The Elongator complex is required to maintain locomotor function**
190 ELPC-2 is a component of the Elongator complex. In *C. elegans,* there are four
191 predicted components of the Elongator complex (ELPC1–4). To test whether functional
192 loss of *elpc-2* causes the locomotor defect independently or as part of the Elongator
193 complex, we measured locomotor activity of strains carrying deletions in *elpc-1* and
194 *elpc-3*. We found that *elpc-1(tm2149)* and *elpc-3(ok2452)* mutant strains also cannot
195 maintain locomotor function during adulthood (Fig. 5A, B). *elpc-1(tm2149);elpc-*
196 *2(ix243)* and *elpc-2(ix243);elpc-3(ok2452)* double mutants did not show additive
197 deficiencies in locomotor function (Fig. 5C; S7A–H). These results suggest that proper
198 functioning of the entire Elongator complex is necessary to maintain locomotor
199 healthspan. We assessed the expression pattern of *elpc-2* by creating an *elpc-2*p*::GFP*
200 transcriptional reporter that expresses GFP under control of the *elpc-2* promoter. The
201 transcriptional reporter was broadly expressed in many tissues including head and body
202 wall muscles, head neurons, pharynx, canal cell, coelomocytes, intestine, and tail (Fig.
203 S8A–C). The expression pattern of *elpc-2* overlaps with previously reported expression
204 of *elpc-1* in the pharynx, head neurons, and body wall muscles (19).
205
206 ***hsf-1* and *elpc-2* mutations may cause a progressive decline in locomotor function**
207 **by overlapping mechanisms**
208 We wondered whether *elpc-2* works together with other known pathways for
209 maintenance of locomotor healthspan in *C. elegans*. HSF-1 and DAF-16 are key
210 transcription factors that have been previously reported to control aging and locomotor
211 healthspan in *C. elegans* (2, 20–23). The *hsf-1(sy441)* mutant, but not the *daf-16(mu86)*
212 mutant, showed significantly greater reductions in maintenance of locomotor function
213 compared to wild type during the first five days of adulthood (Fig. 6A). Other studies
214 have also found that locomotor healthspan is not strongly affected in *daf-16* mutants
215 during early adulthood (6, 24). Therefore, DAF-16 may be involved in maintenance of
216 locomotor healthspan during later stages of adulthood compared to HSF-1.
217 In the *hsf-1(sy441)* and *elpc-2(ix243)* mutant strains, progressive decline in
218 locomotor function occurs during similar time frames from the first to fifth days of
219 adulthood (Fig. 3B; Fig. 6A). Therefore, we tested whether locomotor deficits caused by
220 loss of *elpc-2* and loss of *hsf-1* occur by the same or independent mechanisms. If the
221 two mutations cause locomotor deficits by independent mechanisms, we expect more
222 severe locomotor deficits in the *hsf-1(sy441);elpc-2(ix243)* double mutant than in single
223 mutants. The *hsf-1(sy441);elpc-2(ix243)* double mutants showed no additive
224 deficiencies in locomotor function compared to the *hsf-1(sy441)* mutant strain (Fig. 6B;
225 Fig. S9C, D). The percent change in locomotor activity between adult day 1 and 5 was
226 not significantly different between the single *hsf-1(sy441)* and *elpc-2(ix243)* mutant

227 worms (Fig. 6B; Fig. S9D). These findings suggest that *hsf-1* and *elpc-2* mutations may
228 cause locomotor deficits by overlapping mechanisms. There was however, a significant
229 difference between the locomotor activity of the *elpc-2(ix243)* mutant strain and the *hsf-*
230 *1(sy441)* mutant strain on adult days 1, 3, and 5 (Fig. 6B; Fig. S9B). These results
231 indicate that loss of *hsf-1* has greater effects in development of the neuromuscular
232 system compared to loss of *elpc-2,* while *hsf-1* and *elpc-2* have similar effects on the
233 progressive decline in locomotor activity.
234
235 **The *elpc-2* mutation and the tRNA thiolation mutant *tut-1* have additive effects on**
236 **progressive locomotor decline**
237 Mutants for *elpc-1* and *elpc-3* have previously been reported to modify the
238 wobble uridine of tRNA by adding carbamoylmethyl (ncm) and
239 methoxycarbonylmethyl (mcm) side chains (19, 25). In addition to ncm and mcm
240 modifications, the tRNA wobble uridine is thiolated by TUT-1 (19). We wondered
241 whether tRNA modifications may be involved in the progressive decline in locomotor
242 function observed in the *elpc-2(ix243)* mutant strain. *tut-1(tm1297)* mutant worms also
243 showed a significantly greater decline in locomotor function during adulthood
244 compared to wild-type worms, although the effect of *tut-1(tm1297)* was weaker than
245 that of *elpc-2(ix243)* (Fig. 6C; Fig. S9E, F). tRNA modifications may be involved in
246 maintenance of locomotor healthspan in *C. elegans.*
247 The *elpc-2(ix243)*;*tut-1(tm1297)* double mutant showed synthetic, additive
248 effects for progressive locomotor decline. *elpc-2(ix243)*;*tut-1(tm1297)* double mutant
249 worms showed a significantly greater reduction in maximum velocity and travel
250 distance from the first to fifth days of adulthood relative to either of the single mutants
251 (Fig. 6C; Fig. S9E, F). *elpc-2(ix243)*;*tut-1(tm1297)* double mutant worms also took
252 almost twice as long to reach adulthood (145.4 h) compared to *elpc-2(ix243)* worms
253 (80.2 h) or the *tut-1(tm1297)* worms (82.0 h) (Table S3; Table S5). These results suggest
254 that *elpc-2*-dependent ncm and mcm modifications and *tut-1*-dependent thiolation of
255 tRNA may have non-overlapping roles during development and for the maintenance of
256 locomotor function.
257
258 **Discussion**
259 In this study, we established the Edge Assay to simultaneously measure
260 locomotor function of up to a few hundred worms. For our forward genetic screen, we
261 used the Edge Assay to remove worms with strong developmental locomotor defects, and
262 isolated worms with locomotor deficits that become apparent in adulthood. By carrying
263 out the Edge Assay on the first day of adulthood to remove worms with strong
264 developmental locomotor defects, we were able to overcome the difficulty of
265 distinguishing developmental and progressive locomotor deficit mutants. The Edge Assay
266 can also be used in suppressor screens to search for mutant worms that show

267 improvements in locomotor function of previously characterized *C. elegans* models of
268 neurodegenerative disease. It may also be possible to use the Edge Assay to screen for
269 other types of progressive declines in functional capacity such as sensory or cognitive
270 deficits by replacing the food ring with specific chemicals or learned cues.

271 The *ix241* and *ix243* mutant strains show similar declines in locomotor function,
272 but have different phenotypes in regard to lifespan. This suggests that genes that regulate
273 lifespan and locomotor healthspan may not completely overlap. In terms of improving
274 quality of life, genetic regulators of healthspan may be better therapeutic targets than
275 regulators of lifespan. Further studies and genetic screens that focus on healthspan-related
276 phenotypes may provide novel insights into mechanisms that regulate healthspan and
277 quality of life across many species.

278 In the *ix243* mutant strain, we found that *elpc-2* is required to maintain locomotor
279 healthspan, and works as part of the Elongator complex. The Elongator complex is an
280 evolutionarily conserved protein complex that consists of six subunits in *S. cerevisiae*, *A.*
281 *thaliana*, *M. musculus,* and humans (26, 27). ELP1–ELP3 form the core complex, and
282 ELP4–ELP6 form a sub-complex (26). In *C. elegans*, there are currently only four
283 predicted homologs of the Elongator complex (*elpc-1–4*). From the present study, loss-
284 of-function mutations in *elpc-1*, *elpc-2*, and *elpc-3* caused a shortened locomotor
285 healthspan. Proper functioning of the Elongator complex may require multiple or all
286 components of the complex (27). The present work suggests that the Elongator complex
287 is essential in maintenance of locomotor healthspan.

288 Allelic variants of ELP3, the catalytic subunit of the Elongator complex, were
289 found to be associated with amyotrophic lateral sclerosis (ALS) in three human
290 populations (28). Risk-associated alleles have lower levels of ELP3 in the cerebellum and
291 motor cortex of ALS patients, and protection-associated alleles have higher levels of
292 ELP3 (28). Overexpression of ELP3 reduced levels of axonopathy in the $SOD1^{A4V}$
293 zebrafish model of ALS and $SOD1^{G93A}$ mouse model of ALS (29). The present work
294 complements studies that have been performed in the context of ALS, and suggest that
295 loss of the Elongator complex alone can cause locomotor deficits during adulthood in *C.*
296 *elegans*. Future therapies that target multiple subunits of the Elongator complex may
297 provide more robust effects than therapies that target only the catalytic ELP3 subunit.

298 ELP2 mutations were reported as the causative mutations in a familial form of
299 neurodevelopmental disability (30). Patients who are compound heterozygotes for two
300 different ELP2 missense mutations demonstrate a lack of motor control starting in early
301 development, severe intellectual disability, and progressive loss of locomotor function
302 (30). Loss of ELP2 function may lead to more severe defects in humans compared to *C.*
303 *elegans*. In our newly isolated *elpc-2(ix243)* strain, we also see deficits in locomotor
304 function on the first day of adulthood and a delay in development (Fig. 3A, B; Fig. S3A,
305 B; Table S3). The amino acid sequences of *C. elegans* ELPC-2 and human ELP2 are
306 highly conserved (Fig. S10). This may indicate that some aspects of the

307 neurodevelopmental dysfunctions that result from the human ELP2 mutation may be
308 modeled in our *elpc-2(ix243)* mutant strain.
309 The Elongator complex was originally identified as a transcriptional regulator
310 associated with RNA polymerase II (31). However, follow-up studies have found that the
311 main functions of the Elongator complex may involve tRNA modification (19, 32), and
312 tubulin acetylation (33). The tRNA thiolation mutant, *tut-1(tm1297)*, also showed a
313 progressive decline in locomotor function. In yeast, tRNA modifications are important
314 for proper translation and folding of proteins (25). Modification of tRNA affects
315 translation efficiency and protein folding and may be important in maintenance of
316 locomotor healthspan. In support of this possibility, loss-of-function of both *elpc-2* and
317 *hsf-1* did not cause additive effects in the progressive decline in locomotor function. *hsf-*
318 *1* is a transcription factor, which induces expression of chaperone proteins that aid protein
319 folding (22). Overlapping effects on protein folding may explain why the *hsf-*
320 *1(sy441);elpc-2(ix243)* double mutant did not show any additive deficiencies in
321 locomotor function.
322 In *C. elegans,* mutations in the Elongator complex cause a progressive decline
323 in locomotor function during adulthood. Elongator complex mutations may represent
324 loss-of-function mutations that do not strongly affect development, but have strong
325 effects during maintenance of neuromuscular function. Future studies to characterize
326 genetic mutations and molecular mechanisms in our mutants may provide further insights
327 into how an inherited mutation can cause a delayed onset of disease symptoms.

328

329 **Methods**

330 **Strains**

331 *C. elegans* Bristol N2 strain was used as wild type. Worms were cultivated on
332 Nematode Growth Media (NGM) agar plates with *E. coli* strain OP50 at 20°C (12). See
333 Supplementary Information Appendix for strains used in the present study.

334

335 **Edge Assay**

336 Edge Assay plates were prepared by pouring 16 mL of NGM agar into a circular 9 cm
337 plate. NGM plates were dried overnight with the lid on at 25°C, then kept at 4°C until
338 use. On the day before the Edge Assay, a total of 100 µL of *E. coli* suspension was
339 spotted on four spots near the edge of the NGM plate. The tip of a 50 mL serological
340 pipette was briefly placed over a flame to smoothen the tip. The NGM plate was placed
341 on an inoculating turntable and the smoothened pipette tip was held against the *E.coli*
342 drop. The plate was slowly rotated while holding the pipette tip still. The plate was
343 rotated 360° to spread the *E. coli* around the edge of the whole plate. Plates were
344 incubated overnight at 25°C and used the next day. Synchronized worms were collected

345 and washed twice with M9 buffer containing 0.1% gelatin. Worms were placed on the
346 center of an Edge Assay plate and excess M9 buffer was removed with the edge of a
347 Kimwipe. The number of worms that reached or did not reach the edge were counted at
348 various time points to measure the Edge Assay completion rate.

349

350 **Isolation of mutants manifesting a progressive decline in locomotor function**

351 Wild-type N2 worms were mutagenized and cultured as previously described (12).
352 Larval stage 4 worms were mutagenized by incubation in a 50 mM ethyl
353 methanesulfonate solution for 4 h. EMS-mutagenized F2 adult day 1 worms were
354 collected and washed twice with M9 buffer containing 0.1% aqueous gelatin. Worms
355 were placed at the center of an Edge Assay plate and excess buffer was removed with
356 the edge of a Kimwipe. After 15 min, worms that did not reach the edge were removed
357 using an aspirator. Worms that reached the edge were maintained on the same plate
358 until adult day 3. On adult day 3, worms were collected and washed with M9 buffer
359 containing 0.1% gelatin and the Edge Assay was repeated on a new Edge Assay plate.
360 Worms that were unable to reach the edge were collected as adult day 3 progressive
361 locomotor deficit mutants. Worms that reached the edge were maintained on the same
362 plate until adult day 5. On adult day 5, worms were collected and washed with M9
363 buffer containing 0.1% gelatin and the Edge Assay was repeated on a new Edge Assay
364 plate. Worms that were unable to reach the edge were collected as adult day 5
365 progressive locomotor deficit mutants.

366

367 **Measurements of maximum speed and travel distance**

368 Worms were synchronized by placing five adult day 1 worms onto an NGM plate with
369 food, and allowed to lay eggs for 3 h. When the offspring reached adult day 1, 15
370 worms were picked randomly onto a 6 cm NGM plate without bacteria. After the worms
371 moved away from the initial location with residual food, worms were again moved onto
372 a different NGM plate without bacteria. Movement of worms was recorded for 1.0 min
373 with a charge-coupled device camera INFINITY3-6URM (Lumenera Corporation,
374 Ottawa, Canada). Images were analyzed using ImageJ and wrMTrck software
375 (www.phage.dk/plugins) to produce maximum speed and travel distance (34).
376 Measurements were made with the lid on in a temperature-controlled room set at 20 °C.
377 At least three biological replicate plates of 15 worms each were measured for each
378 strain. Worms that were lost during the video recording were not included in the
379 analysis.

380

381 **Whole-genome DNA sequencing**

382 *C. elegans* DNA was sequenced using the MiSeq platform (Illumina, San Diego, CA).
383 Libraries were prepared with an Illumina TruSeq Library Prep Kit. Mapping was
384 conducted with BWA software (35). Resulting files were converted to bam files, then to
385 pileup format with Samtools (36). Variant analysis was conducted using VarScan and
386 SnpEff available on the Galaxy platform (37–41). Mutation frequencies along the
387 chromosome were calculated and visualized using CloudMap (18).

388

389 **Transcriptional reporter expression**

390 A genomic fragment of 2090-bp immediately upstream of the start codon of the *elpc-2*
391 gene was PCR-amplified using "5' *elpc-2*p overlap ppd95.79 107-" and "3' *elpc-2*p
392 overlap ppd95.79 138-" primers, which have 15-bp overhangs that anneal immediately
393 upstream of the GFP sequence in the pPD95.79 vector (See Supplementary Information
394 for primers used). The pPD95.79 vector containing GFP was linearized by PCR using
395 the "5' ppd95.79 107-" and "3' ppd95.79 138-" primers. The template vector was
396 digested with restriction enzyme *Dpn*I (New England Biolabs, Ipswich, MA), and the
397 linearized vector was purified by Wizard SV Gel and PCR Clean-Up System (Promega,
398 Madison, WI). The pure linearized vector and the *elpc-2* promoter were fused using an
399 In-Fusion HD Cloning Kit (Takara, Kusatsu, Japan) to make the *elpc-2*p*::GFP*
400 transcriptional reporter construct. The construct was microinjected into the gonads of
401 wild-type worms at a concentration of 50 ng/μL. Worms that expressed the reporter
402 construct were immobilized in 25 mM sodium azide and observed under a confocal
403 microscope LSM710 (Carl Zeiss, Oberkochen, Germany). A z-stack image was created
404 from images taken at 1 μm increments.

405

406 **Creation of double mutants**

407 Double mutant strains were created by crossing males of one strain with hermaphrodites
408 of another. Double mutants were checked by extracting their DNA, amplifying a genomic
409 fragment flanking the mutation site by PCR, and sequencing the PCR product by Sanger
410 sequencing. See Supplementary Information for primer details.

411

412 **Statistics**

413 All results are expressed as means with a 95% confidence interval. Student's *t* test was
414 used for pairwise comparisons with Excel 2010 (Microsoft). For multiple comparisons,
415 one-way ANOVA was followed with Dunnett's post hoc test or Tukey's Honest
416 Significant Difference test using R (42). Statistical significance was set at $*P < 0.05$; $**P$
417 <0.01; $***P < 0.001$.
418

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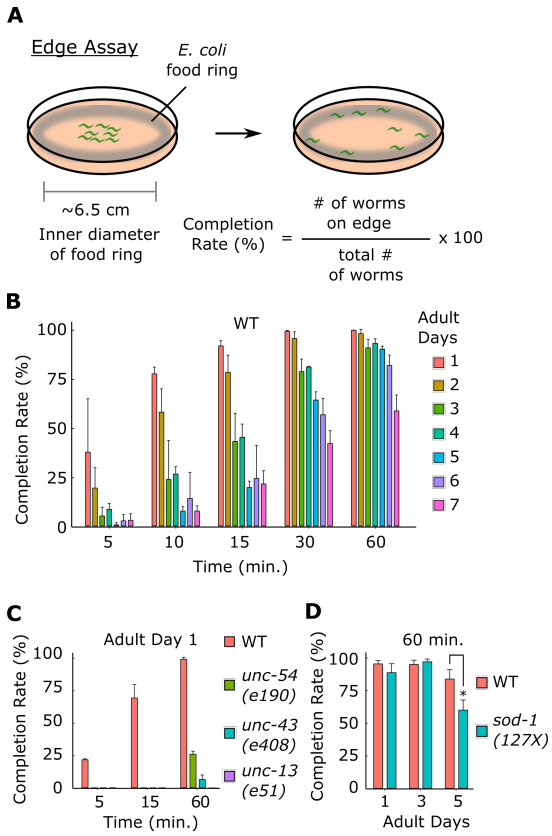

535

536 **Figure 1. "Edge Assay" can measure locomotor ability of worms**

537 (A) (*Left*) Schematic diagram of an Edge Assay plate immediately after placing worms at
538 the center of the plate. (*Right*) Schematic diagram of Edge Assay plate after most worms
539 reached the edge. (B) Edge Assay completion rates of wild-type worms from adult day 1
540 to 7 after 5, 10, 15, 30, and 60 min. (C) Completion rates for WT and developmental
541 mutants deficient in locomotor function, *unc-54(e190)*, *unc-43(e408)*, and *(unc-13(e51)*.
542 (D) Completion rates of WT and a previously reported *C. elegans* model of amyotrophic
543 lateral sclerosis (Hsa-sod-1(127X)). For Edge Assay experiments, n = 3 biological
544 replicate plates with each plate starting with approximately 100 worms per plate on adult
545 day 1. Error bars indicate 95% confidence intervals. *$P < 0.05$; Unpaired Student's *t* test
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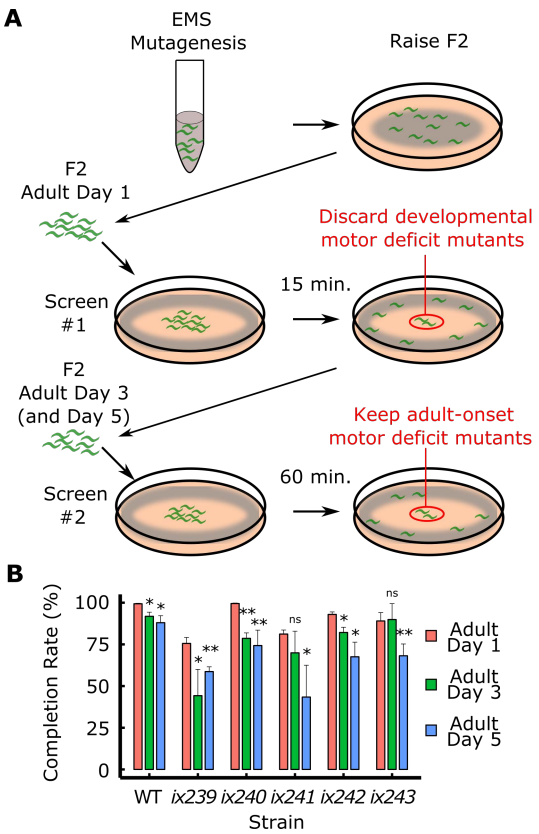

548

549 **Figure 2. Isolation of mutant strains that progressively lose locomotor ability**

550 (A) Schematic description of a forward genetic screen to isolate mutants that
551 progressively lose locomotor ability. (B) Edge Assay completion rates of mutants
552 identified from the screen. Error bars indicate 95% confidence intervals. n = 3 biological
553 replicate plates, with each plate starting with approximately one hundred worms per plate
554 on adult day 1. *$P < 0.05$; **$P < 0.01$; ns, not significant; Paired Student's *t* test vs. adult
555 day 1 completion rate.

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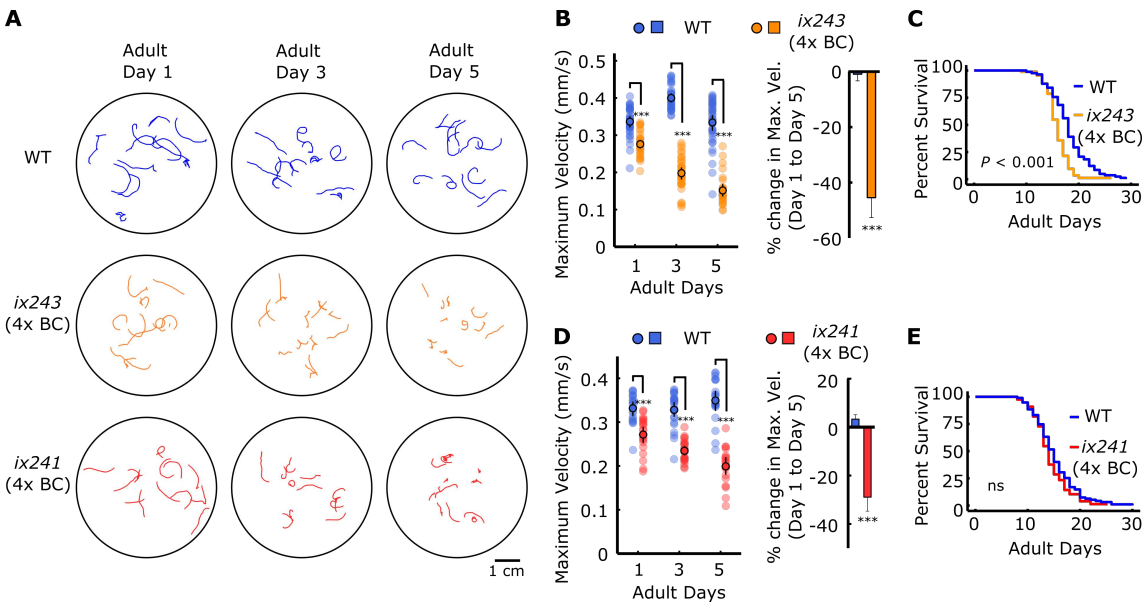
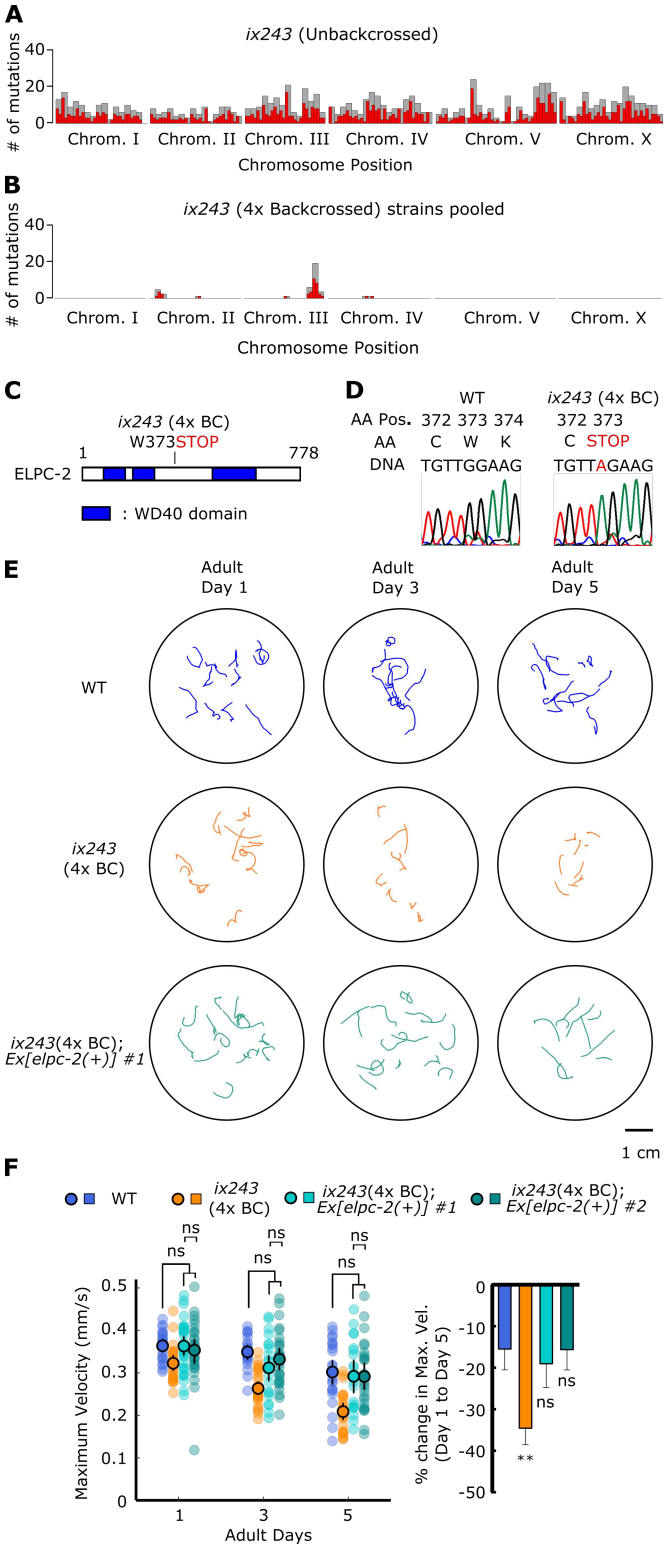


Figure 3. *ix241* and *ix243* worms show progressive locomotor decline after four backcrosses

(A) Representative locomotor tracks from 1-min video recordings of wild-type (WT), *ix243*(backcrossed four times (4x BC)), and *ix241*(4x BC) worms on adult days 1, 3, and 5 on plates with no food. n = 10–15 tracks per plate (some worms were unable to be tracked for a full minute, and were removed from analysis) (B) (*Left*) Maximum velocities of WT and *ix243*(4x BC) worms. (*Right*) Percent change in maximum velocity of WT and *ix243*(4x BC) worms on adult day 5 compared to adult day 1. (C) Survival curve of WT (n = 56 worms) and *ix243*(4x BC) (n = 89) worms.(D) (*Left*) Maximum velocities of WT and *ix241*(4x BC) worms. (*Right*) Change in maximum velocity of WT and *ix241*(4x BC) worms. (E) Survival curve of WT (n = 94) and *ix241*(4x BC) (n = 77) worms. Error bars indicate 95% confidence intervals. For maximum velocity experiments, n = 30–45 worms per strain for each day (10–15 worms from 3 biological replicate plates). For percent change in maximum veloity graphs, n = 3 biological replicate plates. ***$P < 0.001$; ns, not significant; Unpaired Student's *t* test for maximum velocity comparisons; Log-rank test for lifespan comparisons.



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576 **Figure 4. *elpc-2* mutation causes locomotor deficits in the *ix243* mutant strain**

577 (A) Mutation frequency along each chromosome of *ix243* mutant strain before
578 backcrossing. Red bars indicate 0.5-Mb bins and grey bars indicate 1.0-Mb bins. (B)
579 Mutation frequency along each chromosome for pooled *ix243*(4x BC) worms. (C)
580 Schematic diagram of ELPC-2 protein and location of mutation site in *ix243* allele. (D)
581 *ix243* mutation site on ELPC-2 amino acid (AA) sequence and *elpc-2* DNA sequence. (E)
582 Representative locomotor tracks of WT, *ix243*(4x BC), and *ix243*(4x BC);*Ex[elpc-2(+)]*

583 *#1* worms. (F) (*Left*) Maximum velocities of WT, *ix243*(4x BC), *ix243*(4x BC);*Ex[elpc-*
584 *2(+)] #1,* and *ix243*(4x BC);*Ex[elpc-2(+)] #2* worms. n = 30–45 worms per strain for
585 each day (10–15 worms from 3 biological replicate plates). (*Right*) Percent change in
586 maximum velocity of worms from left panel. n = 3 biological replicate plates. Error bars
587 indicate 95% confidence intervals. $^{**}P < 0.01$; ns, not significant ; One-way ANOVA
588 with Dunnett's post hoc test vs. WT.
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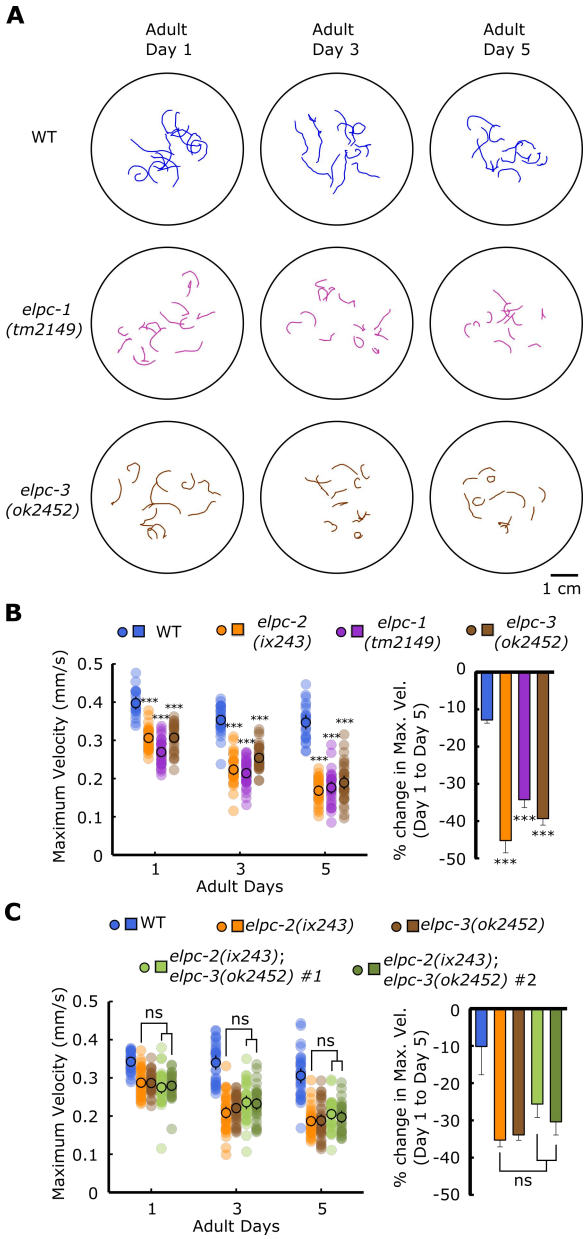

Figure 5. The Elongator complex is required to maintain locomotor function

(A) Representative locomotor tracks of WT, *elpc-1(tm2149)* and *elpc-3(ok2452)* worms. n = 10–15 tracks per plate. (B) (*Left*) Maximum velocities of WT *elpc-1(tm2149)* and *elpc-3(ok2452)* worms. (*Right*) Percent change in maximum velocity of worms from left panel. (C) (*Left*) Maximum velocities of WT, *elpc-2(ix243)*, *elpc-3(ok2452)*, and *elpc-2(ix243);elpc-3(ok2452)* worms. (*Right*) Percent change in maximum velocity of worms from left panel. Error bars indicate 95% confidence intervals. For maximum velocity experiments, n = 30–45 worms per strain for each day (10–15 worms from 3 biological replicate plates). For percent change in maximum veloity graphs, n = 3 biological replicate plates. ***$P < 0.001$; ns, not significant; One-way ANOVA with Dunnett's post hoc test vs. WT for B; One-way ANOVA with Tukey's post hoc test for C.

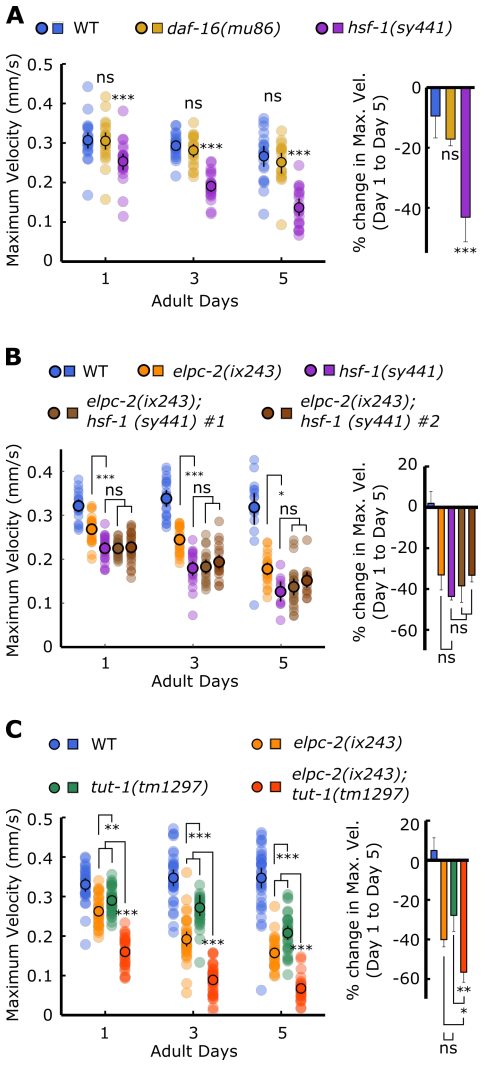

Figure 6. The Elongator complex may work with Heat Shock Factor -1

(A) (*Left*) Maximum velocities of WT, *daf-16(mu86)*, and *hsf-1(sy441)* worms. (*Right*) Percent change in maximum velocity of worms from left panel. (B) (*Left*) Maximum velocities of WT, *elpc-2(ix243)*, *hsf-1(sy441)* and *elpc-2(ix243);hsf-1(sy441)* worms. (*Right*) Percent change in maximum velocity of worms from left panel. (C) (*Left*) Maximum velocities of WT, *elpc-2(ix243)*, *tut-1(tm1297),* and *elpc-2(ix243);tut-1(tm1297)* worms. (*Right*) Percent change in maximum velocity of worms from left panel. Error bars indicate 95% confidence intervals. For maximum velocity experiments, n = 30–45 worms per strain for each day (10–15 worms from 3 biological replicate plates). For percent change in maximum velocity graphs, n = 3 biological replicate plates. *$P < 0.05$; ***$P < 0.001$; ns, not significant; One-way ANOVA with Dunnett's post hoc test vs. WT for A; One-way ANOVA with Tukey's post hoc test for B, C.

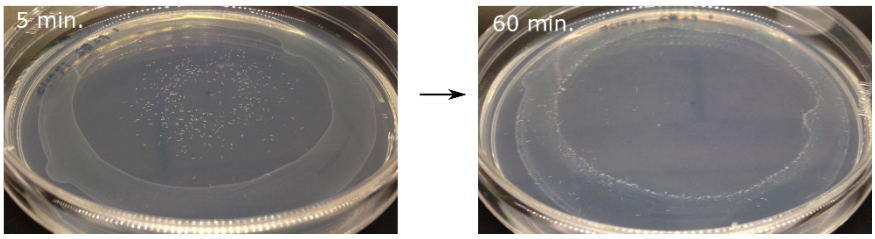

Figure S1. Photos of Edge Assay

(*Left*) Photo of Edge Assay after 5 min with worms moving away from the center. (*Right*) Photo of Edge Assay after 60 min with worms reaching and remaining in the edge.

623 **Table S1. Number of screened genomes and isolated mutants**

	EMS-mutagenesis I			EMS-mutagenesis II			
	Day 1	**Day 3**	**Day 5**	**Day 1**	**Day 3**	**Day 5**	**Total**
Genomes Screened	400			600			
Isolated mutants		13	23		17	17	70
Viable isolated mutants		3	8		9	2	22
Isolated mutants with reproducible deficits		0	3		2	0	5

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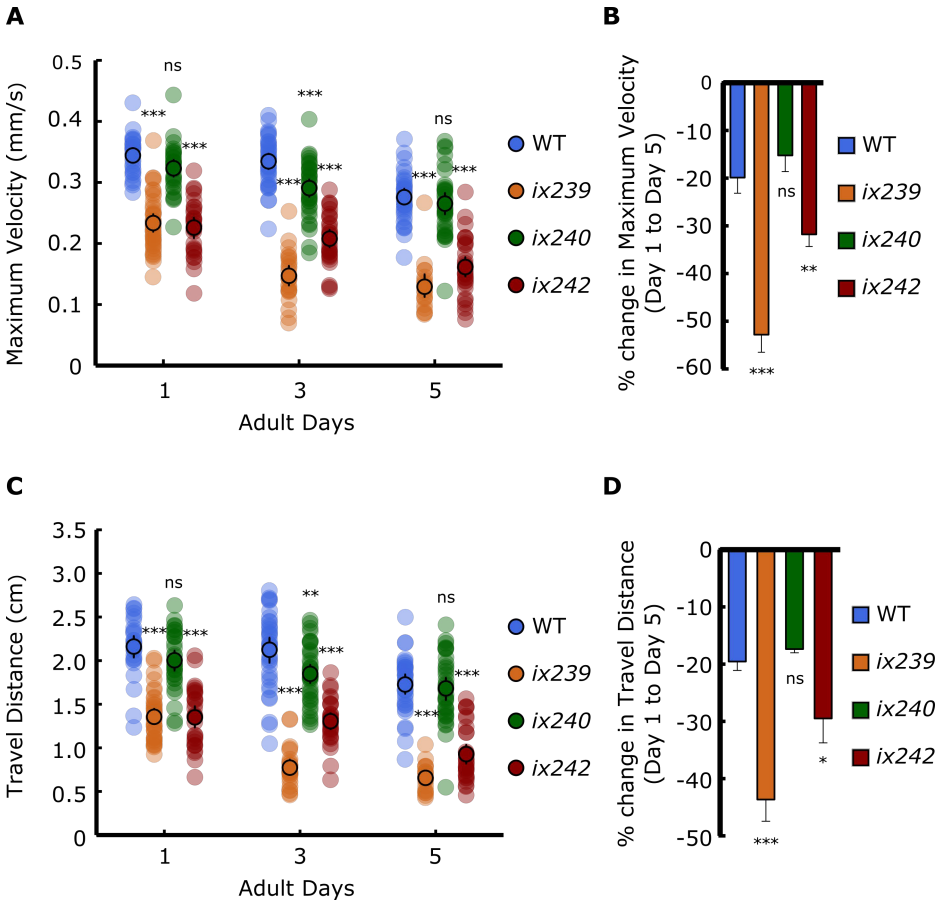

Figure S2. Maximum velocity and travel distance of isolated mutants

(A) Maximum velocities of WT, *ix239, ix240,* and *ix242* worms. (B) Percent change in maximum velocity of worms from A. (C) Travel distances of WT, *ix239, ix240,* and *ix242* worms. (D) Percent change in travel distance of worms from C**.** Error bars indicate 95% confidence intervals. For maximum velocity and travel distance experiments, n = 30–45 worms per strain for each day (10–15 worms from 3 biological replicate plates). For percent change in maximum velocity graphs, n = 3 biological replicate plates. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ns, not significant; One-way ANOVA with Dunnett's post hoc test vs. WT.

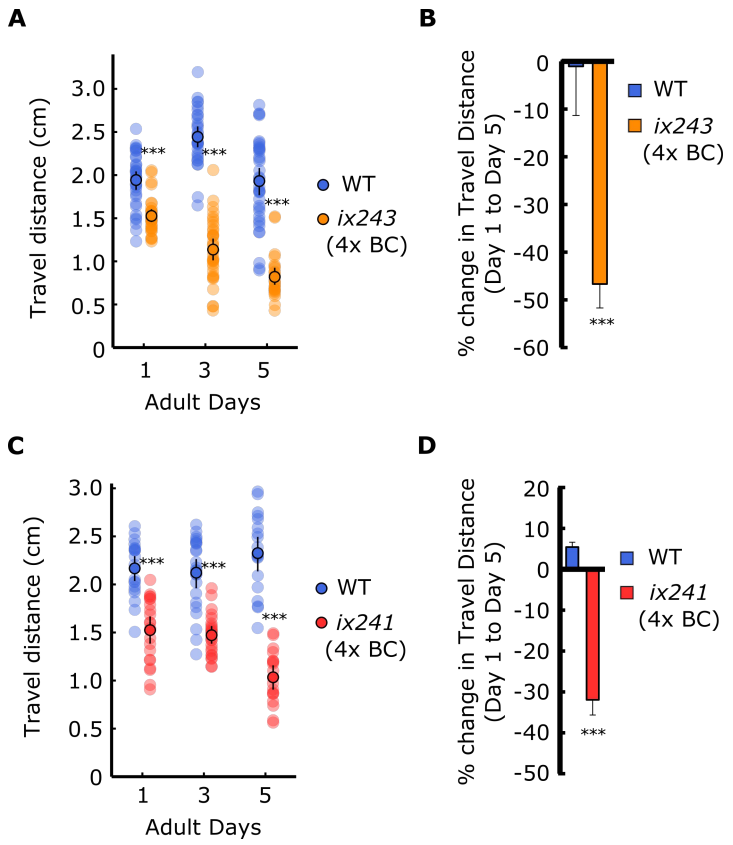

Figure S3. *ix241* and *ix243* worms show progressive locomotor decline after four backcrosses

(A) Travel distances of WT and *ix243* worms (backcrossed four times (4x BC)). (B) Percent change in travel distance of WT and *ix243*(4x BC) worms. (C) Travel distances of WT and *ix241*(4x BC) worms. (D) Percent change in travel distance of WT and *ix241*(4x BC) worms. For travel distance experiments, n = 30–45 worms per strain for each day (10–15 worms from 3 biological replicate plates). For percent change in travel distance graphs, n = 3 biological replicate plates. ***$P < 0.001$; Unpaired Student's *t* test vs. WT.

648 **Table S2. Lifespan Analysis**

Strain	Median Lifespan (adult days)	*P* value vs WT (Log-rank test)	Worms (Counted/Total)	FUDR
N2	18		56/90	25 µg/ml
ix243(4x backcrossed)	16	$P = 0.00078$	89/90	25 µg/ml
N2	18		82/90	25 µg/ml
ix243(4x backcrossed)	16	$P < 0.0001$	84/90	25 µg/ml
N2	18		87/90	25 µg/ml
ix243(4x backcrossed)	16	$P < 0.0001$	87/90	25 µg/ml
N2	17		94/120	0
ix241(4x backcrossed)	16	$P = 0.095$	77/120	0
N2	14		66/90	0
ix241(4x backcrossed)	15	$P = 0.12$	64/90	0
N2	12		67/90	0
ix241(4x backcrossed)	14	$P = 0.024$	74/90	0

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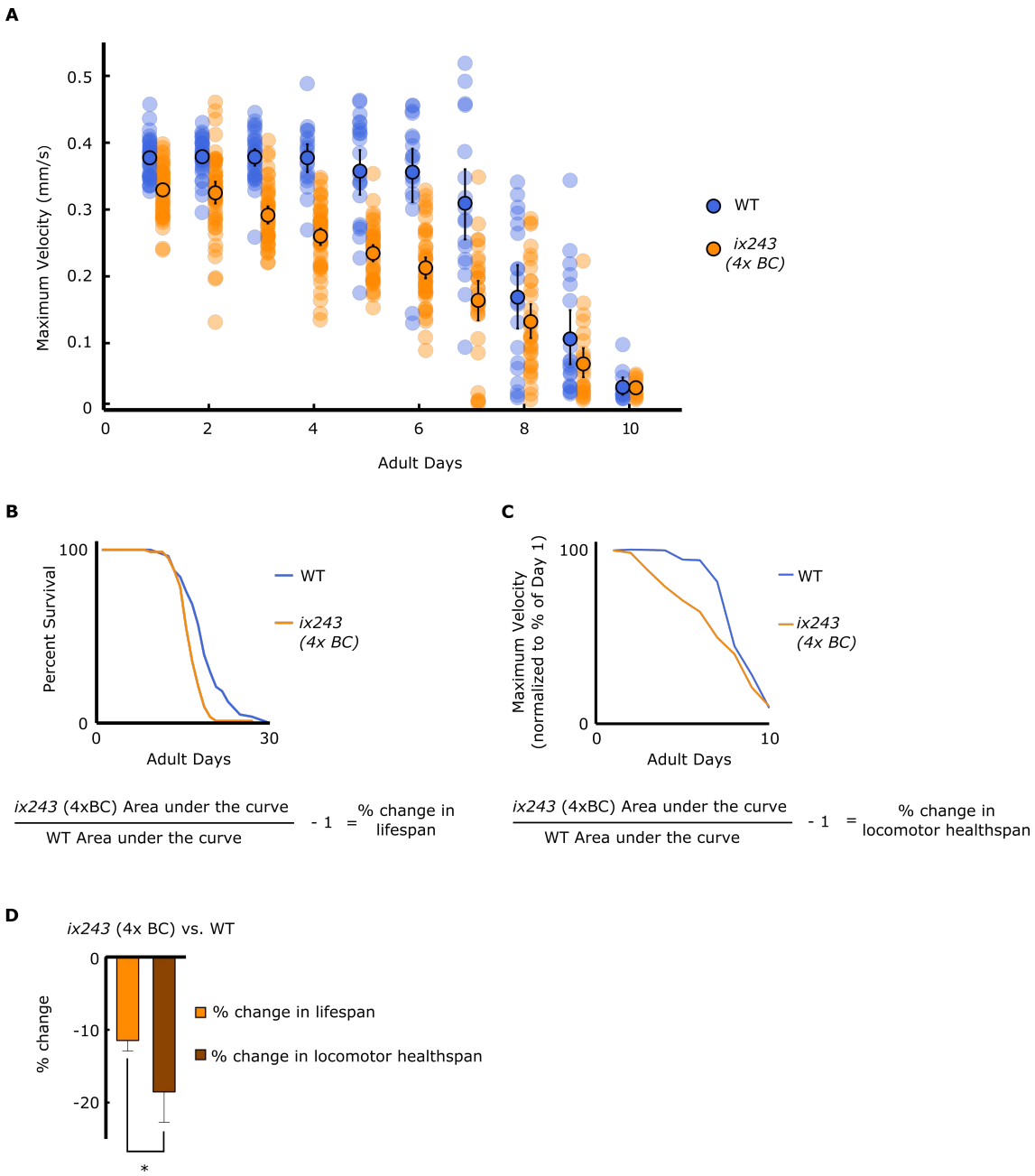

Figure S4. Greater reduction in total locomotor healthspan compared to lifespan in *ix243* worms

(A) Maximum velocities of WT and *ix243* (4x backcrossed (4x BC)) worms. n = 30–45 worms per strain for each day (10–15 worms from 3 biological replicate plates). (B) (Top) Representative survival curve of WT (n = 56 worms) and *ix243*(4x BC) worms (n = 89 worms). (Bottom) Calculation method of percent change in lifespan. (C) (Top) Representative decline in maximum velocity curve of WT and *ix243*(4x BC) worms. n = 30–45 worms per strain for each day (10–15 worms from 3 biological replicate plates). (Bottom) Calculation method of percent change in locomotor healthspan. (D) Percent change in lifespan (n = 3 biological replicate plates for WT and *ix243*(4xBC)) and locomotor healthspan (n = 3 biological replicate plates for WT and *ix243*(4xBC)) of *ix243*(4xBC) worms compared to WT. *$P < 0.05$; Unpaired Student's *t* test.

664 **Table S3. Development times of isolated mutant strains**

665 Development time from egg to first egg-lay (n=5 worms per strain).

Strain	Development Time (h)	% of WT
WT	70.4	100.0%
ix239	74.4	105.7%
ix240	71.2	101.1%
ix241 (4x backcrossed)	73.2	104.0%
ix242	71.8	102.0%
ix243 (4x backcrossed)	80.2	113.9%

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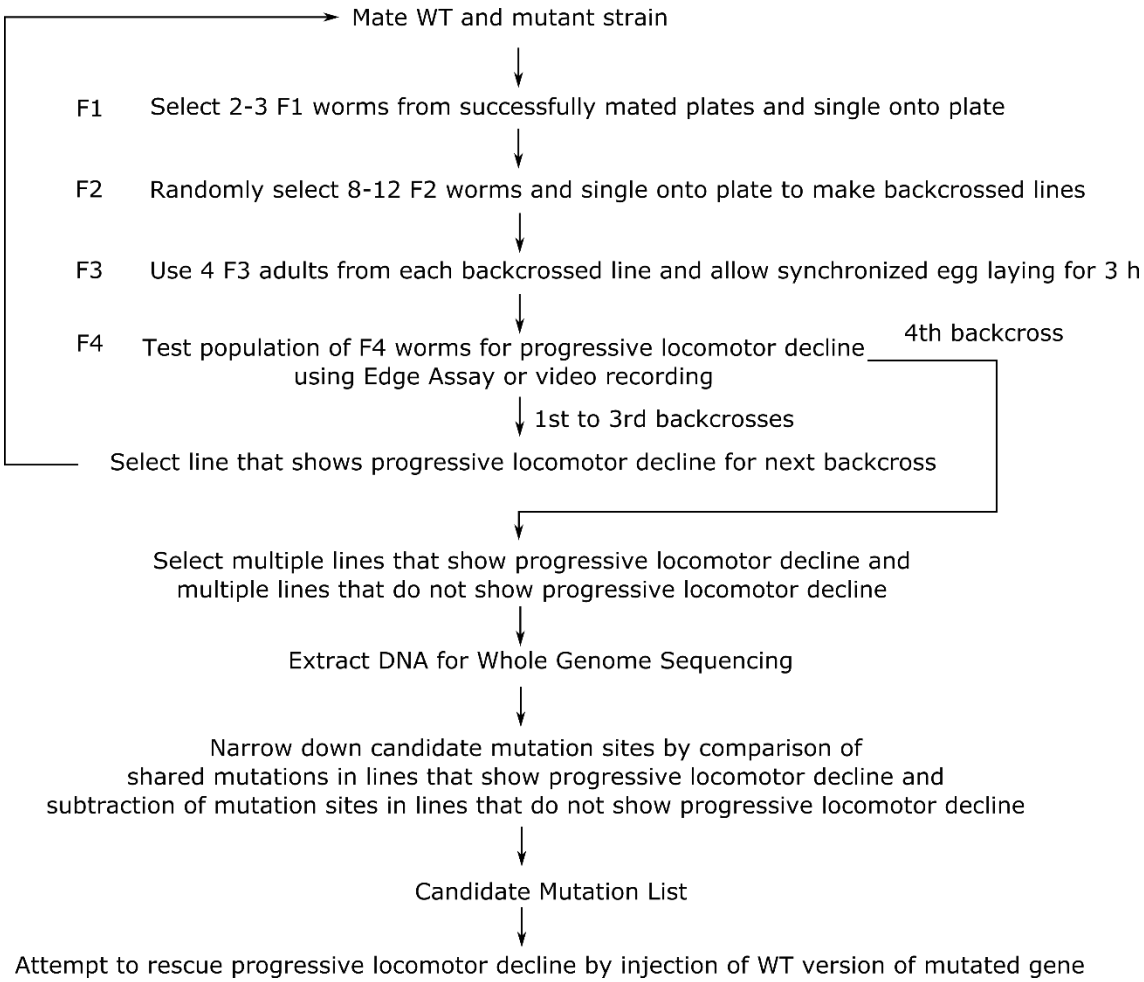

Figure S5. Strategy to identify causative mutation site

671 **Table S4. Remaining mutations in *ix243* mutant strains**

Chrom.	Pos.	Ref.	Alt.	Gene	Mutation Type	Effect
I	3428548	A	AGGCAGACCTAGCCCACCCTG GGCAGACCTAGCCCACCCTG	K03E5.2	downstream gene variant	modifier
I	14448378	G	GCGTCCGGTTTTGGGGTAGGTTT CCACGGCGGCCGACAATTTCCG AGTTTCGCCACTCACTATACTTA ATCAGCAATTTTATAGTGAGTTG CGAAACTCGGAAATTGTCGGCC GTCGTGGATAACTACCCCAAAA CCGGACGACCGGA	Y105E8A.27	downstream gene variant	modifier
II	1465622	C	T	Y51H7C.13	missense variant	moderate
II	1602489	C	T	bath-47	downstream gene variant	modifier
II	1717193	C	T	btb-7	downstream gene variant	modifier
II	1915528	C	T	math-31	upstream gene variant	modifier
II	2339259	G	T	F43C11.6	upstream gene variant	modifier
II	2428896	C	T	F42G2.2	downstream gene variant	modifier
II	2477652	T	A	tsr-1	upstream gene variant	modifier
II	8975684	G	T	tomm-40	upstream gene variant	modifier
III	577387	TAAAAATTT AACAAAA	T	Y55B1AL.1	downstream gene variant	modifier
III	1704981	A	T	Y22D7AR.10	upstream gene variant	modifier
III	7406703	A	T	linc-165	upstream gene variant	modifier
III	7439448	C	T	linc-165-alh-12	intergenic region	modifier
III	11024784	G	A	Y48A6B.16	upstream gene variant	modifier
III	11416475	G	A	Y47D3B.13	upstream gene variant	modifier
III	11549303	G	A	Y66D12A.14	downstream gene variant	modifier
III	11816202	G	A	C18D11.1	upstream gene variant	modifier
III	11895783	G	A	faah-5	upstream gene variant	modifier
III	12134837	G	A	Y75B8A.6	upstream gene variant	modifier
III	12156042	G	A	linc-87	upstream gene variant	modifier
III	12190456	G	A	Y75B8A.54	upstream gene variant	modifier
III	12200963	G	A	Y75B8A.44	downstream gene variant	modifier
III	12223827	G	A	Y75B8A.16	missense variant	moderate
III	12407309	G	A	tat-1	missense variant	moderate
III	12427755	G	A	Y49E10.16	upstream gene variant	modifier
III	12476529	G	A	Y49E10.33	upstream gene variant	modifier
III	12498064	C	T	Y111B2A.3	synonymous variant	low
III	12678743	G	A	elpc-2	stop gained	high
III	12701690	G	A	spin-4	downstream gene variant	modifier
III	12750669	A	T	irld-60	missense variant	moderate
III	12810002	G	A	BE10.5	upstream gene variant	modifier
III	12838123	G	A	Y37D8A.4	synonymous variant	low
III	12892115	G	A	unc-71	upstream gene variant	modifier
III	12923518	G	A	Y37D8A.16	upstream gene variant	modifier
III	12924071	G	A	mrps-10	upstream gene variant	modifier
III	13352002	G	A	F53A2.9	downstream gene variant	modifier
III	13453318	G	A	cua-1	upstream gene variant	modifier
III	13578898	G	A	T05D4.5	upstream gene variant	modifier
IV	6722231	C	T	Y73B6A.6-Y73B6A.2	intergenic region	modifier
IV	7597770	G	A	tag-80	synonymous variant	low
IV	7597783	T	A	tag-80	missense variant	moderate
IV	17276360	A	AT	gln-5	upstream gene variant	modifier

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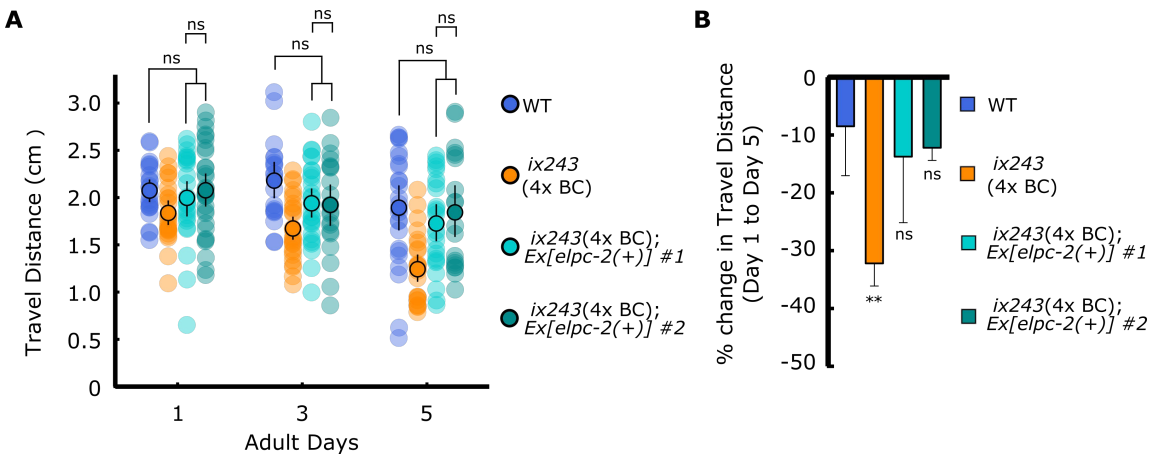

Figure S6. Wild-type *elpc-2* gene rescues progressive decline in locomotor function of the *ix243* mutant strain

(A) Travel distance of WT, *ix243*(4x BC), *ix243*(4x BC);*Ex[elpc-2(+)] #1, and ix243*(4x BC);*Ex[elpc-2(+)] #2* worms. n = 30–45 worms per strain for each day (10–15 worms from 3 biological replicate plates). (B) Percent change in travel distance of worms from A. n = 3 biological replicate plates. $^{**}P < 0.01$; $^{***}P < 0.001$; ns, not significant; One-way ANOVA with Dunnett's post hoc test vs. WT.

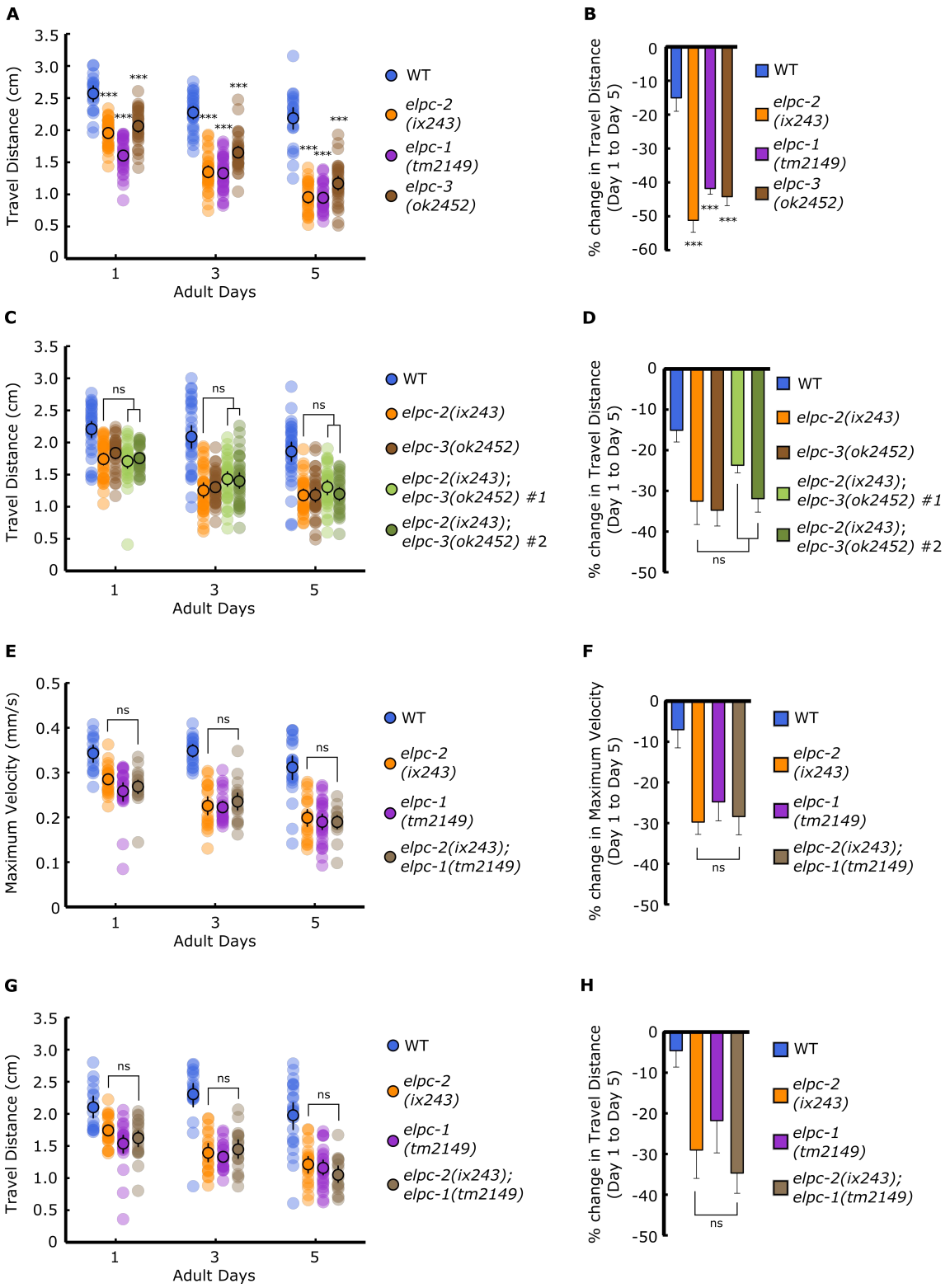

Figure S7. The Elongator complex is required to maintain adult locomotor function (A) Travel distances of WT, *elpc-1(tm2149)* and *elpc-3(ok2452)* worms. (B) Percent change in travel distance of worms from A. (C) Travel distances of WT, *elpc-2(ix243)*, *elpc-3(ok2452)*, and *elpc-2(ix243);elpc-3(ok2452)* worms. (D) Percent change in travel distance of worms from C. (E) Maximum veolcities of WT, *elpc-2(ix243), elpc-1(tm2149), and elpc-1(tm2149);elpc-2(ix243)* worms. (F) Percent change in maximum velocity of

worms from E. (G) Travel distances of WT, *elpc-2(ix243), elpc-1(tm2149), and elpc-1(tm2149);elpc-2(ix243)* worms. (H) Percent change in travel distance of strains from G. For maximum velocity and travel distance experiments, n = 30–45 worms per strain for each day (10–15 worms from 3 biological replicate plates). For percent change in maximum veloity graphs, n = 3 biological replicate plates. ***$P < 0.001$; ns, not significant; One-way ANOVA with Dunnett's post hoc test vs. WT for A, B; One-way ANOVA with Tukey's post hoc test for C–H.

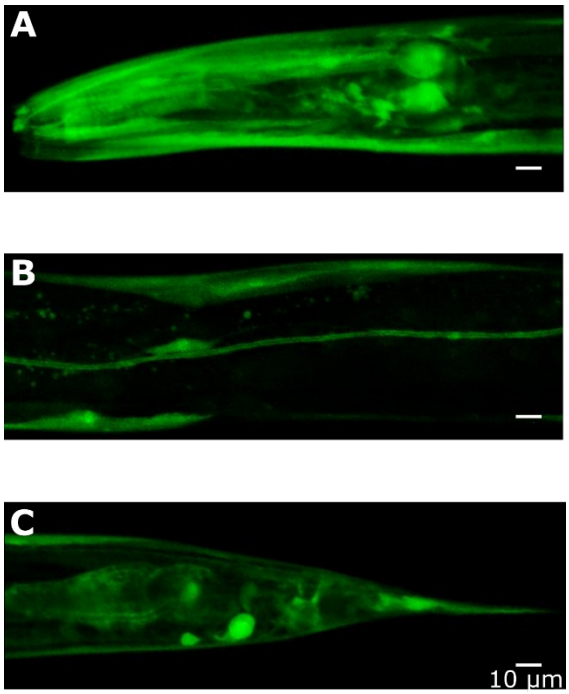

Figure S8. Expression pattern of *elpc-2* transcriptional GFP fusion

(A) *elpc-2p::GFP* expression in pharynx, neurons, and head muscles. (B) *elpc-2p::GFP* expression in body wall muscles and canal cell. (C) *elpc-2p::GFP* expression in coelomocytes, intestine, and tail. Scale bars: 10 µm.

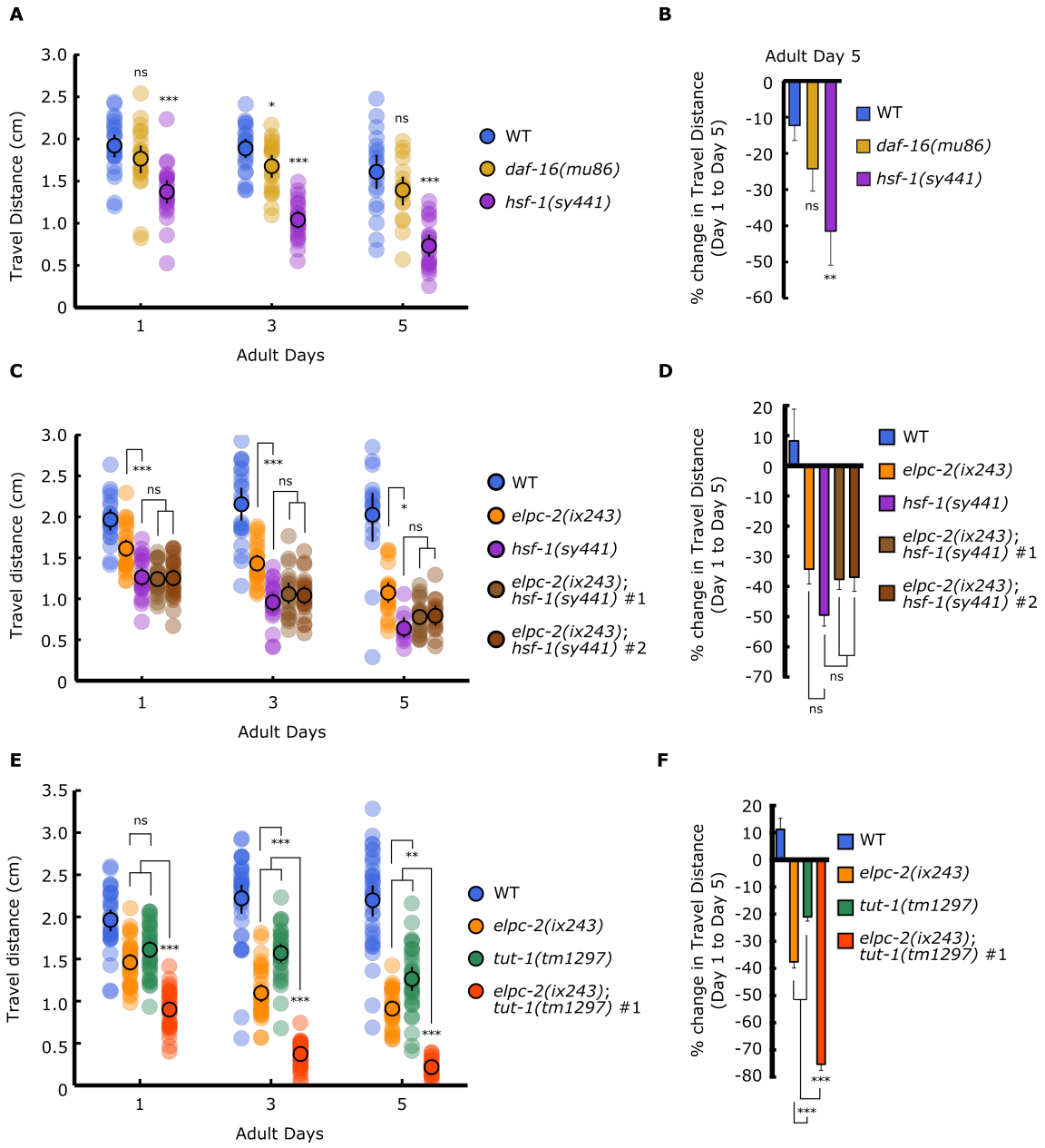

Figure S9. The Elongator complex may work with Heat Shock Factor -1

(A) Travel distances of WT, *daf-16(mu86)*, and *hsf-1(sy441)* worms. (B) Percent change in travel distance of worms from A. (C) Travel distances of WT, *elpc-2(ix243)*, *hsf-1(sy441)* and *elpc-2(ix243);hsf-1(sy441)* worms. (D) Percent change in travel distances of worms from C. (E) Travel distances of WT, *elpc-2(ix243)*, *tut-1(tm1297),* and *elpc-2(ix243);tut-1(tm1297)* worms. (F) Percent change in travel distance of worms from E. Error bars indicate 95% confidence intervals. For travel distance experiments, n = 30–45 worms per strain for each day (10–15 worms from 3 biological replicate plates). For percent change in travel distance graphs, n = 3 biological replicate plates. *$P < 0.05$; ***$P < 0.001$; ns, not significant; One-way ANOVA with Dunnett's post hoc test vs. WT for A, B; One-way ANOVA with Tukey's post hoc test for C–H.

717 **Table S5. Development times of *tut-1(tm1297) and elpc-2(ix243);tut-1(tm1297)***
718 **mutants**
719 Development time from egg to first egg-lay (n = 5 worms per strain).

Strain	Development Time (h)	% of WT
WT	70.4	100.0%
tut-1(tm1297)	82.0	116.5%
elpc-2(ix243);tut-1(tm1297)	145.4	206.5%

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Q6IA86 ELP2_HUMAN      1   -------MVAPVLETSHVF-CCPNRVRGVLNWSSGPRGLLAFGTSCSVVLYD-PLKRVVV    51
Q9NEW7 Q9NEW7_CAEEL    1   MKIEEEFISASVNPRSHCLTACK------------TAPLVAYASSLQIAVQTIPKDDSEV    48
                            : * *   ** : .*                  *:*:.:* .:.:   * .   *

Q6IA86 ELP2_HUMAN     52   -----TNLNGHTARVNCIQWICKQDGSPSTELVSGGSDNQVIHWEIEDNQLLKAVHLQGH   106
Q9NEW7 Q9NEW7_CAEEL   49   GVIKSTSERRHQKPITVL-KRLKSSEIVADEFVTGGVDSRVVLWKLRGEHVEYVADLTGC   107
                                *. . *   :. :    *..   : *:*:** *.:*: *::.:::  ...* *

Q6IA86 ELP2_HUMAN    107   EGPVYAVHAVYQRRTSDPALCTLIVSAAADSAVRLWSKKGPEVMCLQTLNFGNGFALALC   166
Q9NEW7 Q9NEW7_CAEEL  108   DGSVGSVCGCVEDGRK--VVAAAWVSETSNGFHAWWTSSIGDLLNSTEIKL-DHKAFALC   164
                           :* * :* .  :   .  .:.:  ** :::.    *:..  :::    ::: :  *:***

Q6IA86 ELP2_HUMAN    167   LSFLPNTDVPILACGNDDCRIHIFAQ--QNDQFQKVLSLCGHEDWIRGVEWAAFGRDLFL   224
Q9NEW7 Q9NEW7_CAEEL  165   LDAISIQNSVLLAVGTSKRFVELYGESADKKSFSRLISVAGHTDWIHSIAFNDNPDHLLV   224
                           *. :   :  :** *...  :.::.:  ::..*.:::*:.** ***:.: :     .*::

Q6IA86 ELP2_HUMAN    225   ASCSQDCLIRIWKLYIKSTSLETQ--------DDDNIRLKENTFTIENESVKIAFAVTLE   276
Q9NEW7 Q9NEW7_CAEEL  225   ASAGQDTYVRLWAIEPETDEKSENIREDSSTTPPDELTSSANLFSINY----TPYRCSSH   280
                           **..**  :*:* :  :: . . :          *::  . * *.*:       :  : .

Q6IA86 ELP2_HUMAN    277   TVLAGHENWVNAVHWQPVFYKDGVLQQPVRLLSASMDKTMILWAPDEESGVWLEQVRVGE   336
Q9NEW7 Q9NEW7_CAEEL  281   AVMQGHDDWVHSTVWSND---------GRVLLTASSDKTCIIWKE--IDNLWRDDVRLGI   329
                           :*: **::**::. *.                **.** *** *.*     ..:* ::**:*

Q6IA86 ELP2_HUMAN    337   VGGN-TLGFYDCQFN-----------EDGSMIIAHAFHGALHLWKQNTVNPREWTPEIVI   384
Q9NEW7 Q9NEW7_CAEEL  330   VGGGQAAGFFAAVFSSSLDLKDSGEKNAENVVISSSYFGGLHCWKSTDEQKTFWTALPMT   389
                           ***. : **: . *.            :  .::*: ::.*.** **..  :   **   :

Q6IA86 ELP2_HUMAN    385   SGHFDGVQDLVWDP----EGEFIITVGTDQTTRLFAPWKRKDQSQVTWHEIARPQIHGYD   440
Q9NEW7 Q9NEW7_CAEEL  390   GGHVGEVRDVDWHRSDDGDSGFLMSVGQDQTTRVFAKNG----RQQSYVEIARPQVHGHD   445
                           .**.. *:*: *.     :. *:::** *****:**        * :: ******:**:*

Q6IA86 ELP2_HUMAN    441   LKCLAMINRFQFVSGADEKVLRVFSAPRNFVENFCAITGQSLNHVLCNQDSDLPEGATVP   500
Q9NEW7 Q9NEW7_CAEEL  446   MQCLSFVNPSIFVSGAEEKVFRAFRAPKSFVKSLEAISGVPTEKSFGDSD-LAEFGACVP   504
                           ::**:::*   *****:***:*.* **:.**.: **:*   :: : :.*     ** **

Q6IA86 ELP2_HUMAN    501   ALGLSNKAVFQGDIASQPSDEEELLTSTGFEYQQVAFQPSILTEPPTEDHLLQNTLWPEV   560
Q9NEW7 Q9NEW7_CAEEL  505   ALGLSNKPMVEGETVDGE----------HWEEDAFRAAPVVLTSPPTEDTLQQNTLWPEQ   554
                           ******* :.:*: ..            :* : .   * :**.***** * *******

Q6IA86 ELP2_HUMAN    561   QKLYGHGYEIFCVTCNSSKTLLASACKAAKKEHAAIILWNTTSWKQVQNLVFHSLTVTQM   620
Q9NEW7 Q9NEW7_CAEEL  555   HKLYGHGYEVYAVTANPTGNVLATACKSSHVEHSVVMLWSTSNWSKKSEIIGHQLTVTQI   614
                           :********::.**.* : .:**:***::: **:.::**.*:.*.: .::: *.*****:

Q6IA86 ELP2_HUMAN    621   AFSPNEKFLLAVSRDRTWSLWKKQDTISPEFEPVFSLFAFTNKITSVHSRIIWSCDWSPD   680
Q9NEW7 Q9NEW7_CAEEL  615   AWNPSGTRLLTVSRDRTAKLYTEKNGEVDGFDYD-----CVWTSGKQHTRIIWACDWIDD   669
                           *:.*. . **:****** .*:.:::     *:        . .  . *:****.***  *

Q6IA86 ELP2_HUMAN    681   SKYFFTGSRDKKVVVWGECDSTDDCIEHNIGPCSSVLDVGGAVTAVSVCPVLHPSQRYVV   740
Q9NEW7 Q9NEW7_CAEEL  670   EH-FVTASRDQKVIVWAESAGQTAPKAT--------VKLDEPVTA------IAAVSKDVI   714
                           .: *.*.***:**:**.*. .               :.:.   ***      :   .: *:

Q6IA86 ELP2_HUMAN    741   AVGLECGKICLYTWKKTDQVPEINDWTHCVETSQSQSHT--LAIRKLCWKNCSGKTEQKE   798
Q9NEW7 Q9NEW7_CAEEL  715   VAGLQTGELIVLRFDSEGLH--------VIEKIGANRIPIDSAVLRLRFSKNGRK-----   761
                           ..**: *:: :  :.. .           :*.  ::      *: :* :.: . *

Q6IA86 ELP2_HUMAN    799   AEGAEWLHFASCGEDHTVKIHRVNKCAL                                   826
Q9NEW7 Q9NEW7_CAEEL  762   --------LAVATTDAKLRIFNVSQ---                                   778
                                    :* .  * .:*..*.:
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Figure S10. Amino acid alignment of *C. elegans* ELPC-2 and human ELP2

C. elegans ELPC-2 and human ELP2 were aligned using Clustal Omega (43). An asterisk (*) indicates positions that are conserved; colon (:) indicates positions that are strongly similar (> 0.5 in the Gonnet PAM 250 matrix); period (.) indicates positions that are weakly similar (< 0.5 in the Gonnet PAM 250 matrix).

729 **Supplementary Information: Strain list**

Strain	Genotype	Obtained From
CB408	*unc-43(e408) IV*	CGC
CB190	*unc-54(e190) I*	CGC
MT7929	*unc-13(e51)*	CGC
AM725	rmIs290 [*unc-54p* ::Hsa-sod-1 (127X)::YFP]	CGC
VC1937	*elpc-3(ok2452) V*	CGC
PS3551	*hsf-1(sy441) I*	CGC
CF1038	*daf-16(mu86) I*	CGC
n/a	*elpc-1(tm2149) I*	National BioResource Project (Japan)
n/a	*tut-1(tm1297) IV*	National BioResource Project (Japan)
OF1260	*ix239*	This study
OF1261	*ix240*	This study
OF1262	*ix241*	This study
OF1263	*ix241* (4x backcrossed)	This study
OF1264	*ix242*	This study
OF1265	*elpc-2(ix243) III*	This study
OF1266	*elpc-2(ix243) III* (4x backcrossed)	This study
OF1267	*elpc-2(ix243) III;ixEx244[elpc-2(+);lin-44p::RFP]*	This study
OF1268	*elpc-2(ix243) III;ixEx245[elpc-2(+);lin-44p::RFP]*	This study
OF1269	*elpc-2(ix243) III;ixEx246[lin-44p::RFP]*	This study
OF1270	*ixEx247[lin-44p::RFP]*	This study
OF1271	*elpc-1(tm2149) I;elpc-2(ix243) III*	This study
OF1272	*elpc-2(ix243) III;elpc-3(ok2454) V* (#1)	This study
OF1273	*elpc-2(ix243) III;elpc-3(ok2454) V* (#2)	This study
OF1287	*hsf-1(sy441) I;elpc-2(ix243) III* (#1)	This study
OF1288	*hsf-1(sy441) I;elpc-2(ix243) III* (#2)	This study
OF1289	*elpc-2(ix243) III;tut-1(tm1297) IV*	This study

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732 **Supplementary Information: Primer list**

Primer Name	**5'-3' Sequence**
5' *elpc-2p* (2090-bp upstream)	gataagtgacatgccgctgcgtccttac
3' *elpc-2UTR* (851-bp downstream)	aagagacagcgtctgattcttgaaacggta
5' *elpc-2* snp	aaatgaatttttcgccacaaaacccaaaaa
3' *elpc-2* snp	ttcgcgaaaactctcgtagtctgatcctg
5' *elpc-1* del	gaaaagcatcgagttgtccacttgaatcac
3' *elpc-1* del	cttttcagttgaattctggcatctctccaa
5' *elpc-3* del	taatagaacccagatcgagtttggcagatg
3' *elpc-3* del	aatgcatcgtatgtggttaggcggtaaaac
5' *daf-16(e1368)* snp	ccttctcaccatttgtcccttctgattgat
3' *daf-16(e1368)* snp	aaaatgcgttggcaagtgtgagattgtgt
5' *hsf-1(sy441)* snp	agaaaattccgggaaaaactttcaagaagc
3' *hsf-1(sy441)* snp	gcaatagaagatcagcatcatccaacgact
5' *elpc-2p* overlap ppd95.79 107-	gggtaccggtagaaaaaatgaaaatcgaagaagaatttatctc tgctag
3' *elpc-2p* overlap ppd95.79 138-	agttcttctcctttactcatctgggaaacattaaagattctcaattt cgc
5' ppd95.79 107-	atgagtaaaggagaagaacttttcactggag
3' ppd95.79 138-	tttttctaccggtaccctccaagcaagggtc

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