1   LEA motifs promote desiccation tolerance *in vivo*

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28   **Abstract**

29   *Background*

30   Cells and organisms typically cannot survive in the absence of water. However, some

31   animals including nematodes, tardigrades, rotifers, and some arthropods are able to survive

32   near-complete desiccation. One class of proteins known to play a role in desiccation tolerance is

33   the late embryogenesis abundant (LEA) proteins. These largely disordered proteins protect

34   plants and animals from desiccation. A multitude of studies have characterized stress-protective

35   capabilities of LEA proteins *in vitro* and in heterologous systems. However, the extent to which

36   LEA proteins exhibit such functions *in vivo*, in their native contexts in animals, is unclear.

37   Furthermore, little is known about the distribution of LEA proteins in multicellular organisms or

38   tissue-specific requirements in conferring stress protection.

39   *Results*

40   To study the endogenous function of an LEA protein in an animal, we created a null

41   mutant of *C. elegans* LEA-1, as well as endogenous fluorescent reporters of the protein. LEA-1

42   mutant animals formed defective dauer larvae at high temperature. We confirmed that *C.*

43   *elegans* lacking LEA-1 are sensitive to desiccation. LEA-1 mutants were also sensitive to heat

44   and osmotic stress and were prone to protein aggregation. During desiccation, LEA-1

45   expression increased and became more widespread throughout the body. LEA-1 was required

46   at high levels in body wall muscle for animals to survive desiccation and osmotic stress, but

47   expression in body wall muscle alone was not sufficient for stress resistance, indicating a likely

48   requirement in multiple tissues. We identified minimal motifs within *C. elegans* LEA-1 that were

49   sufficient to increase desiccation survival of *E. coli*. To test whether such motifs are central to

50   LEA-1's *in vivo* functions, we then replaced the sequence of *lea-1* with these minimal motifs and

51   found that *C. elegans* dauer larvae formed normally and survived osmotic stress and mild

52   desiccation at the same levels as worms with the full-length protein.

53   *Conclusions*

54   Our results provide insights into the endogenous functions and expression dynamics of

55   an LEA protein in a multicellular animal. The results show that LEA-1 buffers animals from a

56   broad range of stresses. Our identification of LEA motifs that can function in both bacteria and in

57   a multicellular organism *in vivo* suggests the possibility of engineering LEA-1-derived peptides

58   for optimized desiccation protection.

59

60   **Keywords**

61   Late Embryogenesis Abundant (LEA); desiccation; osmotic stress; *C. elegans*; stress

62

63   **Background**

64   Animals regularly encounter abiotic stresses due to fluctuating environments. One such

65   stress is desiccation. Water is essential for cellular metabolism, and without it cellular

66   components including DNA, RNA, proteins, and membranes are unstable. However, certain

67   animals can lose nearly all their internal water and yet survive (anhydrobiosis). This group

68   includes nematodes, rotifers, tardigrades, and certain arthropods such as some crustaceans

69   and insects. Studying the mechanisms by which these organisms are able to survive

70   desiccation is of fundamental interest and may lead to better understanding of biological

71   protectants.

72   One family of common protectants with demonstrated efficacy during desiccation is the

73   late embryogenesis abundant (LEA) proteins. These proteins were originally identified in cotton

74   seeds and were subsequently found to be present in many other plants [1–6]. LEA proteins

75   protect the viability of desiccated seeds [7]. LEA proteins were later identified in the nematode

76   *Aphelenchus avenae* and other anhydrobiotic animals [8–13]. Several families of LEA proteins

77   have been described, each containing distinct motifs [14, 15]. Nematode LEA proteins both in *A.*

78   *avenae* as well as the more common model system *C. elegans* are members of the Group 3

79   LEA proteins. This is the most common LEA variety within anhydrobiotic animals [16]. LEA

80   proteins are thought to be intrinsically disordered, i.e. largely unstructured in hydrated

81   conditions, but under water-limiting conditions the proteins acquire recognizable alpha-helical

82   secondary structure [17–19]. There are several examples in which heterologous expression of

83   LEA proteins confers stress resistance in a number of host cell types, including yeast, bacteria,

84   rice, *D. melanogaster*, and mammalian cell culture [20–26]. Additionally, isolated LEA protein

85   has been demonstrated to protect against protein aggregation and stabilize membranes and

86   liposomes *in vitro* [27–29]. Although the functions of LEA proteins are well-established, most

87   studies have utilized heterologous expression systems. Therefore, we have limited knowledge

88   of the endogenous, *in vivo* roles of LEA proteins.

89   The nematode *C. elegans* is an ideal model for the study of the endogenous functions of

90   LEA proteins. *C. elegans* survive many stresses including desiccation, have a well-resourced

91   genetic toolkit, and contain two LEA family proteins, *lea-1* and *dur-1* (dauer up-regulated) [10,

92   30]. Disruption of *lea-1* has been reported to sensitize worms to desiccation, osmotic stress with

93   sucrose, and larval heat stress [10, 30, 31]. *dur-1* is also required for a robust desiccation

94   response but is apparently unable to compensate for the loss of LEA-1 [30]. Whereas most

95   studies show that heterologous expression is sufficient to protect against stresses in other

96   systems, these *C. elegans* studies provide evidence for necessity in the native function of LEA

97   proteins in animals. However, little is known about the tissues in which LEA proteins are

98   required or the mechanisms by which they function *in vivo* [14, 32, 33].

99   We used LEA-1 of *C. elegans* as a model to study the endogenous function of an LEA

100   protein in a multicellular animal. We confirmed and expanded phenotypes associated with

101   disruption of LEA-1, characterized expression patterns and dynamics in response to

102   desiccation, and identified minimal amino acid motifs within the protein that harbor the

103   biochemical properties to confer desiccation resistance. These results provide insights into the

104   *in vivo*, endogenous functions of an LEA protein in a multicellular animal.

105

106   **Results**

107   **Generation of a null allele of *lea-1***

108   We were motivated to determine the role of LEA-1 in the endogenous organismal

109   context of *C. elegans*. Previous studies have relied on RNAi for gene knockdown and did not

110   fully eliminate *lea-1* mRNA [10] or noted that RNAi seldom fully eliminates the target mRNA [30].

111   One recent study used a deletion allele, but only confirmed the phenotype of desiccation

112   sensitivity [31]. Furthermore, there are many isoforms of LEA-1 (Fig. 1), and the potential for

113   alternative isoform usage makes mutant analysis difficult to interpret. To address these

114   concerns, we created a full-length, 15.8 kb deletion of the *lea-1* locus via CRISPR, and we also

115   cleaned the genetic background of a second, pre-existing mutant allele, *lea-1(tm6452)*, by

116   outcrossing it to wild-type worms for 5 generations [10, 30, 34, 35]. For the full-length deletion

117   (*lea-1Δ*), the deleted gene was replaced with a *pmyo-2*::GFP::*myo-2 3'-UTR* construct as a

118   reporter for the deletion (Fig. 1). The successful deletion was confirmed via visualization of

119   pharyngeal expression of green fluorescent protein (GFP) as well as by PCR (Additional File 1:

120   Fig. S1A-D).

121

122   ***lea-1* mutants exhibit a temperature-dependent defect in forming normal dauer larvae**

123   LEA proteins are known for promoting survival during desiccation. Because *C. elegans*

124   are reported to survive desiccation only when in the dauer state [36], we wanted to determine if

125   LEA-1 impacts dauer formation. Multiple environmental variables influence dauer development,

126   including population density, food availability, and temperature [37]. Additionally, some genetic

127   mutants constitutively form dauer larvae. For example, *daf-2(e1370)* mutant animals are

128   commonly used to obtain dauer larvae as they develop into dauer larvae when grown at 25 ºC

129   [38]. Dauer larvae cease feeding, constrict along the radial axis, form a buccal plug, and

130   synthesize a thickened cuticle with lateral ridges called alae – adaptations that limit exposure to

131   environmental insults [37, 39–41]. For example, dauer larvae are able to survive exposure to the

132 detergent sodium dodecyl sulfate (SDS) [39, 42]. Because it was possible that LEA-1 limits

133 desiccation tolerance by disrupting proper dauer formation, we assessed dauer traits of *lea-1*

134 mutants in both wild-type (N2) and *daf-2(e1370)* backgrounds. When worms were grown at 20

135 ºC, dauer larvae were identifiable as population density increased and food was depleted. *lea-*

136 *1(tm6452)* and *lea-1Δ* dauer larvae that formed in these conditions were resistant to 1% SDS in

137 both wild-type and *daf-2* backgrounds (Fig. 2A). However, *lea-1(tm6452)* and *lea-1Δ* worms

138 cultured at 25 ºC died when exposed to 1% SDS, unlike N2 or *daf-2* control animals cultured at

139 25 ºC (Fig. 2B). Therefore, temperature-dependent sensitivity to 1% SDS was independent of

140 the method of dauer formation (crowding and starvation in an N2 background vs. constitutive

141 dauer formation at 25 ºC in a *daf-2* background).

142   To determine the nature of the dauer defect of *lea-1* mutant animals cultured at 25 ºC,

143 we measured several dauer-specific traits. *lea-1* mutants exhibited normal arrest behavior

144 without feeding. Like control animals, *lea-1Δ*, *lea-1(tm6452)*, *daf-2;lea-1Δ*, and *daf-2;lea-*

145 *1(tm6452)* mutants did not exhibit feeding behavior as dauer-like larvae, as measured by

146 ingestion of fluorescent beads (Fig. 2C). In a *daf-2* background, each mutant allele of *lea-1*

147 caused a modest increase in the width of dauer-like larvae, suggesting a slight defect in radial

148 constriction (Fig 2D). *lea-1* mutant dauer-like larvae appear to arrest similarly to controls, with

149 sealed mouths and lateral alae (Additional File 1: Fig. S2). Because the structure of dauer-

150 specific alae was difficult to assess with DIC optics, we used scanning electron microscopy to

151 assess the ultrastructure of alae. We observed a disruption in the structure of alae, with alae of

152 mutant animals having less-defined ridges (Fig. 2E). Additionally, the cuticle of *lea-1* mutant

153 animals was prone to detachment (Fig. 2F). Cuticle detachment was seen most prominently at

154 bends in the animals. Collectively, these observations define an *in vivo* function for *lea-1* by

155 showing that dauer larvae form in *lea-1* mutants, but in the larvae that form at 25 ºC, cuticle

156 formation and radial constriction are disrupted, and animals are sensitive to 1% SDS.

157

lea-1 deletion mutants are sensitive to multiple stresses

To determine the impact of defective dauer formation on stress resistance, we measured survival of desiccation and osmotic stress in dauer or dauer-like larvae formed at 20 ºC or 25 ºC. We chose to conduct the majority of our experiments in the temperature-sensitive constitutive dauer *daf-2(e1370)* background to facilitate consistent production of dauer worms. In a *daf-2* background, *lea-1Δ* and *lea-1(tm6452)* mutant dauer larvae formed at 20 ºC (SDS-resistant) were only mildly sensitive to desiccation at high relative humidity (97.5%), but were highly sensitive to a more stringent desiccation stress of 60% humidity (Fig. 3A), consistent with published results [10, 30, 31]. When dauer-like larvae formed at 25 ºC were exposed to the same conditions, *daf-2;lea-1Δ* and *daf-2;lea-1(tm6452)* mutant animals had lower survival than controls, even at 97.5% RH (Fig. 3B). Similar results were also observed in an N2 genetic background (Additional File 1: Fig. S3A,B). This suggests that defective dauer formation of *lea-1* mutants at 25 ºC does not fully explain the phenotype of desiccation sensitivity, but can compound its severity.

Additionally, dauer worms of each of these mutant strains were sensitive to osmotic stress in a concentrated salt solution. While there were no differences in survival in worms kept in water for 2 hr, *daf-2;lea-1(tm6452)* mutant dauers formed at 20 ºC had slightly lower survival in 1M NaCl for 2 hr (Fig. 3C). Both *daf-2;lea-1Δ* and *daf-2;lea-1(tm6452)* dauer-like larvae formed at 25 ºC had significantly lower survival when exposed to 1M NaCl for 2 hr (Fig. 3D). Each *lea-1* mutant strain was also sensitive to 1M NaCl in a wild-type (non-*daf-2*) genetic background (Additional File 1: Fig. S3C,D). We also found that *daf-2;lea-1Δ* and *daf-2;lea-1(tm6452)* mutant dauer-like larvae formed at 25 ºC were sensitive to exposure to heat shock at 37 ºC (Fig. 3E).

LEA proteins have been demonstrated to prevent protein aggregation *in vitro* and in cell culture [27, 43–45]. To test for *in vivo* roles in preventing protein aggregation, we assessed the number of polyglutamine aggregates in body wall muscle of *daf-2;lea-1Δ* dauer-like animals (25

184   °C dauer formation). These animals expressed tracts of 35 or 40 glutamines fused to a yellow

185   fluorescent protein (YFP) reporter [46]. Mutants with the Q35::YFP reporter did not have a

186   statistically significant difference from controls (p=0.22); however, *daf-2;lea-1Δ* animals with a

187   Q40::YFP reporter had significantly more aggregates per worm than controls (p=0.02) after 5

188   days of development and dauer formation (Fig. 3F). We conclude that LEA-1 has *in vivo*

189   functions protecting animals against desiccation, osmotic stress, heat stress, and formation of

190   polyglutamine protein aggregates and that temperature-sensitive defects in dauer formation at

191   25 °C can contribute to but do not fully explain the stress sensitivity of *lea-1* mutants.

192

193   **LEA-1 lacks discernable nonredundant functions outside of stress tolerance**

194   Working with a null mutant allowed us to ask if there were any phenotypes associated

195   with deletion of *lea-1* in the context of normal development and physiology. We tested effects on

196   lifespan and brood size as especially sensitive quantitative proxies for diverse effects on

197   physiology or development. Our results suggest that LEA-1 functions specifically in response to

198   stress: In a wild-type (N2) background we did not observe any significant differences in lifespan

199   of *lea-1Δ* animals at 20 °C (Fig. 4A). We observed a modest reduction in lifespan of *lea-*

200   *1(tm6452)* animals (p=0.005), perhaps due to genetic background effects; the lack of reduction

201   in lifespan in *lea-1Δ* animals demonstrates that complete loss of *lea-1* does not decrease

202   lifespan. We also did not observe significant differences in polyglutamine aggregation during

203   aging in these worms when maintained under normal laboratory conditions at 20 °C (Additional

204   File 1: Fig. S4). Because LEA-1 expression is regulated by insulin-like signaling, we also tested

205   for a difference in lifespan in the long-lived *daf-2* mutant background [47, 48]. We found that

206   lifespans of *daf-2;lea-1Δ* and *daf-2;lea-1(tm6452)* were statistically indistinguishable from

207   controls (Fig. 4B). Notably, there was higher mortality of *daf-2;lea-1(tm6452)* during the

208   reproductive period, but this did not significantly influence maximum lifespan (Additional File 1:

209   Table S2). The brood size of *lea-1Δ* and *lea-1(tm6452)* animals was also not significantly

210   different from N2 controls (Fig. 4C). These results indicate that LEA-1 may function primarily

211   during the dauer stage and in response to conditions of external stress.

212

213   **Expression of multiple LEA-1 isoforms is increased in response to desiccation**

214   To examine where LEA-1 is expressed *in vivo*, we used fluorescent mNeonGreen

215   (mNG) and monomeric yellow fluorescent protein for energy transfer (mYPET) tags to label

216   endogenous LEA-1 at two different positions [49, 50]. Sequence for mNG as well as a 3x FLAG

217   and auxin inducible degron sequence were inserted in the N-terminal region of the gene (Fig. 1).

218   Similarly, sequence for mYPET and 3x FLAG were added to the C-terminus of LEA-1 (Fig. 1).

219   As with the *lea-1Δ* mutant, these edits were confirmed via visualization of fluorescence and

220   PCR (Additional File 1: Fig. S1E). Fusion of the fluorescent tags to LEA-1 did not disrupt

221   resistance of dauer larvae formed at 25 ºC to 1% SDS (Fig. 5A). When desiccated, animals

222   expressing the fluorescent tags survived equally as well as *daf-2* control animals (Fig. 5B). We

223   also confirmed that the fluorescent tags did not significantly impact osmotic stress survival in 1M

224   NaCl for 2 hr (Additional File 1: Fig. S5A). We conclude that introduction of either fluorophore

225   did not disrupt functions of the endogenous LEA-1 protein.

226   LEA-1 protein is known to be upregulated in response to desiccation [10, 30]. However,

227   there are many predicted isoforms of LEA-1 (Fig 1), and it is unclear whether all isoforms are

228   upregulated or whether all of LEA-1's protective roles involve such upregulation. To examine the

229   diversity of isoforms that are actively expressed in different conditions we lysed cultures of

230   mixed stage worms, dauer worms, and desiccated dauers to blot with an anti-FLAG antibody to

231   recognize the 3x FLAG epitope appended to each fluorophore. Numerous isoforms appeared

232   tagged with the 5' mNG tag, while fewer isoforms of distinct sizes carrying the 3' mYPET tag

233   were prominent (Fig. 5C). Isoforms labeled with the 5' mNG tag but not the 3' mYPET tag were

234   found to be upregulated during desiccation. These changes in expression were confirmed by

235   quantification of *in vivo* fluorescence intensity in dauer and desiccated worms. Fluorescence

236   from the mNG reporter was significantly increased after 4 days of desiccation at 97.5% relative

237   humidity (Fig. 5D-F). Expression of mNG::LEA-1 was diffuse throughout the body during

238   desiccation (Fig. 5E). In contrast, mYPET fluorescence level was not significantly altered during

239   desiccation (Fig. 5G-I), consistent with the Western blot results. For both reporters, fluorescence

240   intensity was not changed in response to 2 hr of osmotic shock in 1M NaCl (Additional File 1:

241   Fig. S5B-G). We conclude that only some isoforms of LEA-1 are upregulated in response to

242   desiccation, and that *lea-1*'s response to osmotic shock does not appear to involve a similar

243   upregulation.

244

245   **Depletion of LEA-1 in body wall muscle reduces survival of desiccation and osmotic**

246   **stress**

247   To examine the localization of upregulated LEA-1 isoforms in the stage when LEA-1 has

248   a protective role, we visualized mNG::LEA-1 fluorescence in dauer larvae. We found that the

249   protein was prominently expressed in specific tissues: the germline, body wall muscle, pharynx,

250   and excretory cell (Fig. 6A-D). mNG::LEA-1 was localized to both the excretory cell body, which

251   is adjacent to the pharynx (Fig. 6D), as well as excretory canals, which extend laterally along

252   the length of the body (Fig. 6A). Some faint expression was seen in seam cells (lateral

253   hypodermal cells) (Fig. 6B), as well as the intestine (Fig. 6C). Additionally, some unidentified

254   cells in the head were fluorescent. The expression and localization of LEA-1 to particular tissues

255   in dauer worms suggested that its function might be required in only some tissues or required at

256   higher levels in some tissues than in others.

257   To determine the sites of action of LEA-1, we utilized auxin-induced degradation to

258   deplete protein. We used the auxin-inducible degron (AID) sequence that we included on the

259   mNG::3xFLAG tag as a target for protein degradation when the modified F-box protein TIR1 is

260   expressed and worms are exposed to auxin [51]. Expression of TIR1 under the control of

261   different promoters allows for tissue-specific conditional depletion [52]. We used a panel of

262   worms expressing TIR1 in the various tissues in which LEA-1 was expressed to determine the

263   tissue or tissues in which LEA-1 was required for survival of desiccation and osmotic stress (Fig.

264   6E) [52]. These experiments were conducted in a *daf-2* mutant background, for which

265   expression patterns of LEA-1 were not noticeably different from a WT (N2) background

266   (Additional File 1: Fig. S6A). When exposed to auxin, dauer worms showed depletion of

267   mNG::LEA-1 in the expected locations for each promoter used: the intestine (using the *ges-1*

268   promoter, called *ges-1*p), germline (*mex-5*p), pharynx (*myo-2*p), body wall muscle (*myo-3*p),

269   and a combination of intestine, excretory cell and canal, germline, and some other cells in the

270   head (*vha-8*p) (Additional File 1: Fig. S6). Expression of TIR1 with a *col-10* promoter to target

271   hypodermis did not seem to have a significant effect on expression, consistent with the

272   apparently minimal baseline hypodermal expression of mNG::LEA-1 (Additional File 1: Fig.

273   S6C). Additionally, the *myo-2*p::TIR1 strain depleted some pharyngeal mNG::LEA-1 protein, but

274   did not entirely eliminate expression, particularly in the posterior bulb of the pharynx (Additional

275   File 1: Fig. S6F).

276   Depletion of LEA-1 in each of the TIR1 tissue specific strains tested did not disrupt the

277   integrity of dauer larvae formed at 25 ºC, as assessed by survival of 1% SDS (Fig. 6F). When

278   exposed to desiccation at 97.5% relative humidity, LEA-1 depletion in TIR1 expression strains

279   did not significantly reduce survival relative to *daf-2* or *daf-2;*mNG::*lea-1* animals (Fig. 6G).

280   However, when exposed to 60% relative humidity, *myo-3*p::TIR1 worms had significantly

281   reduced survival. This suggests that LEA-1 is required in the body wall muscle to promote

282   desiccation resistance. When exposed to osmotic stress with 1M NaCl for 4 hrs, we observed a

283   similar result. *lea-1Δ* mutants and *myo-3*p::TIR1 worms had significantly reduced survival

284   compared to *daf-2* and *daf-2;*mNG::*lea-1* worms (Fig. 6H). To determine if expression of LEA-1

285   in body wall muscle is sufficient for desiccation and osmotic stress survival, we attempted to

286   rescue survival of *daf-2;lea-1Δ* mutants by expressing a *myo-3p::lea-1a::mNG*

287   extrachromosomal array. Expression of LEA-1a in body wall muscle alone was not sufficient to

11

288   restore SDS resistance, and did not significantly improve tolerance of desiccation or osmotic

289   stress in 1M NaCl (Additional File 1: Fig. S7). Collectively, these results suggest that LEA-1

290   synthesized in the body wall muscle is necessary but not sufficient for survival of multiple

291   stresses, and that other tissues may not require high levels of the tagged LEA-1 isoforms for

292   survival of animals.

293

294   **LEA-1 minimal motifs confer desiccation resistance to bacteria**

295   Having described *in vivo* roles for an animal LEA protein and its localized expression

296   and function, we sought to identify the protein's protective domains. Given the variety of

297   isoforms of *lea-1* in *C. elegans* that are expressed during desiccation (Fig. 1, Fig. 5C), before

298   testing specific domains *in vivo* we employed a heterologous expression approach to efficiently

299   test if different isoforms improved desiccation tolerance in *E. coli* equally, or if some were more

300   effective than others. We selected isoforms A, D, E, F, and K for expression because they have

301   a combination of overlapping and non-overlapping sequence to allow for deduction of regions of

302   interest in the case that some isoforms are more effective at conferring desiccation resistance

303   (Fig. 7A). We confirmed robust expression of each of the 5 different bacterially codon-optimized

304   isoforms in BL21 *E. coli* and found that expression of each isoform conferred stress resistance

305   to the bacteria, relative to cells expressing GFP (Fig. 7B, Additional File 1: Fig. S8). Expression

306   of a truncated version of GFP or actin (*C. elegans act-2*) did not increase desiccation resistance

307   of bacteria (Fig. 7B).

308   Because all five isoforms tested were sufficient to improve bacterial desiccation

309   resistance, we hypothesized that motifs shared by these isoforms might be sufficient for

310   conferring bacterial desiccation resistance. Group 3 LEA proteins commonly have repeated

311   motifs of 11 amino acids that are capable of protecting heterologous cells against stresses

312   including low pH, high salt, and desiccation-induced protein aggregation [8, 21, 45, 53–56].

313   These motifs are predicted to form alpha helices, and circular dichroism of LEA proteins and

314   motif-containing peptides has confirmed alpha helix formation during conditions when water is

315   limiting [17, 18, 57]. We used MEME Suite to detect a repeated 11-mer from the protein

316   sequence of one of the longest *C. elegans* isoforms, LEA-1K [58]. An 11-mer was found that is

317   predicted to form an amphipathic alpha-helix (Fig. 7C). When search parameters were relaxed

318   to include motifs of other lengths, a 97-mer peptide encompassing multiple 11-mers was also

319   identified. In the 1397 amino acid protein, there were five occurrences of the 97-mer and 61

320   instances of the 11-mer (Fig. 7D). To determine if these motifs alone from *C. elegans* LEA-1

321   were sufficient to improve desiccation tolerance, we expressed codon-optimized versions of

322   these idealized motifs in *E. coli*. We included the base 11-mer, repeats containing two (22-mer)

323   and four (44-mer) copies of its sequence, and the 97-mer. Expression of each of these minimal

324   motifs significantly improved desiccation survival of the bacteria (Fig. 7E). Thus, it is likely that

325   any of LEA-1's isoforms that contain such repeats would improve survival of desiccation.

326

327   **LEA-1 motifs are sufficient for desiccation tolerance and osmotic stress survival *in vivo***

328   Because motifs of LEA-1 improved desiccation survival of bacteria, we were motivated

329   to test if minimal motifs of LEA-1 are sufficient for stress tolerance *in vivo*. To specifically

330   determine if minimal LEA-1 motifs are sufficient for desiccation tolerance in *C. elegans*, we

331   deleted nearly all possible exons of *lea-1* and replaced them with sequence encoding single

332   codon-optimized 44-mer or 97-mer motifs fused to mNG to allow visual confirmation of

333   expression (Fig. 8A). Genomic sequence encoding the most N-terminal predicted exons for

334   some isoforms (like the short N-terminal exons of isoforms K and N as shown in Fig. 8A) was

335   left intact because these regions likely overlap with the promoter region or other regulatory

336   sequence. These short predicted exons do not encode any 11-mer motifs. We confirmed

337   genomic edits by PCR genotyping (Additional File 1: Fig. S9A,B). We also determined that the

338   expression patterns of each inserted motif::mNG fusion are similar to the overall pattern

339   observed for tagged versions of the endogenous protein, suggesting that these genomic edits

340   did not significantly disrupt gene regulation (Additional File 1: Fig. S9C,D).

341   Expression of either the 44 amino acid motif or 97 amino acid motif was sufficient to

342   replace full-length LEA-1's role in dauer development. Each of the motif expression lines formed

343   dauers at 25 ºC that were resistant to 1% SDS (Fig. 8B). Expression of the 44-mer and 97-mer

344   motifs also promoted desiccation tolerance of animals at 97.5% relative humidity (Fig. 8C).

345   When exposed to 60% relative humidity, the protective capacity of these motifs was limited, and

346   survival was reduced to levels comparable to *lea-1Δ* mutants. Expression of each motif in lieu of

347   the complete LEA-1 protein was also sufficient to promote survival of osmotic stress in 1M NaCl

348   (Fig. 8D). Survival of motif-expressing animals was indistinguishable from controls and

349   significantly improved relative to *lea-1Δ* mutants. Expression of these motifs specifically in body

350   wall muscle was not sufficient to restore SDS resistance, desiccation tolerance, or osmotic

351   stress survival to *daf-2;lea-1Δ* animals (Additional File 1: Fig. S9). The ability of short repetitive

352   motif sequences under endogenous control to substitute for multiple functions of full-length LEA-

353   1 suggests that these motifs may account for much of the functionality of the full-length LEA-1

354   protein during osmotic stress and desiccation.

355

356   **Discussion**

357   LEA proteins are commonly found in animals with interesting extremotolerant abilities

358   but with limited genetic tools. Therefore, a common approach has been to study LEA proteins

359   (and fragments thereof) in heterologous systems [20, 21, 23, 26, 55, 56, 59–62]. While such

360   approaches have revealed many potential functions of LEA proteins, there has been limited

361   evidence to bridge protective abilities observed *in vitro* and in heterologous systems to the

362   endogenous, *in vivo* context in an animal [10, 30, 63]. Therefore, we were motivated to use *C.*

363   *elegans* as a model to test for *in vivo* functions of LEA-1 in its native context.

364   Previous studies of LEA-1 in *C. elegans* have often relied on RNAi phenotypes [10, 30].

365   We generated a null mutant (*lea-1Δ*) that lacks the entire gene, in order to eliminate the

366   possibility of residual LEA-1 expression or function. Comparison of this mutant to the insertion

367   and deletion mutant *lea-1(tm6452)* reveals similar sensitivities to a variety of stresses, including

368   desiccation, osmotic stress, and heat. We also found that at 25 ºC *lea-1* mutants formed dauer-

369   like larvae that were sensitive to 1% SDS and had visible cuticle defects. The stress sensitive

370   phenotypes assessed in dauer larvae were present even in SDS-resistant dauer larvae formed

371   at 20 ºC, but in some cases were more severe in the dauer-like larvae formed at 25 ºC. The

372   cuticle limits the rate of water loss, so it is possible that the same defects that make *lea-1*

373   mutants sensitive to SDS could allow more rapid loss of water and poorer desiccation survival.

374   The function of multiple LEA-1 isoforms and amino acid motifs in improving desiccation

375   tolerance of bacteria provides evidence that function of LEA-1 does not depend entirely on its

376   role in dauer development, but likely functions as a protectant within cells during desiccation

377   and related stresses.

378   We focused on measuring stress resistance in dauer larvae, in which LEA-1 is

379   expressed more highly at both the level of mRNA and protein [10, 36]. DUR-1, the other group 3

380   LEA protein of *C. elegans*, is also upregulated in dauer larvae, but is apparently unable to

381   compensate for the loss of LEA-1 [30]. LEA-1 does not significantly impact lifespan or brood

382   size (Fig. 4), which demonstrates the importance of the protein specifically in the context of

383   stress resistance. Similarly, accumulation of polyglutamine protein aggregates was exacerbated

384   by deletion of LEA-1 in dauer worms, but not in aging adult worms (Fig. 3F, Additional File 1:

385   Fig. S4). Effects of LEA-1 on protein aggregation have previously been demonstrated *in vitro*

386   and in heterologous contexts [27, 43, 64]. We present evidence that LEA-1 can affect

387   proteostasis *in vivo* in a multicellular animal. It would be fascinating to further explore any

388   differential effects of LEA-1 with other types of aggregation-prone proteins and in tissues

389   beyond the body wall muscle. Because LEA-1 is expressed highly in body wall muscle and is

390   required there for desiccation survival and osmotic stress resistance, it is possible that

391   improving proteostasis in this tissue contributes to survival of these stresses.

392   Adding endogenous, single-copy fluorescent tags to LEA-1 without disrupting LEA-1

393   function revealed the sites of its expression and *in vivo* dynamics in response to stress. In dauer

394   larvae, mNG-tagged LEA-1 is expressed most notably in the germline, body wall muscle,

395   pharynx, excretory cell, as well as some unidentified cells in the head. Expression patterns were

396   similar in worms tagged with mYPET, although some sites of expression were more faint.

397   Surprisingly, blotting for the 3x FLAG epitope included in both mNG and mYPET tags revealed

398   significantly fewer isoforms tagged by mYPET than expected based on isoform predictions if all

399   isoforms were expressed at high levels (Fig. 1, Fig. 5C). It is possible that the C-terminal exons

400   are not actually included in as many isoforms as expected, or that some isoforms are expressed

401   at low levels. Another possibility is that LEA-1 protein is processed by cleavage into shorter

402   peptides [65]. The difference in the number of prominent isoforms labeled by each tag is further

403   reflected in the significant increase in expression of mNG-labeled proteins but not mYPET-

404   labeled proteins during desiccation (Fig. 5C-I). The upregulation of mNG-tagged proteins does

405   not seem to be specific to a single isoform, but rather, multiple isoforms are expressed more

406   highly during desiccation (Fig. 5C). Therefore, the increased fluorescence seen *in vivo* likely

407   reflects the summation of these isoforms of varying sizes and compositions. LEA-1 has been

408   reported to be regulated by transcription factors including *daf-16*/FOXO and *skn-1*/Nrf, but

409   whether such regulation is tissue-specific or unique to some isoforms remains unexplored [47,

410   66]. Expression of LEA-1 in several tissues, and the upregulation of multiple isoforms led to two

411   questions: 1) In which tissue or tissues is LEA-1 required for stress resistance? 2) Are particular

412   isoforms more effective at conferring stress resistance?

413   To address the first question we utilized the auxin-inducible degradation system to

414   deplete LEA-1 in various tissues [52]. By expressing TIR1 under the control of different

415   promoters, we had control over the tissues in which proteins containing the auxin-inducible

416   degron (AID) tag were degraded. The AID sequence was included on the mNG tag and

417   therefore should deplete the same isoforms containing the 3x FLAG tag (Fig. 5C). It is possible

418   that some predicted isoforms that are not tagged with the mNG construct could escape

419   degradation (for example isoforms C, D, and E, Fig. 1), although we do not know if such

420   isoforms are expressed or their relative abundance. Furthermore, even though we observed

421   decreased fluorescence in most cases (Additional File 1: Fig. S6), depletion of AID-containing

422   proteins may be incomplete. Incomplete degradation could result from insufficient levels of TIR1

423   (based on the strength of the promoter driving it) in tissues with high levels of LEA-1. For

424   example, with *myo-2p* driven TIR1, some expression of mNG::LEA-1 remained in the pharynx –

425   particularly in the posterior bulb (Fig S6F). Thus, we cannot rule out the possibility that LEA-1 is

426   required in the pharynx for survival of desiccation and osmotic stress. In contrast, depletion of

427   LEA-1 in body wall muscle resulted in significantly reduced survival of both desiccation and

428   osmotic stress (Fig. 6G,H). The consensus site of action from these two stresses lends

429   confidence to the result. However, given the caveats that some untagged isoforms may remain,

430   and that there could be incomplete degradation (even if fluorescence is depleted below

431   detectable levels), we cannot formally conclude that LEA-1 is not required in other tissues.

432   Rather, we present evidence that LEA-1 is required at high levels in at least body wall muscle to

433   promote desiccation and osmotic stress tolerance. Yet, expression in muscle alone is

434   insufficient for stress resistance (Additional File 1: Fig. S7, Additional File 1: Fig. S10). Overall,

435   our data suggest that other tissues can survive at least with reduced levels of the LEA-1

436   isoforms that are induced in dauer larvae. For robust survival during desiccation and osmotic

437   stress LEA-1 is necessary in body wall muscle, but also likely required in some combination of

438   other tissues.

439   There are several possible explanations for the relative importance of LEA-1 in body wall

440   muscle. It is possible that the location of body wall muscle, which covers a significant area along

441   the circumference of dauer larvae, could function as a sub-cuticular sheath to protect the animal

442   [67]. However, it is also conceivable that the absolute abundance of LEA-1 rather than a

443   particular tissue-specific distribution is essential for survival. Because body wall muscle is a

444   large tissue and expresses LEA-1 at high levels, depletion from this site of production could

445   more significantly deplete the overall pool of LEA-1 in the animal. A third possibility is that

446   decreased proteostasis in body wall muscle (as suggested by protein aggregation in Fig. 3F)

447   could lead to paralysis and death.

448   To answer the second question of which LEA-1 isoforms are most functional in

449   conferring stress resistance, we initially employed a heterologous expression approach. *E. coli*

450   provides an ideal system to facilitate expression of proteins and rapidly screen for desiccation

451   resistance. The fact that multiple LEA-1 isoforms could improve desiccation tolerance of

452   bacteria turned our attention to conserved motifs contained within these isoforms. Similar motifs

453   from LEA proteins of other organisms have been demonstrated to function in stress resistance

454   [21, 45, 55, 56, 68]. These LEA motifs acquire secondary structure during desiccation-like

455   conditions and specifically are thought to form amphipathic alpha-helices [19, 57, 69]. The 11-

456   mer we identified is likely to form an amphipathic helix based on the amino acid composition

457   and locations as depicted in Fig. 7C. Although the amino acid identity is different from other

458   similarly identified LEA motifs, the putative structural similarities and charge distribution hint at

459   the relative importance of these biochemical properties over sequence identity [12, 69].

460   Expression of the 11-mer alone was sufficient to improve bacterial desiccation survival.

461   Increased numbers of motif repeats may improve survival, although the importance of multiple

462   motifs occurring in the same contiguous peptide versus the possibility of oligomerization

463   remains unclear. Notably, in bacteria the expression levels of LEA proteins and motifs are likely

464   much higher than in endogenous contexts.

465   Inserting motifs into the endogenous *lea-1* locus of *C. elegans* allowed us to test if the

466   identified motifs are sufficient to confer desiccation *in vivo*. LEA motifs were highly effective

467   substitutes for the full-length protein for phenotypes of osmotic stress resistance and

468   desiccation at 97.5% RH (Fig. 8). This suggests that the function of LEA-1 can in large part be

469   explained by the presence of these amino acid repeats. The inability of these motifs to fully

470   rescue desiccation survival at 60% humidity could be a result of the appended fluorescent tag

471   disrupting some aspect of the motif's function. It is also possible that larger order repeats are

472   necessary during more severe desiccation. Unique combinations of motifs or longer contiguous

473   sequences may allow for increased functionality – particularly in the context of more severe

474   desiccation. This may further explain the apparent complexity and variety of isoforms of the

475   protein (Fig. 1, Fig. 5C). If there is limited selective pressure for maintenance of a single long

476   contiguous sequence, then many functional exon combinations may arise. This could contribute

477   to the large number of proteins produced by this gene. An optimal number of motifs and the

478   most functional combination of motif variants appended to each other remain to be

479   characterized.

480   The ability of LEA motifs to function similarly in a single-celled prokaryote (*E. coli*) and a

481   multicellular eukaryote (*C. elegans*) encourages the possibility of engineering broadly functional

482   desicco-protectants. LEA proteins and motifs that can function in cells with fundamentally

483   different subcellular organization likely harbor basic biochemical properties that promote cell

484   and organismal survival. Relative to bacteria, multicellular animals face further challenges

485   during desiccation including coordination of a response across multiple tissue types and

486   protecting diverse organelles and subcellular compartments. *C. elegans* is a prime animal

487   model for continued study of these *in vivo* mechanisms of desiccation tolerance.

488

489   **Conclusions**

490   In summary, we created a null mutant of LEA-1 to study its role *in vivo* and characterized

491   a temperature-sensitive dauer formation defect and several phenotypes of stress sensitivity.

492   LEA-1 is expressed in multiple tissues, and it is required at high levels in body wall muscle to

493   carry out its functions. We identified LEA-1 motifs that are sufficient to improve bacterial

494   desiccation survival. These motifs also function in place of full-length LEA-1 to promote

495   desiccation tolerance *in vivo*. Future work is needed to dissect the principles by which the

496   peptide repeat sequences function. Still, the conserved function of LEA-based peptides in a

497   single-celled prokaryote and a multicellular animal suggests the possibility of engineering LEA-

498   1-derived peptides that broadly promote desiccation tolerance.

499

500   **Methods**

501   *CRISPR editing and generation of extrachromosomal array lines in C. elegans*

502   To create a deletion of *lea-1* and to insert mNG and mYPET fluorescent tags into the

503   endogenous gene locus, CRISPR methods were employed [34, 35]. Using the self-excising

504   cassette (SEC) system, sgRNA sequences were added to the sgRNA-Cas9 plasmid pDD162

505   using the NEB Q5 site-directed mutagenesis kit. Homology arms of ~500-700bp were added to

506   plasmids carrying the SEC and repair templates. To insert a myo-2p::GFP::myo-2 3'UTR

507   reporter to track the null deletion of *lea-1*, homology arms were cloned into plasmid pDD317. To

508   insert mNG or mPYET tags, homology arms were cloned into pUA77 and pDD283 respectively.

509   To replace the endogenous *lea-1* sequence with LEA motifs, sequence was first codon-

510   optimized for *C. elegans* and synthesized by Integrated DNA Technologies (IDT). These

511   stretches were cloned into pUA77 with the same upstream homology arm for insertion of mNG

512   to the endogenous locus and the same downstream homology arm for insertion of mYPET into

513   the endogenous locus. The same sgRNAs for each of the initial mNG and mYPET insertions

514   were used in combination to excise the *lea-1* locus.

515   Worms were injected with 50 ng/μL of plasmid containing the sgRNA and Cas9, and 10-

516   20 ng/μL plasmid containing the repair template, along with a co-injection mix [35]. 2-3 days

517   after injection worms were treated with Hygromycin and selected for transgene-carrying rollers

518   lacking red co-injection mix extra-chromosomal arrays. L1 worms were heat shocked at 32 °C

519   for 5 hr to excise the SEC. Genomic edits were confirmed by visualization of fluorescent

520   reporters and with PCR genotyping.

521   Construction of most TIR1-expressing strains is described in [52]. The *col-10p*::TIR1 line

522   was generated by cloning to combine the *col-10* promoter with the TIR1 construct of pDD356

523   (NEB Hifi Assembly Master Mix). Plasmid pAP082 was used to express Cas9 and a sgRNA to

524   target the chromosome I insertion site. Worms were injected with 50 ng/μL of pAP082 and 20

525   ng/μL plasmid containing the repair template.

526   To establish extrachromosomal array lines expressing *lea-1a* or motifs specifically in

527   body wall muscle, the *myo-3* promoter and an mNG fluorescent tag was cloned onto each of

528   *lea-1* isoform A, a 44-mer motif sequence, and a 97-mer motif sequence. DNA was injected into

529   the gonads of LP852 *daf-2(e1370);lea-1Δ(cp423[myo-2p::GFP::myo-2 3'UTR])* animals at a

530   concentration of 50 ng/μL. Progeny reliably expressing and transmitting the mNG labeled

531   construct in body wall muscle were selected and established as transgenic array lines.

532

533   *Genotyping*

534   Template genomic DNA was create by lysing worms in lysis buffer with proteinase K.

535   Worms were picked into 0.2mL tubes and briefly frozen at -80 ºC, then heated at 65 ºC for 1hr

536   followed by 95 ºC for 15 minutes. This lysate was used for PCR genotyping. Primers were

537   designed to confirm CRISPR genome modifications and to genotype the pre-existing allele *lea-*

538   *1(tm6452)* during backcrossing. Sequences of these primers can be found in Additional File 1:

539   Supplemental Table 1. PCR genotyping was carried out using either Gotaq or Q5 High Fidelity

540   Polymerase (NEB). Annealing temperature and extension time were adjusted based on the

541   melting temperature of the primers and size of the amplicon.

542

543   *C. elegans maintenance*

544   Worms were maintained according to standard laboratory conditions on nematode

545   growth media (NGM) plates, fed OP50 *E. coli*, and stored at 20 ºC. Standard methods were

546   employed for crossing worms, and males were generated by keeping L4 worms at 32 ºC for 5-6

547   hr and 25 ºC overnight. The following strains and alleles were used: N2, *lea-1(tm6452)*

548   (backcrossed 5x into N2), AM140 *rmIs132[unc-54p::Q35::YFP]*, AM141 *rmIs133[unc-*

549   *54p::Q40::YFP]*, CB1370 *daf-2(e1370)*, LP847 *lea-1Δ(cp423[myo-2p::GFP::myo-2 3'UTR])*,

550   LP852 *daf-2(e1370);lea-1Δ(cp423[myo-2p::GFP::myo-2 3'UTR])*, LP858 *lea-1(cp431[mNG::3x*

551   *FLAG::AID::lea-1])*, LP859 *lea-1(cp430[lea-1::mYPET::3x FLAG])*, LP860 *daf-2(e1370);lea-*

552   *1(cp431[mNG::3x FLAG::AID::lea-1])*, LP861 *daf-2(e1370);lea-1(cp430[lea-1::mYPET::3x*

553   *FLAG])*, LP862 *rmIs132[unc-54p::Q35::YFP];lea-1Δ(cp423[myo-2p::GFP::myo-2 3'UTR])*,

554   LP863 *rmIs133[unc-54p::Q40::YFP];lea-1Δ(cp423[myo-2p::GFP::myo-2 3'UTR])*, LP865

555   cpSi171*[vha-8p::TIR1::F2A::mTagBFP::AID*::NLS];daf-2(e1370);lea-1(cp431[mNG::3x*

556   *FLAG::AID*::lea-1])*, LP866 cpSi172*[myo-2p::TIR1::F2A::mTagBFP::AID::NLS];daf-*

557   *2(e1370);lea-1(cp431[mNG::3x FLAG::AID*::lea-1])*, LP867 cpSi173*[col-*

558   *10p::TIR1::F2A::mTagBFP::AID*::NLS];daf-2(e1370);lea-1(cp431[mNG::3x FLAG::AID::lea-1])*,

559   LP868 cpSi174*[myo-3p::TIR1::F2A::mTagBFP::AID*::NLS];daf-2(e1370);lea-1(cp431[mNG::3x*

560   *FLAG::AID::lea-1])*, LP875 *daf-2(e1370);lea-1(tm6452, 5x backcrossed)*, LP876 *reSi5[ges-*

561   *1p::TIR1::F2A::BFP::AID*::NLS::tbb-2 3'UTR];daf-2(e1370);lea-1(cp431[mNG::3x*

562   *FLAG::AID::lea-1])*, LP877 *wrdSi18[mex-5p::TIR1::F2A::BFP::AID*::NLS::tbb-2 3'UTR];daf-*

563   *2(e1370);lea-1(cp431[mNG::3x FLAG::AID::lea-1])*, LP882 *rmIs132[unc-54p::Q35::YFP];daf-*

564   *2(e1370);lea-1Δ(cp423[myo-2p::GFP::myo-2 3'UTR])*, LP883 *rmIs133[unc-54p::Q40::YFP];daf-*

565   *2(e1370);lea-1Δ(cp423[myo-2p::GFP::myo-2 3'UTR])*, LP885 *daf-2(e1370);lea-1(cp432[lea-*

566   *1p::44-mer::mNG::3x FLAG::AID]*, LP887 *daf-2(e1370);lea-1(cp433[lea-1p::97-mer::mNG::3x*

567   *FLAG::AID]*.

568

569   *Analysis of dauer larvae*

570   To assess SDS resistance of larvae we picked dauer or dauer-like larvae into 1mL of 1%

571   SDS. After 15 minutes, worms were scored for viability by movement in response to gentle

572   prodding.

573   To determine if worms were actively feeding, we employed a fluorescent bead-eating

574   assay [70]. Cultures of *E. coli* OP50 were grown overnight at 37 ºC with shaking, and 10 mL of

575   culture were centrifuged at 5000 rpm for 10 minutes and resuspended in 1mL LB. Plates were

576   prepared by adding 1 μL of red fluorescent latex beads (Sigma L3280) to the concentrated

577   bacteria and plating ~60 μL onto NGM plates. Worms were added to the lawns of bacteria on

578   dry plates and allowed to feed for 30 min. The number of worms with ingested beads was

579   scored by visual inspection using a Zeiss Axio Zoom.V16 to identify red fluorescence in the

580   pharynx or intestine of each worm.

581   Representative images of larvae were taken using DIC optics on a Nikon Eclipse E800

582   microscope and a pco.panda sCMOS camera. To calculate the width of larvae as a measure of

583   radial constriction, images were analyzed in FIJI. The width of worms was measured

584   immediately posterior to the pharyngeal bulb.

585

586   *Scanning electron microscopy*

587   *C. elegans* were fixed in 2% paraformaldehyde/2.5% glutaraldehyde in 0.15M sodium

588   phosphate buffer, pH 7.4, for 1 hr at room temperature and stored at 4 ºC. Following three

589   washes in 0.15M sodium phosphate buffer, pH 7.4, the samples were post-fixed in 1% osmium

590   tetroxide in 0.15M sodium phosphate buffer, pH 7.4 for 1hr and gently washed three times in

591   deionized water. Samples were then deposited onto a poly d lysine coverslip or transferred to a

592   microporous chamber and gradually dehydrated with ethanol (30%, 50%, 75%, 100%, 100%).

593   Coverslips and microporous capsules were then transferred to a Samdri-795 critical point dryer

594   and dried using carbon dioxide as the transitional solvent (Tousimis Research Corporation,

595   Rockville, MD). Samples were mounted on 13 mm aluminum stubs with double-sided carbon

23

596   adhesive and sputter coated with 5 nm of gold-palladium alloy (60 Au:40 Pd, Cressington

597   Sputter Coater 208HR, model 8000-220, Ted Pella, Redding, CA). Specimens were observed

598   and images taken using a Zeiss Supra 25 FESEM operating at 5 kV, using the SE2 detector,

599   5mm working distance, and 30 µm aperture (Carl Zeiss SMT Inc., Peabody, MA).

600

601   *C. elegans desiccation, heat, and osmotic stress*

602   *C. elegans* were desiccated according to a previously established protocol [30, 36].

603   Briefly, desiccation chambers were made with varying ratios of glycerol:water in order to

604   produce relative humidity (RH) of either 97.5% or 60%[71]. Dauer worms were formed by

605   moving *daf-2(e1370)* mutant embryos to 25 ºC. Dauer worms were picked into ~1.5-2 μL

606   droplets of water and initially exposed to 97.5% for 4 days as preconditioning. Worms were

607   either rehydrated in M9 or moved to 60% RH desiccation chambers for 1 day before recovery.

608   Survival was scored as the percentage of worms moving or responsive to physical stimulus.

609   Heat stress was implemented by exposing dauer worms to 37 ºC. Multiple plates

610   containing dauer larvae were moved to a 37 ºC incubator for defined periods of time. Worms

611   were scored for survival based on movement or responsiveness to physical stimulus.

612   To measure osmotic stress tolerance, dauer worms were added to water or 1M NaCl in

613   a 96-well or 24-well plate for 2 hr or 4 hr. Worms were then transferred to unseeded NGM plates

614   and survival was scored by movement or responsiveness to physical stimulus.

615

616   *C. elegans lifespan and brood size*

617   For lifespan assays 10 L1 worms per plate were picked to an NGM plate. For each

618   genotype five plates of ten worms were included to start each experiment. Worms were

619   maintained at 20 ºC and transferred to new plates every other day until they were no longer

620   reproductive. Worms that crawled onto the sides of the dishes or were otherwise missing were

621   censored. Survival was scored every other day by movement or responsiveness to touch with a

622   platinum wire. Three independent biological replicates were conducted.

623   To determine brood size, individual embryos were added to NGM plates. 15 worms were

624   included to start in each of three experiments. Worms were transferred to new plates every

625   other day. After transfer, progeny on plates were allowed to develop for ~24 hr and were then

626   counted. Total brood was calculated as the sum of all progeny produced by an individual worm.

627   Worms that failed to hatch or crawled off the plate were censored.

628

629   *Polyglutamine protein aggregation*

630   Strains AM140 and AM141 express polyglutamine tracts of 35 or 40 repeated glutamine

631   residues fused to yellow fluorescent protein (YFP) [46]. These strains were crossed with *lea-1Δ*

632   (LP847). To assess protein aggregation during aging the worms were picked to plates and

633   allowed to develop for 4 or 8 days. Worms were periodically transferred to fresh plates to

634   separate them from progeny. The number of aggregates per worm was scored by imaging

635   worms and counting the number of fluorescent puncta in the body wall muscle of each worm.

636   To determine the number of aggregates in dauer worms the AM140 and AM141

637   reporters were crossed into *daf-2(e1370);lea-1Δ* (LP852) to put the reporter in a *daf-2* mutant

638   background. Dauer formation was induced as before by growth at 25 ºC. The number of

639   aggregates per worm was counted after 5 days (including development and arrest as dauer

640   larvae).

641

642   *Fluorescent imaging and quantification*

643   Worms expressing mNG::LEA-1 or LEA-1::mYPET were imaged on a Nikon TiE stand

644   with CSU-X1 spinning disk head (Yokogawa), 514 nm solid state laser, and ImagEM

645   EMCCD camera (Hamamatsu). Metamorph software was used for image acquisition. To

646   quantify fluorescence in control dauers, desiccated dauers, and dauers exposed to 1 M

647   NaCl for 2 hr, worms were imaged with a 10x objective. Images were imported into FIJI

648   for analysis. Whole worms were outlined manually. The total fluorescence intensity was

649   measured. The outline of the worm was moved to background area of the image to

650   obtain a background measurement. The fluorescence intensity was calculated by

651   subtracting the background from that of the worm. For representative images of worms

652   higher magnification objectives (20x or 60x) were used.

653

654   *Western blotting*

655   Protein for western blotting was obtained from worms expressing mNG::3x FLAG::LEA-1

656   or LEA-1::mYPET::3x FLAG. Mixed stage populations were washed from 3-6 NGM plates,

657   dauer worms from plates at 25 ºC, and desiccated worms from plastic dishes at 97.5% RH for 4

658   days. Large quantities (>20,000) of synchronous worms were obtained for dauer formation and

659   desiccation by standard hypochlorite treatment. Worms were collected, washed in M9, pelleted,

660   and resuspended in ~100 μL lysis buffer containing 50 mM HEPES (pH 7.4), 1mM EGTA, 1mM

661   MgCl$_2$, 100 mM KCl, 10% glycerol, 0.05% NP-40, DTT, and an EDTA-free protease inhibitor

662   tablet (added fresh to 12 mL of buffer) [72]. Worms in lysis buffer were briefly frozen at -80 ºC,

663   then sonicated on ice with a Branson Sonifier 250 wand sonicator at 50% duty until no intact

664   worms remained (2-5 minutes in individual bouts of no longer than 1-2 minutes). The

665   concentration of protein in lysates was determined with the Biorad Protein Assay (Bradford)

666   according to specifications, measuring the OD595 in a total volume of 1mL in cuvettes.

667   A 4-12% Bis-Tris NuPAGE minigel (Invitrogen), loaded with 10 μg of protein in sample

668   buffer (3x concentrate contains 6% SDS, 240mM Tris pH 6.8, 30% glycerol, 0.04% w/v

669   Bromophenol blue, and 50 μL 2-ME per mL) was run in 1x NuPAGE MOPS running buffer

670   (Novex). 10 μL of Precision Plus Protein Kaleidoscope Standard (Biorad) was loaded in lanes

671   adjacent to samples. Gels were run at 140V for 90 minutes. Protein was transferred to a PVDF

672   membrane activated in methanol at 90V for 90 min (in NuPAGE transfer buffer, at 4 ºC).

673   Membranes were rinsed in PBST, and blocked in PBST + 5% BSA for 1 hr with rocking.

674   Membranes were soaked in a 1:1000 dilution of primary antibody (mouse anti-FLAG, Sigma Cat

675   #F1804) overnight at 4 ºC with rocking. Membranes were washed 3x in PBST, then soaked in a

676   1:5000 dilution of secondary antibody (goat anti-mouse IgG, Invitrogen Cat #31432) for 1 hr with

677   rocking. Membranes were washed 3 times and visualized after addition of SuperSignal West

678   Pico PLUS Chemiluminescent Substrate (Thermo Scientific).

679   After initial visualization, membranes were stripped and re-blotted for tubulin as a

680   loading control. Membranes were incubated in mild stripping buffer (15 g glycine, 1 g SDS, 10

681   mL Tween20, bring volume to 1 L with H_2O, pH 2.2) for ten minutes with rocking. Buffer was

682   replaced, and allowed to soak for another ten minutes. Membranes were then washed 3x in

683   PBST, blocked for 1 hr in PBST with 5% BSA, and soaked in a 1:500 dilution of primary

684   antibody (rat anti-tubulin, Invitrogen Cat #MA1-80189) overnight at 4 ºC. After washing 3x in

685   PBST, secondary antibody (goat anti-rat IgG, Thermo Fisher Cat #31470) was added in a

686   1:5000 dilution and membranes soaked for 1 hr with rocking and visualized as before. A single

687   band was detected indicating antibody specificity for tubulin.

688

689   *Protein depletion with auxin-induced degradation*

690   Worms expressing TIR1 under various promoters were grown on plates containing 1mM

691   auxin (indole-3-acetic acid) [51]. Embryos or L1s were picked to NGM + auxin plates and

692   allowed to develop into dauer larvae at 25 ºC for at least 5 days before exposure to desiccation

693   or osmotic stress. Degradation of LEA-1 tagged with mNG::3x FLAG::AID was assessed by

694   imaging worms.

695

696   *Heterologous expression in bacteria*

697   A bacterial codon-optimized version of *lea-1* isoform K was synthesized by Genewiz.

698   Isoforms A, D, E, and F were subcloned from that construct. 30bp of homology was added by

699   PCR to expression constructs, and they were cloned into a PCR-linearized pDest17 using NEB

700   Hifi Assembly Master Mix. Similarly, to clone motifs, 30bp homology was added synthesized

701   codon-optimized DNA fragments (from Integrated DNA Technologies), and they were cloned

702   into PCR-linearized pDest17 with NEB HiFi Assembly Master Mix. GFP and truncated GFP

703   controls were cloned by the same method, using plasmid pFCcGi as a template to obtain the

704   GFP sequence [73]. The *C. elegans act-2* gene was also cloned as a control from cDNA. All

705   plasmids were transformed into NEB 5-alpha cells and correct inserts were verified by

706   sequencing.

707   For desiccation experiments, plasmids containing expression constructs were

708   transformed into *E. coli* BL21 AI (Invitrogen). Individual colonies were picked into 3 mL LB with

709   100 µg/mL ampicillin (Amp) and grown overnight (~12-16 hrs) at 37 ºC in a shaker incubator.

710   Cultures were then diluted 1:20 and 0.2% L-arabinose was added to induce protein expression

711   for 4 hr. Protein expression was confirmed with a Coomassie gel. Bacteria were pelleted and

712   briefly boiled to generate a lysate. Protein concentration was quantified using the Biorad Protein

713   Assay (Bradford) according to specifications. From these protein concentrations an appropriate

714   volume was determined to load 20 ug of total protein per well. 5 µL of pageruler prestained

715   ladder was loaded. A 4-12% BT NuPAGE minigel was run in 1x NuPAGE MOPS running buffer.

716   The gel was stained with Coomassie and de-stained overnight. GFP-expressing bacteria

717   provide a convenient visual confirmation of protein expression in each experiment.

718   To obtain equivalent numbers of starting bacteria to measure desiccation survival,

719   OD600 of 4 hr induced cultures was measured and volumes equivalent to an OD600 of 1.5

720   were added to 1.5 mL Eppendorf tubes. Bacteria were washed once in 0.85% NaCl. Bacteria

721   were then resuspended in 1 mL 0.85% NaCl. A 20 µL sample was removed and used for 10-fold

722   dilution of the culture. A 10-fold dilution series was plated on LB + Amp agar plates by spotting

28

723   10 µL. These control plates were grown at 37 ºC overnight. The remaining 980 µL supernatant

724   was aspirated and tubes were desiccated in a speedvac overnight (~12-16hr). Bacteria were

725   rehydrated in 980 µL of LB + Amp and given 1-2 hr to recover before plating a 10-fold dilution

726   series, as with controls the day before. Bacteria on plates were grown overnight at 37 ºC.

727   Survival was calculated by dividing cfu of each desiccated sample by its control.

728

729   *Motif analysis*

730   The MEME tool of the MEME suite (http://meme-suite.org/tools/meme) was used to

731   identify conserved motifs within LEA-1 [58]. The protein sequence of Isoform K was used as a

732   template since it contains nearly all possible exons of the protein. Position weight matrices,

733   consensus motifs, and distribution of motifs throughout the protein were obtained from this

734   analysis. The pepwheel program (https://www.bioinformatics.nl/cgi-bin/emboss/pepwheel) was

735   used to generate the diagram of the putative alpha-helical structure formed by the 11-mer.

736   Coloration of amino acids was added by hand.

737

738   *Statistical analysis*

739   Statistical tests were carried out as described in figure legends. Unpaired t-tests were

740   conducted in Microsoft Excel. ANOVAs and post-hoc Dunnett's tests were conducted in

741   RStudio. For analysis of bacterial desiccation survival, percent survival was log transformed to

742   standardize variance for statistical analysis. Variance was assessed with Bartlett's test. Lifespan

743   analysis was conducted on aggregate data from three biological replicates with OASIS 2, and

744   data were plotted with GraphPad Prism [74].

745

746   **Abbreviations**

747   AID: auxin-inducible degron, GFP: green fluorescent protein, LEA: late embryogenesis

748   abundant, mNG: mNeonGreen, mYPET: monomeric yellow fluorescent protein for energy

749   transfer, SEC: self-excising cassette, YFP: yellow fluorescent protein.

750

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759

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767

768   **Availability of Data and Materials**

769   All data generated or analyzed during this study are included in this published article and

770   supplementary information files. Raw data are included in Additional File 2. *C. elegans* strains

771   generated in this study will be deposited to the *Caenorhabditis Genetics Center*. Plasmids and

772   other reagents will be made available upon request.

773

Authors' Contributions

JDH conceived of the study, conducted experiments, and analyzed data under the

supervision of BG. JDH wrote a first draft of the manuscript and BG reviewed and edited the

manuscript. Both authors read and approved the final manuscript.

Consent for Publication

Not applicable.

Ethics Approval and Consent to Participate

Not applicable.

Competing Interests

The authors declare that they have no competing interests.

Figures

Figure 1. Predicted isoform structure of LEA-1 and generation of a null allele and two

endogenous fluorescent tags. Isoform annotations were combined from the UCSC genome

browser (http://genome.ucsc.edu) from the Feb. 2013 release (WBcel235/ce11) and Wormbase

version WS278. Locations for the existing tm6452 indel mutation, the newly created CRISPR

null mutation, and the insertion of mNeonGreen and mYPET fluorescent tags are indicated.

Figure 2. *lea-1* mutants have a temperature-sensitive dauer formation defect. **A)** Dauer larvae

formed at 20 ºC are resistant to 1% SDS. **B)** Dauer larvae of *lea-1Δ* and *lea-1(tm6452)* mutants

formed at 25 ºC are sensitive to 1% SDS in both a wild-type (N2) background and a *daf-*

2(e1370) mutant background. **C)** *lea-1Δ*, *lea-1(tm6452)*, *daf-2;lea-1Δ* and *daf-2;lea-1(tm6452)*

799   mutant dauer-like larvae formed at 25 ºC do not ingest fluorescent beads. Non-dauer larvae of

800   each genotype did exhibit feeding behavior and ingest beads. **D)** *daf-2*; *lea-1Δ* and *daf-2; lea-*

801   *1(tm6452)* mutants at 25 ºC have limited radial constriction relative to *daf-2* mutant dauers. A

802   one-way ANOVA indicates significant differences across genotypes (p<0.0001) and a post-hoc

803   Dunnett's test indicates that both *daf-2;lea-1Δ* and *daf-2;lea-1(tm6452)* had increased width

804   relative to *daf-2* controls (p=0.04, p<0.0001, respectively). The width of dauer larvae was

805   measured posterior to the pharynx. Points represent the width of individual worms and black

806   bars depict the mean. The distribution of widths is shown above in density plots. **E)** SEM images

807   highlight the ultrastructure of alae of *daf-2*, *daf-2; lea-1Δ* and *daf-2; lea-1(tm6452)* mutants. *daf-*

808   *2* mutant dauers display normal alae consisting of five distinct ridges. Each of the *lea-1* mutants

809   have disrupted alae that **F)** DIC images show *daf-2*; *lea-1Δ* and *daf-2; lea-1(tm6452)* dauer-like

810   larvae with detached cuticles, as indicated by arrowheads.

811

812   Figure 3. LEA-1 is required for resistance to multiple stresses. **A)** *daf-2; lea-1Δ* and *daf-2; lea-*

813   *1(tm6452)* dauer larvae formed at 20 ºC are sensitive to desiccation. Survival is modestly

814   reduced in worms exposed to 97.5% relative humidity for 4 days (p=0.03, p=0.10 respectively,

815   n=4, unpaired T-test) and more significantly reduced after an additional day at 60% RH

816   (p<0.0001, p=0.009 respectively, n=3, unpaired T-test). **B)** Dauer-like larvae of *daf-2;lea-1Δ* and

817   *daf-2;lea-1(tm6452)* mutants formed at 25 ºC are sensitive to desiccation. Survival is

818   significantly reduced in worms exposed to 97.5% relative humidity for 4 days (p=0.0002,

819   p=0.0001 respectively, n=4, unpaired T-test) as well as 60% for 1 day after 4 days at 97.5%

820   (p<0.0001, p<0.0001, n=4, unpaired T-test). **C)** *lea-1* mutant dauers formed at 20 ºC exhibit mild

821   sensitivity to osmotic stress in 1M NaCl for 2 hr (*daf-2;lea-1Δ* p=0.17, *daf-2;lea-1(tm6452)*

822   p=0.02, n=4, unpaired T-test). **D)** Dauer-like larvae of *daf-2;lea-1Δ* and *daf-2;lea-1(tm6452)*

823   mutants are sensitive to osmotic stress in 1M NaCl for 2 hr (p<0.0001 for each genotype

824   compared to control, n=4, unpaired T-test). Data points in A-D represent results from individual

825   experiments and bars represent mean survival. **E)** Dauer larvae of *lea-1Δ* and *lea-1(tm6452)*

826   mutants are sensitive to heat stress at 37°C (p<0.0001 for each mutant allele relative to control,

827   n=4-5 replicates per timepoint, 2-way ANOVA). Lines depict mean survival and error bars

828   represent SEM. Data points depict survival from individual experiments. **F)** *daf-2;lea-1Δ* mutants

829   have a higher number of polyglutamine protein aggregates. The total number of aggregates in

830   the body wall muscle of individual worms from three independent biological replicates is shown.

831   Thick bars indicate the mean number of aggregates and error bars depict 95% confidence

832   intervals. Worms carrying a transgene expressing a 35-glutamine repeat (Q35) with a YFP for

833   visualization have only marginally more aggregates than controls (p=0.22, n=3, unpaired T-test),

834   while worms carrying a Q40::YFP transgene have significantly more aggregates than controls

835   (p=0.02, n=3, unpaired T-test). * indicates p<0.05, *** indicates p<0.001.

836

837   Figure 4. Phenotypes of LEA-1 are specific to conditions of stress. **A)** Lifespan of *lea-1Δ*

838   mutants is not significantly different from WT (N2) animals (p=0.40, log-rank test). *lea-1(tm6452)*

839   mutants were slightly short-lived relative to WT (N2) animals (p=0.005, log-rank test). **B)**

840   Lifespan is not significantly altered by *lea-1Δ* or *lea-1(tm6452)* mutations in a *daf-2* mutant

841   background (p=0.11, p=0.07, respectively, log-rank test). **C)** There are no significant differences

842   in brood size of *lea-1Δ* (p=0.97, n=3, unpaired T-test) or *lea-1(tm6452)* mutants (p=0.41, n=3,

843   unpaired T-test) relative to N2 worms. Brood size of individual worms from independent

844   replicates are shown. The mean is also shown and error bars represent 95% confidence

845   intervals.

846

847   Figure 5. Functional endogenous fluorescent tags reveal increased LEA-1 protein expression of

848   multiple isoforms during desiccation. **A)** Endogenous fluorescent tags do not disrupt the function

849   of LEA-1 in forming dauers that are resistant to 1% SDS (dauers formed at 25 ºC). **B)**

850   Endogenous fluorescent tags do not disrupt the function of LEA-1 in desiccation survival. *lea-1Δ*

33

851   are sensitive to desiccation at 97.5% relative humidity (p<0.0001, n=4, unpaired T-test) and

852   60% relative humidity (p=0.0002, n=4, unpaired T-test). Survival of mNeonGreen tagged LEA-1

853   worms (mNG::*lea-1*) and mYPET tagged worms (*lea-1*::mYPET) was not significantly different

854   from *daf-2(e1370)* controls after exposure to 97.5% or 60% RH. **C)** Protein samples extracted

855   from of mixed stage cultures, dauer worms, and desiccated worms were blotted with an anti-

856   FLAG antibody indicates multiple isoforms tagged by the two independent tags. The membrane

857   was stripped and blotted for tubulin as a loading control. Total protein in each lane was

858   quantified and normalized to the tubulin loading control. Normalized proteins expression

859   measurements, relative to mixed stage mNG::*lea-1* animals, are listed at the bottom of each

860   lane (Norm. Exp.). **D)** A representative dauer worm expressing mNG::*lea-1*. **E)** A representative

861   desiccated dauer larvae expressing mNG::*lea-1*. **F)** mNG fluorescence is significantly increased

862   in desiccated worms relative to non-desiccated controls (p=0.001, n=3 replicates with 14-15

863   worms per condition, unpaired T-test). **G)** A representative dauer worm expressing *lea-*

864   *1*::mYPET. **H)** A desiccated dauer expressing *lea-1*::mYPET. **I)** Fluorescent intensity is not

865   significantly different between control and desiccated dauer worms expressing *lea-1*::mYPET

866   (p=0.39, n=3 replicates with 15 worms per condition, unpaired T-test). Note that all worms were

867   in a *daf-2(e1370)* background. ** indicates p<0.01, *** indicates p<0.001.

868

869   Figure 6. LEA-1 is required in body wall muscle to survive desiccation and osmotic stress. **A-D)**

870   Representative images of an mNG::*lea-1* worm depict the major sites of expression. There is

871   prominent fluorescence in the germline, body wall muscle, excretory cell, and pharynx. There is

872   also some apparent expression in seam cells (B) and faint fluorescence in the intestine (C). A

873   zoomed in image of the excretory cell is shown in (D). **E)** A cartoon depicts tissues in which

874   LEA-1 is expressed that were targeted for protein depletion by driving TIR1 under the control of

875   different promoters. **F)** Survival in 1% SDS is plotted for worms with tissue specific LEA-1

876   depletion by expression of TIR1 under various promoters and exposure to 1mM auxin. Dauer

877   larvae were formed at 25 ºC. Depletion of LEA-1 did not sensitize worms to SDS. **G)**

878   Desiccation survival is plotted for worms with tissue-specific LEA-1. Depletion of the mNG::*lea-1*

879   protein utilizing auxin-induced degradation reduces desiccation survival in worms expressing

880   TIR1 under a *myo-3* (body wall muscle-specific) promoter relative to *daf-2* controls (p=0.0001,

881   n=3, unpaired T-test) and worms expressing only mNG::*lea-1* and not TIR1 (p=0.001, n=3,

882   unpaired T-test). No other site of TIR1 expression significantly altered desiccation survival at

883   60% RH. **H)** Survival of osmotic stress in 1M NaCl for 4 hrs is plotted for the same strains as in

884   (E). *lea-1Δ* mutants and worms expressing TIR1 under a *myo-3* promoter were the only two

885   strains that with significant differences in survival relative to both control and mNG::*lea-1-*

886   expressing animals (*daf-2*; *lea-1Δ*: vs. *daf-2* p=0.006, vs. *daf-2;* mNG::*lea-1* p=0.01; *daf-2;*

887   mNG::*lea-1; myo-3p*::TIR1: vs. *daf-2* p=0.0001, vs. *daf-2;* mNG::*lea-1* p=0.0009, unpaired T-

888   tests, n=3). Note, the images in A-D show a worm with an N2 background, whereas worms in F-

889   H are in a *daf-2(e1370)* background. ** indicates p<0.01, *** indicates p<0.001.

890

891   Figure 7. Heterologous expression of *C. elegans lea-1* isoforms and motifs improves bacterial

892   desiccation survival. **A)** Select isoforms of LEA-1 used for bacterial expression. **B)** Desiccation

893   survival of BL21 *E. coli* expressing codon-optimized *C. elegans* LEA-1 isoforms. Heterologous

894   expression of each isoform increased desiccation survival (1-way ANOVA p<0.0001, post-hoc

895   Dunnett's test: Isoform A p<0.0001, Isoform D p<0.0001, Isoform E p=0.0008, Isoform F

896   p<0.0001, Isoform K p<0.0001, each isoform compared to GFP control, n=6). **C)** A consensus

897   11-mer motif found in LEA-1 likely forms an amphipathic alpha helix. The position weight matrix

898   of amino acids in the motif is shown, as well as a wheel diagram depicting the relative position

899   of each consensus amino acid in an alpha helical conformation. **D)** Consensus amino acid

900   sequences are shown for the 11-mer as well as a 97-mer motif that was also detected with an

901   expanded motif window size. The frequency and distribution of occurrences of these motifs

902   within isoform K are shown. **E)** Desiccation survival of BL21 *E. coli* is significantly increased by

903   expression of motifs of LEA-1. Codon-optimized sequences for the 11-mer, as well as

904   concatenated repeats of the 11-mer sequence (2x=22-mer, 4x=44-mer), and the 97-mer motif

905   were expressed. Expression of each peptide increased survival of desiccated bacteria (1-way

906   ANOVA p<0.0001, post-hoc Dunnett's test: 11-mer p<0.0001, 22-mer p<0.0001, 44-mer

907   p<0.0001, 97-mer p<0.0001, compared to GFP control, n=7). *** indicates p<0.001.

908

909   Figure 8. Minimal LEA-1 motifs promote desiccation and osmotic stress survival *in vivo*. **A)**

910   Endogenous *lea-1* sequence was largely deleted and replaced with sequence encoding codon

911   optimized, mNG-tagged, idealized motifs of 44 amino acids (4x 11-mer) or 97 amino acids. **B)**

912   Expression of 44 and 97 amino acid LEA-1 motifs is sufficient for the formation of dauer larvae

913   (25 ºC) that are resistant to 1% SDS. **C)** Desiccation survival of *daf-2*, *daf-2;lea-1Δ*, *daf-2;44-*

914   mer::mNG, and *daf-2;*97-mer::mNG animals is shown. *daf-2;lea-1Δ* mutants had significantly

915   lower survival than control animals at both 97.5% RH (p<0.0001, unpaired T-test, n=4) and 60%

916   RH (p<0.0001, unpaired T-test, n=4). At 97.5% RH motif-expressing worms had survival that

917   was statistically indistinguishable from control, and significantly improved relative to *daf-2;lea-1Δ*

918   animals (44-mer p=0.36 , 97-mer p=0.78 , unpaired T-tests, n=4). When dehydrated at 60% RH,

919   motif-expressing worms each had significantly reduced survival (*daf-2;*44-mer::mNG p=0.0003,

920   *daf-2;*97-mer::mNG p<0.0001, unpaired T-tests relative to *daf-2*, n=4). **D)** *daf-2;lea-1Δ* mutants,

921   but not motif-expressing animals, were sensitive to osmotic stress in 1M NaCl for 4 hr (*daf-*

922   *2;lea-1Δ* p<0.0001, *daf-2;*44-mer::mNG p=0.53, *daf-2;*97-mer::mNG p=0.87 unpaired T-test vs.

923   *daf-2*, n=4). Worms expressing LEA motifs had significantly improved survival relative to *daf-2;*

924   *lea-1Δ* animals (*daf-2;*44-mer::mNG p<0.0001, *daf-2;*97-mer::mNG p<0.0001, unpaired T-test,

925   n=4). *** indicates p<0.001.

926

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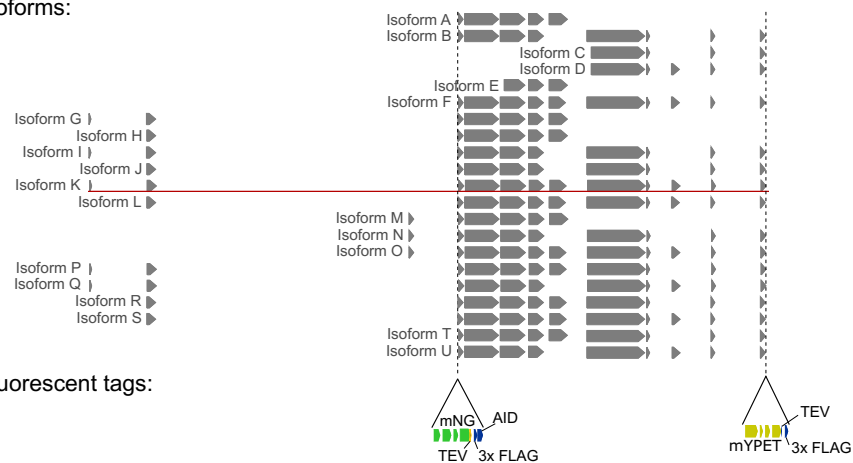1025   pheromone, food, and temperature. Dev Biol. 1984;102:368–78.
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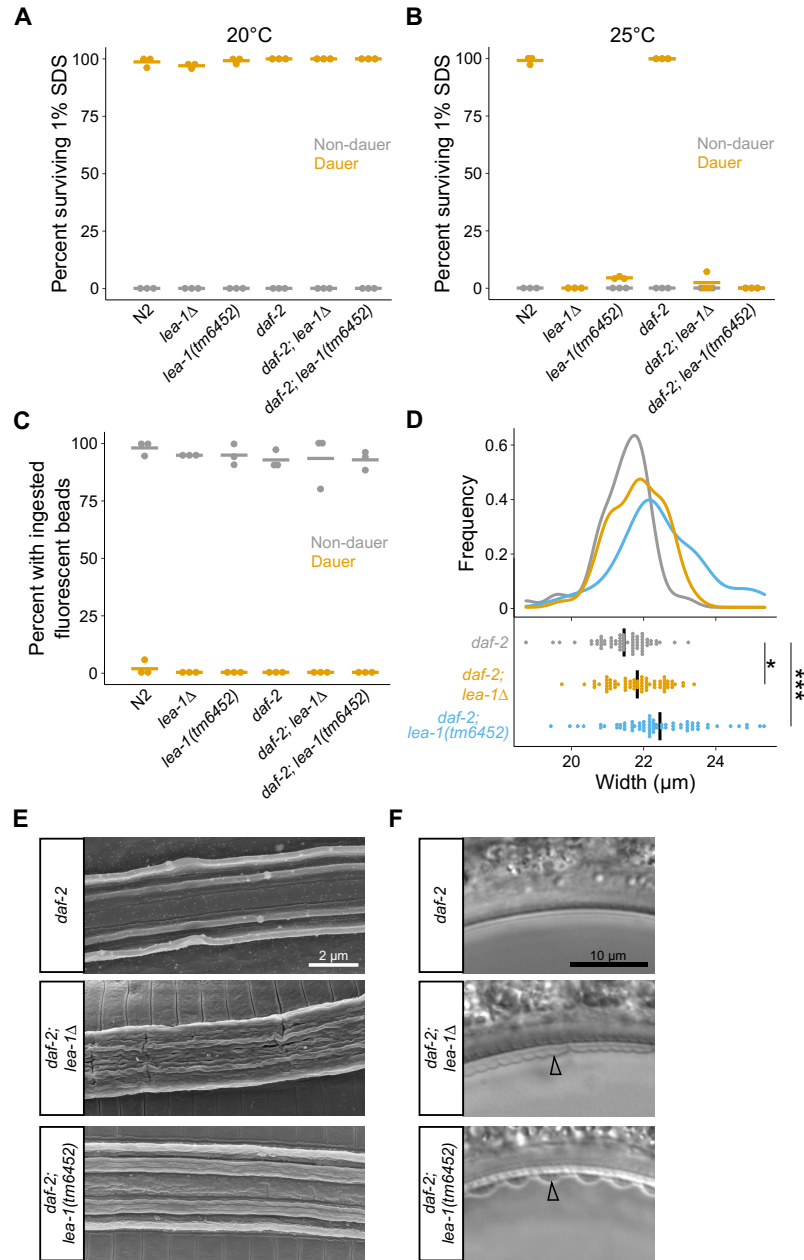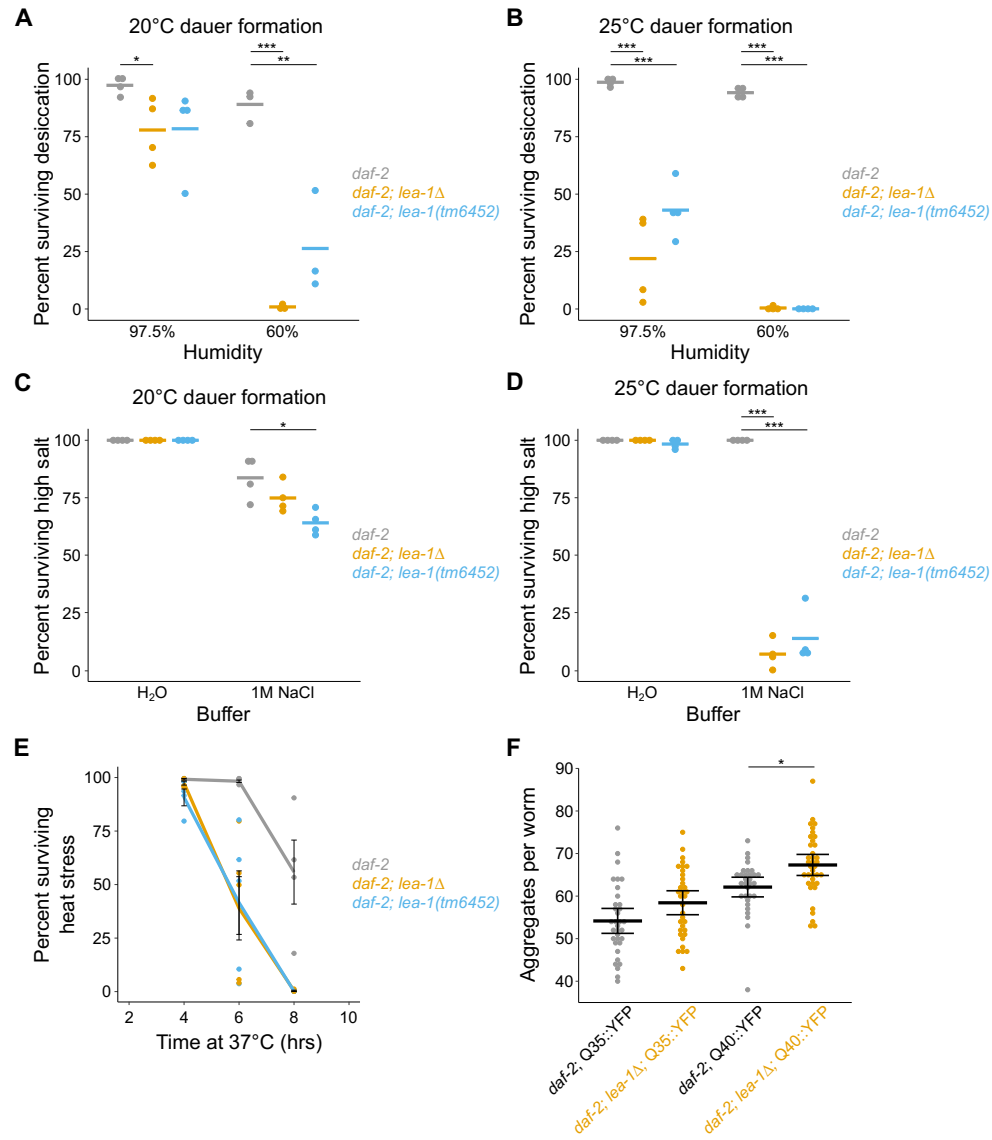1124

Figure 1

Isoforms:

Isoform A
Isoform B
Isoform C
Isoform D
Isoform E
Isoform F
Isoform G
Isoform H
Isoform I
Isoform J
Isoform K
Isoform L
Isoform M
Isoform N
Isoform O
Isoform P
Isoform Q
Isoform R
Isoform S
Isoform T
Isoform U

Fluorescent tags:

mNG   AID
TEV   3x FLAG

TEV
mYPET   3x FLAG

Deletions:

CRISPR deletion   tm6452 Indel

myo-2p GFP myo-2 3'UTR

0 kb   2 kb   4 kb   6 kb   8 kb   10 kb   12 kb   14 kb   16 kb

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Figure 2

Figure 3

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Figure 4

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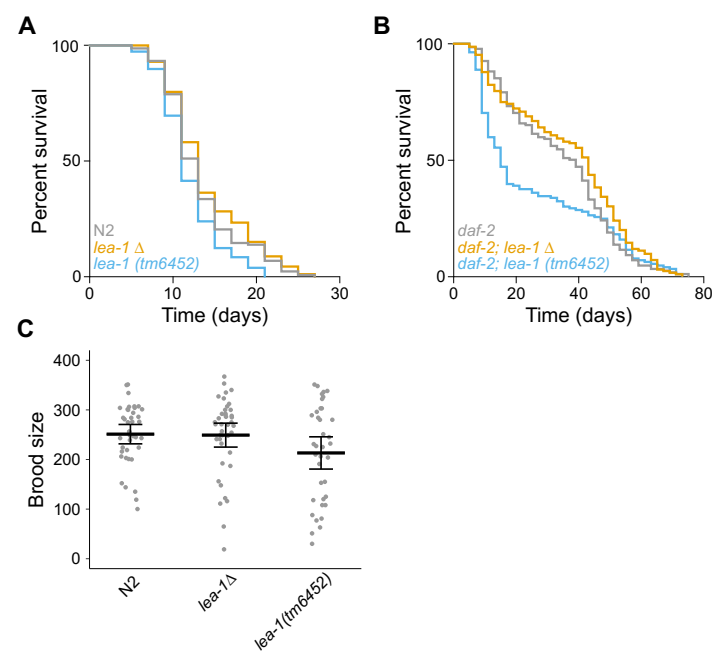
Figure 5

Figure 6

Figure 7

A

Isoform A

Isoform D

Isoform E

Isoform F

Isoform K

0 kb 2 kb 4 kb 6 kb 8 kb 10 kb 12 kb 14 kb 16 kb

B

Percent surviving desiccation

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***
***
***
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10

1

0.1

0.01

0.001

0.0001

GFP, truncated GFP, act-2, lea-1 isoform A, lea-1 isoform D, lea-1 isoform E, lea-1 isoform F, lea-1 isoform K

C

bits

Hydrophilic

D D D
S   S
K   K
W   A
   A A

Hydrophobic

D

DKASDAWDSAK

AKDKTKSLTEKVGDAISGAYDSVKEKASDVA
DSFKAHSTDSKDNVENKAADAYNSAKDKAS
DAWDKTKDKAGEAKDKAGDAWDSTKDKAG
DAWDSTK

Isoform K

E

Percent surviving desiccation

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100
10
1
0.1
0.01
0.001
0.0001
0.00001
0.000001

GFP, truncated GFP, act-2, 11-mer, 22-mer, 44-mer, 97-mer

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Figure 8

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1134 **Additional File 1**

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1136 **LEA motifs promote desiccation tolerance *in vivo***

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Figure S1

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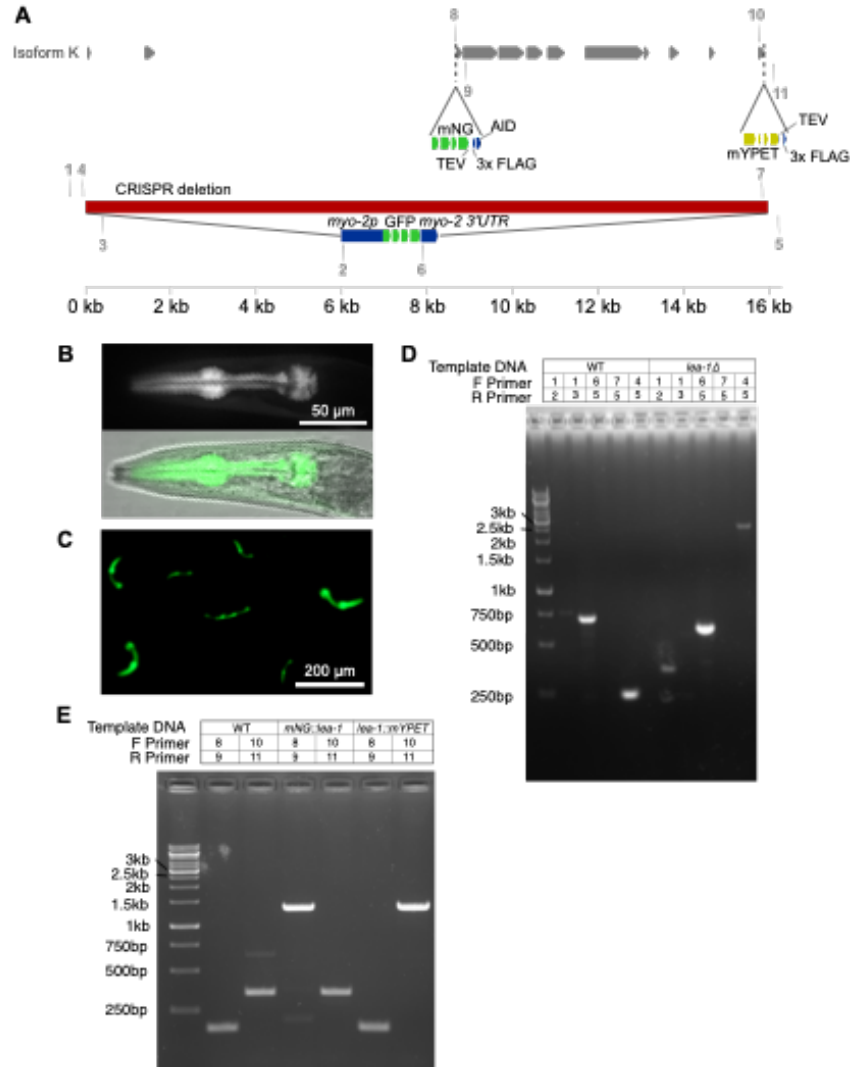
1161 **Additional File 1: Figure S1.** Genotyping LEA-1 alleles. **A)** A simplified schematic depicting

1162 locations of primers to genotype the various genome edits. Primers are indicated with numbers.

1163 Sequences can be found in Table S1. **B)** A representative image depicts pharyngeal GFP

1164 expression in an *lea-1Δ* mutant in which 15.8 kb of genomic sequence was replaced with *myo-*

1165 *2p*::GFP::*myo-2 3'UTR* as a visual marker for the deletion. **C)** A representative image shows the

1166 plate level phenotype of pharyngeal GFP expression in *lea-1Δ* mutants. **D)** Primers as indicated

1167 by numbers in Figure 1 were used to amplify genomic DNA and confirm deletion of the

1168 endogenous *lea-1* locus and insertion of *myo-2p*::GFP::*myo-2 3'UTR* in the *lea-1Δ* mutant. **E)**

1169 The indicated primers were used to amplify across the loci in genomic DNA at which the mNG

1170 and mYPET tags were inserted. Genotyping confirms insertion of the mNG::3xFLAG::AID tag in

1171 a relatively N-terminal position of *lea-1* and insertion of the mYPET::3xFLAG tag at the C-

1172 terminus of the gene.

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Figure S2
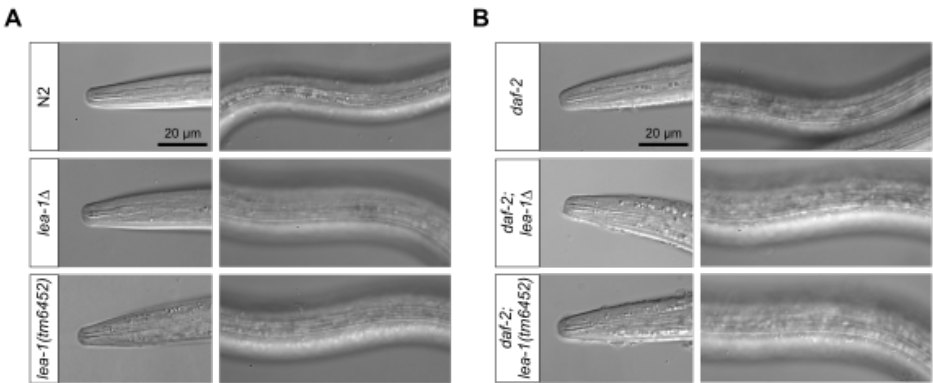
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Additional File 1: Figure S2. Representative images of dauer-like larvae of *lea-1* mutants. **A)**
The mouth and alae of N2, *lea-1Δ*, and *lea-1(tm6452)* worms are shown. **B)** The mouth and
alae of *daf-2*, *daf-2;lea-1Δ*, and *daf-2;lea-1(tm6452)* worms are shown. Dauer-like larvae were
formed at 25 ºC in both A and B.
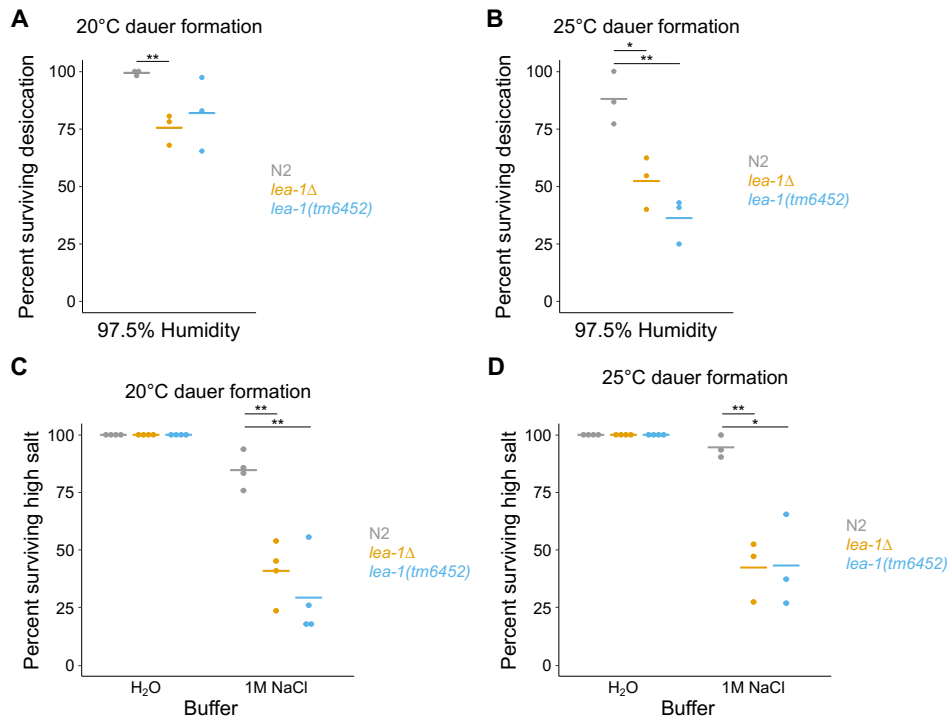
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Figure S3

Additional File 1: Figure S3. Mutations in *lea-1* impact wild-type worms (N2) similarly to *daf-2*

mutants. **A)** *lea-1Δ* dauer larvae formed at 20 ºC had reduced desiccation survival at 97.5% RH

(p=0.003, unpaired T-test vs. N2, n=3). Desiccation survival of *lea-1(tm6452)* mutants was

statistically indistinguishable from N2 (p=0.13, unpaired T-test, n=3). **B)** Both *lea-1Δ* and *lea-*

1(tm6452) dauer-like larvae formed at 25 ºC have reduced desiccation survival at 97.5% RH

(p=0.02, p=0.004 respectively, unpaired T-tests vs. N2, n=3). **C)** *lea-1Δ* and *lea-1(tm6452)*

dauer larvae formed at 20 ºC have reduced survival in 1M NaCl for 2 hr (p=0.001, p=0.001,

unpaired T-tests vs. N2, n=4). **D)** *lea-1Δ* and *lea-1(tm6452)* dauer-like larvae formed at 25 ºC

have reduced survival in 1M NaCl for 2 hr (p=0.003, p=0.01 respectively, unpaired T-tests vs.

N2, n=3).

Figure S4
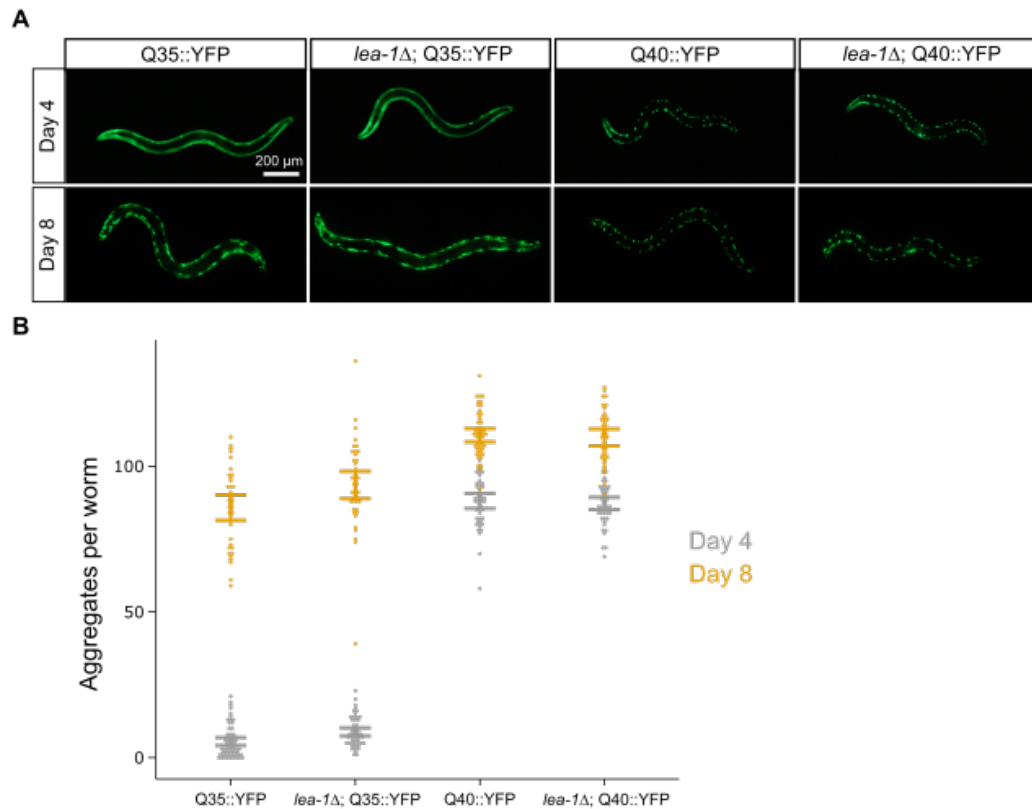
Additional File 1: Figure S4. LEA-1 does not significantly alter polyglutamine protein

aggregation due to age. **A)** Representative images of 4 and 8 day old worms expressing

polyglutamine::YFP constructs in body wall muscle. **B)** There are no significant differences in

the number of polyglutamine aggregates between control (N2 background) and *lea-1Δ* animals

for either Q35::YFP (day 4 p=0.23, day 8 p=0.42, n=3, unpaired T-test) or Q40::YFP (day 4

p=0.81, day 8 p=0.32, n=3, unpaired T-test).

Figure S5
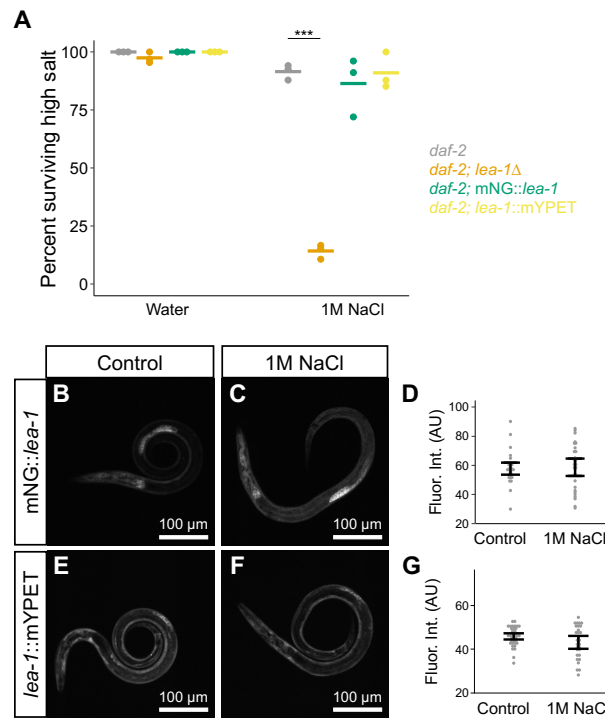
Additional File 1: Figure S5. LEA-1 expression does not increase in response to short-term osmotic stress. **A)** Fluorescent tags to not disrupt function of LEA-1 during osmotic stress. Survival is plotted for worms exposed to either water or 1M NaCl for 2 hr. Bars represent mean survival. Neither *daf-2;* mNG::*lea-1* nor *daf-2; lea-1*::mYPET worms were significantly different from control *daf-2* worms exposed to 1M NaCl. **B)** Representative image of a control *daf-2; mNG::lea-1* dauer worm. **D)** A *daf-2;* mNG::*lea-1* dauer larvae exposed to 1M NaCl for 2 hr. **E)** mNG fluorescence is not significantly altered in worms exposed to 1M NaCl relative to controls (p=0.86, n=3 replicates, unpaired T-test). **F)** A representative *daf-2* dauer worm expressing *lea-1*::mYPET. **G)** A *daf-2; lea-1*::mYPET dauer after 2 hr in 1M NaCl. **H)** Fluorescent intensity is not significantly different between controls worms expressing *lea-1*::mYPET (p=0.36, n=3 replicates, unpaired T-test). *** indicates p<0.001.
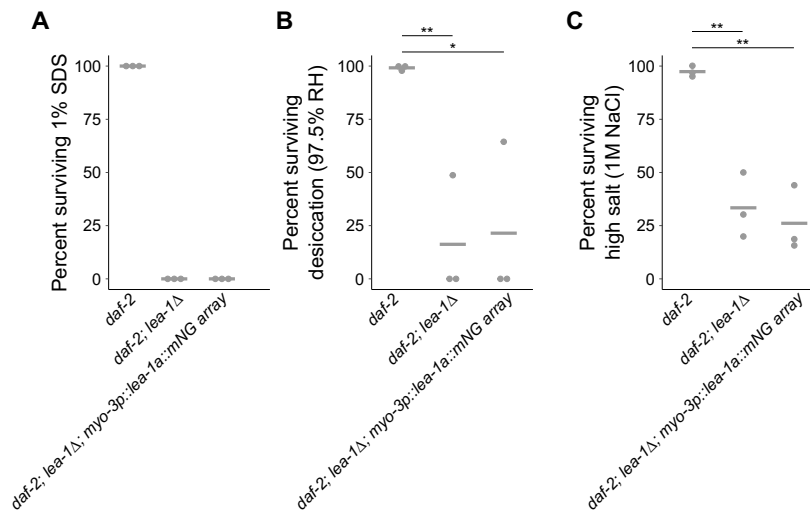
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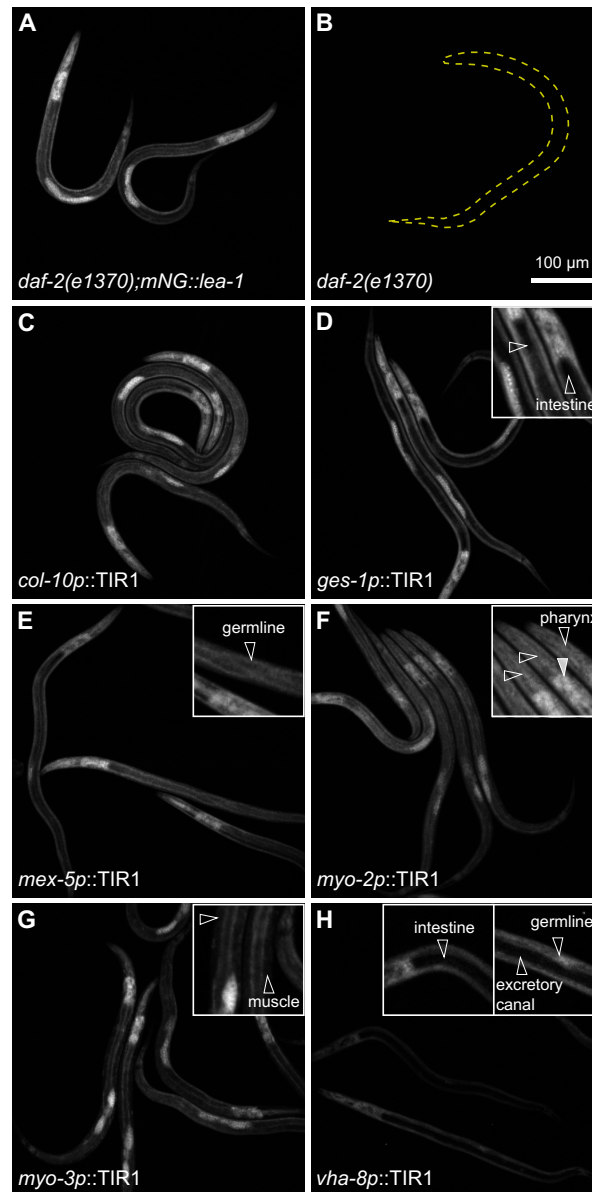
Figure S6

Additional File 1: Figure S6. Representative images of auxin-induced depletion of mNG::*lea-1* in TIR1 expressing strains. **A)** Baseline expression of mNG::LEA-1 in the absence of TIR1 expression. **B)** *daf-2* mutant animals have no detectable baseline fluorescence. Yellow dashed lines indicate the outline of a worm. **C)** TIR1 expressed from the *col-10* promoter should deplete LEA-1 in the hypodermis. It had a minimal effect on expression levels in worms. **D)** TIR1 driven by the *ges-1* promoter reduced LEA-1 in the intestine. **E)** TIR1 under the control of the *mex-5* promoter depleted LEA-1 in the germline. Some worms retained a significantly reduced amount of germline LEA-1. **F)** *myo-2p*::TIR1 worms have reduced LEA-1 in the pharynx. This construct did not totally deplete LEA-1. In particular, expression levels are still relatively high in the

1247   posterior bulb of the pharynx (solid arrowhead). Depletion in the more anterior regions of the

1248   pharynx was more pronounced (open arrowheads). **G)** Expression of *myo-3p*::TIR1 significantly

1249   reduced LEA-1 levels in body wall muscle. **H)** TIR1 driven by the *vha-8* promoter is expressed in

1250   multiple tissues and depletes LEA-1 in the excretory cell (and canal), intestine, and germline.

1251   TIR-1 expression was also observed in hypodermis and some cells of the head. The 100 µm

1252   scale bar applies to all images. All images were taken with the same microscope settings and

1253   are shown on the same intensity scale. For the 2x magnified insets the brightness was adjusted

1254   to facilitate visualization. All worms were grown on plates containing 1mM auxin. Open

1255   arrowheads indicate sites of LEA-1 depletion in each strain.

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Figure S7

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1275 **Additional File 1: Figure S7.** Overexpression of *lea-1a* in body wall muscle does not rescue

1276 SDS sensitivity, desiccation tolerance, or osmotic stress resistance. **A)** Expression of mNG-

1277 tagged *lea-1a* in body wall muscle (*myo-3*p::*lea-1a*::mNG) does not increase 1% SDS survival

1278 of dauer-like larvae formed at 25 ℃. **B)** Body wall expression of mNG-tagged *lea-1a* does not

1279 improve desiccation survival at 97.5% RH. *daf-2;lea-1Δ* and *myo-3p*::*lea-1a*::mNG worms each

1280 have reduced desiccation survival at 97.5% RH relative to *daf-2* controls (p=0.007, p=0.02,

1281 respectively, n=3, unpaired T-test) **C)** Survival of osmotic stress in 1M NaCl for 2 hr is not

1282 improved by expression of *myo-3p::lea-1a::*mNG. Both *daf-2;lea-1Δ* and *myo-3p*::*lea-1a*::mNG

1283 worms are sensitive to 1M NaCl (p=0.002, p=0.001 respectively vs. *daf-2*, n=3, unpaired T-test).

1284 * indicates p<0.05, ** indicates p<0.01.

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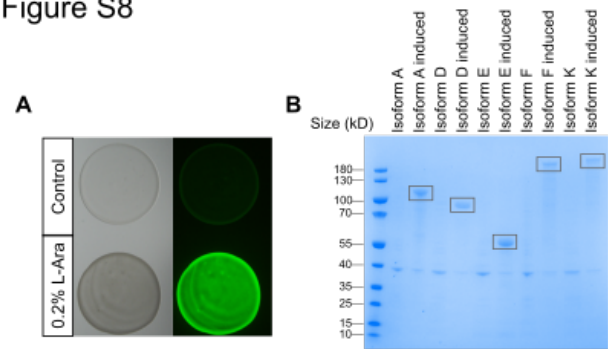
Figure S8

Additional File 1: Figure S8. Controls for heterologous expression of proteins in bacteria. **A)** BL21 *E.coli* carrying pDest17 driving expression of GFP are induced by growth in media with 0.2% L-arabinose. Samples of bacteria from liquid cultured were spotted onto LB agar plates for imaging. **B)** A Coomassie stained 4-12% Bis-Tris gel shows expression of *C. elegans* LEA-1 isoforms A, D, E, F, and K in BL21 *E. coli* when induced with 0.2% L-arabinose. 20 µg of total protein was loaded per lane.

Figure S9
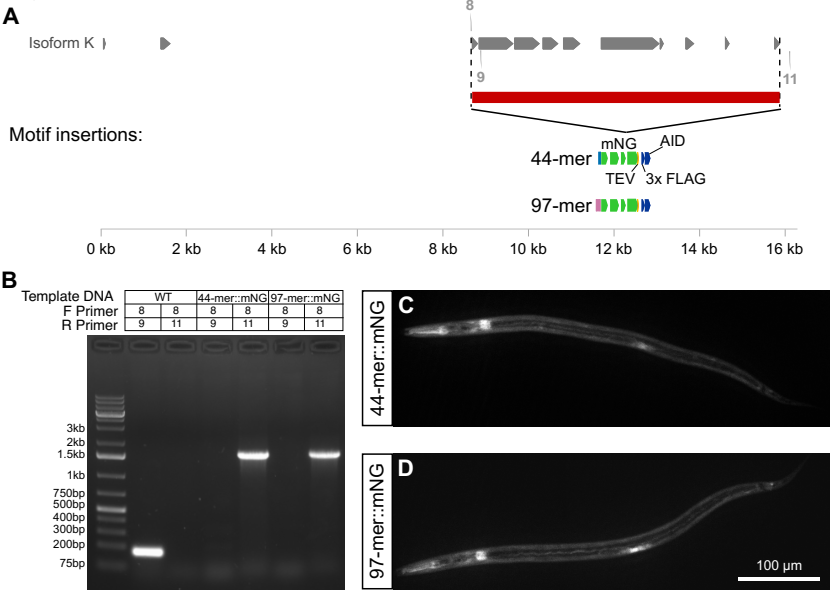
Additional File 1: Figure S9. Characterization of LEA-1 motif-expressing worms. **A)** A
schematic depicts the regions of genomic deletion and insertions and the primers used for
genotyping these edits. **B)** PCR genotyping confirms deletion of the majority of LEA-1 exons
and insertion of sequence encoding 44-mer::mNG or 97-mer::mNG. **C)** A representative image
depicts *in vivo* expression of the 44-mer::mNG fusion protein in a dauer worm. **D)** A
representative image depicts *in vivo* expression of the 97-mer::mNG fusion protein in a dauer
worm. Worms in C and D were in a *daf-2* background.

Figure S10
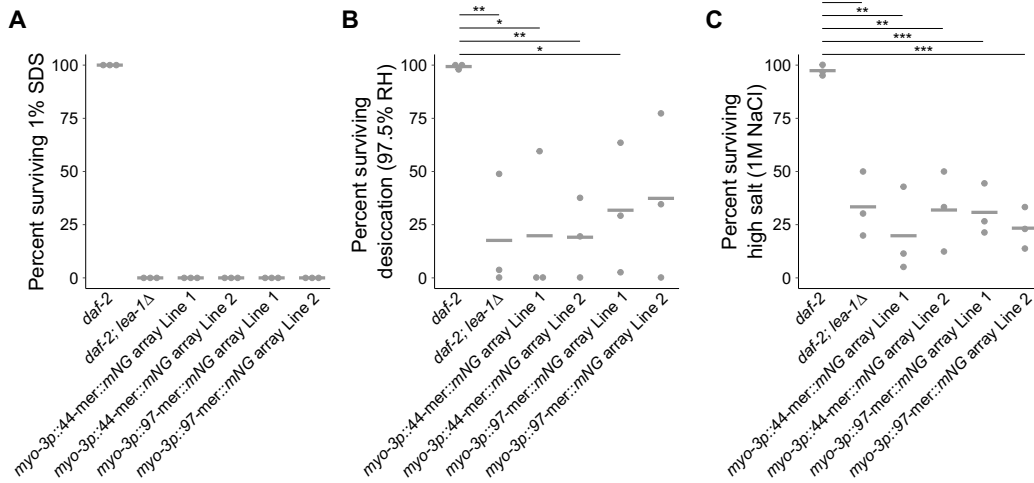
Additional File 1: Figure S10. Expression of LEA-1 motifs in body wall muscle does not

improve SDS resistance, desiccation tolerance, or osmotic stress survival. **A)** Overexpression

array lines carrying *myo-3p*::44-mer::mNG or *myo-3p*::97-mer::mNG transgenes remain

sensitive to 1% SDS. **B)** *myo-3p*::44-mer::mNG and *myo-3p*::97-mer::mNG array lines do not

rescue 97.5% RH desiccation survival of *daf-2;lea-1Δ* animals. Strains have reduced survival

relative to *daf-2* controls (*daf-2;lea-1Δ* p=0.006, *myo-3p*::44-mer::mNG Line 1 p=0.02, *myo-*

3p::44-mer::mNG Line 2 p=0.002, *myo-3p*::97-mer::mNG Line 1 p=0.02, *myo-3p*::97-mer::mNG

Line 2 p=0.05, n=3, unpaired T-test). None of the array carrying lines is statistically

distinguishable from *daf-2;lea-1Δ*. **C)** Muscle-specific expression of LEA-1 motifs does not

improve survival of 2 hr osmotic stress in 1M NaCl. Survival of each array carrying line was not

significantly different from *daf-2; lea-1Δ*. Rather, strains remain sensitive to 1M NaCl (*daf-2;lea-*

1Δ p=0.002, *myo-3p*::44-mer::mNG Line 1 p=0.003, *myo-3p*::44-mer::mNG Line 2 p=0.004,

myo-3p::97-mer::mNG Line 1 p=0.0007, *myo-3p*::97-mer::mNG Line 2 p=0.0002 vs. *daf-2*, n=3,

unpaired T-test). * indicates p<0.05, ** indicates p<0.01, *** indicates p<0.001.

1356   **Additional File 1: Table S1.** Genotyping primers for *lea-1* genomic edits.

Primer number	Sequence	Genotyping information
1	CGATGGTACCACAATGACCA	For use with primers #2 and #3.
2	TTTATGGACATTTAAAGCAAAGGA	Paired with #1 will give 417 bp band in *lea-1Δ* but no band in WT.
3	TCACTTGAGAGCCCGAACTT	Paired with #1 will give 742 bp band in WT but no band in *lea-1Δ*.
4	GATCACCATCTCCACCAACC	Paired with #5 will give 2,569 bp band in *lea-1Δ* but no band in WT.
5	GGGCAATCCAAAAAGATTGA	For use with primers #4, #6, and #7.
6	ACCCGTTTCTCTTCCCCTAC	Paired with #5 will give 620 bp band in *lea-1Δ* but no band in WT.
7	GGACACTCTTCGCTCGACTC	Paired with #5 will give 256 bp band in WT but no band in *lea-1Δ*.
8	CAACAAAATGAGCTTTATGGATAAAG	For use with primer #9.
9	ATCTTTTCACCGACGGTGTTG	Paired with #8 will give 170 bp band in WT and 1,364 bp band in mNG::*lea-1* but no band in 44-mer::mNG or 97-mer::mNG.
10	ACTTGAAAACTGGAACTCTTCCATC	For use with primer #11.
11	CTACAACAAAGAATTAACAG	Paired with #10 will give 347 bp band in WT and 1,412 bp band in *lea-1*::mYPET. When paired with #8 will give 1,612 bp band in 44-mer::mNG and 1,771 bp band in 97-mer::mNG.

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1366 **Additional File 1: Table S2.** Lifespan analysis of *lea-1* mutants.

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Genotype	n	Mean Survival (days)	SE	95% CI	Maximum Survival (days)
N2	151	13.22	0.38	12.47 ~ 13.96	27
lea-1(tm6452)	150	11.94	0.3	11.35 ~ 12.53	21
lea-1Δ	150	13.75	0.42	12.91 ~ 14.58	27
daf-2	141	34.36	1.47	31.48 ~ 37.23	75
daf-2;lea-1(tm6452)	144	26.29	1.77	22.83 ~ 29.76	73
daf-2;lea-1Δ	150	36.89	1.56	33.84 ~ 39.95	73

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