LEA motifs promote desiccation tolerance *in vivo*

Jonathan D. Hibshman[a] and Bob Goldstein[a,b]

[a]Biology Department, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

[b]Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

*Corresponding author, Jonathan D. Hibshman

Biology Department

616 Fordham Hall

The University of North Carolina at Chapel Hill

Chapel Hill, NC 27599-3280

E-mail: hibsjo01@email.unc.edu

Abstract

Background

Cells and organisms typically cannot survive in the absence of water. However, there are some notable exceptions, including animals such as nematodes, tardigrades, rotifers, and some arthropods. One class of proteins known to play a role in desiccation resistance is the late embryogenesis abundant (LEA) proteins. These largely disordered proteins protect plants and animals from desiccation. A multitude of studies have characterized stress-protective capabilities of LEA proteins *in vitro* and in heterologous systems. However, the extent to which LEA proteins exhibit such functions *in vivo*, in their native contexts in animals, is unclear. Furthermore, little is known about the distribution of LEA proteins in multicellular organisms or tissue-specific requirements in conferring stress protection.

Results

To study the endogenous function of an LEA protein in an animal, we created a true null mutant of *C. elegans* LEA-1, as well as endogenous fluorescent reporters of the protein. We confirmed that *C. elegans* lacking LEA-1 are sensitive to desiccation. LEA-1 mutant animals were also sensitive to heat and osmotic stress and were prone to protein aggregation. During desiccation, LEA-1 expression increased and became more widespread throughout the body. LEA-1 was required at high levels in body wall muscle for animals to survive desiccation and osmotic stress. We identified minimal motifs within *C. elegans* LEA-1 that are sufficient to increase desiccation survival of *E. coli*. To test whether such motifs are central to LEA-1's *in vivo* functions, we then replaced the sequence of *lea-1* with these minimal motifs and found that *C. elegans* survived mild desiccation and osmotic stress at the same levels as worms with the full-length protein.

Conclusions

Our results provide insights into the endogenous functions and expression dynamics of an LEA protein in a multicellular animal. The results show that LEA-1 buffers animals from a

54 broad range of stresses. Our identification of LEA motifs that can function in both bacteria and in
55 a multicellular organism suggests the possibility of engineering LEA-1-derived peptides for
56 optimized desiccation protection.
57
58 **Background**
59 Animals regularly encounter abiotic stresses due to fluctuating environments. One such
60 stress is desiccation. Water is essential for cellular metabolism, and without it cellular
61 components including DNA, RNA, proteins, and membranes are unstable. However, certain
62 animals can lose nearly all their internal water and yet survive (anhydrobiosis). This group
63 includes nematodes, rotifers, tardigrades, and certain arthropods such as some crustaceans
64 and insects. Studying the mechanisms by which these organisms are able to survive
65 desiccation is of fundamental interest and may lead to better understanding of biological
66 protectants.
67 One family of common protectants with demonstrated efficacy during desiccation is the
68 late embryogenesis abundant (LEA) proteins. These proteins were originally identified in cotton
69 seeds and were subsequently found to be present in many other plants [1–6]. LEA proteins
70 protect the viability of desiccated seeds [7]. Animal LEA proteins were later identified in the
71 nematode *Aphelenchus avenae* and other anhydrobiotic animals [8–13]. Several families of LEA
72 proteins have been described, each containing distinct motifs [14, 15]. Nematode LEA proteins
73 both in *A. avenae* as well as the more common model system *C. elegans* are members of the
74 Group 3 LEA proteins. This is the most common LEA variety within anhydrobiotic animals [16].
75 LEA proteins are thought to be intrinsically disordered, i.e. largely unstructured in hydrated
76 conditions, but under water-limiting conditions the proteins acquire recognizable alpha-helical
77 secondary structure [17–19]. There are several examples in which heterologous expression of
78 LEA proteins confers stress resistance in a number of host cell types, including yeast, bacteria,
79 rice, and mammalian cell culture [20–25]. Additionally, isolated LEA protein has been

80 demonstrated to protect against protein aggregation and stabilize membranes and liposomes *in*
81 *vitro* [26–28]. Although the functions of LEA proteins are well-established, most studies have
82 utilized heterologous expression systems. Therefore, we have limited knowledge of the
83 endogenous, *in vivo* roles of LEA proteins.

84 The nematode *C. elegans* is an ideal model for the study of the endogenous functions of
85 LEA proteins. *C. elegans* survive many stresses including desiccation, have a well-resourced
86 genetic toolkit, and contain two LEA family proteins, *lea-1* and *dur-1* (dauer up-regulated) [10,
87 29]. Disruption of *lea-1* has been reported to sensitize worms to desiccation, osmotic stress with
88 sucrose, and larval heat stress [10, 29, 30]. *dur-1* is also required for a robust desiccation
89 response but is apparently unable to compensate for the loss of LEA-1 [29]. Whereas most
90 studies show that heterologous expression is sufficient to protect against stresses in other
91 systems, these *C. elegans* studies provide evidence for necessity in the native function of LEA
92 proteins in animals. However, little is known about the tissues in which LEA proteins are
93 required or the mechanisms by which they function *in vivo* [14, 31, 32].

94 We used LEA-1 of *C. elegans* as a model to study the endogenous function of an LEA
95 protein in a multicellular animal. We confirmed and expanded phenotypes associated with
96 disruption of LEA-1, characterized expression patterns and dynamics in response to
97 desiccation, and identified minimal amino acid motifs within the protein that harbor the
98 biochemical properties to confer desiccation resistance. These results provide insights into the
99 *in vivo*, endogenous functions of an LEA protein in a multicellular animal.

100

101 **Results**

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

103 We were motivated to determine the role of LEA-1 in the endogenous organismal
104 context of *C. elegans*. Previous studies have relied on RNAi for gene knockdown and did not
105 fully eliminate *lea-1* mRNA [10] or noted that RNAi seldom fully eliminates the target mRNA [29].

106 One recent study used a deletion allele, but only confirmed the phenotype of desiccation
107 sensitivity [30]. Furthermore, there are many isoforms of LEA-1 (Fig. 1), and the potential for
108 alternative isoform usage makes mutant analysis difficult to interpret. To address these
109 concerns, we created a full-length, 15.8 kb deletion of the *lea-1* locus via CRISPR, and we also
110 cleaned the genetic background of a second, pre-existing mutant allele, *lea-1(tm6452)*, by
111 outcrossing it to wild-type worms for 5 generations [10, 29, 33, 34]. For the full-length deletion
112 (*lea-1Δ*), the deleted gene was replaced with a *pmyo-2*::GFP::*myo-2 3'-UTR* construct as a
113 reporter for the deletion (Fig. 1). The successful deletion was confirmed via visualization of
114 pharyngeal GFP as well as by PCR (Fig. S1A-D).
115
116 ***lea-1* deletion mutants are sensitive to multiple stresses**
117 LEA proteins are most well known for promoting survival during desiccation. Because
118 worms are reported to survive desiccation only when in the dauer state [35], and *daf-2(e1370)*
119 offers a temperature-sensitive constitutive dauer phenotype when worms are grown at 25 ºC
120 [36], we chose to conduct the majority of our experiments in this *daf-2* background to facilitate
121 consistent production of dauer worms. In a *daf-2(e1370)* background, we confirmed that both
122 *lea-1Δ* and *lea-1(tm6452)* dauer worms were sensitive to desiccation at high relative humidity
123 (97.5%) and at a more stringent desiccation stress of 60% humidity (Fig. 2A), consistent with
124 published results [10, 29, 30]. Additionally, dauer worms of each of these mutant strains were
125 sensitive to osmotic stress in a concentrated salt solution. While there were no differences in
126 survival in worms kept in water for 2 hr, both *lea-1Δ* and *lea-1(tm6452)* worms had significantly
127 lower survival when exposed to 1M NaCl for 2 hr (Fig. 2B). Both mutants were also sensitive to
128 exposure to heat shock at 37 ºC (Fig. 2C).
129 LEA proteins have been demonstrated to prevent protein aggregation *in vitro* and in cell
130 culture [26, 37–39]. To test for *in vivo* roles in preventing protein aggregation, we assessed the
131 number of polyglutamine aggregates in body wall muscle of *lea-1Δ* animals. These animals

132 expressed tracts of 35 or 40 glutamines fused to a yellow fluorescent protein (YFP) reporter
133 [40]. Mutants with the Q35::YFP reporter did not have a statistically significant difference from
134 controls (p=0.22); however, *lea-1Δ* animals with a Q40::YFP reporter had significantly more
135 aggregates per worm than controls (p=0.02) after 5 days of development and dauer formation
136 (Fig. 2D). We conclude that LEA-1 has *in vivo* functions protecting animals against desiccation,
137 osmotic stress, heat stress, and formation of polyglutamine protein aggregates.
138

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

140 Working with a true null mutant allowed us to ask if there were any phenotypes
141 associated with deletion of *lea-1* in the context of normal development and physiology. We
142 tested effects on lifespan and brood size as especially sensitive quantitative proxies for diverse
143 effects on physiology or development. Our results suggest that LEA-1 functions specifically in
144 response to stress: In a wild-type (N2) background we did not observe any significant
145 differences in lifespan or brood size of *lea-1Δ* animals (Fig. 3A,B). We also did not observe
146 significant differences in polyglutamine aggregation during aging in these worms when
147 maintained under normal laboratory conditions (Fig. S2). These results indicate that LEA-1 may
148 function primarily during the dauer stage and in response to conditions of external stress.
149

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

151 To examine where LEA-1 is expressed *in vivo*, we used fluorescent monomeric
152 NeonGreen (mNG) and monomeric yellow fluorescent protein (mYPET) tags to label
153 endogenous LEA-1 at two different positions [41, 42]. Sequence for mNG as well as a 3x FLAG
154 and auxin inducible degron sequence were inserted in the N-terminal region of the gene (Fig. 1).
155 Similarly, sequence for mYPET and 3x FLAG were added to the C-terminus of LEA-1 (Fig. 1).
156 As with the *lea-1Δ* mutant, these edits were confirmed via visualization of fluorescence and
157 PCR (Fig. S1E). When desiccated, animals expressing the fluorescent tags survived equally as

158 well as *daf-2* control animals (Fig. 4A). We also confirmed that the fluorescent tags did not
159 significantly impact osmotic stress survival in 1M NaCl for 2 hr (Fig. S3A). We conclude that
160 introduction of either fluorophore did not disrupt functions of the endogenous LEA-1 protein.
161 LEA-1 protein is known to be upregulated in response to desiccation [10, 29]. However,
162 there are many predicted isoforms of LEA-1 (Fig 1), and it is unclear whether all isoforms are
163 upregulated or whether all of LEA-1's protective roles involve such upregulation. To examine the
164 diversity of isoforms that are actively expressed in different conditions we lysed cultures of
165 mixed stage worms, dauer worms, and desiccated dauers to blot with an anti-FLAG antibody to
166 recognize the 3x FLAG epitope appended to each fluorophore. Numerous isoforms appeared
167 tagged with the 5' mNG tag, while fewer isoforms of distinct sizes carrying the 3' mYPET tag
168 were prominent (Fig. 3B). Isoforms labeled with the 5' mNG tag but not the 3' mYPET tag were
169 found to be upregulated during desiccation. These changes in expression were confirmed by
170 quantification of *in vivo* fluorescence intensity in dauer and desiccated worms. Fluorescence
171 from the mNG reporter was significantly increased after 4 days of desiccation at 97.5% relative
172 humidity (Fig. 4C-E). Expression of mNG::LEA-1 was diffuse throughout the body during
173 desiccation (Fig. 4D). In contrast, mYPET fluorescence level was not significantly altered during
174 desiccation (Fig. 4F-H), consistent with the Western blot results. For both reporters,
175 fluorescence intensity was not changed in response to 2 hr of osmotic shock in 1M NaCl (Fig.
176 S3B-G). We conclude that only some isoforms of LEA-1 are upregulated in response to
177 desiccation, and that *lea-1*'s response to osmotic shock does not appear to involve a similar
178 upregulation.
179
180 **Depletion of LEA-1 in body wall muscle reduces survival of desiccation and osmotic**
181 **stress**
182 To examine the localization of upregulated LEA-1 isoforms in the stage when LEA-1 has
183 a protective role, we visualized mNG::LEA-1 fluorescence in dauer larvae. We found that the

184 protein was prominently expressed in specific tissues: the germline, body wall muscle, pharynx,
185 and excretory cell (Fig. 5A-D). mNG::LEA-1 was localized to both the excretory cell body, which
186 is adjacent to the pharynx (Fig. 5D), as well as excretory canals, which extend laterally along
187 the length of the body (Fig. 5A). Some faint expression was seen in seam cells (Fig. 5B), as well
188 as the intestine (Fig. 5C). Additionally, some unidentified cells in the head were fluorescent. The
189 expression and localization of LEA-1 to particular tissues in dauer worms suggested that its
190 function might be required in only some tissues or required at higher levels in some tissues than
191 in others.

192 To determine the sites of action of LEA-1, we utilized auxin-induced degradation to
193 deplete protein. We used the auxin-induced degron (AID) sequence that we included on the
194 mNG::3xFLAG tag as a target for protein degradation when the modified F-box protein TIR1 is
195 expressed and worms are exposed to auxin [43]. Expression of TIR1 under the control of
196 different promoters allows for tissue-specific conditional depletion [44]. We used a panel of
197 worms expressing TIR1 in the various tissues in which LEA-1 was expressed to determine the
198 tissue or tissues in which LEA-1 was required for survival of desiccation and osmotic stress (Fig.
199 5E) [44]. These experiments were conducted in a *daf-2* mutant background, for which
200 expression patterns of LEA-1 were not noticeably different from a WT (N2) background (Fig.
201 S4A). When exposed to auxin, dauer worms showed depletion of mNG::LEA-1 in the expected
202 locations for each promoter used: the intestine (using the *ges-1* promoter, called *ges-1*p),
203 germline (*mex-5*p), pharynx (*myo-2*p), body wall muscle (*myo-3*p), and a combination of
204 intestine, excretory cell and canal, germline, and some other cells in the head (*vha-8*p) (Fig.
205 S4). Expression of TIR1 with a *col-10* promoter to target hypodermis did not seem to have a
206 significant effect on expression, consistent with the apparently minimal baseline hypodermal
207 expression of mNG::LEA-1 (Fig. S4C). Additionally, the *myo-2*p::TIR1 strain depleted some
208 pharyngeal mNG::LEA-1 protein, but did not entirely eliminate expression, particularly in the
209 posterior bulb of the pharynx (Fig. S4F).

210 When exposed to desiccation at 97.5% relative humidity, no TIR1 expression strain had
211 significantly reduced survival relative to controls (*daf-2*) or worms expressing mNG::LEA-1 alone
212 (Fig. 5F). However, when exposed to 60% relative humidity, *myo-3*p::TIR1 worms had
213 significantly reduced survival. This suggests that LEA-1 is required in the body wall muscle to
214 promote desiccation resistance. When exposed to osmotic stress with 1M NaCl for 4 hrs, we
215 observed a similar result. *lea-1Δ* mutants and *myo-3*p::TIR1 worms had significantly reduced
216 survival compared to control (*daf-2*) and mNG::LEA-1-expressing worms (Fig. 5G). Collectively,
217 these results suggest that LEA-1 synthesized in the body wall muscle is required for survival of
218 multiple stresses, and that other tissues do not require high levels of the tagged LEA-1 isoforms
219 for survival of animals.
220
221 **LEA-1 minimal motifs confer desiccation resistance to bacteria**
222 Having described *in vivo* roles for an animal LEA protein and its localized expression
223 and function, we sought to identify the protein's protective domains. Given the variety of
224 isoforms of *lea-1* in *C. elegans* that are expressed during desiccation (Fig. 1, Fig. 4B), before
225 testing specific domains *in vivo* we employed a heterologous expression approach to efficiently
226 test if different isoforms improved desiccation tolerance in *E. coli* equally, or if some were more
227 effective than others. We selected isoforms A, D, E, F, and K for expression because they have
228 a combination of overlapping and non-overlapping sequence to allow for deduction of regions of
229 interest in the case that some isoforms are more effective at conferring desiccation resistance
230 (Fig. 6A). We confirmed robust expression of each of the 5 different bacterially codon-optimized
231 isoforms in BL21 *E. coli* and found that expression of each isoform conferred stress resistance
232 to the bacteria, relative to cells expressing GFP (Fig. 6B, Fig. S5). Expression of a truncated
233 version of GFP or actin (*C. elegans act-2*) did not increase desiccation resistance of bacteria
234 (Fig. 6B).

235 Because all five isoforms tested were sufficient to improve bacterial desiccation
236 resistance, we hypothesized that motifs shared by these isoforms might be sufficient for
237 conferring bacterial desiccation resistance. Group 3 LEA proteins commonly have repeated
238 motifs of 11 amino acids that are capable of protecting heterologous cells against stresses
239 including low pH, high salt, and desiccation-induced protein aggregation [8, 21, 39, 45–48].
240 These motifs are predicted to form alpha helices, and circular dichroism of LEA proteins and
241 motif-containing peptides has confirmed alpha helix formation during conditions when water is
242 limiting [17, 18, 49]. We used MEME Suite to detect a repeated 11-mer from the protein
243 sequence of one of the longest *C. elegans* isoforms, LEA-1K [50]. An 11-mer was found that is
244 predicted to form an amphipathic alpha-helix (Fig. 6C). When search parameters were relaxed
245 to include motifs of other lengths, a 97-mer peptide encompassing multiple 11-mers was also
246 identified. In the 1397 amino acid protein, there were five occurrences of the 97-mer and 61
247 instances of the 11-mer (Fig. 6D). To determine if these motifs alone from *C. elegans* LEA-1
248 were sufficient to improve desiccation tolerance, we expressed codon-optimized versions of
249 these idealized motifs in *E. coli*. We included the base 11-mer, repeats containing two (22-mer)
250 and four (44-mer) copies of its sequence, and the 97-mer. Expression of each of these minimal
251 motifs significantly improved desiccation survival of the bacteria (Fig. 6E). Thus, it is likely that
252 any of LEA-1's isoforms that contain such repeats would improve survival of desiccation.
253

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

255 Because motifs of LEA-1 improved desiccation survival of bacteria, we were motivated
256 to test if minimal motifs of LEA-1 are sufficient for stress tolerance *in vivo*. To specifically
257 determine if minimal LEA-1 motifs are sufficient for desiccation tolerance in *C. elegans*, we
258 deleted nearly all possible exons of *lea-1* and replaced them with sequence encoding single
259 codon-optimized 44-mer or 97-mer motifs fused to mNG to allow visual confirmation of
260 expression (Fig. 7A). Genomic sequence encoding the most N-terminal predicted exons for

261 some isoforms (like the short N-terminal exons of isoforms K and N as shown in Fig. 7A) was
262 left intact because these regions likely overlap with the promoter region or other regulatory
263 sequence. These short predicted exons do not encode any 11-mer motifs. We confirmed
264 genomic edits by PCR genotyping (Fig. S6A,B). We also determined that the expression
265 patterns of each inserted motif::mNG fusion are similar to the overall pattern observed for
266 tagged versions of the endogenous protein, suggesting that these genomic edits did not
267 significantly disrupt gene regulation (Fig. S6C,D).

268 Expression of either the 44 amino acid motif or 97 amino acid motif was sufficient to
269 replace full-length LEA-1's role in promoting desiccation tolerance of animals at 97.5% relative
270 humidity (Fig. 7B). When exposed to 60% relative humidity, the protective capacity of these
271 motifs was limited, and survival was reduced to levels comparable to *lea-1Δ* mutants.
272 Expression of each motif in lieu of the complete LEA-1 protein was also sufficient to promote
273 survival of osmotic stress in 1M NaCl (Fig. 7C). Survival of motif-expressing animals was
274 indistinguishable from controls and significantly improved relative to *lea-1Δ* mutants. These
275 results indicate that short repetitive motif sequences likely account for much of the functionality
276 of the full length LEA-1 protein during osmotic stress and desiccation.

277

278 **Discussion**

279 LEA proteins are commonly found in animals with interesting extremotolerant abilities
280 but with limited genetic tools. Therefore, a common approach has been to study LEA proteins
281 (and fragments thereof) in heterologous systems [20, 21, 23, 47, 48, 51–54]. While such
282 approaches have revealed many potential functions of LEA proteins, there has been limited
283 evidence to bridge protective abilities observed *in vitro* and in heterologous systems to the
284 endogenous, *in vivo* context in an animal [10, 29, 55]. Therefore, we were motivated to use *C.*
285 *elegans* as a model to test for *in vivo* functions of LEA-1 in its native context.

286 Previous studies of LEA-1 in *C. elegans* have often relied on RNAi phenotypes [10, 29].
287 We generated a true null mutant (*lea-1Δ*) that lacks the entire gene, in order to eliminate the
288 possibility of residual LEA-1 expression or function. Comparison of this mutant to the insertion
289 and deletion mutant *lea-1(tm6452)* reveals similar sensitivities to a variety of stresses, including
290 desiccation, osmotic stress, and heat. Importantly, these phenotypes were assessed in dauer
291 larvae, in which LEA-1 is expressed more highly at both the level of mRNA and protein [10, 35].
292 DUR-1, the other group 3 LEA protein of *C. elegans*, is also upregulated in dauer larvae, but is
293 apparently unable to compensate for the loss of LEA-1 [29]. LEA-1 does not significantly impact
294 lifespan or brood size (Fig. 3), which demonstrates the importance of the protein specifically in
295 the context of stress resistance. Similarly, accumulation of polyglutamine protein aggregates
296 was exacerbated by deletion of LEA-1 in dauer worms, but not in normally aging worms (Fig.
297 2D, Fig. S2). Effects of LEA-1 on protein aggregation have previously been demonstrated *in*
298 *vitro* and in heterologous contexts [26, 37, 56]. We present evidence that LEA-1 can affect
299 proteostasis *in vivo* in a multicellular animal. It would be fascinating to further explore any
300 differential effects of LEA-1 with other types of aggregation-prone proteins and in tissues
301 beyond the body wall muscle. Because LEA-1 is expressed highly in body wall muscle and is
302 required there for desiccation survival and osmotic stress resistance, it is possible that
303 improving proteostasis in this tissue contributes to survival of these stresses.
304 Adding endogenous, single-copy fluorescent tags to LEA-1 without disrupting LEA-1
305 function revealed the sites of its expression and *in vivo* dynamics in response to stress. In dauer
306 larvae, mNG-tagged LEA-1 is expressed most notably in the germline, body wall muscle,
307 pharynx, excretory cell, as well as some unidentified cells in the head. Expression patterns were
308 similar in worms tagged with mYPET, although some sites of expression were more faint.
309 Surprisingly, blotting for the 3x FLAG epitope included in both mNG and mYPET tags revealed
310 significantly fewer isoforms tagged by mYPET than expected based on isoform predictions if all
311 isoforms were expressed at high levels (Fig. 1, Fig. 4B). It is possible that the C-terminal exons

312 are not actually included in as many isoforms as expected, or that some isoforms are expressed
313 at low levels. The difference in the number of prominent isoforms labeled by each tag is further
314 reflected in the significant increase in expression of mNG-labeled proteins but not mYPET-
315 labeled proteins during desiccation (Fig. 4B-H). The upregulation of mNG-tagged proteins does
316 not seem to be specific to a single isoform, but rather, multiple isoforms are expressed more
317 highly during desiccation (Fig. 4B). Therefore, the increased fluorescence seen *in vivo* likely
318 reflects the summation of these isoforms of varying sizes and compositions. LEA-1 has been
319 reported to be regulated by transcription factors including *daf-16*/FOXO and *skn-1*/Nrf, but
320 whether such regulation is tissue-specific or unique to some isoforms remains unexplored [57,
321 58]. Expression of LEA-1 in several tissues, and the upregulation of multiple isoforms led to two
322 questions: 1) In which tissue or tissues is LEA-1 required for stress resistance? 2) Are particular
323 isoforms more effective at conferring stress resistance?

324 To address the first question we utilized the auxin-inducible degradation system to
325 deplete LEA-1 in various tissues [44]. By expressing TIR1 under the control of different
326 promoters, we had control over the tissues in which proteins containing the auxin inducible
327 degron (AID) tag were degraded. The AID sequence was included on the mNG tag and
328 therefore should deplete the same isoforms containing the 3x FLAG tag (Fig. 4B). It is possible
329 that some predicted isoforms that are not tagged with the mNG construct could escape
330 degradation (for example isoforms C, D, and E, Fig. 1), although we do not know if such
331 isoforms are expressed or their relative abundance. Furthermore, even though we observed
332 decreased fluorescence in most cases (Fig. S4), depletion of AID-containing proteins may be
333 incomplete. Incomplete degradation could result from insufficient levels of TIR1 (based on the
334 strength of the promoter driving it) in tissues with high levels of LEA-1. For example, with *myo-*
335 *2p* driven TIR1, some expression of mNG::LEA-1 remained in the pharynx – particularly in the
336 posterior bulb (Fig S4E). Thus, we cannot rule out the possibility that LEA-1 is required in the
337 pharynx for survival of desiccation and osmotic stress. In contrast, depletion of LEA-1 in body

338 wall muscle resulted in significantly reduced survival of both desiccation and osmotic stress
339 (Fig. 5F,G). The consensus site of action from these two stresses lends confidence to the result.
340 However, given the caveats that some untagged isoforms may remain, and that there could be
341 incomplete degradation (even if fluorescence is depleted below detectable levels), we cannot
342 formally conclude that LEA-1 is not required in other tissues. Rather, we present evidence that
343 LEA-1 is required in at least body wall muscle to survive desiccation and osmotic stress. Our
344 data suggest that other tissues can survive at least with reduced levels of the LEA-1 isoforms
345 that are induced in dauer larvae.

346 To answer the second question of which LEA-1 isoforms are most functional in
347 conferring stress resistance, we initially employed a heterologous expression approach. *E. coli*
348 provides an ideal system to facilitate expression of proteins and rapidly screen for desiccation
349 resistance. The fact that multiple LEA-1 isoforms could improve desiccation tolerance of
350 bacteria turned our attention to conserved motifs contained within these isoforms. Similar motifs
351 from LEA proteins of other organisms have been demonstrated to function in stress resistance
352 [21, 39, 47, 48, 59]. These LEA motifs acquire secondary structure during desiccation-like
353 conditions and specifically are thought to form amphipathic alpha-helices [19, 49, 60]. The 11-
354 mer we identified is likely to form an amphipathic helix based on the amino acid composition
355 and locations as depicted in Fig. 6C. Although the amino acid identity is different from other
356 similarly identified LEA motifs, the putative structural similarities and charge distribution hint at
357 the relative importance of structure and charge distribution over sequence identity [12, 60].
358 Expression of the 11-mer alone was sufficient to improve bacterial desiccation survival. A longer
359 sequence (44-mer) marginally improved survival relative to the 11-mer ($p=0.02$, unpaired T-test,
360 $n=7$), similar to a 97-mer ($p=0.05$, unpaired T-test, $n=7$). Thus, an increased number of motif
361 repeats may improve survival. The importance of multiple motifs occurring in the same
362 contiguous peptide versus the possibility of oligomerization remains unclear. Notably, in bacteria

363 the expression levels of LEA proteins and motifs are likely much higher than in endogenous
364 contexts.

365 Inserting motifs into the endogenous *lea-1* locus of *C. elegans* allowed us to test if the
366 identified motifs are sufficient to confer desiccation *in vivo*. LEA motifs were highly effective
367 substitutes for the full-length protein for phenotypes of osmotic stress resistance and
368 desiccation at 97.5% RH (Fig. 7). This suggests that the function of LEA-1 can in large part be
369 explained by the presence of these amino acid repeats. Unique combinations of motifs or longer
370 contiguous sequences may allow for increased functionality – particularly in the context of more
371 severe desiccation. This may further explain the apparent complexity and variety of isoforms of
372 the protein (Fig. 1, Fig. 4B). If there is limited selective pressure for maintenance of a single long
373 contiguous sequence, then many functional exon combinations may arise. This could contribute
374 to the large number of proteins produced by this gene. An optimal number of motifs and the
375 most functional combination of motif variants appended to each other remain to be
376 characterized.

377 The ability of LEA motifs to function similarly in a single-celled prokaryote (*E. coli*) and a
378 multicellular eukaryote (*C. elegans*) encourages the possibility of engineering broadly functional
379 desicco-protectants. LEA proteins and motifs that can function in cells with fundamentally
380 different subcellular organization likely harbor basic biochemical properties that promote cell
381 and organismal survival. Relative to bacteria, multicellular animals face further challenges
382 during desiccation including coordination of a response across multiple tissue types and
383 protecting diverse organelles and subcellular compartments. *C. elegans* is a prime animal
384 model for continued study of these *in vivo* mechanisms of desiccation tolerance.

385

386 **Conclusions**

387 In summary, we created a true null mutant of LEA-1 to study its role *in vivo* and
388 characterized several phenotypes of stress sensitivity. LEA-1 is expressed in multiple tissues,

389 and it is required at high levels in body wall muscle to carry out its functions. We identified LEA-
390 1 motifs that are sufficient to improve bacterial desiccation survival. These motifs also function
391 in place of full length LEA-1 to promote desiccation tolerance *in vivo*. Future work is needed to
392 dissect the principles by which the peptide repeat sequences function. Still, the conserved
393 function of LEA-based peptides in a single-celled prokaryote and a multicellular animal suggests
394 the possibility of engineering LEA-1-derived peptides that broadly promote desiccation
395 tolerance.
396
397 **Methods**
398 *CRISPR editing in C. elegans*
399 To generate a deletion of *lea-1* and to insert mNG and mYPET fluorescent tags into the
400 endogenous gene locus, CRISPR methods were employed [33, 34]. Using the self-excising
401 cassette (SEC) system, sgRNA sequences were added to the sgRNA-Cas9 plasmid pDD162
402 using the NEB Q5 site-directed mutagenesis kit. Homology arms of ~500-700bp were added to
403 plasmids carrying the SEC and repair templates. To insert a myo-2p::GFP::myo-2 3'UTR
404 reporter to track the null deletion of *lea-1*, homology arms were cloned into plasmid pDD317. To
405 insert mNG or mPYET tags, homology arms were cloned into pUA77 and pDD283 respectively.
406 To replace the endogenous *lea-1* sequence with LEA motifs, sequence was first codon-
407 optimized for *C. elegans* and synthesized by Integrated DNA Technologies (IDT). These
408 stretches were cloned into pUA77 with the same upstream homology arm for insertion of mNG
409 to the endogenous locus and the same downstream homology arm for insetion of mYPET into
410 the endogenous locus. The same sgRNAs for each of the initial mNG and mYPET insertions
411 were used in combination to excise the *lea-1* locus.
412 Worms were injected with 50 ng/μL of plasmid containing the sgRNA and Cas9, and 10-
413 20 ng/μL plasmid containing the repair template, along with a co-injection mix [34]. 2-3 days

414 after injection worms were treated with Hygromycin and selected for transgene-carrying rollers
415 lacking red co-injection mix extra-chromosomal arrays. L1 worms were heat shocked at 32 °C
416 for 5 hr to excise the SEC. Genomic edits were confirmed by visualization of fluorescent
417 reporters and with PCR genotyping.
418 Construction of most TIR1-expressing strains is described in [44]. The *col-10p*::TIR1 line
419 was generated by cloning to combine the *col-10* promoter with the TIR1 construct of pDD356
420 (NEB Hifi Assembly Master Mix). Plasmid pAP082 was used to express Cas9 and a sgRNA to
421 target the chromosome I insertion site. Worms were injected with 50 ng/μL of pAP082 and 20
422 ng/μL plasmid containing the repair template.
423
424 *Genotyping*
425 Template genomic DNA was create by lysing worms in lysis buffer with proteinase K.
426 Worms were picked into 0.2mL tubes and briefly frozen at -80 ºC, then heated at 65 ºC for 1hr
427 followed by 95 ºC for 15 minutes. This lysate was used for PCR genotyping. Primers were
428 designed to confirm CRISPR genome modifications and to genotype the pre-existing allele *lea-*
429 *1(tm6452)* during backcrossing. Sequences of these primers can be found in Supplemental
430 Table 1. PCR genotyping was carried out using either Gotaq or Q5 High Fidelity Polymerase
431 (NEB). Annealing temperature and extension time were adjusted based on the melting
432 temperature of the primers and size of the amplicon.
433
434 *C. elegans maintenance*
435 Worms were maintained according to standard laboratory conditions on nematode
436 growth media (NGM) plates, fed OP50 *E. coli*, and stored at 20 ºC. Standard methods were
437 employed for crossing worms, and males were generated by keeping L4 worms at 32 ºC for 5-6
438 hr and 25 ºC overnight. The following strains and alleles were used: N2, *lea-1(tm6452)*

439 (backcrossed 5x into N2), AM140 *rmIs132[unc-54p::Q35::YFP]*, AM141 *rmIs133[unc-*
440 *54p::Q40::YFP]*, CB1370 *daf-2(e1370)*, LP847 *lea-1Δ(cp423[myo-2p::GFP::myo-2 3'UTR])*,
441 LP852 *daf-2(e1370);lea-1Δ(cp423[myo-2p::GFP::myo-2 3'UTR])*, LP858 *lea-1(cp431[mNG*::*3x*
442 *FLAG::AID::lea-1])*, LP859 *lea-1(cp430[lea-1::mYPET::3x FLAG])*, LP860 *daf-2(e1370)*;*lea-*
443 *1(cp431[mNG*::*3x FLAG::AID::lea-1])*, LP861 *daf-2(e1370)*;*lea-1(cp430[lea-1::mYPET::3x*
444 *FLAG])*, LP862 *rmIs132[unc-54p::Q35::YFP];lea-1Δ(cp423[myo-2p::GFP::myo-2 3'UTR])*,
445 LP863 *rmIs133[unc-54p::Q40::YFP];lea-1Δ(cp423[myo-2p::GFP::myo-2 3'UTR])*, LP865
446 cpSi171*[vha-8p::TIR1::F2A::mTagBFP::AID*::NLS];daf-2(e1370);lea-1(cp431[mNG*::*3x*
447 *FLAG::AID*::lea-1])*, LP866 cpSi172*[myo-2p::TIR1::F2A::mTagBFP::AID::NLS];daf-*
448 *2(e1370);lea-1(cp431[mNG*::*3x FLAG::AID*::lea-1])*, LP867 cpSi173*[col-*
449 *10p::TIR1::F2A::mTagBFP::AID*::NLS];daf-2(e1370);lea-1(cp431[mNG*::*3x FLAG::AID::lea-1])*,
450 LP868 cpSi174*[myo-3p::TIR1::F2A::mTagBFP::AID*::NLS];daf-2(e1370);lea-1(cp431[mNG*::*3x*
451 *FLAG::AID::lea-1])*, LP875 *daf-2(e1370);lea-1(tm6452, 5x backcrossed)*, LP876 *reSi5[ges-*
452 *1p::TIR1::F2A::BFP::AID*::NLS::tbb-2 3'UTR];daf-2(e1370);lea-1(cp431[mNG*::*3x*
453 *FLAG::AID::lea-1])*, LP877 *wrdSi18[mex-5p::TIR1::F2A::BFP::AID*::NLS::tbb-2 3'UTR];daf-*
454 *2(e1370);lea-1(cp431[mNG*::*3x FLAG::AID::lea-1])*, LP882 *rmIs132[unc-54p::Q35::YFP]*;*daf-*
455 *2(e1370);lea-1Δ(cp423[myo-2p::GFP::myo-2 3'UTR])*, LP883 *rmIs133[unc-54p::Q40::YFP];daf-*
456 *2(e1370);lea-1Δ(cp423[myo-2p::GFP::myo-2 3'UTR])*, LP885 *daf-2(e1370);lea-1(cp432[lea-*
457 *1p::44-mer::mNG::3x FLAG::AID]*, LP887 *daf-2(e1370);lea-1(cp433[lea-1p::97-mer::mNG::3x*
458 *FLAG::AID]*.
459
460 *C. elegans desiccation, heat, and osmotic stress*
461 *C. elegans* were desiccated according to a previously established protocol [29, 35].
462 Briefly, desiccation chambers were made with varying ratios of glycerol:water in order to
463 produce relative humidity (RH) of either 97.5% or 60%[61]. Dauer worms were formed by
464 moving *daf-2(e1370)* mutant embryos to 25º C. Dauer worms were picked into ~1.5-2 μL

465 droplets of water and initially exposed to 97.5% for 4 days as preconditioning. Worms were
466 either rehydrated in M9 or moved to 60% RH desiccation chambers for 1 day before recovery.
467 Survival was scored as the percentage of worms moving or responsive to physical stimulus.
468 Heat stress was implemented by exposing dauer worms to 37 ºC. Multiple plates
469 containing dauer larvae were moved to a 37 ºC incubator for defined periods of time. Worms
470 were scored for survival based on movement or responsiveness to physical stimulus.
471 To measure osmotic stress tolerance, dauer worms were added to water or 1M NaCl in
472 a 96-well or 24-well plate for 2 hr or 4 hr. Worms were then transferred to unseeded NGM plates
473 and survival was scored by movement or responsiveness to physical stimulus.
474
475 *C. elegans lifespan and brood size*
476 For lifespan assays 10 L1 worms per plate were picked to an NGM plate. For each
477 genotype five plates of ten worms were included to start each experiment. Worms were
478 transferred to new plates every other day until they were no longer reproductive. Worms that
479 crawled onto the sides of the dishes or were otherwise missing were censored. Survival was
480 scored every other day by movement or responsiveness to touch with a platinum wire. Three
481 independent biological replicates were conducted.
482 To determine brood size, individual embryos were added to NGM plates. 15 worms were
483 included to start in each of three experiments. Worms were transferred to new plates every
484 other day. After transfer, progeny on plates were allowed to develop for ~24 hr and were then
485 counted. Total brood was calculated as the sum of all progeny produced by an individual worm.
486 Worms that failed to hatch or crawled off the plate were censored.
487
488 *Polyglutamine protein aggregation*
489 Strains AM140 and AM141 express polyglutamine tracts of 35 or 40 repeated glutamine
490 residues fused to YFP [40]. These strains were crossed with *lea-1Δ* (LP847). To assess protein

491 aggregation during aging the worms were picked to plates and allowed to develop for 4 or 8
492 days. Worms were periodically transferred to fresh plates to separate them from progeny. The
493 number of aggregates per worm was scored by imaging worms and counting the number of
494 fluorescent puncta in the body wall muscle of each worm.

495 To determine the number of aggregates in dauer worms the AM140 and AM141
496 reporters were crossed into *daf-2(e1370);lea-1Δ* (LP852) to put the reporter in a *daf-2* mutant
497 background. Dauer formation was induced as before by growth at 25 ºC. The number of
498 aggregates per worm was counted after 5 days (including development and arrest as dauer
499 larvae).

500

501 *Fluorescent imaging and quantification*

502 Worms expressing mNG::LEA-1 or LEA-1::mYPET were imaged on a Nikon TiE stand
503 with CSU-X1 spinning disk head (Yokogawa), 514 nm solid state laser, and ImagEM
504 EMCCD camera (Hamamatsu). Metamorph software was used for image acquisition. To
505 quantify fluorescence in control dauers, desiccated dauers, and dauers exposed to 1 M
506 NaCl for 2 hr, worms were imaged with a 10x objective. Images were imported into FIJI
507 for analysis. Whole worms were outlined manually. The total fluorescence intensity was
508 measured. The outline of the worm was moved to background area of the image to
509 obtain a background measurement. The fluorescence intensity was calculated by
510 subtracting the background from that of the worm. For representative images of worms
511 higher magnification objectives (20x or 60x) were used.

512

513 *Western blotting*

514 Protein for western blotting was obtained from worms expressing mNG::3x FLAG::LEA-1
515 or LEA-1::mYPET::3x FLAG. Mixed stage populations were washed from 3-6 NGM plates,

516 dauer worms from plates at 25 ºC, and desiccated worms from plastic dishes at 97.5% RH for 4
517 days. Large quantities (>20,000) of synchronous worms were obtained for dauer formation and
518 desiccation by standard hypochlorite treatment. Worms were collected, washed in M9, pelleted,
519 and resuspended in ~100 µL lysis buffer containing 50 mM HEPES (pH 7.4), 1mM EGTA, 1mM
520 $MgCl_2$, 100 mM KCl, 10% glycerol, 0.05% NP-40, DTT, and an EDTA-free protease inhibitor
521 tablet (added fresh to 12 mL of buffer) [62]. Worms in lysis buffer were briefly frozen at -80 ºC,
522 then sonicated on ice with a Branson Sonifier 250 wand sonicator at 50% duty until no intact
523 worms remained (2-5 minutes in individual bouts of no longer than 1-2 minutes). The
524 concentration of protein in lysates was determined with the Biorad Protein Assay (Bradford)
525 according to specifications, measuring the OD595 in a total volume of 1mL in cuvettes.
526 A 4-12% Bis-Tris NuPAGE minigel (Invitrogen), loaded with 10 µg of protein in sample
527 buffer (3x concentrate contains 6% SDS, 240mM Tris pH 6.8, 30% glycerol, 0.04% w/v
528 Bromophenol blue, and 50 µL 2-ME per mL) was run in 1x NuPAGE MOPS running buffer
529 (Novex). 10 µL of Precision Plus Protein Kaleidoscope Standard (Biorad) was loaded in lanes
530 adjacent to samples. Gels were run at 140V for 90 minutes. Protein was transferred to a PVDF
531 membrane activated in methanol at 90V for 90 min (in NuPAGE transfer buffer, at 4 ºC).
532 Membranes were rinsed in PBST, and blocked in PBST + 5% BSA for 1 hr with rocking.
533 Membranes were soaked in a 1:1000 dilution of primary antibody (mouse anti-FLAG, Sigma Cat
534 #F1804) overnight at 4 ºC with rocking. Membranes were washed 3x in PBST, then soaked in a
535 1:5000 dilution of secondary antibody (goat anti-mouse IgG, Invitrogen Cat #31432) for 1 hr with
536 rocking. Membranes were washed 3 times and visualized after addition of SuperSignal West
537 Pico PLUS Chemiluminescent Substrate (Thermo Scientific).
538 After initial visualization, membranes were stripped and re-blotted for tubulin as a
539 loading control. Membranes were incubated in mild stripping buffer (15 g glycine, 1 g SDS, 10
540 mL Tween20, bring volume to 1 L with H_20, pH 2.2) for ten minutes with rocking. Buffer was
541 replaced, and allowed to soak for another ten minutes. Membranes were then washed 3x in

542 PBST, blocked for 1 hr in PBST with 5% BSA, and soaked in a 1:500 dilution of primary
543 antibody (rat anti-tubulin, Invitrogen Cat #MA1-80189) overnight at 4 ºC. After washing 3x in
544 PBST, secondary antibody (goat anti-rat IgG, Thermo Fisher Cat #31470) was added in a
545 1:5000 dilution and membranes soaked for 1 hr with rocking and visualized as before. A single
546 band was detected indicating antibody specificity for tubulin.
547
548 *Protein depletion with auxin-induced degradation*
549 Worms expressing TIR1 under various promoters were grown on plates containing 1mM
550 auxin (indole-3-acetic acid) [43]. Embryos or L1s were picked to NGM + auxin plates and
551 allowed to develop into dauer larvae at 25 ºC for at least 5 days before exposure to desiccation
552 or osmotic stress. Degradation of LEA-1 tagged with mNG::3x FLAG::AID was assessed by
553 imaging worms.
554
555 *Heterologous expression in bacteria*
556 A bacterial codon-optimized version of *lea-1* isoform K was synthesized by Genewiz.
557 Isoforms A, D, E, and F were subcloned from that construct. 30bp of homology was added by
558 PCR to expression constructs, and they were cloned into a PCR-linearized pDest17 using NEB
559 Hifi Assembly Master Mix. Similarly, to clone motifs, 30bp homology was added synthesized
560 codon-optimized DNA fragments (from Integrated DNA Technologies), and they were cloned
561 into PCR-linearized pDest17 with NEB HiFi Assembly Master Mix. GFP and truncated GFP
562 controls were cloned by the same method, using plasmid pFCcGi as a template to obtain the
563 GFP sequence [63]. The *C. elegans act-2* gene was also cloned as a control from cDNA. All
564 plasmids were transformed into NEB 5-alpha cells and correct inserts were verified by
565 sequencing.
566 For desiccation experiments, plasmids containing expression constructs were
567 transformed into *E. coli* BL21 A1 (Invitrogen). Individual colonies were picked into 3 mL LB with

568 100 µg/mL ampicillin (Amp) and grown overnight (~12-16 hrs) at 37 ºC in a shaker incubator.
569 Cultures were then diluted 1:20 and 0.2% L-arabinose was added to induce protein expression
570 for 4 hr. Protein expression was confirmed with a Coomassie gel. Bacteria were pelleted and
571 briefly boiled to generate a lysate. Protein concentration was quantified using the Biorad Protein
572 Assay (Bradford) according to specifications. From these protein concentrations an appropriate
573 volume was determined to load 20 ug of total protein per well. 5 µL of pageruler prestained
574 ladder was loaded. A 4-12% BT NuPAGE minigel was run in 1x NuPAGE MOPS running buffer.
575 The gel was stained with Coomassie and de-stained overnight. GFP-expressing bacteria
576 provide a convenient visual confirmation of protein expression in each experiment.
577 To obtain equivalent numbers of starting bacteria to measure desiccation survival,
578 OD600 of 4 hr induced cultures was measured and volumes equivalent to an OD600 of 1.5
579 were added to 1.5 mL Eppendorf tubes. Bacteria were washed once in 0.85% NaCl. Bacteria
580 were then resuspended in 1 mL 0.85% NaCl. A 20 µL sample was removed and used for 10-fold
581 dilution of the culture. A 10-fold dilution series was plated on LB + Amp agar plates by spotting
582 10 µL. These control plates were grown at 37 ºC overnight. The remaining 980 µL supernatant
583 was aspirated and tubes were desiccated in a speedvac overnight (~12-16hr). Bacteria were
584 rehydrated in 980 µL of LB + Amp and given 1-2 hr to recover before plating a 10-fold dilution
585 series, as with controls the day before. Bacteria on plates were grown overnight at 37 ºC.
586 Survival was calculated by dividing cfu of each desiccated sample by its control.
587
588 *Motif analysis*
589 The MEME tool of the MEME suite (http://meme-suite.org/tools/meme) was used to
590 identify conserved motifs within LEA-1 [50]. The protein sequence of Isoform K was used as a
591 template since it contains nearly all possible exons of the protein. Position weight matrices,
592 consensus motifs, and distribution of motifs throughout the protein were obtained from this
593 analysis. The pepwheel program (https://www.bioinformatics.nl/cgi-bin/emboss/pepwheel) was

594 used to generate the diagram of the putative alpha-helical structure formed by the 11-mer.
595 Coloration of amino acids was added by hand.
596
597 *Statistical analysis*
598 Statistical tests were carried out as described in figure legends. Unpaired t-tests were
599 conducted in Microsoft Excel and ANOVAs were conducted in RStudio. Lifespan analysis was
600 conducted using GraphPad Prism.
601
602 **Acknowledgements**
603 We thank members of the Goldstein lab for helpful discussions and feedback. Ari Pani
604 offered guidance on plasmid design and CRISPR editing. Priya Hibshman provided advice and
605 assistance with Western blotting. Rob Dowen provided a plasmid from which the promoter of
606 *col-10* was cloned. JDH was supported by the National Institutes of Health (NIH)
607 1F32GM131577. This work was also supported by NSF grant IOS 2028860 awarded to BG.
608 Some strains were provided by the CGC, which is funded by NIH Office of Research
609 Infrastructure Programs (P40 OD010440). The *tm6452* allele of *lea-1* was obtained from the
610 National Bioresource Project in Tokyo, Japan [64].
611
612 **Availability of Data and Materials**
613 *C. elegans* strains generated in this study will be deposited to the *Caenorhabditis*
614 *Genetics Center*. Plasmids and other reagents will be made available upon request.
615
616 **Declarations**
617 The authors declare that they have no competing interests.
618
619

620 **Figures**

621 Figure 1. Predicted isoform structure of LEA-1 and generation of a null allele and two
622 endogenous fluorescent tags. Isoform annotations were combined from the UCSC genome
623 browser (http://genome.ucsc.edu) from the Feb. 2013 release (WBcel235/ce11) and Wormbase
624 version WS278. Locations for the existing tm6452 indel mutation, the newly created CRISPR
625 null mutation, and the insertion of mNeonGreen and mYPET fluorescent tags are indicated.

626

627 Figure 2. *lea-1* is required for tolerance of desiccation and osmotic stress. **A)** Dauer larvae of
628 *lea-1Δ* and *lea-1(tm6452)* mutants are sensitive to desiccation. Survival is significantly reduced
629 in worms exposed to 97.5% relative humidity for 4 days (p=0.0002, p=0.0001 respectively, n=4,
630 unpaired T-test) as well as 60% for 1 day after 4 days at 97.5% ($p=2.5x10^{-10}$, $p=1.8x10^{-10}$
631 respectively, n=4, unpaired T-test). Data points represent results from individual experiments
632 and bars represent mean survival. **B)** Dauer larvae of *lea-1Δ* and *lea-1(tm6452)* mutants are
633 sensitive to osmotic stress in 1M NaCl for 2 hr ($p=8.8x10^{-8}$, $p=6.1x10^{-6}$ respectively, n=4,
634 unpaired T-test). Data points represent results from individual experiments and bars represent
635 mean survival. **C)** Dauer larvae of *lea-1Δ* and *lea-1(tm6452)* mutants are sensitive to heat stress
636 at 37°C (p<0.0001 for each mutant allele relative to control, n=4-5 replicates per timepoint, 2-
637 way ANOVA). Lines depict mean survival and error bars represent SEM. Data points depict
638 survival from individual experiments. **D)** *lea-1Δ* mutants have a higher number of polyglutamine
639 protein aggregates. The total number of aggregates in the body wall muscle of individual worms
640 from three independent biological replicates is shown. Thick bars indicate the mean number of
641 aggregates and error bars depict 95% confidence intervals. Worms carrying a transgene
642 expressing a 35-glutamine repeat (Q35) with a YFP for visualization have only marginally more
643 aggregates than controls (p=0.22, n=3, unpaired T-test), while worms carrying a Q40::YFP
644 transgene have significantly more aggregates than controls (p=0.02, n=3, unpaired T-test).

645 Note, all worms were in a *daf-2(e1370)* background to facilitate dauer formation at 25°C. *
646 indicates p<0.05, *** indicates p<0.001.
647
648 Figure 3. Phenotypes of LEA-1 are specific to conditions of stress. **A)** Lifespan is not
649 significantly altered in either *lea-1Δ* or *lea-1(tm6452)* mutants in a WT (N2) background. **B)**
650 There are no significant differences in brood size of *lea-1Δ* (p=0.97, n=3, unpaired T-test) or *lea-*
651 *1(tm6452)* mutants (p=0.41, n=3, unpaired T-test) relative to N2 worms. Brood size of individual
652 worms from independent replicates are shown. The mean is also shown and error bars
653 represent 95% confidence intervals. Lifespan and brood size experiments were carried out in a
654 WT (N2) background.
655
656 Figure 4. Functional endogenous fluorescent tags reveal increased LEA-1 protein expression of
657 multiple isoforms during desiccation. **A)** Endogenous fluorescent tags do not disrupt the function
658 of LEA-1 in desiccation survival. *lea-1Δ* are sensitive to desiccation at 97.5% relative humidity
659 ($p=6.6x10^{-10}$, n=4, unpaired T-test) and 60% relative humidity (p=0.0002, n=4, unpaired T-test).
660 Survival of mNeonGreen tagged LEA-1 worms (mNG::*lea-1*) and mYPET tagged worms (*lea-*
661 *1*::mYPET) was not significantly different from *daf-2(e1370)* controls after exposure to 97.5% or
662 60% RH. **B)** Protein samples extracted from of mixed stage cultures, dauer worms, and
663 desiccated worms were blotted with an anti-FLAG antibody indicates multiple isoforms tagged
664 by the two independent tags. The membrane was stripped and blotted for tubulin as a loading
665 control. Total protein in each lane was quantified and normalized to the tubulin loading control.
666 Normalized proteins expression measurements, relative to mixed stage mNG::*lea-1* animals,
667 are listed at the bottom of each lane (Norm. Exp.). **C)** A representative dauer worm expressing
668 mNG::*lea-1*. **D)** A representative desiccated dauer larvae expressing mNG::*lea-1*. **E)** mNG
669 fluorescence is significantly increased in desiccated worms relative to non-desiccated controls
670 (p=0.001, n=3 replicates with 14-15 worms per condition, unpaired T-test). **F)** A representative

671 dauer worm expressing *lea-1*::mYPET. **G)** A desiccated dauer expressing *lea-1*::mYPET. **H)**
672 Fluorescent intensity is not significantly different between control and desiccated dauer worms
673 expressing *lea-1*::mYPET (p=0.39, n=3 replicates with 15 worms per condition, unpaired T-test).
674 Note that all worms were in a *daf-2(e1370)* background. ** indicates p<0.01, *** indicates
675 p<0.001.
676
677 Figure 5. LEA-1 is required in body wall muscle to survive desiccation and osmotic stress. **A-D)**
678 Representative images of an mNG::*lea-1* worm depict the major sites of expression. There is
679 prominent fluorescence in the germline, body wall muscle, excretory cell, and pharynx. There is
680 also some apparent expression in seam cells (B) and faint fluorescence in the intestine (C). A
681 zoomed in image of the excretory cell is shown in (D). **E)** A cartoon depicts tissues in which
682 LEA-1 is expressed that were targeted for protein depletion by driving TIR1 under the control of
683 different promoters. **F)** Desiccation survival is plotted for worms with tissue-specific LEA-1
684 depletion by expression of TIR1 under various promoters and exposure to 1mM auxin.
685 Depletion of the mNG::*lea-1* protein utilizing auxin-induced degradation reduces desiccation
686 survival in worms expressing TIR1 under a *myo-3* (body wall muscle-specific) promoter relative
687 to controls (p=0.0001, n=3, unpaired T-test) and worms expressing only mNG::*lea-1* and not
688 TIR1 (p=0.001, n=3, unpaired T-test). No other site of TIR1 expression significantly altered
689 desiccation survival at 60% RH. **G)** Survival of osmotic stress in 1M NaCl for 4 hrs is plotted for
690 the same strains as in (E). *lea-1Δ* mutants and worms expressing TIR1 under a *myo-3* promoter
691 were the only two strains that with significant differences in survival relative to both control and
692 mNG::*lea-1*-expressing animals (*lea-1Δ*: vs. control p=0.006, vs. mNG::*lea-1* p=0.01; *myo-3p*:
693 vs. control p=0.0001, vs. mNG::*lea-1* p=0.0009, unpaired T-tests, n=3). Note, the images in A-D
694 show a worm with an N2 background, whereas worms in (E) and (F) are in a *daf-2(e1370)*
695 background (control). ** indicates p<0.01, *** indicates p<0.001.
696

697 Figure 6. Heterologous expression of *C. elegans lea-1* isoforms and motifs improves bacterial
698 desiccation survival. **A)** Select isoforms of LEA-1 used for bacterial expression. **B)** Desiccation
699 survival of BL21 *E. coli* expressing codon-optimized *C. elegans* LEA-1 isoforms. Heterologous
700 expression of each isoform increased desiccation survival (Isoform A $p=2x10^{-5}$, Isoform D
701 p=0.0002, Isoform E p=0.003, Isoform F $p=9x10^{-5}$, Isoform K $p=7x10^{-7}$, unpaired T-tests vs.
702 GFP, n=6). **C)** A consensus 11-mer motif found in LEA-1 likely forms an amphipathic alpha
703 helix. The position weight matrix of amino acids in the motif is shown, as well as a wheel
704 diagram depicting the relative position of each consensus amino acid in an alpha helical
705 conformation. **D)** Consensus amino acid sequences are shown for the 11-mer as well as a 97-
706 mer motif that was also detected with an expanded motif window size. The frequency and
707 distribution of occurrences of these motifs within isoform K are shown. **E)** Desiccation survival of
708 BL21 *E. coli* is significantly increased by expression of motifs of LEA-1. Codon-optimized
709 sequences for the 11-mer, as well as concatenated repeats of the 11-mer sequence (2x=22-
710 mer, 4x=44-mer), and the 97-mer motif were expressed. Expression of each peptide increased
711 survival of desiccated bacteria (11-mer $p=2x10^{-6}$, 22-mer $p=5x10^{-6}$, 44-mer $p=1x10^{-7}$, 97-mer
712 $p=1x10^{-7}$, unpaired T-tests vs. GFP, n=7).
713
714 Figure 7. Minimal LEA-1 motifs promote desiccation and osmotic stress survival *in vivo*. **A)**
715 Endogenous *lea-1* sequence was largely deleted and replaced with sequence encoding codon
716 optimized, mNG-tagged, idealized motifs of 44 amino acids (4x 11-mer) or 97 amino acids. **B)**
717 Desiccation survival of control (*daf-2*), *lea-1Δ*, 44-mer::mNG, and 97-mer::mNG animals is
718 shown. *lea-1Δ* mutants had significantly lower survival than controls at both 97.5% RH
719 ($p=2.3x10^{-6}$, unpaired T-test, n=4) and 60% RH ($p=4.2x10^{-7}$, unpaired T-test, n=4). At 97.5%
720 RH motif-expressing worms had survival that was statistically indistinguishable from control, and
721 significantly improved relative to *lea-1Δ* animals (44-mer p=0.36 , 97-mer p=0.78 , unpaired T-
722 tests, n=4). When dehydrated at 60% RH, motif-expressing worms each had significantly

723 reduced survival (44-mer::mNG p=2.7x10^{-4} , 97-mer::mNG p=7.4x10^{-5} , unpaired T-tests relative
724 to control, n=4). **C)** *lea-1Δ* mutants, but not motif-expressing animals, were sensitive to osmotic
725 stress in 1M NaCl for 4 hr (*lea-1Δ* p=7.5x10^{-7}, 44-mer::mNG p=unpaired T-test vs. control, n=4).
726 Experiments in B and C were conducted in dauer worms in a *daf-2(e1370)* background. ***
727 indicates p<0.001.
728
729 Figure S1. Genotyping LEA-1 alleles. **A)** A simplified schematic depicting locations of primers to
730 genotype the various genome edits. Primers are indicated with numbers. Sequences can be
731 found in Table S1. **B)** A representative image depicts pharyngeal GFP expression in an *lea-1Δ*
732 mutant in which 15.8 kb of genomic sequence was replaced with *myo-2p*::GFP::*myo-2 3'UTR* as
733 a visual marker for the deletion. **C)** A representative image shows the plate level phenotype of
734 pharyngeal GFP expression in *lea-1Δ* mutants. **D)** Primers as indicated by numbers in Figure 1
735 were used to amplify genomic DNA and confirm deletion of the endogenous *lea-1* locus and
736 insertion of *myo-2p*::GFP::*myo-2 3'UTR* in the *lea-1Δ* mutant. **E)** The indicated primers were
737 used to amplify across the loci in genomic DNA at which the mNG and mYPET tags were
738 inserted. Genotyping confirms insertion of the mNG::3xFLAG::AID tag in a relatively N-terminal
739 position of *lea-1* and insertion of the mYPET::3xFLAG tag at the C-terminus of the gene.
740
741 Figure S2. LEA-1 does not significantly alter polyglutamine protein aggregation due to age. **A)**
742 Representative images of 4 and 8 day old worms expressing polyglutamine::YFP constructs in
743 body wall muscle. **B)** There are no significant differences in the number of polyglutamine
744 aggregates between control and *lea-1Δ* animals for either Q35::YFP (day 4 p=0.23, day 8
745 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).
746
747 Figure S3. LEA-1 expression does not increase in response to short-term osmotic stress. **A)**
748 Fluorescent tags to not disrupt function of LEA-1 during osmotic stress. Survival is plotted for

749 worms exposed to either water or 1M NaCl for 2 hr. Bars represent mean survival. Neither
750 mNG::*lea-1* nor *lea-1*::mYPET worms were significantly different from control worms exposed to
751 1M NaCl. **B)** Representative image of a control mNG::*lea-1* dauer worm. **D)** A mNG::*lea-1* dauer
752 larvae exposed to 1M NaCl for 2 hr. **E)** mNG fluorescence is not significantly altered in worms
753 exposed to 1M NaCl relative to controls (p=0.86, n=3 replicates, unpaired T-test). **F)** A
754 representative control dauer worm expressing *lea-1*::mYPET. **G)** An *lea-1*::mYPET dauer after 2
755 hr in 1M NaCl. **H)** Fluorescent intensity is not significantly different between controls worms
756 expressing *lea-1*::mYPET (p=0.36, n=3 replicates, unpaired T-test). All worms were in a *daf-*
757 *2(e1370)* background. *** indicates p<0.001.
758
759 Figure S4. Representative images of auxin-induced depletion of mNG::*lea-1* in TIR1 expressing
760 strains. **A)** Baseline expression of mNG::LEA-1 in the absence of TIR1 expression. **B)** *daf-2*
761 mutant animals have no detectable baseline fluorescence. Yellow dashed lines indicate the
762 outline of a worm. **C)** TIR1 expressed from the *col-10* promoter should deplete LEA-1 in the
763 hypodermis. It had a minimal effect on expression levels in worms. **D)** TIR1 driven by the *ges-1*
764 promoter reduced LEA-1 in the intestine. **E)** TIR1 under the control of the *mex-5* promoter
765 depleted LEA-1 in the germline. Some worms retained a significantly reduced amount of
766 germline LEA-1. **F)** *myo-2p*::TIR1 worms have reduced LEA-1 in the pharynx. This construct did
767 not totally deplete LEA-1. In particular, expression levels are still relatively high in the posterior
768 bulb of the pharynx (solid arrowhead). Depletion in the more anterior regions of the pharynx was
769 more pronounced (open arrowheads). **G)** Expression of *myo-3p*::TIR1 significantly reduced
770 LEA-1 levels in body wall muscle. **H)** TIR1 driven by the *vha-8* promoter is expressed in multiple
771 tissues and depletes LEA-1 in the excretory cell (and canal), intestine, and germline. TIR-1
772 expression was also observed in hypodermis and some cells of the head. The 100 µm scale bar
773 applies to all images. All images were taken with the same microscope settings and are shown
774 on the same intensity scale. For the 2x magnified insets the brightness was adjusted to facilitate

775 visualization. All worms were grown on plates containing 1mM auxin. Open arrowheads indicate
776 sites of LEA-1 depletion in each strain.
777
778 Figure S5. Controls for heterologous expression of proteins in bacteria. **A)** BL21 *E.coli* carrying
779 pDest17 driving expression of GFP are induced by growth in media with 0.2% L-arabinose.
780 Samples of bacteria from liquid cultured were spotted onto LB agar plates for imaging. **B)** A
781 Coomassie stained 4-12% Bis-Tris gel shows expression of *C. elegans* LEA-1 isoforms A, D, E,
782 F, and K in BL21 *E. coli* when induced with 0.2% L-arabinose. 20 µg of total protein was loaded
783 per lane.
784
785 Figure S6. Characterization of LEA-1 motif-expressing worms. **A)** A schematic depicts the
786 regions of genomic deletion and insertions and the primers used for genotyping these edits. **B)**
787 PCR genotyping confirms deletion of the majority of LEA-1 exons and insertion of sequence
788 encoding 44-mer::mNG or 97-mer::mNG. **C)** A representative image depicts *in vivo* expression
789 of the 44-mer::mNG fusion protein in a dauer worm. **D)** A representative image depicts *in vivo*
790 expression of the 97-mer::mNG fusion protein in a dauer worm. Worms in C and D were in a
791 *daf-2(e1370)* background.

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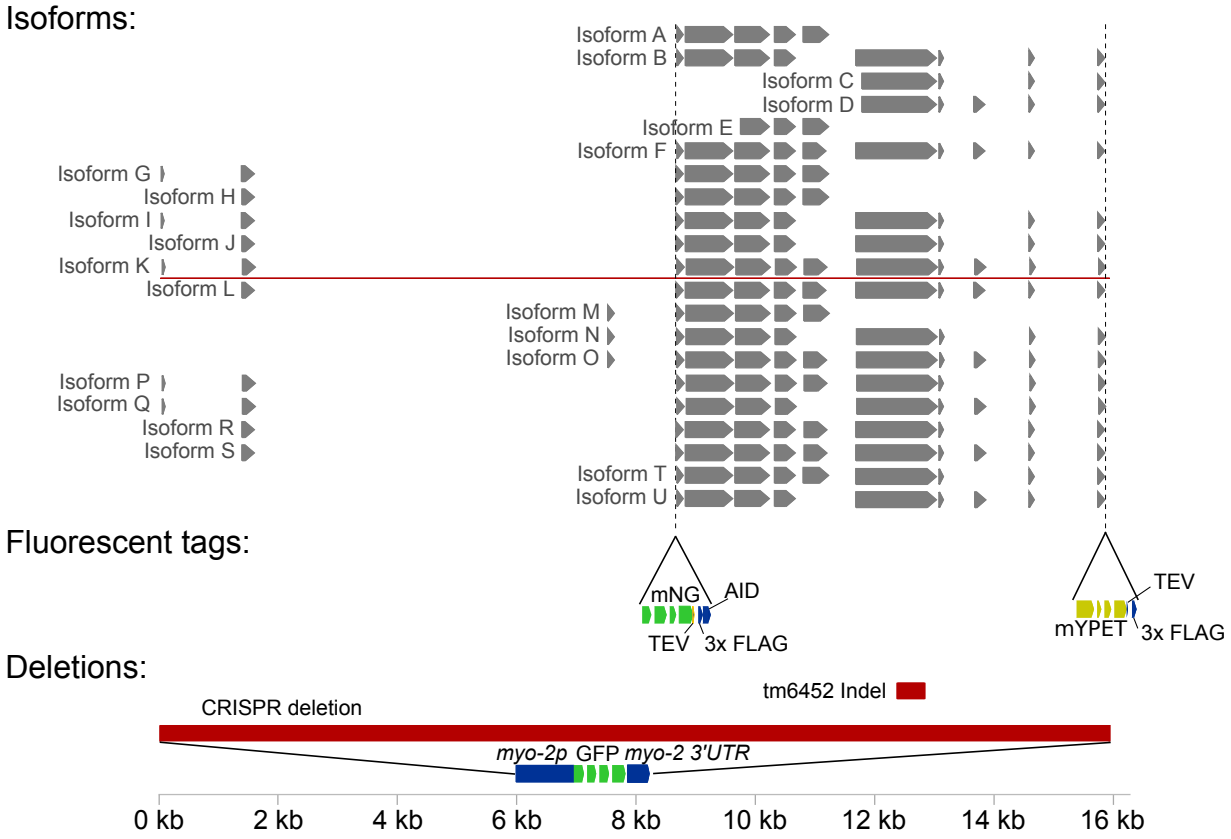
966

Figure 1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Isoform S
Isoform T
Isoform U
Fluorescent tags:
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AID
TEV
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TEV
mYPET
3x FLAG
Deletions:
tm6452 Indel
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Figure 2

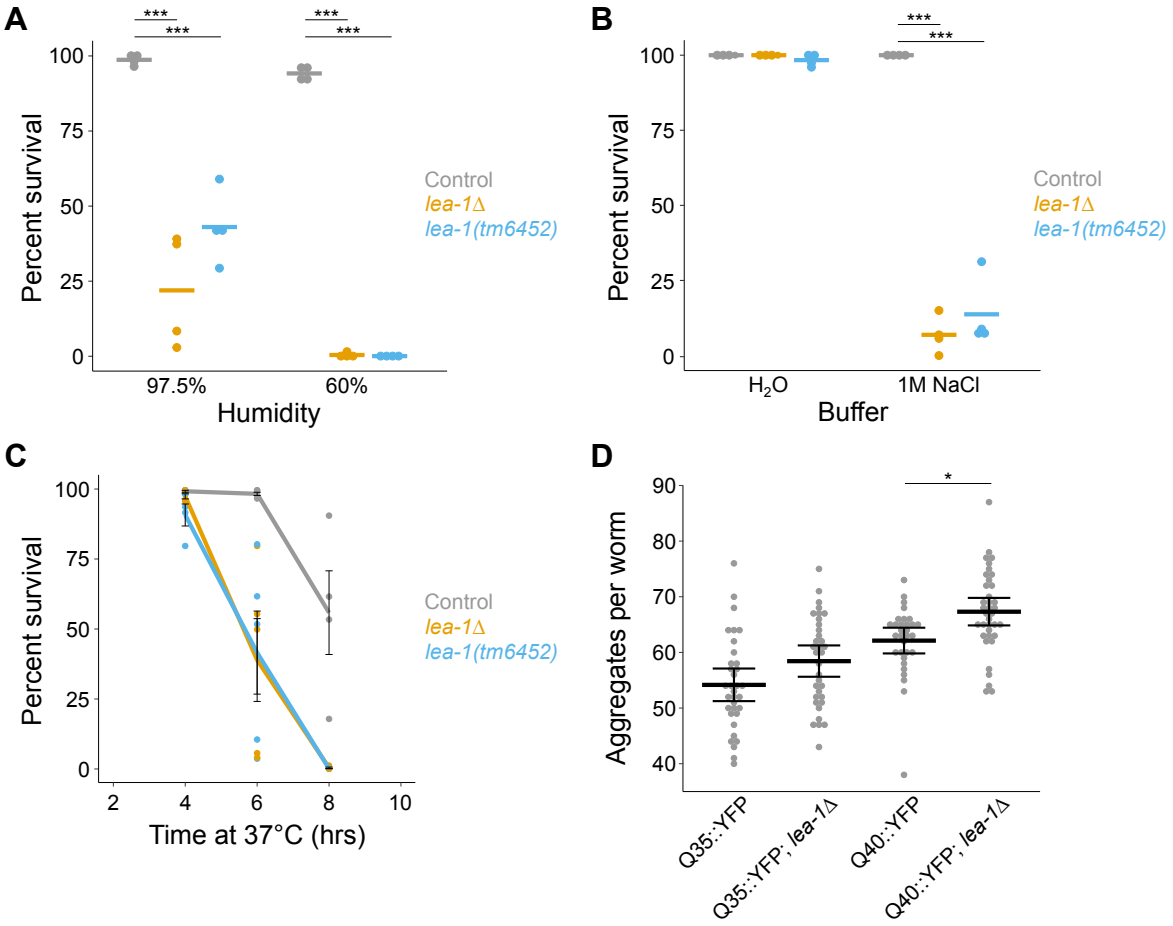

A
***
***
***
***
100
75
50
25
0
Percent survival
97.5%
60%
Humidity
Control
lea-1Δ
lea-1(tm6452)
B
***
***
100
75
50
25
0
Percent survival
H2O
1M NaCl
Buffer
Control
lea-1Δ
lea-1(tm6452)
C
100
75
50
25
0
Percent survival
2
4
6
8
10
Time at 37°C (hrs)
Control
lea-1Δ
lea-1(tm6452)
D
*
90
80
70
60
50
40
Aggregates per worm
Q35::YFP
Q35::YFP; lea-1Δ
Q40::YFP
Q40::YFP; lea-1Δ

Figure 3

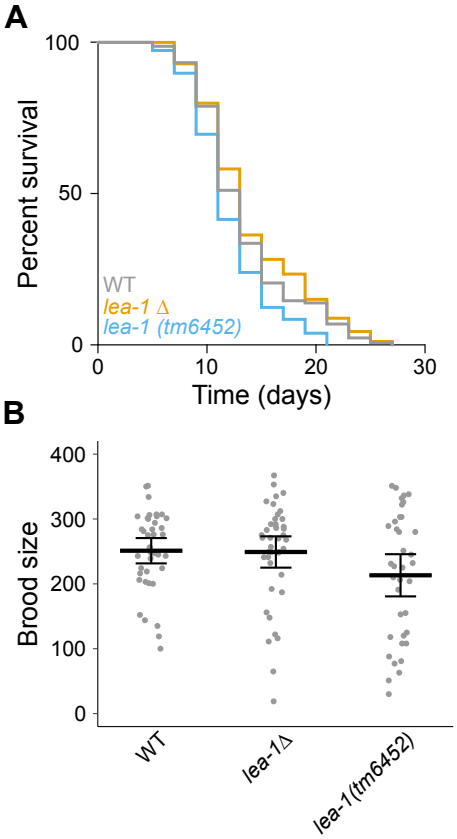

A
100
50
0
Percent survival
WT
lea-1 Δ
lea-1 (tm6452)
0
10
20
30
Time (days)
B
400
300
200
100
0
Brood size
WT
lea-1Δ
lea-1(tm6452)

Figure 4

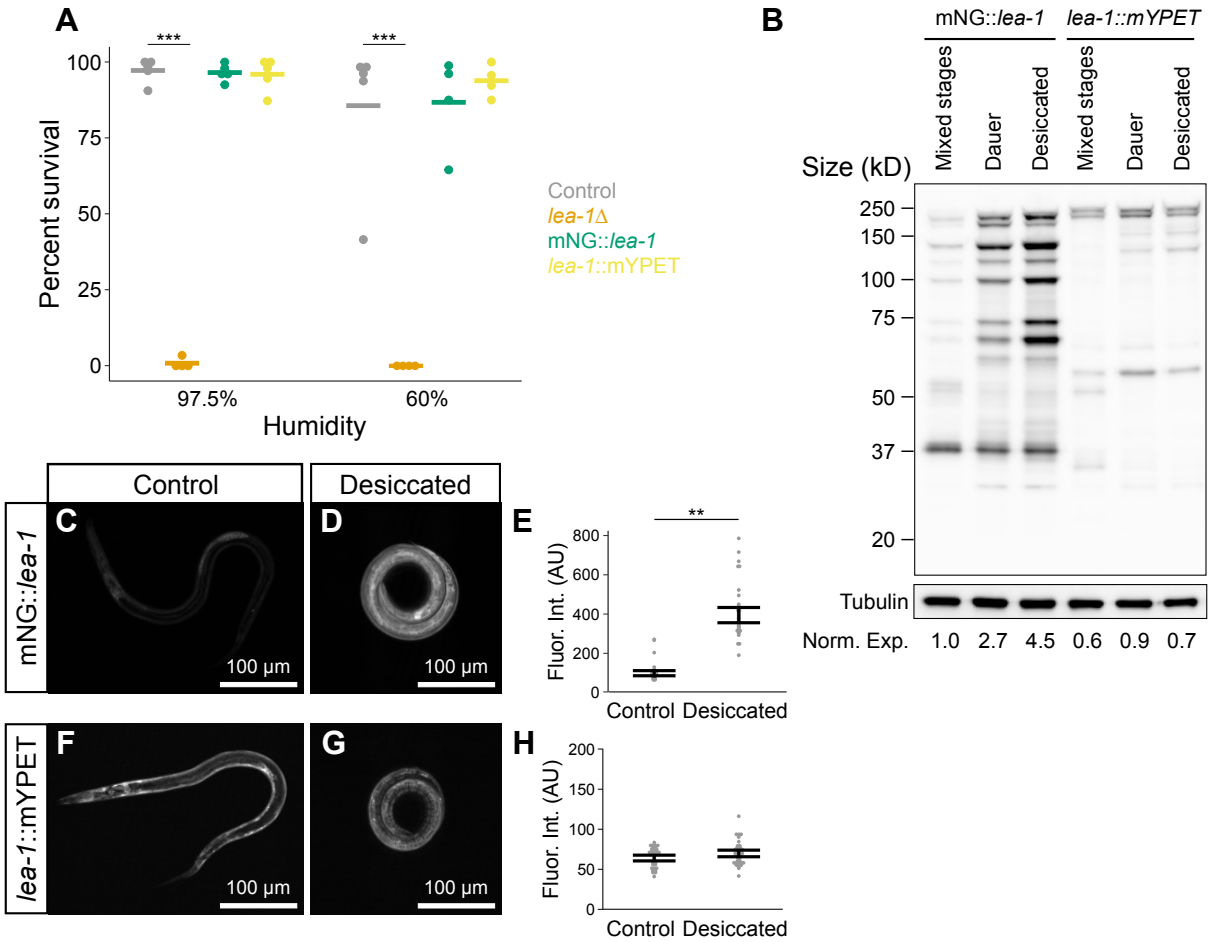
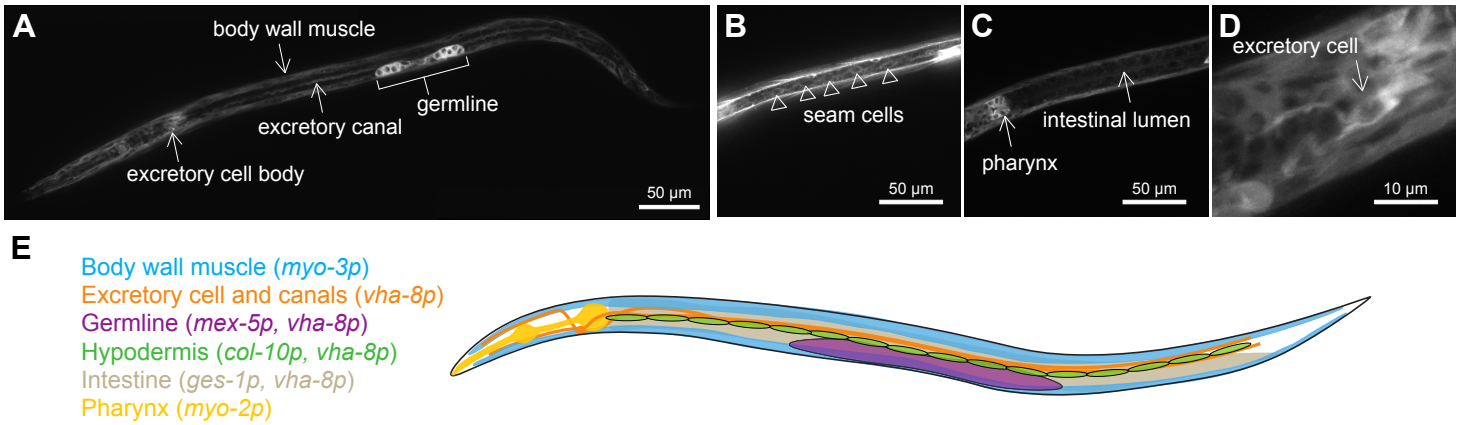

A
***
***
Percent survival
100
75
50
25
0
97.5%
60%
Humidity
Control
lea-1Δ
mNG::lea-1
lea-1::mYPET
B
mNG::lea-1
lea-1::mYPET
Mixed stages
Dauer
Desiccated
Mixed stages
Dauer
Desiccated
Size (kD)
250
150
100
75
50
37
20
Tubulin
Norm. Exp.
1.0
2.7
4.5
0.6
0.9
0.7
Control
Desiccated
mNG::lea-1
lea-1::mYPET
C
D
E
F
G
H
100 µm
**
Fluor. Int. (AU)
800
600
400
200
0
Control
Desiccated
200
150
100
50
0

Figure 5


A
body wall muscle
germline
excretory canal
excretory cell body
50 µm
B
seam cells
50 µm
C
intestinal lumen
pharynx
50 µm
D
excretory cell
10 µm
E
Body wall muscle (myo-3p)
Excretory cell and canals (vha-8p)
Germline (mex-5p, vha-8p)
Hypodermis (col-10p, vha-8p)
Intestine (ges-1p, vha-8p)
Pharynx (myo-2p)

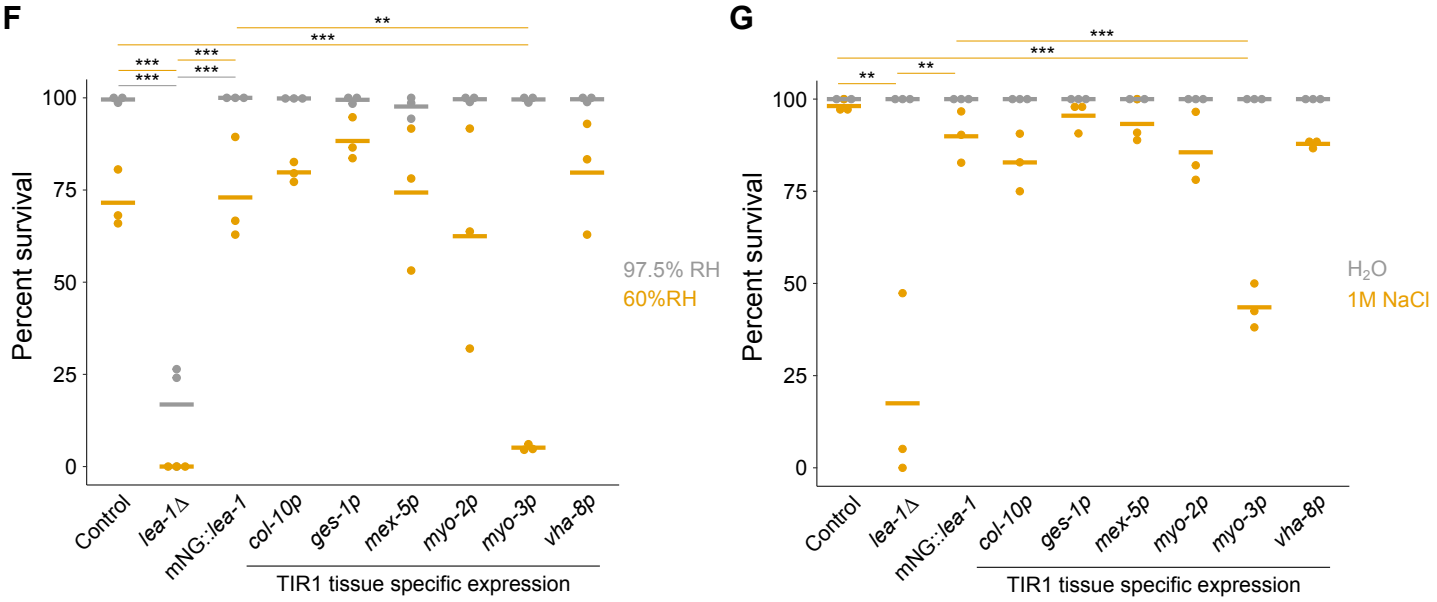

F
Percent survival
100
75
50
25
0
97.5% RH
60%RH
Control
lea-1Δ
mNG::lea-1
col-10p
ges-1p
mex-5p
myo-2p
myo-3p
vha-8p
TIR1 tissue specific expression
G
Percent survival
H2O
1M NaCl
TIR1 tissue specific expression

Figure 6

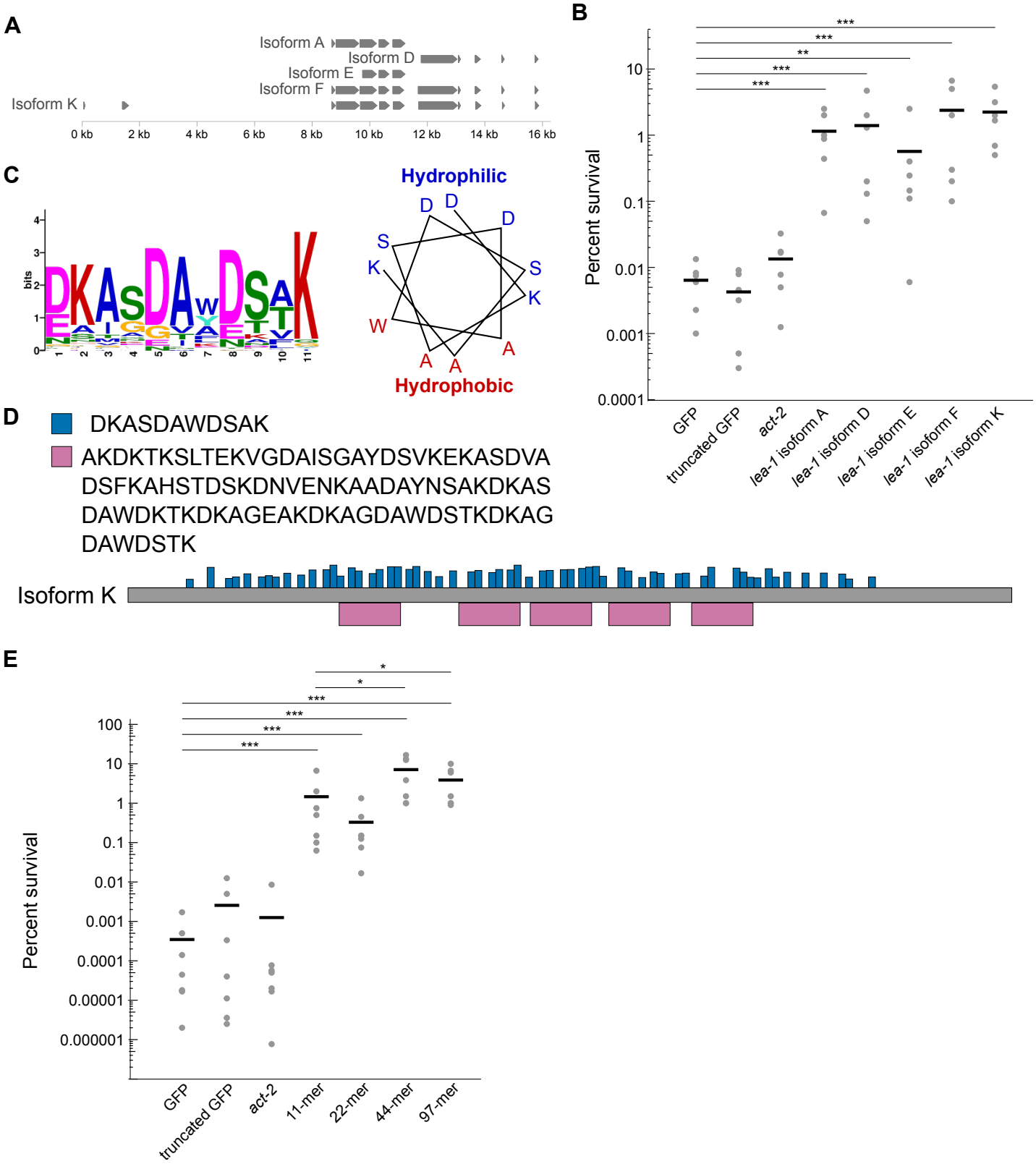

A
Isoform A
Isoform D
Isoform E
Isoform F
Isoform K
0 kb
2 kb
4 kb
6 kb
8 kb
10 kb
12 kb
14 kb
16 kb
B
Percent survival
GFP
truncated GFP
act-2
lea-1 isoform A
lea-1 isoform D
lea-1 isoform E
lea-1 isoform F
lea-1 isoform K
C
bits
Hydrophilic
Hydrophobic
D
DKASDAWDSAK
AKDKTKSLTEKVGDAISGAYDSVKEKASDVA
DSFKAHSTDSKDNVENKAADAYNSAKDKAS
DAWDKTKDKAGEAKDKAGDAWDSTKDKAG
DAWDSTK
Isoform K
E
Percent survival
GFP
truncated GFP
act-2
11-mer
22-mer
44-mer
97-mer

Figure 7

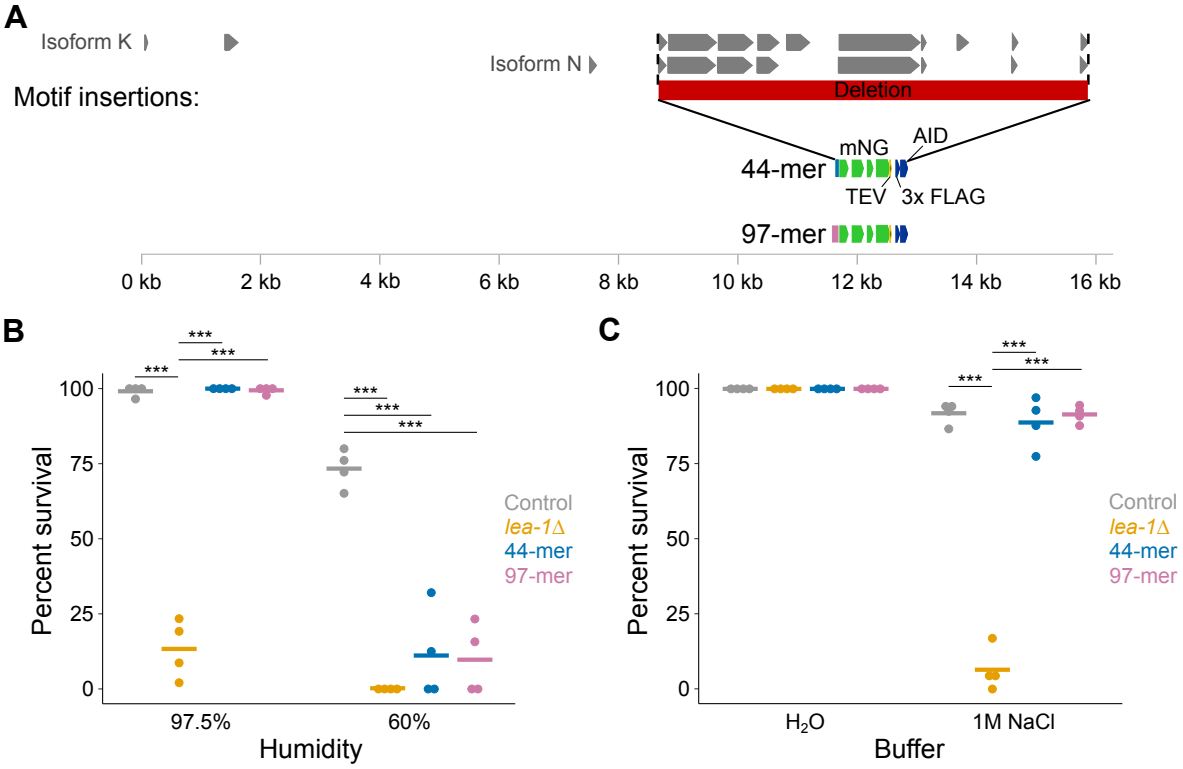
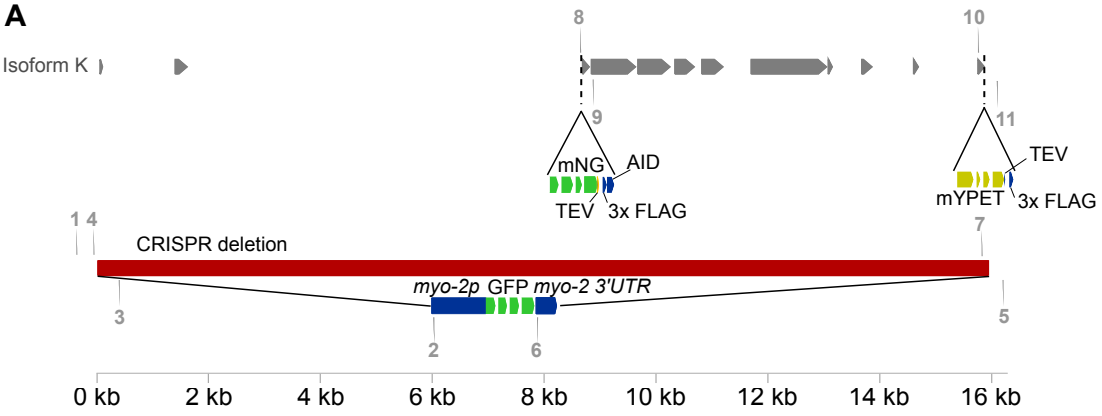
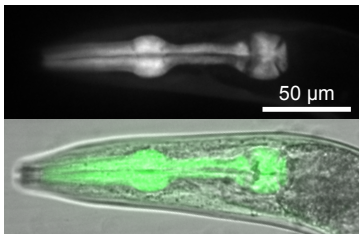

A
Isoform K
Isoform N
Motif insertions:
Deletion
mNG
AID
44-mer
TEV
3x FLAG
97-mer
0 kb
2 kb
4 kb
6 kb
8 kb
10 kb
12 kb
14 kb
16 kb
B
Percent survival
100
75
50
25
0
***
97.5%
60%
Humidity
Control
lea-1Δ
44-mer
97-mer
C
Percent survival
H2O
1M NaCl
Buffer

Figure S1

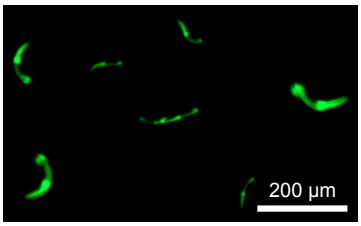
A



B

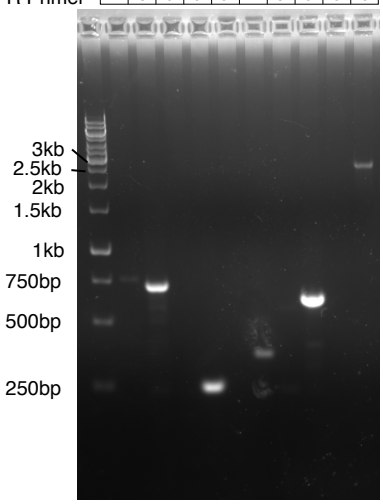


C



D

Template DNA	WT					*lea-1Δ*				
F Primer	1	1	6	7	4	1	1	6	7	4
R Primer	2	3	5	5	5	2	3	5	5	5



E

Template DNA	WT		*mNG::lea-1*		*lea-1::mYPET*	
F Primer	8	10	8	10	8	10
R Primer	9	11	9	11	9	11

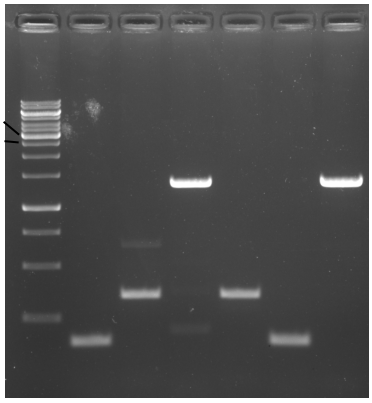
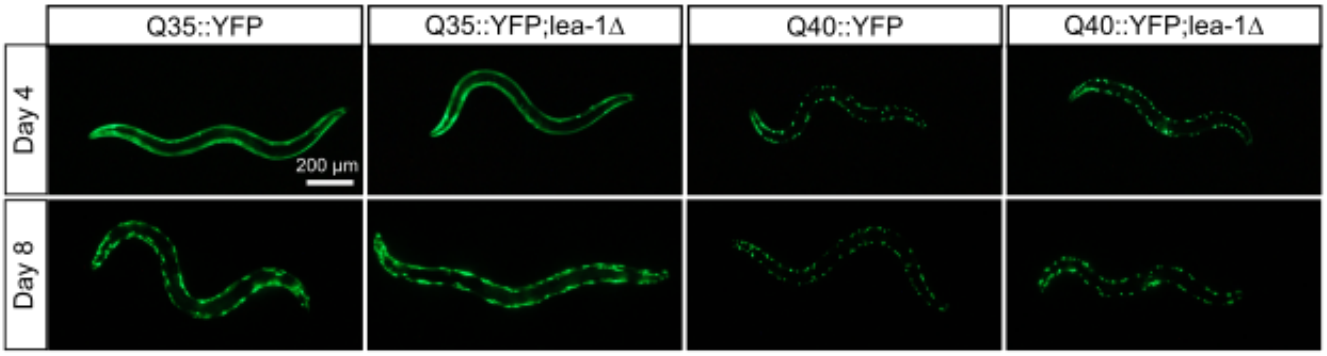


Figure S2

A


Q35::YFP
Q35::YFP;lea-1Δ
Q40::YFP
Q40::YFP;lea-1Δ
Day 4
Day 8
200 µm

B

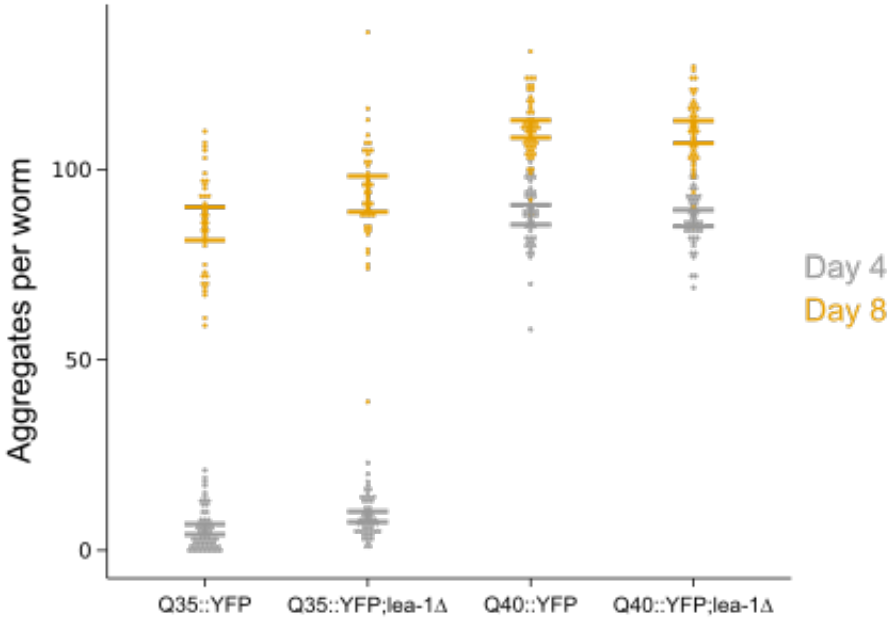

Aggregates per worm
100
50
0
Q35::YFP
Q35::YFP;lea-1Δ
Q40::YFP
Q40::YFP;lea-1Δ
Day 4
Day 8

Figure S3

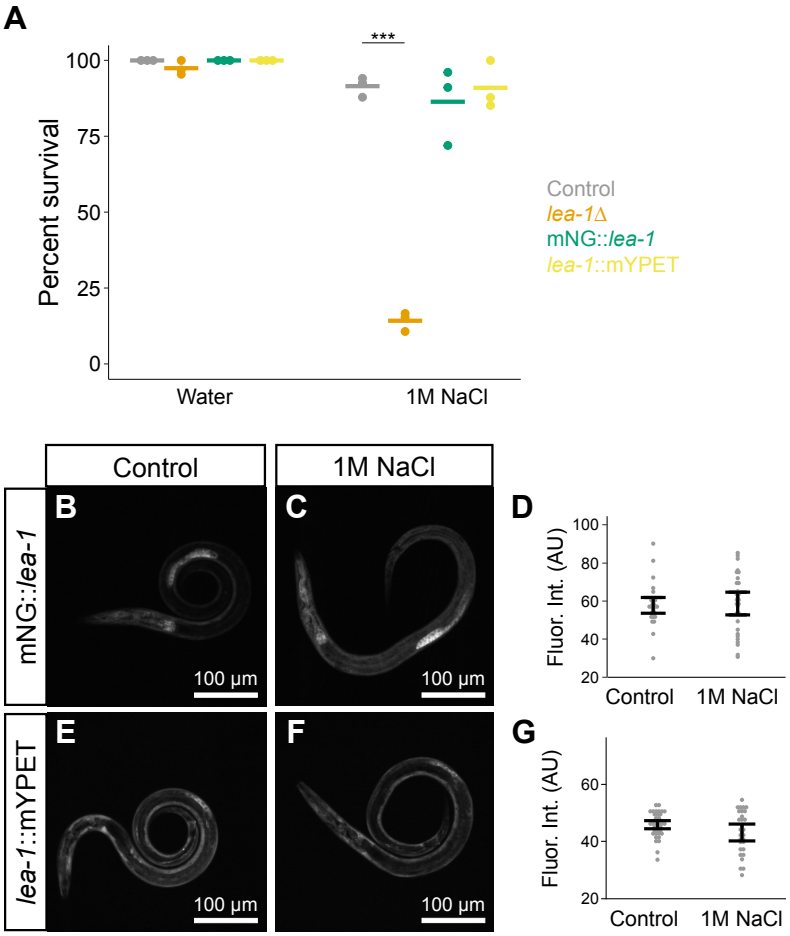

A
***
Percent survival
100
75
50
25
0
Water
1M NaCl
Control
lea-1Δ
mNG::lea-1
lea-1::mYPET
Control
1M NaCl
mNG::lea-1
lea-1::mYPET
B
C
D
E
F
G
100 µm
Fluor. Int. (AU)
100
80
60
40
20
Control
1M NaCl

Figure S4

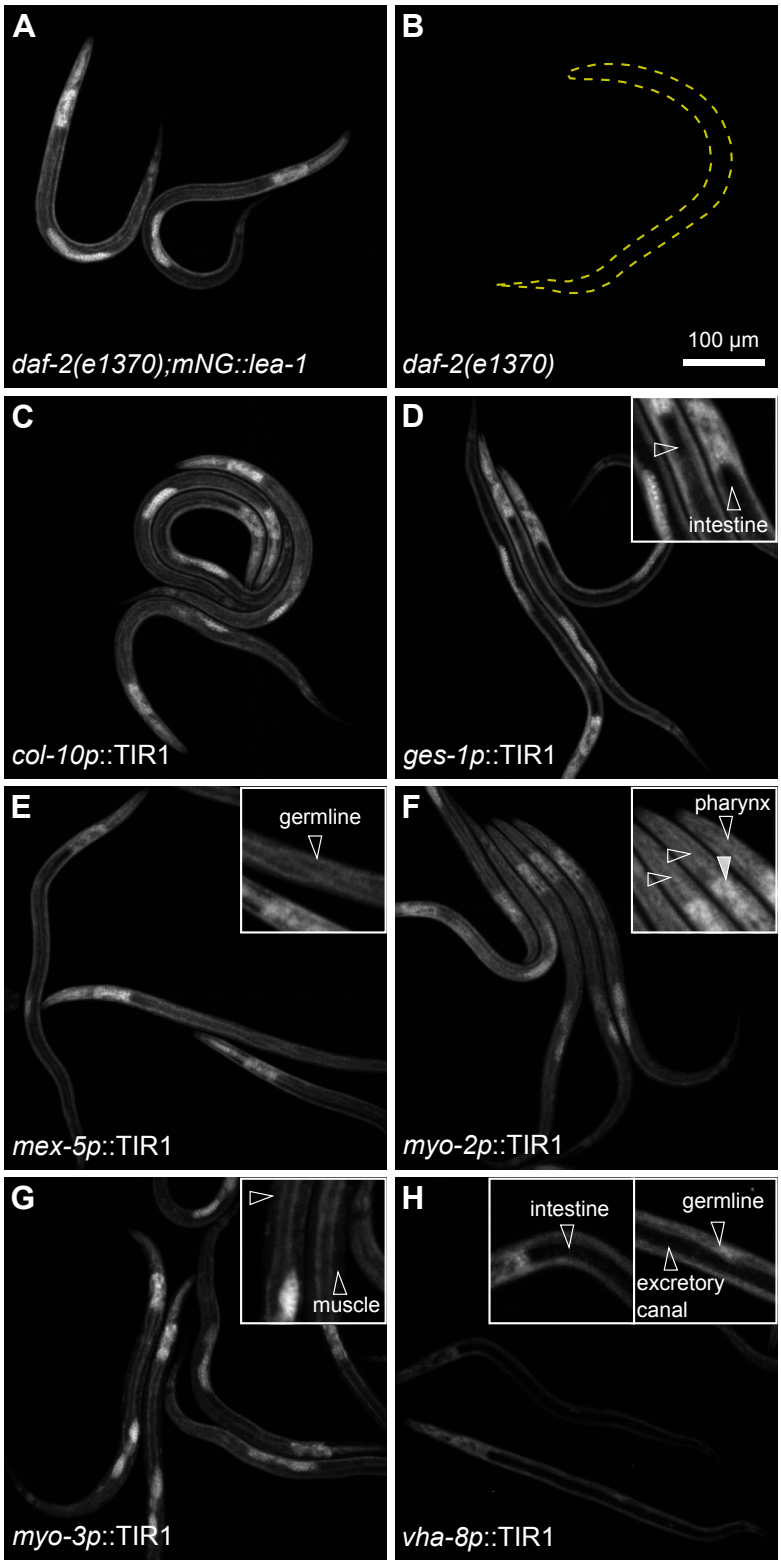
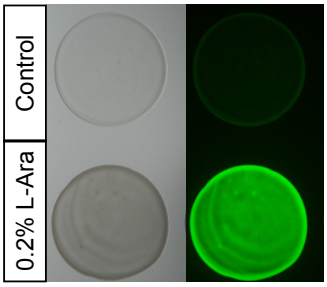

A
daf-2(e1370);mNG::lea-1
B
daf-2(e1370)
100 µm
C
col-10p::TIR1
D
intestine
ges-1p::TIR1
E
germline
mex-5p::TIR1
F
pharynx
myo-2p::TIR1
G
muscle
myo-3p::TIR1
H
intestine
germline
excretory canal
vha-8p::TIR1

Figure S5


A
Control
0.2% L-Ara

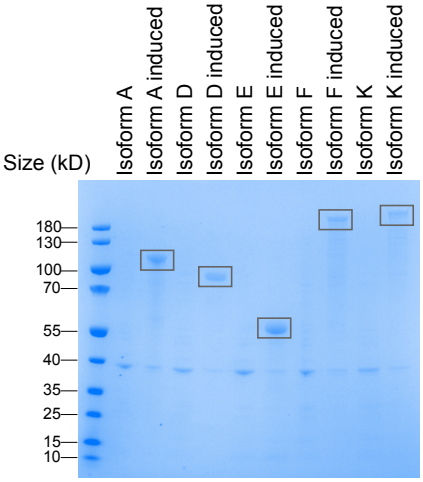
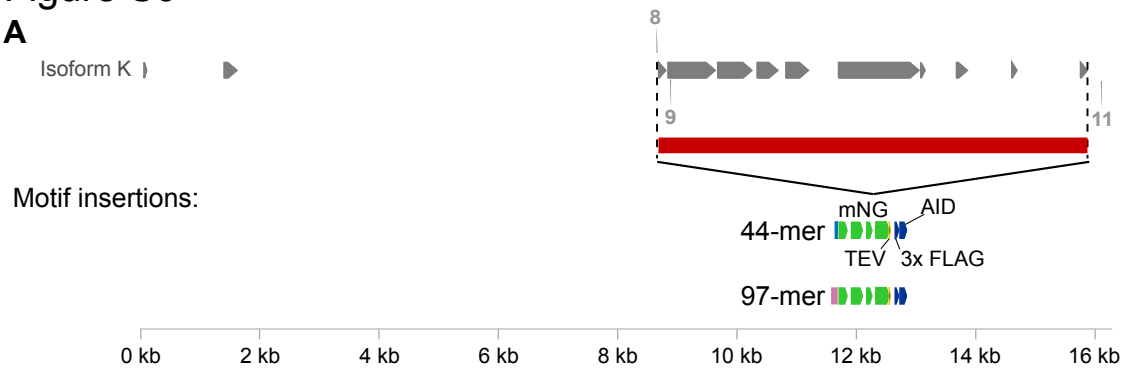

B
Size (kD)
Isoform A
Isoform A induced
Isoform D
Isoform D induced
Isoform E
Isoform E induced
Isoform F
Isoform F induced
Isoform K
Isoform K induced
180
130
100
70
55
40
35
25
15
10

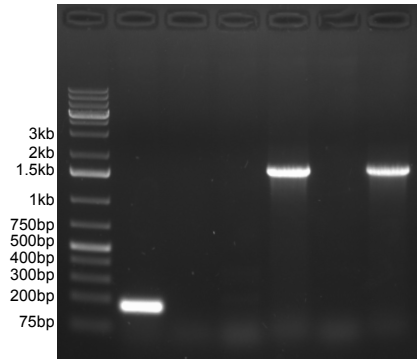
Figure S6

A



B

Template DNA	WT		44-mer::mNG		97-mer::mNG	
F Primer	8	8	8	8	8	8
R Primer	9	11	9	11	9	11



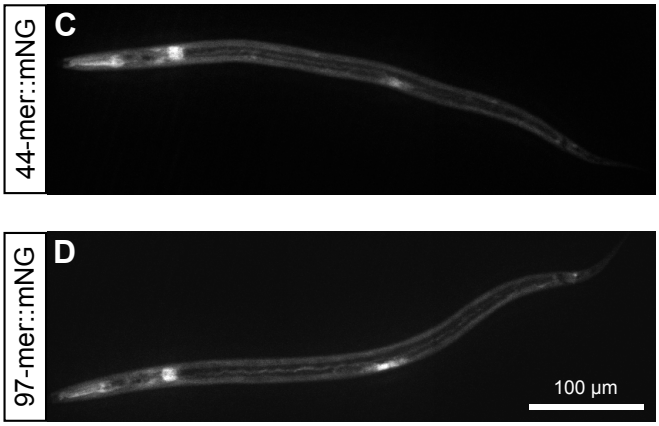


Table S1. Genotyping primers for *lea-1* genomic edits.

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-1*Δ but no band in WT.
3	TCACTTGAGAGCCCGAACTT	Paired with #1 will give 742 bp band in WT but no band in *lea-1*Δ.
4	GATCACCATCTCCACCAACC	Paired with #5 will give 2,569 bp band in *lea-1*Δ 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-1*Δ but no band in WT.
7	GGACACTCTTCGCTCGACTC	Paired with #5 will give 256 bp band in WT but no band in *lea-1*Δ.
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.