Intrinsically aggregation-prone proteins form amyloid-like aggregates and contribute to tissue aging in *C. elegans*.

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Abstract:

Reduced protein homeostasis leading to increased protein instability is a common molecular feature of aging, but it remains unclear whether this is a cause or consequence of the aging process. In neurodegenerative diseases and other amyloidoses, specific proteins self-assemble into amyloid fibrils and accumulate as pathological aggregates in different tissues. More recently, widespread protein aggregation has been described during normal aging. Until now, an extensive characterization of the nature of age-dependent protein aggregation has been lacking. Here, we show that age-dependent aggregates are rapidly formed by newly synthesized proteins and have an amyloid-like structure resembling that of protein aggregates observed in disease. We then demonstrate that age-dependent protein aggregation accelerates the functional decline of different tissues in *C. elegans*. Together, these findings imply that amyloid-like aggregates contribute to the aging process and therefore could be important targets for strategies designed to maintain physiological functions in the late stages of life.

Introduction:

Aging is a gradual decline in physiological functions and organ integrity and indeed diminished physical capacity and cognitive functions are already apparent before midlife (Belsky et al., 2015). The aging process greatly enhances the risk for chronic diseases leading to long-term disabilities in the elderly population and often premature death (Kaeberlein, 2013). A better understanding of what drives aging and in particular functional decline holds the promise of identifying targets to maintain quality of life with old age. It is generally agreed that the ultimate root causes of aging occur at the molecular level (Kaeberlein, 2013). One of the most universal hallmarks of aging is increased protein instability (Golubev, Hanson, & Gladyshev, 2017; Gorisse et al., 2016). In young individuals, an efficient protein homeostasis

41 (proteostasis) network prevents the accumulation of damaged proteins. However, overwhelming

42 evidence points to a collapse in the proteostasis network with age and consequently a decline in the

43 ability to cope with protein physical and chemical instability (Taylor & Dillin, 2011). Still the role of

44 protein instability in aging is unclear (Finch & Crimmins, 2016; Hekimi, Lapointe, & Wen, 2011).

45 During aging, an important cause of protein instability is cumulative damage through non-enzymatic

46 posttranslational modifications occurring through reactions with metabolites and reactive oxygen

47 species (Golubev et al., 2017). Destabilizing mutations, transcriptional and translational inefficacy are

48 also a significant source of protein instability (Nedialkova & Leidel, 2015; Vermulst et al., 2015). In a

49 disease context, a specific form of protein instability, namely protein aggregation, is a common feature

50 of amyloidoses and many neurodegenerative diseases. In these diseases, a specific protein converts from

51 its native soluble conformation into an insoluble state by self-assembly into a cross-β sheet filamentous

52 structure termed amyloid fibril. Amyloid fibrils accumulate as pathological deposits in a variety of

53 different tissues. Although most proteins contain amyloid-promoting sequences and form amyloid fibrils

54 in appropriate conditions *in vitro* (Chiti et al., 1999; Goldschmidt, Teng, Riek, & Eisenberg, 2010), there is

55 little evidence for this disease-related conformational state in metazoa in the absence of disease. Global

56 proteomic characterizations of age-dependent protein insolubility, a hallmark of disease-associated

57 protein aggregation, have been carried out in wild-type *C. elegans* (David et al., 2010; Reis-Rodrigues et

58 al., 2012; Walther et al., 2015). Several hundred proteins enriched in distinct structural and functional

59 characteristics were identified that lose their native structure in aged animals and become highly-

60 insoluble in strong detergents (David et al., 2010; Lechler et al., 2017; Reis-Rodrigues et al., 2012). *In vivo*

61 data demonstrate that these proteins accumulate in solid/immobile structures in aged animals.

62 However, until now it is not known whether these age-dependent protein aggregates contain amyloid

63 fibrils. Elucidating the structural nature of this novel type of protein instability would help to understand

64 the causes and consequences of protein aggregation during aging and to explain potential interactions

65 with disease-aggregating proteins.

66 Since the initial discovery in *C. elegans*, age-dependent protein aggregation has been demonstrated in

67 different organs in mammals (Ayyadevara, Balasubramaniam, et al., 2016; Ayyadevara, Mercanti, et al.,

68 2016; Groh, Buhler, et al., 2017; Leeman et al., 2018; Tanase et al., 2016). Importantly, age-dependent

69 protein aggregation is likely to be relevant in neurodegenerative processes. Indeed, the over-

70 representation of proteins prone to aggregate with age among proteins sequestered in Alzheimer's

71 disease inclusions of amyloid plaques and tau neurofibrillary tangles highlights a possible contribution to

72 pathological processes (Ciryam, Tartaglia, Morimoto, Dobson, & Vendruscolo, 2013; David et al., 2010).

73 Moreover, recent evidence shows that aggregates from wild-type aged mouse brains are a potent

74 heterologous seed for amyloid-β (Aβ) aggregation (Groh, Buhler, et al., 2017). However, because of the

75 difficulties to distinguish the effects of protein aggregation from other consequences of aging, it is still

76 not clear whether age-dependent protein aggregation plays a role in accelerating the aging process

77 (David, 2012). A previous study has shown that *C. elegans* treated with RNAi targeting genes encoding

78 aggregation-prone proteins tend to live longer than randomly chosen targets (Reis-Rodrigues et al.,

79 2012). However, the interpretation of these results is complicated by the loss-of-function of these

80 proteins. Recently, we found that aged animals with high aggregation levels of an RNA-binding protein

81 (RBP) with a low-complexity prion-like domain were shorter lived, significantly smaller and less motile

82 than animals with low RBP aggregation levels (Lechler et al., 2017). Still this evidence does not provide a

83 definite answer to whether age-dependent protein aggregation plays a causal role in aging rather than

84 being a simple by-product. To understand whether protein aggregation is protective or harmful, it is

85 informative to look at how long-lived animals modulate protein solubility. Longevity mechanisms and

86 enhanced proteostasis are tightly coupled (Taylor & Dillin, 2011). However, whereas several studies

87 show reduced age-dependent protein aggregation in long-lived animals (David et al., 2010; Demontis &

88   Perrimon, 2010; Lechler et al., 2017), a recent study suggests that enhancing protein aggregation could

89   be a strategy to promote longevity (Walther et al., 2015).

90   In the current study, we use a combination of transmission electron microscopy (TEM), fluorescence

91   lifetime imaging (FLIM) (Kaminski Schierle et al., 2011), Thioflavin T (ThT) staining and structured

92   illumination microscopy (SIM) (Young, Strohl, & Kaminski, 2016) to reveal amyloid-like structures in age-

93   dependent protein aggregates *in vivo*. Unlike protein chemical instability caused by cumulative damage,

94   age-dependent protein aggregates are formed by intrinsically aggregation-prone proteins, even shortly

95   after their synthesis. We demonstrate that age-dependent protein aggregation is toxic and contributes

96   to functional decline in *C. elegans*.

97

98   **Results:**

99   **Age-dependent aggregating proteins are intrinsically prone to aggregate in certain tissues.**

100   Previously, we performed an extensive characterization of the aggregation of two proteins, casein kinase

101   I isoform alpha (KIN-19) and Ras-like GTP-binding protein rhoA (RHO-1). Both KIN-19 and RHO-1 were

102   identified among the proteins with the highest propensity to become insoluble with age in wild-type *C.*

103   *elegans* somatic tissues (David et al., 2010). *In vivo* analysis of animals expressing these proteins fused to

104   fluorescent tags showed the appearance of immobile deposits with age (David et al., 2010). Among the

105   insoluble proteome, the enrichment of certain physico-chemical features such as high aliphatic amino

106   acid content or propensity to form β-sheet-rich structures shows that age-dependent protein

107   aggregation is not random (David et al., 2010; Lechler et al., 2017; Walther et al., 2015). To understand

108   whether KIN-19 and RHO-1 have an intrinsic capacity to aggregate similar to disease-associated proteins

109   or whether a progressive accumulation of protein damage caused by non-enzymatic posttranslational

110   modifications is required to induce their aggregation, we evaluated the dynamics of protein aggregation

111   *in vivo*. Protein labeling with mEOS2, a green-to-red photoconvertible fluorescent protein, has been

112   successfully used to track protein dynamics (McKinney, Murphy, Hazelwood, Davidson, & Looger, 2009).

113   In the present case, we used the mEOS2 tag to investigate how fast newly synthesized KIN-19 and RHO-1

114   aggregate. For this purpose, we generated transgenic animals expressing KIN-19::mEOS2 in either the

115   pharynx or in the body-wall muscles and transgenic animals expressing RHO-1::mEOS2 in the pharynx.

116   The mEOS2 tag did not disrupt the aggregation potential of KIN-19, as the absence of fluorescence

117   recovery after photobleaching confirms that both KIN-19::mEOS2 puncta in the pharynx and body-wall

118   muscle are highly immobile structures (Figure supplement 1 A-D).

119   To follow newly synthesized proteins, we set-up a system to perform irreversible photoconversion of the

120   mEOS2 tag present in live animals from green to red by exposing them to intense blue light. At a defined

121   time-point, we photoconverted the mEOS present in aggregates to red. After the photoconversion,

122   newly synthesized proteins emitted green fluorescence and could thus easily be distinguished from old

123   (photoconverted/red) aggregates. This method allowed us to follow the rate of new aggregate formation

124   and the rate of old aggregate removal in a population of transgenic animals over time. We observed

125   photoconversion of all aggregates, but we also noted that the core region of some larger aggregates

126   continued to emit green fluorescence (Figure 1 A, B). Interestingly, we observed a doubling in the

127   number of animals with newly-formed green aggregates, 24 hours after the conversion at day 5 of

128   adulthood in both the pharyngeal and body-wall muscles. Conversely, analysis of the red aggregates

129   suggested a slow removal of old aggregates (Figure 1 A, B, Supplementary file 1). Blocking translation

130   shortly before conversion reduced the formation of new aggregates (Figure 1 B). Conversion in young

131   animals and blocking translation produced similar results (Figure supplement 1 E). Confocal imaging

132   shows that newly synthesized KIN-19 associate with pre-existing aggregates (Figure 1 C). However, we

133   also observed large aggregates emitting only green fluorescence suggesting that seeding is not a

134   prerequisite for KIN-19 aggregate formation (Figure 1 C). We observed similar aggregation kinetics in

135   animals with RHO-1::mEOS2 expressed in the pharynx (Figure supplement 1 F). Together, these results

136   reveal that aggregate formation can proceed rapidly after protein synthesis.

137

138   **Age-dependent protein aggregates contain amyloid-like structures *in vivo*.**

139   Both KIN-19 and RHO-1 are nearly identical to their human orthologues (sequence identity over 87%). X-

140   ray diffraction shows that the human orthologues are globular proteins formed by a series of α-helices

141   and β-strands. The ability of KIN-19 and RHO-1 to aggregate shortly after synthesis suggests that their

142   aggregation may be associated with the presence of folding intermediates. Notably, both proteins have

143   several segments with a high amyloid propensity (Figure supplement 2 A, B) (Goldschmidt et al., 2010).

144   This feature raises the possibility that KIN-19 and RHO-1 gain an amyloid-like conformation during their

145   aggregation with age. We have previously shown that FLIM can be used to determine whether certain

146   proteins are likely to form amyloid-like aggregates as the formation of amyloid fibrils leads to a

147   significant drop in the fluorescence lifetime of the fluorescent-tagged amyloid proteins due to quenching

148   (Chen et al., 2017; Kaminski Schierle et al., 2011; Murakami et al., 2015). In the current study, we applied

149   FLIM to determine whether KIN-19 and RHO-1 form amyloid-like aggregates in live *C. elegans*. For this,

150   we generated transgenic animals expressing translational fusions with the yellow fluorescent protein

151   Venus in the pharynx. We first confirmed that there is an age-dependent increase in aggregate formation

152   by KIN-19::Venus and RHO-1::Venus in the pharynx (Figure supplement 3 A, B) (Lechler et al., 2017).

153   Using FLIM, we found that pharyngeal KIN-19 and RHO-1::Venus display a significantly decreased

154   fluorescence lifetime compared to Venus only control worms (Figure 2 A-C, Figure supplement 4 A).

155   Areas with the strongest drop in fluorescence lifetime co-localize with fluorescent-labeled aggregates

156   (Figure 2 A). Of note, we also observed a similar drop in the fluorescence lifetime of pharyngeal RHO-1

157   labeled with tagRFP compared to tagRFP alone (Figure supplementary 4 B). In both models with KIN-19

158   and RHO-1, we observed a significant drop in the fluorescence lifetime already at day 1, consistent with

159   the appearance of aggregates in young animals due to protein overexpression. In particular, between

160   day 1 and day 7 of adulthood, we measured a dramatic decrease in fluorescence lifetime of RHO-1

161   aggregates and a more modest decrease in the fluorescence lifetime of KIN-19 aggregates. This result is

162   consistent with the age-dependent increase in aggregate formation by both proteins and the fact that

163   RHO-1 puncta tend to be larger and more solid than KIN-19 puncta. Indeed, all RHO-1 puncta evaluated

164   showed no recovery after photobleaching whereas 30% of KIN-19 puncta showed some recovery as

165   previously described (Figure supplement 3 C-E compared to (David et al., 2010)). In addition to

166   evaluating the fluorescence lifetime, we assessed whether RHO-1 aggregates are stained by the Congo

167   red derivative X34 previously used to detect amyloid deposits (Styren, Hamilton, Styren, & Klunk, 2000).

168   Consistent with the amyloid-like nature of the aggregates, we observed co-localization of X34 with RHO-

169   1 aggregates *in vivo* (Figure supplementary 4C-E).

170   To gain more insight into the capacity of RHO-1 to form amyloid-like fibrils, we expressed and purified

171   recombinant RHO-1 (Figure supplement 5 A, B) and aggregated it over time by constant shaking in

172   stabilizing buffer to induce fibrillisation at 37˚C for a week. We obtained low quantities of RHO-1 fibrils,

173   which we were able to analyze by TEM. Analysis of RHO-1 by TEM revealed fibril-like structures which

174   resemble amyloid fibrils such as formed by huntingtin (Htt) (Figure supplement 5 C) (Reif, Chiki, Ricci, &

175   Lashuel, 2018). Indeed, RHO-1 fibrils had a morphology more similar to Htt fibrils which are shorter and

176   more branch-like compared to fibrils formed from other amyloids such as alpha-synuclein, which are

177   long and flexible. Additionally, RHO-1 and Htt fibrils display no twisting which can be observed in alpha-

178   synuclein fibrils, suggesting that the packing of the monomeric structure and the interaction between

179   protofibrils may be different (Lyubchenko, Krasnoslobodtsev, & Luca, 2012). Importantly, already at day

180   1, we found comparable fibrillary structures in extracts from RHO-1::Venus transgenic animals (Figure

181   supplement 5 D). In order to confirm that similar fibril structures can be found in the absence of a

182   fluorescent tag, we have isolated fibrils by affinity purification from day 7 old transgenic worms

183   expressing pharyngeal RHO-1::HisAvi and KIN-19::HisAvi and imaged them by TEM (Figure supplement

184   5E). Moreover, we have stained isolated RHO-1::HisAvi day 7 fibrils, RHO-1::tagRFP day 2 fibrils and

185   extracts from non-aggregated control worms day 2 with amyloid-binding molecule ThT. Using SIM, we

186   found ThT positive RHO-1 fibrils as shown previously for polyglutamine and Aß fibrils in cells (Lu et al.,

187   2019; Young et al., 2016) (Figure supplement 6).

188   Together, these findings strongly suggest that Casein kinase I isoform alpha and Ras-like GTP-binding

189   protein rhoA aggregates contain amyloid-like structures *in vivo*.

190

191   **Age-dependent protein aggregation accelerates functional decline.**

192   A reliable indicator of human aging is a decline in physical capacity such as decreased muscle strength

193   and coordination (Belsky et al., 2015). Similarly, *C. elegans* displays an age-related decline in pharyngeal

194   pumping and body movement (Huang, Xiong, & Kornfeld, 2004). To investigate whether age-dependent

195   protein aggregation accelerates functional decline with age, we measured pharyngeal pumping and

196   swimming in liquid (thrashing) in transgenic animals overexpressing fluorescent-labeled KIN-19 and RHO-

197   1 either in the pharynx or the body-wall muscles. In all models, protein aggregation increased with age,

198   as measured by the change from a diffuse distribution to the formation of specific puncta by the

199   fluorescent-tagged proteins (Figure supplement 7). Among the transgenic models generated, the largest

200   age-dependent changes were observed for *C. elegans* expressing KIN-19::tagRFP in the pharynx (Figure

201   supplement 7 A, E) (David et al., 2010). Whereas the majority of these transgenic animals have no

202   aggregation at day 2, this dramatically changes with age and at day 8, the majority display high levels of

203   KIN-19::tagRFP aggregation. Importantly, pumping frequency was strongly reduced in day 7 aged animals

204   with the highest levels of pharyngeal KIN-19::tagRFP aggregation compared to those with the lowest

205   aggregation levels (Figure 3 A, Supplementary file 2). The detrimental effect of age-dependent protein

206   aggregation was also apparent in animals with KIN-19::tagRFP aggregation in the body-wall muscles,

207   since we observed an earlier decline in swimming frequency associated with KIN-19::tagRFP aggregation

208   in the body-wall muscle (Figure supplement 8 A). This effect was amplified in animals with the highest

209   level of aggregation (Figure 3 B). Of note, the fluorescent tagRFP expressed alone in the pharynx or in the

210   body-wall muscle did not affect muscle function (Figure supplement 8 A, C). Decreased body movement

211   with age is associated with increased sarcopenia characterized by disordered sarcomeres and reduced F-

212   actin filaments (F-actin) (Baird et al., 2014; Herndon et al., 2002). To evaluate whether the presence of

213   KIN-19 aggregates is linked to muscle damage at the cellular level, we assessed F-actin staining in animals

214   with KIN-19 aggregation in the body-wall muscles. We found that animals with aggregates tended to

215   have higher levels of disrupted sarcomers compared to controls (Figure supplement 9 A, B).

216

217   To further show that protein aggregation is the cause of the functional decline in our transgenic animal

218   models rather than other co-occurring aging factors, we examined the effects of protein aggregation in

219   the absence of aging. Most likely due to the high level of overexpression in the pharyngeal muscles, RHO-

220   1::tagRFP aggregated abundantly already in young animals (Figure supplement 7 C, E). We found that

221   these high levels of RHO-1::tagRFP aggregation strongly impaired pharyngeal pumping in young animals

222   (day 2) (Figure 3 C). Notably, these young animals displayed pumping rates normally observed only in

223   aged animals. Moreover, RHO-1 aggregation disrupted actin filament structure in the pharyngeal

224   muscles (Figure supplement 9C, D). To exclude that RHO-1 overexpression itself is toxic to *C. elegans*, we

225   examined another transgenic model where RHO-1::Venus is expressed under the strong *C. elegans* body-

226   wall muscle promoter *Punc-54*. These animals have similar levels of transgene expression compared to

227   animals with body-wall muscle KIN-19::tagRFP (Figure supplement 7 F). Yet, whereas KIN-19 aggregates

228   abundantly in the body-wall muscle, RHO-1::Venus hardly aggregates in this tissue (Figure supplement 7

229   D). Thus, these transgenics are a suitable control to identify RHO-1 toxicity caused by overexpression.

230   Importantly, we did not observe reduced thrashing in animals with body-wall muscle RHO-1,

231   demonstrating that overexpressed of RHO-1 without concomittant aggregation is not toxic (Figure 3 D).

232   Similarly, the observation that only animals with the highest levels of pharyngeal KIN-19::tagRFP

233   aggregation have functional impairment shows that KIN-19::tagRFP overexpression alone does not cause

234   pumping defects (Figure 3 A and Figure supplement 8 B). Together, these positive and negative controls

235   reveal that protein aggregation itself is detrimental in *C. elegans*.

236   Collectively, these findings show that animals with accelerated protein aggregation experience an earlier

237   onset of functional decline in the tissues affected.

238

239   **Discussion:**

240   Widespread protein aggregation in the context of normal aging has been observed in *C. elegans* (David et

241   al., 2010; Reis-Rodrigues et al., 2012; Walther et al., 2015), *Drosophila* (Demontis & Perrimon, 2010),

242   *Saccharomyces cerevisiae* (Peters et al., 2012) and in mammals, notably in neural stem cells (Leeman et

243   al., 2018), heart (Ayyadevara, Mercanti, et al., 2016) and skeletal muscles (Ayyadevara,

244   Balasubramaniam, et al., 2016), bone marrow and spleen (Tanase et al., 2016). Whereas these hundreds

245   of proteins are maintained in a functional and soluble state in young animals, they lose their functional

246   structure with age and accumulate into insoluble aggregates. The detergent insoluble properties and

247   solid nature of the aggregates indicate similarities with disease-associated protein aggregation. However,

248   until now it has not been known whether age-dependent protein aggregates display amyloid-like

249   structures, a key characteristic of disease-associated protein aggregation. In this study, we focus on two

250   normally globular proteins, Casein kinase I isoform alpha and Ras-like GTP-binding protein rhoA. We

251   show that aggregates of either proteins display distinct fluorescence quenching properties characteristic

252   of amyloid structures. In neurodegenerative diseases and amyloidosis, protein aggregation is a crucial

253 part of the pathological process. We demonstrate that aggregates formed by proteins prone to

254 aggregation with age contribute to functional decline in the tissues affected. Our results predict that

255 even if only a proportion of the hundreds of proteins becoming insoluble with age form harmful amyloid-

256 like aggregates, this would be a significant cause of tissue aging for a variety of organs.

257 The speed of aggregate formation and the presence of amyloid-like structure brings significant insight

258 into the aggregation process occurring during normal aging. We demonstrate that newly synthesized

259 proteins rapidly assemble into large aggregates. The recruitment of newly synthesized proteins into

260 existing aggregates indicates a seeding effect. However, we also observed the rapid formation of new

261 aggregates entirely made up of newly synthesized proteins. This result shows that molecular aging of the

262 protein caused by progressive accumulation of damage is not required for proteins to aggregate with

263 age. Moreover, amyloid-like aggregates forming already in young animals indicate an intrinsic

264 aggregation propensity. Therefore, it is likely that these proteins self-assemble into amyloid structures

265 directly from unfolded or partially folded states occurring during or shortly after translation rather than

266 undergoing unfolding from their natively folded state. This conclusion is consistent with the view that

267 protein folding intermediates are particularly at risk of aggregating as exemplified by a recent study

268 revealing that newly synthesized proteins constitute the majority of the insoluble fraction prompted by

269 thermal stress (Xu et al., 2016). Furthermore, artificially increasing ribosome pausing during translation

270 causes widespread protein aggregation (Nedialkova & Leidel, 2015). Both Casein kinase I isoform alpha

271 and Ras-like GTP-binding protein rhoA contain several hexapeptides with high propensity for fibrillation

272 which are normally buried in the fully folded protein (Figure supplement 2). Interestingly, the most

273 prominent amyloid-promoting sequence for both proteins is localized near the N-terminus. Therefore,

274 aberrant interactions may start already during translation. As protein aggregation typically results in loss

275 of function, there is a strong evolutionary pressure to avoid this and molecular chaperones have evolved

276 to recognize specifically sequences of high aggregation propensity (Rousseau, Serrano, & Schymkowitz,

277   2006). Our findings predict that age-dependent protein aggregation would result from decreased levels

278   of molecular chaperones linked to protein synthesis rather than molecular chaperones induced by stress

279   (Albanese, Yam, Baughman, Parnot, & Frydman, 2006; Pechmann, Willmund, & Frydman, 2013).

280   Impaired proteasome-mediated removal of unfolded proteins directly after synthesis (Schubert et al.,

281   2000) could also significantly contribute to age-dependent protein aggregation. Interestingly, the relative

282   lack of Ras-like GTP-binding protein rhoA aggregation in the body-wall muscles compared to the

283   pharyngeal muscles shows that this protein only aggregates within a specific cellular environment. Thus

284   tissue-specific factors could be crucial for the aggregation process. These could be differences in the

285   tissue proteostasis network and reliance on certain proteostasis components with age (Hamer,

286   Matilainen, & Holmberg, 2010; Kern, Ackermann, Clement, Duerk, & Behl, 2010; Sala, Bott, & Morimoto,

287   2017) but also age-related changes in the local tissue environment. For example, tissue-specific changes

288   in ATP levels (Patel et al., 2017), availability of certain ions such as Ca^{2+} (Lautenschlager et al., 2018),

289   redox state (Kirstein et al., 2015), could explain why certain proteins aggregate in one tissue but not

290   another.

291   The resemblance between age-dependent protein aggregation and disease-associated protein

292   aggregation raises the question whether they have similar proteotoxic mechanisms. Our results show

293   that there is a strong correlation between the presence of large aggregates and impaired tissue function.

294   In disease, intracellular aggregate toxicity is caused, at least in part, by sequestration and the resulting

295   loss of function of essential cellular proteins (Hosp et al., 2017; Olzscha et al., 2011; Yu et al., 2014).

296   Notably, proteostasis network components are significantly enriched in the age-dependent insoluble

297   proteome (David et al., 2010). Therefore loss-of-function of proteins responsible for preventing

298   aggregation could be a key source of toxicity. The correlation between large aggregates and

299   proteotoxicity observed in the present study, does not exclude that intermediate forms on the path to

300   age-dependent protein aggregation are also proteotoxic. Indeed, disease-associated pre-fibrillar species

301 or oligomers can be highly toxic by interacting through their hydrophobic side chains with other cellular

302 components and in particular lipid membranes (Fusco et al., 2017; Knowles, Vendruscolo, & Dobson,

303 2014; Walsh et al., 2002). Further experiments will be needed to characterize which types of

304 intermediate species occur during age-dependent protein aggregation and to evaluate their potential

305 toxicity. Conversely, it remains possible that some forms of age-dependent protein aggregation could be

306 protective or manipulated to form harmless non-amyloid aggregates. Finally, it is possible that age-

307 dependent protein aggregation occurring in one tissue will induce accelerated aging in another tissue.

308 Following the evidence for seeding, self-propagation and cell-to-cell transfer of amyloid aggregating

309 species in a disease context (Jucker & Walker, 2013), it is intriguing to speculate that the same

310 mechanisms may arise with proteins aggregating during normal aging.

311 The proteotoxicity and amyloid conformation of age-dependent protein aggregation has important

312 implications for diseases associated with protein aggregation. Accelerated functional decline and the

313 overload of the proteostasis network caused by age-dependent protein aggregation could indirectly

314 enhance disease-associated pathogenesis. There is also evidence for a direct connection between

315 disease and aging-related aggregation. Indeed, a significant proportion of the proteins sequestered in

316 disease pathological deposits are prone to aggregate with age (Ciryam et al., 2013; David et al., 2010).

317 Notably, Casein kinase I isoform alpha is present in tau aggregates (Kannanayakal, Mendell, & Kuret,

318 2008; Kuret et al., 1997). Therefore, age-dependent aggregation-prone proteins interact with disease-

319 aggregating proteins in humans. Furthermore, we have demonstrated that minute amounts of insoluble

320 proteins from aged wild-type mouse brains or aged *C. elegans* are sufficient to cross-seed amyloid-β

321 aggregation *in vitro* (Groh, Buhler, et al., 2017). One possibility is that the highly hydrophobic nature of

322 the amyloid-like structures in age-dependent protein aggregates provides a destabilizing surface that can

323 promote the conformational conversion of disease-associated aggregating proteins. If the composition of

324 the insoluble proteome is cell-specific, increases in age-dependent protein aggregation, for example

325 through higher expression or somatic aggregation-promoting mutations (Freer et al., 2016; Lodato et al.,

326 2018), could sensitize specific cells to disease-associated pathogenesis.

327 In summary, our study demonstrates that aggregation of proteins during normal aging resembles

328 pathological protein aggregation observed in neurodegenerative diseases and amyloidosis. We then

329 show that age-dependent protein aggregation causes early functional decline, a read-out of accelerated

330 aging. These findings emphasize age-dependent amyloid-like aggregation as an important target to

331 restore physical capacity and to promote healthy aging. Already promising results reveal that lysosome

332 activation in the germline and in aged neural stem cells clears protein aggregates and rejuvenates the

333 cells (Bohnert & Kenyon, 2017; Leeman et al., 2018).

334

335 **Methods:**

336 Key Resources Table :

Reagent type (species) or resource	Designation	Source or reference	Identifiers	Additional information
Gene (*C. elegans*)	*kin-19*	Wormbase	WBGene0000220 2	
Gene (*C. elegans*)	*rho-1*	Wormbase	WBGene0000435 7	
Strain (*E. coli*)	OP50-1	Caenorhabditis genetic center (CGC)	**RRID:WB-STRAIN:OP50-1**	Streptomycin resistant strain of OP50
Genetic reagent (*C. elegans*)	*muEx473[Pkin-19::kin-19::tagrfp + Ptph-1::gfp]*	doi: 10.1371/journal.pbio .1000450	**RRID:WB-STRAIN:CF3166**	N2 background
Genetic reagent (*C. elegans*)	*muEx512[pkin-19::tagRFP + Ptph-1::GFP]*	doi: 10.1371/journal.pbio .1000450	CF3317	N2 background
Genetic reagent (*C. elegans*)	*muIs209[Pmyo-3::kin-19::tagrfp + Ptph-1::gfp]*	doi: 10.1371/journal.pbio .1000450	CF3649	N2 background

Genetic reagent (*C. elegans*)	*muIs210[pmyo-3::tagrfp + ptph-1::GFP]*	doi: 10.1371/journal.pbio.1000450	CF3650	N2 background
Genetic reagent (*C. elegans*)	*muEx587[Pkin-19::kin-19::meos2 + Punc-122::gfp]*	this paper	CF3706	N2 background
Genetic reagent (*C. elegans*)	*uqIs9[Pmyo-2::rho-1::tagrfp + Ptph-1::gfp]*	this paper	DCD13	N2 background
Genetic reagent (*C. elegans*)	*uqEx4[Pmyo-3::kin-19::meos2]*	this paper	DCD69	N2 background
Genetic reagent (*C. elegans*)	*uqEx[Pmyo-2::rho-1::meos2 + Punc-122::gfp + cb-unc-119(+)]*	this paper	DCD83	injected into EG6699 containing ttTi5605II; unc-119(ed3)III
Genetic reagent (*C. elegans*)	*uqEx22[Punc-54::rho-1::venus]*	this paper	DCD127	N2 background
Genetic reagent (*C. elegans*)	*uqIs12[Pmyo-2::rho-1::venus]*	this paper	DCD146	N2 background
Genetic reagent (*C. elegans*)	*uqEx37[Pkin-19::kin-19::venus + punc-122::gfp]*	doi: 10.1016/j.celrep.2016.12.033	DCD179	N2 background
Genetic reagent (*C. elegans*)	*uqIs22 [pkin19::kin19::hisavi + pkin19::birAtagrfp]*	this paper	DCD242	fem-1(hc17ts)IV background
Genetic reagent (*C. elegans*)	*uqIs19 [pmyo2::rho1::hisavi + pmyo2::birAtagrfp]*	this paper	DCD243	fem-1(hc17ts)IV background
Genetic reagent (*C. elegans*)	*uqEx49[pkin-19::meos]*	this paper	DCD245	N2 background
Genetic reagent (*C. elegans*)	*uqEx51[pmyo-2::venus]*	this paper	DCD248	N2 background
Genetic reagent (*C. elegans*)	*rmIs126[Punc-54::YFP]*	https://doi.org/10.1073/pnas.152161099	**RRID:WB-STRAIN:AM134**	N2 background
Genetic reagent (*C. elegans*)	*fem-1(hc17ts)IV*	CGC	**WB Cat# BA17, RRID:WB-STRAIN:BA17**	
recombinant DNA reagent	tagRFP	Evrogen, pTagRFP-N, FP142		
recombinant DNA reagent	pKA1062 containing mEOS2	other		Kaveh Ashrafi, UCSF, USA
antibody	Phalloidin-iFluor™ 488 conjugate	ABD-23115, AAT Bioquest, Biomol, Germany		"1:50"

chemical compound, drug	X34	SML1954, Sigma-Aldrich, Germany		1mM final
chemical compound, drug	thioflavin T	#ab120751, abcam, UK		50 µM
commercial assay or kit	5-Hydroxytryptamine creatinine sulfate complex	H7752, Sigma-Aldrich, Germany		10 µM
commercial assay or kit	Nickel Sepharose 6 Fast Flow beads from HisTrap FF Crude column	GE Healthcare, Uppsala, Sweden		

337

338   Cloning and Strain Generation:

339   Cloning was carried out using the Gateway system (Life Technologies, Darmstadt, Germany). Pmyo-2

340   promoter and pKA1062 mEOS2 translational vector were kindly provided by Dr. Brian Lee and Dr. Kaveh

341   Ashrafi, UCSF. *rho-1* cDNA was amplified from a cDNA library prepared from total RNA isolated from N2

342   worms. Plasmid containing biotinylation enzyme birA was kindly provided by Dr. Ekkehard Schulze

343   (University Freiburg). All constructs contain the unc-54 3' UTR. The tagrfp vector was obtained from

344   Evrogen (AXXORA, San Diego, CA, USA). Venus was generated by targeted mutation of the yfp gene.

345   HisAvi tagged KIN-19 and RHO-1 were generated by cloning at the C-terminus a RGSH6 tag together with

346   a bacterially derived polypeptide serving as a biotinylation signal *in vivo* as previously described (Schaffer

347   et al., 2010; Tagwerker et al., 2006). Constructs were sequenced at each step. Transgenics were

348   generated by microinjection of the constructs at concentrations between 10 to 50 ng/µl into N2 animals.

349   Stable lines were generated by irradiating the animals containing the extrachromosomal array in a CL-

350   1000 Ultraviolet Crosslinker (UVP) with 275µJ x 100. 100% transmission lines was backcrossed at least

351   four times into the wild-type N2 strain.

352

353   Maintenance:

354   All strains were kept at 15°C on NGM plates inoculated with OP50 using standard techniques. Age-

355   synchronization was achieved by transferring adults of the desired strain to 20°C and selecting their

356   progeny at L4 stage. All experiments were performed at 20°C. Day 1 of adulthood starts 24h after L4.

357

358   Photoconversion of mEOS2-tag and quantification of fluorescence levels:

359   For photoconversion, worms were transferred onto a small (diameter 35 mm) NGM plate without food.

360   The plate was placed 0.5 cm below a collimator (Collimator High-End Lumencor, Leica, Germany) fitted

361   with a filter for blue fluorescence (387/11 BrightLine HC, diameter 40mm) and illuminated by a

362   Lumencor Sola SE II (AHF, Tübingen). Conversion of mEOS2 in transgenic animals was performed four

363   times for five minutes, with 2 minutes pauses between exposures. To reduce translation, worms were

364   placed two hours before conversion on bacterial seeded plates with 500 µg/ml cycloheximide and kept

365   after conversion on plates with cycloheximide for 48h during aggregation quantification.

366

367   Aggregation quantification *in vivo*:

368   Aggregation levels were determined using Leica fluorescence microscope M165 FC with a Planapo 2.0x

369   objective. Aggregation was quantified following pre-set criteria adapted to the transgene expression

370   pattern and levels in the different transgenic *C. elegans* models: Animals expressing *Pkin-19::KIN-*

371   *19::mEOS2, Pkin-19::KIN-19::Venus or Pkin-19::KIN-19::TagRFP* were divided into less than 10 puncta

372   (low aggregation), between 10 and 100 puncta (medium aggregation) and over 100 puncta in the

373   anterior pharyngeal bulb (high aggregation). Animals overexpressing *Pmyo-2::RHO-1::Venus* were

374   divided into less than 10 puncta in anterior or posterior pharyngeal bulb (low aggregation), over 10

375   puncta in either bulbs (medium aggregation) and over 10 puncta in both bulbs (high aggregation).

376   Because of extensive RHO-1 aggregation in animals overexpressing *Pmyo-2::RHO-1::TagRFP*, aggregation

377   was only quantified in the isthmus: animals with no aggregation (low aggregation), animals with

378   aggregation in up to 50% (medium aggregation) and animals with aggregation in more than 50% (high

379   aggregation) of the isthmus. Animals overexpressing *Pmyo-3::KIN-19::TagRFP* were divided into over 15

380   puncta in the head or the middle body region (low aggregation), over 15 puncta in the head and the

381   middle body region (medium aggregation) and over 15 puncta in head, middle body and tail region (high

382   aggregation). The same categories defined for animals overexpressing *Pmyo-3::KIN-19::TagRFP* were

383   used to evaluate animals overexpressing *Pmyo-3::KIN-19::mEOS2* with a cutoff of 10 puncta instead of 15

384   to account for slightly lower aggregation levels. Animals overexpressing *Punc-54::RHO-1::Venus* were

385   divided into two categories: less than 15 puncta in the whole animal (low aggregation) and over 15

386   puncta in the whole animal (medium aggregation). Counting was done in a blind fashion. Two-tailed

387   Fisher's exact test using an online tool (https://www.socscistatistics.com/tests/fisher/default2.aspx) was

388   performed for statistical analysis.

389

390   Confocal imaging:

391   For confocal analysis using a Leica SP8 confocal microscope with the HC PL APO CS2 63x / 1.30 NA

392   glycerol objective, worms were mounted onto slides with 2% agarose pads using 2 µM levamisole for

393   anaesthesia. Worms were examined using the Leica HyD hybrid detector. The tag mEOS2 was detected

394   using 506nm as excitation and an emission range from 508-525nm for green fluorescence and 571nm as

395   excitation and an emission range from 573-602nm for red fluorescence. 3D reconstructions were

396   performed using the Leica Application Suite (LAS X). For X34 imaging, X34 was excited with a 405nm laser

397   and detected with an emission window between 470-520nm and RHO-1::tagRFP using 555nm as

398   excitation and an emission range from 565nm-620nm. For muscle structure imaging, phalloidin was

399   visualized by excitation at 488nm and with an emission window between 506-551nm and KIN-

400   19::tagRFP, RHO-1::tagRFP and tagRFP were visualized by excitation at 555nm and with an emission

401   window between 560-650nm.

402   FRAP analysis was performed as previously described (David et al., 2010) using the Leica SP8 confocal

403   microscope PMT detector. Relative fluorescence intensity (RFI) was analysed as described previously

404   following the equation RFI = (Tt/Ct)/(T0/C0), where T0 is the intensity in the region of interest (ROI)

405   before photobleaching; Tt, the intensity in the ROI at a defined time after photobleaching; C0, the

406   intensity in the non-bleached part of the puncta before photobleaching; and Ct, the intensity in the non-

407   bleached part of the puncta after bleaching (Brignull, Morley, Garcia, & Morimoto, 2006).

408

409   Fluorescence lifetime imaging *in vivo*:

410   For fluorescence lifetime imaging, transgenic *C. elegans* were mounted on microscope slides with 2.5%

411   agarose pads using 25 mM NaN3 as anaesthetic. All samples were assayed on a modified confocal-based

412   platform (Olympus FV300-IX70) equipped with a 60x oil objective (PLAPON 60XOSC2 1.4NA, Olympus,

413   Germany) and integrated with time-correlated single photon counting (TCSPC) FLIM implementation. A

414   pulsed supercontinuum (WL-SC-400-15, Fianium Ltd., UK) at 40MHz repetition rate served as the

415   excitation source. YFP was excited at 510nm using a tuneable filter (AOTFnC-400.650, Quanta Tech, New

416   York, USA). The excitation light was filtered with FF03-510/20 and the fluorescence emission was filtered

417   with FF01-542/27 (both bandpass filters from Semrock Inc., New York, USA) before reaching the

418   photomultiplier tube (PMC-100, Becker & Hickl GmBH, Berlin, Germany). Photons were recorded by a

419   SPC-830 (Becker and Hickl GmBH, Germany) module that permits sorting photons from each pixel into a

420   histogram according to the photon arrival times. Photons were acquired for two minutes to make a

421   single 256 X 256 FLIM image and photobleaching was verified to be negligible during this time. Photon

422   count rates were always kept below 1% of the laser repetition rate to avoid pulse pileup. All raw FLIM

423   images were fitted with a single exponential decay function using FLIMfit (Warren et al., 2013) and

424   exported to MATLAB (Mathworks, Inc., Natick, Massachusetts, USA) to obtain an intensity weighted

425 lifetime average for each image. Statistical analysis was carried out using two-way ANOVA followed by

426 Sidak's multiple comparisons test in Graphpad Prism software (La Jolla, California, USA).

427

428 X34 staining:

429 Worms were incubated in 1 mM X-34 in 10 mM Tris-HCl pH 8 for two hours, gently shaking at room

430 temperature as previously described (Link et al., 2001). Worms were then transferred to bacteria seeded

431 NGM plates to destain overnight before confocal imaging.

432

433 Pharyngeal pumping analysis:

434 Electrical activity of the pharyngeal pumping was measured using the NemaMetrix ScreenChip System

435 (NemaMetrix, Eugene OR). To record the electrical activity of pharynx pumping, the NemaMetrix

436 ScreenChip System (NemaMetrix, Eugene OR) was used. The entire setup is housed in a laboratory that

437 maintained a temperature of approximately 21°. Baseline noise was typically between 5 and 25 µV.

438 For each experiment, 50 worms were picked in 1.5 ml of M9 + 0.01% Triton and washed 3 times via low-

439 speed centrifugation. Worms were resuspended in 1.5 ml M9 + 0.01% Triton + 10 µM 5-

440 Hydroxytryptamine creatinine sulfate complex (Serotonin creatinine sulfate monohydrate) (Sigma,

441 H7752) and incubated for 20 minutes. The ScreenChip system was placed on a stereoscope and loaded

442 with a fresh screen chip. The screen chip was then vacuum-filled with M9 + 0.01% Triton +10 µM 5-

443 Hydroxytryptamine creatinine sulfate complex and the NemAquire software initiated for baseline noise

444 checking. The animals were loaded into the recording channel of the screen chip via vacuum. After

445 loading each animal, we waited at least 30 seconds or until the pumping became regular before starting

446 to record. Each animal was recorded for approximately 2 minutes regardless of whether pumping activity

447 was observed or not. Between 20-40 animals were recorded for each condition.

448 The recordings were analysed by NemAnalysis v0.2 software using the "Brute Force" optimization

449 method. The ideal settings were chosen automatically from all combinations of the bounds settings

450 (Minimum SNR from 1.4 (low) to 2.0 (high), with a Step size of 0.1; Highpass Cutoff from 10 (low) to 20

451 (high), with a Step size of 5) and applied to produce the analysis results. Data was exported into Excel for

452 statistical analysis. The student's t test was used for statistical analysis.

453

454 Thrashing analysis:

455 To quantify movement in terms of body-bends-per-second, movies of worms swimming in liquid were

456 acquired with high frame rates (15 frames per second) using a high-resolution monochrome camera (JAI

457 BM-500 GE, Stemmer imaging GmbH, Puchheim, Germany). For each condition, around 40 animals were

458 filmed (see Supplementary figure 1 for exact numbers). Worms were picked from cultivation plate and

459 allowed to swim in a small plastic petri dish filled with M9 + 0.01% Triton. Petri dish containing worms

460 was placed on a transparent platform and illuminated from bottom up with a flat backlight (CCS TH-

461 211/200-RD, Stemmer imaging GmbH, Puchheim, Germany) to achieve homogeneous, high contrast

462 lighting. The entire setup is housed in a laboratory that maintained a temperature of approximately 21°.

463 Movies were taken 10 minutes after placing the animals in the liquid. Five consecutive 30-second movies

464 were made for each group of worms. The movies were then analysed using the ImageJ wrMTrck plugin

465 (Nussbaum-Krammer, Neto, Brielmann, Pedersen, & Morimoto, 2015). The wrMTrck plugin tracked

466 individual worms in the movies and counted the numbers of body-bends. The input values of

467 wrMTrck_Batch are detailed in Supplementary file 3. Mann-Whitney test was used for statistical analysis

468 (GraphPad Prism 7).

469

470 Analysis of muscle structure:

471   To analyse the muscle structures in the presence of aggregates, worms were collected at day 4 (for body-

472   wall muscle KIN-19::tagRFP strain CF3649 and tagRFP control strain CF3650) and at day 2 (for pharyngeal

473   RHO-1::tagrfp strain DCD13 and tagRFP control strain CF3317) and fixed in 4% PFA for 10 minutes at

474   room temperature (RT). Worms were stained with phalloidin to visualize F-actin following a modified

475   protocol by Karady et al. (Karady et al., 2013). Briefly, worms were washed in phosphate buffered saline

476   (PBS), incubated for 30 minutes in PBS with 2% Tween and reduced for 30 minutes in Tris-Triton β-

477   mercaptoethanol solution (5% β-mercaptoethanol, 1% triton X-100, 130mM Tris pH 6.8). After washing

478   with PBS, worms were stained with phalloidin (1:50 in PBS, 0.5% triton X-100; Phalloidin-iFluor™ 488

479   conjugate from AAT Bioquest), washed and mounted on slides for confocal imaging.

480   Image analysis of muscle struture was performed in a blind fashion. The body-wall muscle structure was

481   considered normal when the actin filaments are smooth and tightly arranged with no empty space

482   between, modest defective when some of the filaments were slightly distorted or less densely packed.

483   Finally, body-wall muscles were considered severe defective when the filaments appeared significantly

484   thinner or highly wrinkled, or with large empty space between. The pharyngeal muscle structure was

485   considered severely defective when the actin filament structure showed large holes.

486

487   Plasmid generation for RHO-1 recombinant expression:

488   *C. elegans* RHO-1 cDNA was cloned into pET32a expression vector using restriction sites BamHI and

489   HindIII (NEB, UK). The open reading frame encoded the RHO-1 fusion protein comprising of a thioredoxin

490   protein, 6xHis tag and the Tobacco etch virus (TEV) cleavage recognition site with the sequence

491   ENLYFQA, where TEV cleaves between Q and A, which was also the N-terminal residue of the Rho-1

492   protein sequence, followed by the RHO-1 protein. The plasmid was confirmed by DNA sequencing

493   (Source Bioscience, Cambridge, UK).

494

495   Expression and Isolation of Recombinant RHO-1 from Inclusion Bodies:

496   Reagents were purchased from Sigma-Aldrich, UK unless stated. BL21 DE3 STAR™ E. coli (Thermo Fisher

497   Scientific, USA) were transformed with pET32a:RHO-1. 3 L cultures of E. coli in Lysogeny Broth containing

498   carbenicillin (100 ug/mL) were grown at 37°C at 250 rpm and induced for expression of the RHO-1 fusion

499   protein with 1 mM isopropyl-β-thiogalactopyranoside (IPTG) for four hours. *E. coli* were pelleted by

500   centrifuge at 8000 x g for 15 minutes before being washed by resuspension in PBS with 1% Triton X-110

501   and 1 mM Tris(2-carboxyethyl)phosphine hydrochloride (TCEP) and centrifuged again. The pelleted *E. coli*

502   were either stored at -20°C until further use or lysed straight away. The RHO-1 fusion protein forms in

503   inclusion bodies. To release the inclusion bodies, the *E. coli* were resuspended in 20 mL of lysis buffer per

504   1 L of culture (50 mM Tris, 500 mM NaCl, 5 mM MgCl2, 1 mM phenylmethylsulfonyl fluoride (PMSF),

505   protease inhibitor tablets (cOmplete™, Mini EDTA-free, Roche), 1% Triton X-110, 1 mM TCEP, pH 8 at

506   4°C) and sonicated on ice using three rounds of 30 s on and 30 s off 70% sonication power. The

507   suspension was centrifuged at 10,000 x g for 10 minutes at 4°C. The pellet containing RHO-1 inclusion

508   bodies was resuspended in 30 mL per 1 L culture of wash buffer 1 (Lysis buffer + 2 M urea, pH 8 at 4°C).

509   The inclusion bodies were sonicated for four rounds of 10 s on, 20 s off and centrifuged at 10,000 x g for

510   10 minutes at 4°C, this wash was then repeated again. Wash number 3 and 4 used the same sonication

511   and centrifuge parameters, but pellets were washed with wash buffer 2 (Lysis buffer, 2 M urea, 1 mM

512   TCEP, without Triton-110 or protease inhibitors). The final pellet became paler and more chalk-like.

513   These wash steps are important to lead to a purer final RHO-1 protein. The inclusion bodies were then

514   solublised in solublising buffer using 10 mL per 1 L of culture for 1 hour using a magnetic stirrer (50 mM

515   Tris, 500 mM NaCl, 5 mM MgCl2, 6 M guanidinium hydrochloride (GuHCl), 1 mM TCEP, (6 M Urea can

516   also be used in place of GuHCl, but GuHCl gives a slightly higher final protein yield)). Insoluble material

517   was removed by centrifuge at 16,000 x g for 10 minutes at room temperature (RT).

518

519   Purification of Recombinant RHO-1:

520   The RHO-1 fusion protein was purified using two linked-together 1 mL HisTrap Crude FF columns on an

521   ÄKTA Pure (GE Healthcare, Sweden). The columns were equilibrated with solublising buffer before 10 mL

522   of protein was loaded onto the column by the sample pump. The columns were washed in wash buffer

523   (50 mM Tris, 500 mM NaCl, 5 mM $MgCl_2$, 6 M Urea, 20 mM imidazole) before being eluted against a

524   linear gradient of elution buffer (50 mM Tris, 500 mM NaCl, 5 mM $MgCl_2$, 6 M Urea, 500 mM imidazole)

525   over 16 column volumes of a 1 x 1 mL column, i.e. 16 mL. Multiple purification runs were performed until

526   all RHO-1 fusion protein was purified. Fractions from all runs containing the RHO-1 fusion protein were

527   pooled and dialysed overnight in 2 L stabilising buffer modified from ((Healthcare; Nelson, Lee, &

528   Fremont, 2014; Thomson, Olson, Jackson, & Schrader, 2012) (50 mM Tris, 250 mM NaCl, 100 mM

529   arginine, 5 mM reduced glutathione, 0.5 mM oxidised glutathione, 5 mM MgCl2, 5 µM guanosine 5'-

530   diphosphate (GDP) (Generon, UK) pH 7.2). The RHO-1 fusion protein was then concentrated to 1 mL per

531   1 L culture using Spectra/Gel Absorbent (Spectrum Labs, USA) through a 10 kDa MWCO Slide-A-Lyzer™

532   dialysis cassette (Thermo Fisher Scientific). The RHO-1 fusion protein was then incubated overnight with

533   recombinant TEV protease to cleave the RHO-1 from the fusion tag in a 1:50 ration of TEV to RHO-1

534   fusion protein based on absorbance at 280 nm. Recombinant TEV was produced by Dr Marielle Wälti

535   using methods from (Tropea, Cherry, & Waugh, 2009). The cleaved RHO-1 was separated from the fusion

536   tag and TEV protein by purification using the two linked-together 1 mL HisTrap Crude FF columns on an

537   ÄKTA Pure. The columns were equilibrated in stabilising buffer and 1 mL of the protein loaded onto the

538   columns by injection. Cleaved RHO-1 was eluted in stabilisation buffer during the column wash. The

539   fusion tag and the His-tagged TEV were retained on the column and eluted with 100% elution buffer over

540   10 CV (stabilisation buffer with 500 mM imidazole). Protein concentration was monitored throughout

541   purification using the extinction coefficient 0.862 M^{-1} cm^{-1} for the fusion protein, 0.849 M^{-1} cm^{-1} for the

542   refolded fusion protein and 0.875 M^{-1} cm^{-1} for the cleaved refolded RHO-1 protein. Quantitative analysis

543 of protein purity was performed in FIJI image analysis software (Schindelin et al., 2012) by profiling

544 protein band intensity of the stained gel mass spectrometry confirmed the purification of RHO-1. To

545 note, RHO-1 precipitated in NaP, Tris, NaCl buffers. RHO-1 protein mass was confirmed by mass

546 spectrometry.

547

548 Fibrillisation of RHO-1 and analysis by Transmission Electron Microscopy (TEM):

549 RHO-1 was fibrillised by incubating 20 µM in stabilising buffer during a Thioflavin-T (ThT) based-assay

550 with 10 µM ThT (AbCam, UK) in non-binding, clear bottom, black 96-well plate (PN 655906 Greiner Bio-

551 One GmbH, Germany). The plate was incubated at 37°C with constant shaking at 300 rpm for 7 days.

552 RHO-1 was taken from the wells in the microplate for imaging by TEM. All samples also contained 0.05%

553 NaN$_3$ to prevent bacterial growth. Samples were incubated in an oven rotating at maximum speed (UVP

554 HB-1000 Hybridizer, Fisher Scientific) at 37°C for five weeks.

555 Fibrillised RHO-1 samples were centrifuged for 20 minutes at 21,000 x g and the supernatant removed

556 leaving 10 µL of sample. Each 10 µL sample was incubated on a glow-discharged copper grid for 1

557 minute. Excess liquid was blotted off and the grid washed in twice in dH2O for 15 seconds. 2% uranyl

558 acetate was used to negatively stain the samples for 30 s before imaging on the Tecnai G2 80-200kv TEM

559 at the Cambridge Advanced Imaging Centre.

560

561 Preparation and analysis of worm lysates for fibrils by TEM:

562 *C. elegans* DCD146 expressing RHO-1::Venus, DCD242 expressing KIN-19::HisAvi and DCD243 expressing

563 RHO-1::HisAvi were grown to confluency on high growth medium plates and bleached to obtain a

564 synchronized population of worms as previously described (Sulston, 1988). L1s were transferred into a

565 liquid culture with complete S basal supplemented with OP50-1 (OP50 with Streptomycin resistance) and

566 grown at 20°C or 25°C (to induce sterility of DCD242 and DCD243) as previously described (Groh, Buhler,

567 et al., 2017; Groh, Gallotta, et al., 2017). At day 1 of adulthood (DCD146) or at day 7 of adulthood

568 (DCD242 and DCD243), worms were allowed to sediment in a separation funnel and washed with cold

569 M9. The worm pellet was resuspended in PBS with 2x protease inhibitor tablets (cOmplete™, Mini EDTA-

570 free, Roche) and frozen in liquid nitrogen. For TEM with RHO-1::Venus, frozen worms were resuspended

571 in radioimmunoprecipitation assay (RIPA) buffer with protease inhibitor tablets and lysed by 20-25

572 passages through a cell homogeniser (Isobiotec, Germany) using a tungsten carbide ball with 16 μm

573 clearance. Cuticle fragments and unlysed worms were removed by centrifugation for 5 min at 835 g and

574 4 °C. After careful removal of the supernatant, the insoluble fraction was collected by centrifugation for

575 30 min at 21,000 g and 4 °C. The supernatant was removed and the pellet was resuspended in PBS with

576 protease inhibitor tablets by homogenising with a needle (27G, Sterican). To perform TEM with fibrils

577 isolated by affinity, we pulled down His-tagged RHO-1 and KIN-19 by nickel beads. For this, 250 μL worm

578 extract was added to 250 μL RIPA buffer (Invitrogen) with protease inhibitors and sonicated for 10s

579 twice. Cuticle fragments and unlysed worms were removed by centrifugation for 1 minute at 800 x g. The

580 supernatant was removed and centrifuged at 21 k x g for 20 minutes. The pellet was resuspended in 250

581 μL PBS with protease inhibitors and passed through a 30 G needle three times. 20 μL of precharged

582 Nickel Sepharose 6 Fast Flow beads taken from a HisTrap FF Crude column (GE Healthcare, Uppsala,

583 Sweden) were incubated with the resuspended pellet overnight at 4°C on a fixed speed rotator at 20 rpm

584 (SB2, Stuart, Staffordshire, UK). To separate the beads from unbound proteins the tubes were

585 centrifuged at 800 x g for 1 min and the supernatant removed. 60 μL of 500 mM imidazole in PBS pH 8

586 was added to the beads and incubated overnight at 4°C on a fixed speed rotator at 20 rpm. To isolate

587 eluted fibrils the tubes were centrifuged at 800 x g for 1 min and the supernatant containing the fibrils

588 was used for TEM experiments. 10 μL sample was applied to a carbon coated grid, and 2% uranyl acetate

589   was used for negative staining. Imaging was performed on the Tecnai G2 80-200kv TEM at the

590   Cambridge Advanced Imaging Centre.

591

592   Preparation and analysis of worm lysates for fibrils by SIM:

593   Nunc® Lab-Tek® II Chamber Slide™ (Sigma, Dorset, UK) were coated for 30 min with 0.01 % poly-L-Lysine

594   (P4707, Sigma) before incubation for one hour with either nickel bead extracted fibrils or resuspended

595   worm pellets prepared as described above. RHO-1::tagRFP expressing transgenics (DCD13) and *fem-1(-)*

596   mutants (CF2137; the non-aggregated control worm) extracts were incubated with 50 μM thioflavin T

597   (ThT) (#ab120751, abcam, UK) for one hour and washed three times in PBS before imaging. To visualize

598   amyloids from worm extracts, we used our custom-built SIM providing a spatial resolution approaching

599   90 nm at frame rates reaching 22 Hz (Young et al., 2016). Hardware control and image reconstruction

600   were performed with software written in LabView and Matlab (Strohl & Kaminski, 2015). For

601   visualization, ImageJ was used.

602

603

608

609 **References:**

610 Albanese, V., Yam, A. Y., Baughman, J., Parnot, C., & Frydman, J. (2006). Systems analyses reveal two
611   chaperone networks with distinct functions in eukaryotic cells. *Cell, 124*(1), 75-88.
612   doi:10.1016/j.cell.2005.11.039
613 Ayyadevara, S., Balasubramaniam, M., Suri, P., Mackintosh, S. G., Tackett, A. J., Sullivan, D. H., . . . Dennis,
614   R. A. (2016). Proteins that accumulate with age in human skeletal-muscle aggregates contribute
615   to declines in muscle mass and function in Caenorhabditis elegans. *Aging (Albany NY), 8*(12),
616   3486-3497. doi:10.18632/aging.101141
617 Ayyadevara, S., Mercanti, F., Wang, X., Mackintosh, S. G., Tackett, A. J., Prayaga, S. V., . . . Mehta, J. L.
618   (2016). Age- and Hypertension-Associated Protein Aggregates in Mouse Heart Have Similar
619   Proteomic Profiles. *Hypertension, 67*(5), 1006-1013. doi:10.1161/HYPERTENSIONAHA.115.06849
620 Baird, N. A., Douglas, P. M., Simic, M. S., Grant, A. R., Moresco, J. J., Wolff, S. C., . . . Dillin, A. (2014). HSF-
621   1-mediated cytoskeletal integrity determines thermotolerance and life span. *Science, 346*(6207),
622   360-363. doi:10.1126/science.1253168
623 Belsky, D. W., Caspi, A., Houts, R., Cohen, H. J., Corcoran, D. L., Danese, A., . . . Moffitt, T. E. (2015).
624   Quantification of biological aging in young adults. *Proc Natl Acad Sci U S A, 112*(30), E4104-4110.
625   doi:10.1073/pnas.1506264112
626 Bohnert, K. A., & Kenyon, C. (2017). A lysosomal switch triggers proteostasis renewal in the immortal C.
627   elegans germ lineage. *Nature, 551*(7682), 629-633. doi:10.1038/nature24620
628 Brignull, H. R., Morley, J. F., Garcia, S. M., & Morimoto, R. I. (2006). Modeling polyglutamine
629   pathogenesis in C. elegans. *Methods Enzymol, 412*, 256-282. doi:10.1016/S0076-6879(06)12016-
630   9
631 Chen, W., Young, L. J., Lu, M., Zaccone, A., Strohl, F., Yu, N., . . . Kaminski, C. F. (2017). Fluorescence Self-
632   Quenching from Reporter Dyes Informs on the Structural Properties of Amyloid Clusters Formed
633   in Vitro and in Cells. *Nano Lett, 17*(1), 143-149. doi:10.1021/acs.nanolett.6b03686
634 Chiti, F., Webster, P., Taddei, N., Clark, A., Stefani, M., Ramponi, G., & Dobson, C. M. (1999). Designing
635   conditions for in vitro formation of amyloid protofilaments and fibrils. *Proc Natl Acad Sci U S A,*
636   *96*(7), 3590-3594.
637 Ciryam, P., Tartaglia, G. G., Morimoto, R. I., Dobson, C. M., & Vendruscolo, M. (2013). Widespread
638   aggregation and neurodegenerative diseases are associated with supersaturated proteins. *Cell*
639   *Rep, 5*(3), 781-790. doi:10.1016/j.celrep.2013.09.043
640 David, D. C. (2012). Aging and the aggregating proteome. *Front Genet, 3*, 247.
641   doi:10.3389/fgene.2012.00247
642 David, D. C., Ollikainen, N., Trinidad, J. C., Cary, M. P., Burlingame, A. L., & Kenyon, C. (2010). Widespread
643   protein aggregation as an inherent part of aging in C. elegans. *PLoS Biol, 8*(8), e1000450.
644   doi:10.1371/journal.pbio.1000450
645 Demontis, F., & Perrimon, N. (2010). FOXO/4E-BP signaling in Drosophila muscles regulates organism-
646   wide proteostasis during aging. *Cell, 143*(5), 813-825. doi:10.1016/j.cell.2010.10.007
647 Finch, C. E., & Crimmins, E. M. (2016). Constant molecular aging rates vs. the exponential acceleration of
648   mortality. *Proc Natl Acad Sci U S A, 113*(5), 1121-1123. doi:10.1073/pnas.1524017113
649 Freer, R., Sormanni, P., Vecchi, G., Ciryam, P., Dobson, C. M., & Vendruscolo, M. (2016). A protein
650   homeostasis signature in healthy brains recapitulates tissue vulnerability to Alzheimer's disease.
651   *Sci Adv, 2*(8), e1600947. doi:10.1126/sciadv.1600947
652 Fusco, G., Chen, S. W., Williamson, P. T. F., Cascella, R., Perni, M., Jarvis, J. A., . . . De Simone, A. (2017).
653   Structural basis of membrane disruption and cellular toxicity by alpha-synuclein oligomers.
654   *Science, 358*(6369), 1440-1443. doi:10.1126/science.aan6160

655 Goldschmidt, L., Teng, P. K., Riek, R., & Eisenberg, D. (2010). Identifying the amylome, proteins capable of
656   forming amyloid-like fibrils. *Proc Natl Acad Sci U S A, 107*(8), 3487-3492.
657   doi:10.1073/pnas.0915166107
658 Golubev, A., Hanson, A. D., & Gladyshev, V. N. (2017). Non-enzymatic molecular damage as a prototypic
659   driver of aging. *J Biol Chem, 292*(15), 6029-6038. doi:10.1074/jbc.R116.751164
660 Gorisse, L., Pietrement, C., Vuiblet, V., Schmelzer, C. E., Kohler, M., Duca, L., . . . Gillery, P. (2016). Protein
661   carbamylation is a hallmark of aging. *Proc Natl Acad Sci U S A, 113*(5), 1191-1196.
662   doi:10.1073/pnas.1517096113
663 Groh, N., Buhler, A., Huang, C., Li, K. W., van Nierop, P., Smit, A. B., . . . David, D. C. (2017). Age-
664   Dependent Protein Aggregation Initiates Amyloid-beta Aggregation. *Front Aging Neurosci, 9*,
665   138. doi:10.3389/fnagi.2017.00138
666 Groh, N., Gallotta, I., Lechler, M. C., Huang, C., Jung, R., & David, D. C. (2017). Methods to Study Changes
667   in Inherent Protein Aggregation with Age in Caenorhabditis elegans. *J Vis Exp*(129).
668   doi:10.3791/56464
669 Hamer, G., Matilainen, O., & Holmberg, C. I. (2010). A photoconvertible reporter of the ubiquitin-
670   proteasome system in vivo. *Nat Methods, 7*(6), 473-478. doi:10.1038/nmeth.1460
671 Healthcare, G. E. Purifying Challenging Proteins Principales and Methods. *GE Healthcare*
672   *Handbook*(Handbook 28-9095-31 AA).
673 Hekimi, S., Lapointe, J., & Wen, Y. (2011). Taking a "good" look at free radicals in the aging process.
674   *Trends Cell Biol, 21*(10), 569-576. doi:10.1016/j.tcb.2011.06.008
675 Herndon, L. A., Schmeissner, P. J., Dudaronek, J. M., Brown, P. A., Listner, K. M., Sakano, Y., . . . Driscoll,
676   M. (2002). Stochastic and genetic factors influence tissue-specific decline in ageing C. elegans.
677   *Nature, 419*(6909), 808-814. doi:10.1038/nature01135
678 Hosp, F., Gutierrez-Angel, S., Schaefer, M. H., Cox, J., Meissner, F., Hipp, M. S., . . . Mann, M. (2017).
679   Spatiotemporal Proteomic Profiling of Huntington's Disease Inclusions Reveals Widespread Loss
680   of Protein Function. *Cell Rep, 21*(8), 2291-2303. doi:10.1016/j.celrep.2017.10.097
681 Huang, C., Xiong, C., & Kornfeld, K. (2004). Measurements of age-related changes of physiological
682   processes that predict lifespan of Caenorhabditis elegans. *Proc Natl Acad Sci U S A, 101*(21),
683   8084-8089. doi:10.1073/pnas.0400848101
684 Jucker, M., & Walker, L. C. (2013). Self-propagation of pathogenic protein aggregates in
685   neurodegenerative diseases. *Nature, 501*(7465), 45-51. doi:10.1038/nature12481
686 Kaeberlein, M. (2013). Longevity and aging. *F1000Prime Rep, 5*, 5. doi:10.12703/P5-5
687 Kaminski Schierle, G. S., Bertoncini, C. W., Chan, F. T. S., van der Goot, A. T., Schwedler, S., Skepper, J., . . .
688   Kaminski, C. F. (2011). A FRET sensor for non-invasive imaging of amyloid formation in vivo.
689   *Chemphyschem, 12*(3), 673-680. doi:10.1002/cphc.201000996
690 Kannanayakal, T. J., Mendell, J. R., & Kuret, J. (2008). Casein kinase 1 alpha associates with the tau-
691   bearing lesions of inclusion body myositis. *Neurosci Lett, 431*(2), 141-145.
692   doi:10.1016/j.neulet.2007.11.066
693 Karady, I., Frumkin, A., Dror, S., Shemesh, N., Shai, N., & Ben-Zvi, A. (2013). Using Caenorhabditis elegans
694   as a model system to study protein homeostasis in a multicellular organism. *J Vis Exp*(82),
695   e50840. doi:10.3791/50840
696 Kern, A., Ackermann, B., Clement, A. M., Duerk, H., & Behl, C. (2010). HSF1-controlled and age-associated
697   chaperone capacity in neurons and muscle cells of C. elegans. *PLoS One, 5*(1), e8568.
698   doi:10.1371/journal.pone.0008568
699 Kirstein, J., Morito, D., Kakihana, T., Sugihara, M., Minnen, A., Hipp, M. S., . . . Morimoto, R. I. (2015).
700   Proteotoxic stress and ageing triggers the loss of redox homeostasis across cellular
701   compartments. *EMBO J, 34*(18), 2334-2349. doi:10.15252/embj.201591711
702 Knowles, T. P., Vendruscolo, M., & Dobson, C. M. (2014). The amyloid state and its association with
703   protein misfolding diseases. *Nat Rev Mol Cell Biol, 15*(6), 384-396. doi:10.1038/nrm3810

704   Kuret, J., Johnson, G. S., Cha, D., Christenson, E. R., DeMaggio, A. J., & Hoekstra, M. F. (1997). Casein
705   kinase 1 is tightly associated with paired-helical filaments isolated from Alzheimer's disease
706   brain. *J Neurochem, 69*(6), 2506-2515.
707   Lautenschlager, J., Stephens, A. D., Fusco, G., Strohl, F., Curry, N., Zacharopoulou, M., . . . Schierle, G. S. K.
708   (2018). C-terminal calcium binding of alpha-synuclein modulates synaptic vesicle interaction. *Nat
709   Commun, 9*(1), 712. doi:10.1038/s41467-018-03111-4
710   Lechler, M. C., Crawford, E. D., Groh, N., Widmaier, K., Jung, R., Kirstein, J., . . . David, D. C. (2017).
711   Reduced Insulin/IGF-1 Signaling Restores the Dynamic Properties of Key Stress Granule Proteins
712   during Aging. *Cell Rep, 18*(2), 454-467. doi:10.1016/j.celrep.2016.12.033
713   Leeman, D. S., Hebestreit, K., Ruetz, T., Webb, A. E., McKay, A., Pollina, E. A., . . . Brunet, A. (2018).
714   Lysosome activation clears aggregates and enhances quiescent neural stem cell activation during
715   aging. *Science, 359*(6381), 1277-1283. doi:10.1126/science.aag3048
716   Link, C. D., Johnson, C. J., Fonte, V., Paupard, M., Hall, D. H., Styren, S., . . . Klunk, W. E. (2001).
717   Visualization of fibrillar amyloid deposits in living, transgenic Caenorhabditis elegans animals
718   using the sensitive amyloid dye, X-34. *Neurobiol Aging, 22*(2), 217-226.
719   Lodato, M. A., Rodin, R. E., Bohrson, C. L., Coulter, M. E., Barton, A. R., Kwon, M., . . . Walsh, C. A. (2018).
720   Aging and neurodegeneration are associated with increased mutations in single human neurons.
721   *Science, 359*(6375), 555-559. doi:10.1126/science.aao4426
722   Lu, M., Williamson, N., Mishra, A., Michel, C. H., Kaminski, C. F., Tunnacliffe, A., & Kaminski Schierle, G. S.
723   (2019). Structural progression of amyloid-beta Arctic mutant aggregation in cells revealed by
724   multiparametric imaging. *J Biol Chem, 294*(5), 1478-1487. doi:10.1074/jbc.RA118.004511
725   Lyubchenko, Y. L., Krasnoslobodtsev, A. V., & Luca, S. (2012). Fibrillogenesis of huntingtin and other
726   glutamine containing proteins. *Subcell Biochem, 65*, 225-251. doi:10.1007/978-94-007-5416-
727   4_10
728   McKinney, S. A., Murphy, C. S., Hazelwood, K. L., Davidson, M. W., & Looger, L. L. (2009). A bright and
729   photostable photoconvertible fluorescent protein. *Nat Methods, 6*(2), 131-133.
730   doi:10.1038/nmeth.1296
731   Murakami, T., Qamar, S., Lin, J. Q., Schierle, G. S., Rees, E., Miyashita, A., . . . St George-Hyslop, P. (2015).
732   ALS/FTD Mutation-Induced Phase Transition of FUS Liquid Droplets and Reversible Hydrogels
733   into Irreversible Hydrogels Impairs RNP Granule Function. *Neuron, 88*(4), 678-690.
734   doi:10.1016/j.neuron.2015.10.030
735   Nedialkova, D. D., & Leidel, S. A. (2015). Optimization of Codon Translation Rates via tRNA Modifications
736   Maintains Proteome Integrity. *Cell, 161*(7), 1606-1618. doi:10.1016/j.cell.2015.05.022
737   Nelson, C. A., Lee, C. A., & Fremont, D. H. (2014). Oxidative refolding from inclusion bodies. *Methods Mol
738   Biol, 1140*, 145-157. doi:10.1007/978-1-4939-0354-2_11
739   Nussbaum-Krammer, C. I., Neto, M. F., Brielmann, R. M., Pedersen, J. S., & Morimoto, R. I. (2015).
740   Investigating the spreading and toxicity of prion-like proteins using the metazoan model
741   organism C. elegans. *J Vis Exp*(95), 52321. doi:10.3791/52321
742   Olzscha, H., Schermann, S. M., Woerner, A. C., Pinkert, S., Hecht, M. H., Tartaglia, G. G., . . . Vabulas, R.
743   M. (2011). Amyloid-like aggregates sequester numerous metastable proteins with essential
744   cellular functions. *Cell, 144*(1), 67-78. doi:10.1016/j.cell.2010.11.050
745   Patel, A., Malinovska, L., Saha, S., Wang, J., Alberti, S., Krishnan, Y., & Hyman, A. A. (2017). ATP as a
746   biological hydrotrope. *Science, 356*(6339), 753-756. doi:10.1126/science.aaf6846
747   Pechmann, S., Willmund, F., & Frydman, J. (2013). The ribosome as a hub for protein quality control. *Mol
748   Cell, 49*(3), 411-421. doi:10.1016/j.molcel.2013.01.020
749   Peters, T. W., Rardin, M. J., Czerwieniec, G., Evani, U. S., Reis-Rodrigues, P., Lithgow, G. J., . . . Hughes, R.
750   E. (2012). Tor1 regulates protein solubility in Saccharomyces cerevisiae. *Mol Biol Cell, 23*(24),
751   4679-4688. doi:10.1091/mbc.E12-08-0620

752   Reif, A., Chiki, A., Ricci, J., & Lashuel, H. A. (2018). Generation of Native, Untagged Huntingtin Exon1
753   Monomer and Fibrils Using a SUMO Fusion Strategy. *J Vis Exp*(136). doi:10.3791/57506
754   Reis-Rodrigues, P., Czerwieniec, G., Peters, T. W., Evani, U. S., Alavez, S., Gaman, E. A., . . . Hughes, R. E.
755   (2012). Proteomic analysis of age-dependent changes in protein solubility identifies genes that
756   modulate lifespan. *Aging Cell, 11*(1), 120-127. doi:10.1111/j.1474-9726.2011.00765.x
757   Rousseau, F., Serrano, L., & Schymkowitz, J. W. (2006). How evolutionary pressure against protein
758   aggregation shaped chaperone specificity. *J Mol Biol, 355*(5), 1037-1047.
759   doi:10.1016/j.jmb.2005.11.035
760   Sala, A. J., Bott, L. C., & Morimoto, R. I. (2017). Shaping proteostasis at the cellular, tissue, and
761   organismal level. *J Cell Biol, 216*(5), 1231-1241. doi:10.1083/jcb.201612111
762   Schaffer, U., Schlosser, A., Muller, K. M., Schafer, A., Katava, N., Baumeister, R., & Schulze, E. (2010).
763   SnAvi--a new tandem tag for high-affinity protein-complex purification. *Nucleic Acids Res, 38*(6),
764   e91. doi:10.1093/nar/gkp1178
765   Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., . . . Cardona, A. (2012).
766   Fiji: an open-source platform for biological-image analysis. *Nat Methods, 9*(7), 676-682.
767   doi:10.1038/nmeth.2019
768   Schubert, U., Anton, L. C., Gibbs, J., Norbury, C. C., Yewdell, J. W., & Bennink, J. R. (2000). Rapid
769   degradation of a large fraction of newly synthesized proteins by proteasomes. *Nature,*
770   *404*(6779), 770-774. doi:10.1038/35008096
771   Strohl, F., & Kaminski, C. F. (2015). A joint Richardson-Lucy deconvolution algorithm for the
772   reconstruction of multifocal structured illumination microscopy data. *Methods Appl Fluoresc,*
773   *3*(1), 014002. doi:10.1088/2050-6120/3/1/014002
774   Styren, S. D., Hamilton, R. L., Styren, G. C., & Klunk, W. E. (2000). X-34, a fluorescent derivative of Congo
775   red: a novel histochemical stain for Alzheimer's disease pathology. *J Histochem Cytochem, 48*(9),
776   1223-1232. doi:10.1177/002215540004800906
777   Sulston, J. H., J. . (1988). *The Nematode Caenorhabditis elegans* Cold Spring Harbor: Cold Spring Harbor
778   Laboratory Press.
779   Tagwerker, C., Flick, K., Cui, M., Guerrero, C., Dou, Y., Auer, B., . . . Kaiser, P. (2006). A tandem affinity tag
780   for two-step purification under fully denaturing conditions: application in ubiquitin profiling and
781   protein complex identification combined with in vivocross-linking. *Mol Cell Proteomics, 5*(4), 737-
782   748. doi:10.1074/mcp.M500368-MCP200
783   Tanase, M., Urbanska, A. M., Zolla, V., Clement, C. C., Huang, L., Morozova, K., . . . Santambrogio, L.
784   (2016). Role of Carbonyl Modifications on Aging-Associated Protein Aggregation. *Sci Rep, 6*,
785   19311. doi:10.1038/srep19311
786   Taylor, R. C., & Dillin, A. (2011). Aging as an event of proteostasis collapse. *Cold Spring Harb Perspect*
787   *Biol, 3*(5). doi:10.1101/cshperspect.a004440
788   Thomson, C. A., Olson, M., Jackson, L. M., & Schrader, J. W. (2012). A simplified method for the efficient
789   refolding and purification of recombinant human GM-CSF. *PLoS One, 7*(11), e49891.
790   doi:10.1371/journal.pone.0049891
791   Tropea, J. E., Cherry, S., & Waugh, D. S. (2009). Expression and purification of soluble His(6)-tagged TEV
792   protease. *Methods Mol Biol, 498*, 297-307. doi:10.1007/978-1-59745-196-3_19
793   Vermulst, M., Denney, A. S., Lang, M. J., Hung, C. W., Moore, S., Moseley, M. A., . . . Erie, D. A. (2015).
794   Transcription errors induce proteotoxic stress and shorten cellular lifespan. *Nat Commun, 6*,
795   8065. doi:10.1038/ncomms9065
796   Walsh, D. M., Klyubin, I., Fadeeva, J. V., Cullen, W. K., Anwyl, R., Wolfe, M. S., . . . Selkoe, D. J. (2002).
797   Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term
798   potentiation in vivo. *Nature, 416*(6880), 535-539. doi:10.1038/416535a

799   Walther, D. M., Kasturi, P., Zheng, M., Pinkert, S., Vecchi, G., Ciryam, P., . . . Hartl, F. U. (2015).
800   Widespread Proteome Remodeling and Aggregation in Aging C. elegans. *Cell, 161*(4), 919-932.
801   doi:10.1016/j.cell.2015.03.032
802   Warren, S. C., Margineanu, A., Alibhai, D., Kelly, D. J., Talbot, C., Alexandrov, Y., . . . French, P. M. (2013).
803   Rapid global fitting of large fluorescence lifetime imaging microscopy datasets. *PLoS One, 8*(8),
804   e70687. doi:10.1371/journal.pone.0070687
805   Xu, G., Pattamatta, A., Hildago, R., Pace, M. C., Brown, H., & Borchelt, D. R. (2016). Vulnerability of newly
806   synthesized proteins to proteostasis stress. *J Cell Sci, 129*(9), 1892-1901. doi:10.1242/jcs.176479
807   Young, L. J., Strohl, F., & Kaminski, C. F. (2016). A Guide to Structured Illumination TIRF Microscopy at
808   High Speed with Multiple Colors. *J Vis Exp*(111). doi:10.3791/53988
809   Yu, A., Shibata, Y., Shah, B., Calamini, B., Lo, D. C., & Morimoto, R. I. (2014). Protein aggregation can
810   inhibit clathrin-mediated endocytosis by chaperone competition. *Proc Natl Acad Sci U S A,
811   111*(15), E1481-1490. doi:10.1073/pnas.1321811111

812

813

Figure legends

815 **Figure 1: Newly synthesized KIN-19 rapidly transitions into aggregates in aged animals**

816 (A, B) Following photoconversion at day 5 in the pharynx or in the body-wall muscle, the number of

817 animals with newly synthesized green-emitting non-converted KIN-19::mEOS2 aggregates doubles over

818 24 hours. Conversely levels of red-emitting converted aggregates slowly declines. Blocking translation

819 with cycloheximide (CHX) prevents new aggregate formation (B). Aggregation is evaluated in *Pkin-*

820 *19::KIN-19::mEOS2* and *Pmyo-3::KIN-19::mEOS2* transgenic animals on the basis of the fluorescence

821 intensity in puncta, representing aggregation (see methods). Quantification was done in the same

822 population of *C. elegans* before conversion (BC), after conversion (AC), 24h after conversion and 48h

823 after conversion. Fisher's exact test comparing low versus medium + high aggregation levels to after

824 conversion: *$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$. Fisher's exact test comparing low + medium versus

825 high aggregation levels to after conversion: °°$p < 0.01$. See source data including independent biological

826 repeat in supplementary file 1.

827 (C) 24 hours after photoconversion at day 7, newly synthesized KIN-19::mEOS2 (green emitting) forms

828 new aggregates and associates around older aggregates (red emitting). 3D reconstruction in the

829 pharyngeal anterior bulb region. Arrow heads highlight large new aggregates formed independently of

830 previous aggregates. Scale bar 2 μm.

831

832 **Figure supplement 1 (related to Figure 1): Newly synthesized KIN-19 and RHO-1 rapidly transitions into**

833 **aggregates in young animals**

834 (A) KIN-19::mEOS2 puncta visible in the body wall muscle contain immobile protein, demonstrated by

835 lack of fluorescent recovery after photobleaching (FRAP) after 5 minutes. Area bleached enlarged in

836 caption. Scale bar 2 μm.

837   (B) KIN-19::mEOS2 puncta visible in the pharynx contain immobile protein, demonstrated by FRAP after 8

838   minutes. Area bleached enlarged in caption. Scale bar 2 μm.

839   (C) FRAP analysis of KIN-19::mEOS2 puncta present in aged worms. Quantification of relative

840   fluorescence intensity (RFI) over time. Number of animals = 4, puncta evaluated = 6, mean ± SD is

841   represented. See source data in supplementary file 1.

842   (D) FRAP analysis of mEOS2 puncta/areas of fluorescence present in aged worms. Number of animals = 3,

843   puncta evaluated = 6, mean ± SD is represented. Only a few puncta are present in a population of

844   animals overexpressing *Pkin-19::mEOS2* (one in 80 transgenics at day 9 had more than 10 puncta in

845   anterior bulb).

846   (E) In young animals, aggregates are formed by newly synthesized KIN-19 and inhibition of translation (by

847   cycloheximide (CHX)) reduces new aggregate formation. Aggregation is evaluated in *Pkin-19::KIN-*

848   *19::mEOS2* transgenic animals on the basis of the fluorescence intensity in puncta, representing

849   aggregation (see methods). Quantification was done in the same population of *C. elegans* before

850   conversion (BC) at day 2, after conversion (AC), 24h after conversion and 48h after conversion. Fisher's

851   exact test comparing low versus medium + high aggregation levels to after conversion: $*p < 0.05$, $**p <$

852   0.01, $****p < 0.0001$. See source data in supplementary file 1.

853

854   (F) RHO-1::mEOS2 aggregates strongly already at day 1 and forms new aggregates (green) 24 h after

855   photoconversion and associates with older aggregates (red). Scale bar 15 μm, 7 μm in zoom.

856

857   **Figure 2: Drop in fluorescence lifetime reveals amyloid-like structure of KIN-19 and RHO-1 aggregates**

858   **in live *C. elegans*.**

859   (A) Representative intensity-weighted FLIM images of Venus only (control), RHO-1::Venus and KIN-

860   19::Venus worms. Scale = 25 µm. Inset shows zoomed-in images (intensity and FLIM) of the anterior bulb

861   for Venus only, RHO-1::Venus and KIN-19::Venus worms at day 12. Scale = 25 µm.

862   (B) Scatter plot shows a drop in the intensity-weighted fluorescence lifetime averages in adult worms

863   expressing RHO-1::Venus and KIN-19::Venus compared to worms expressing Venus only (control) in the

864   pharynx. n = 7-10, 2 independent biological repeats. Data is shown as mean lifetime +/- SEM and the

865   statistical analysis was performed using two-way ANOVA with Sidak´s multiple comparisons test: **

866    $p<0.01$, **** $p<0.0001$. See source data in supplementary file 1.

867   (C) Normalised histograms of intensity-weighted fluorescence lifetimes for RHO-1::Venus and KIN-

868   19::Venus show a population shift towards lower lifetimes compared to Venus only (control) worms. The

869   histograms contain information from all pixels of all images acquired for each condition.

870

871   **Figure supplement 2 (related to Figure 2): Fibrillation propensity calculate by ZipperDB**

872   (A) Fibrillation propensity profile for KIN-19

873   (B) Fibrillation propensity profile for RHO-1

874   Table inlet shows hexapeptides with Composite scores below -45.

875   ZipperDB: https://services.mbi.ucla.edu/zipperdb/

876

877   **Figure supplement 3 (related to Figure 2): Aggregation with age of RHO-1::Venus and KIN-19::Venus**

878   (A) Increased pharyngeal KIN-19 aggregation with age in animals expressing *Pkin-19::KIN-19::Venus*.

879   Panel reproduced from https://doi.org/10.1016/j.celrep.2016.12.033.

880   (B) Increased pharyngeal RHO-1 aggregation with age in animals expressing *Pmyo-2::RHO-1::Venus*.

881   Numbers of worms indicated in the bars. Significance calculated low + medium versus high aggregation

882   levels compared to day 2 of adulthood; Fisher's exact test: ****p< 0.0001. See source data in

883   supplementary file 1.

884   (C) RHO-1::Venus puncta visible in the pharynx contain immobile protein, demonstrated by absence of

885   fluorescence recovery after 4 minutes. Scale bar 2 µm.

886   (D) RHO-1::Venus aggregates are highly immobile. Table summarizes FRAP experiments carried out at

887   different ages.

888   (E) FRAP analysis of RHO-1::Venus puncta present in aged worms compared to Venus alone.

889   Quantification of RFI over time. Number of animals = 5, puncta evaluated = 6, mean ± SD is represented.

890   See source data in supplementary file 1.

891

892   **Figure supplement 4 (related to Figure 2): Staining of RHO-1::tagRFP with Congo red derivative X34**

893   (A) Representative fluorescence decay curves, lifetime fits (with associated chi^2 values) and fit residuals

894   for Venus only (control), RHO-1::Venus and KIN-19::Venus adult worms at day 12. The black trace

895   represents the instrument response function (IRF).

896   (B) Drop in fluorescence lifetime upon RHO-1::tagRFP aggregation. Scatter plot showing the intensity-

897   weighted mean fluorescence lifetime values of day 1 adult worms expressing RHO-1::tagRFP in the

898   pharynx compared to worms expressing tagRFP only. n = 7-8, 2 independent biological repeats

899   combined. t-test with Welch's correction for unequal standard deviations: **** p<0.0001.

900   See source data in supplementary file 1.

901   (C) RHO-1 aggregates are stained by Congo red derivative X34. Day 2 old animals expressing *Pmyo-*

902   *2::RHO-1::tagRFP*. Scale bar 30 µm. Zoom scale bar 15 µm.

903   (D) Absence of X34 staining in wild-type pharynx. Day 3 old wild-type animals (N2). Scale bar 30 µm.

904   Zoom scale bar 15 µm.

905   (E) RHO-1::tagRFP fluorescence does not interfer with X34 fluorescence as demonstrated by eliminating

906   tagRFP fluorescence by bleaching (in white square). Scale bar 3 µm.

907

908   **Figure supplement 5 (related to Figure 2): Recombinant RHO-1 forms amyloid-like fibrils *in vitro* and *in***

909   ***vivo***

910   (A) Purification steps of recombinant RHO-1 analyzed by SDS-PAGE gel electrophoresis and Coomassie

911   staining. The RHO-1 fusion protein consisting of the thioredoxin protein, His tag and Tobacco etch virus

912   (TEV) cleavage recognition site was purified on a Crude FF column and eluted with imidazole. It ran

913   slightly quicker than the expected 40 kDa predicted size (lane 1). The fusion protein was incubated with

914   recombinant TEV protease overnight in a 1:50 ratio (lane 2). The cleaved RHO-1 protein did not bind to

915   the Crude FF column and eluted in the flow through (lane 3). The TEV protease which has a 6xHis tag and

916   the remaining uncleaved fusion protein were eluted from the column with imidazole (lane 4).

917   (B) Matrix Assisted Laser Desorption/Ionization (MADLI) mass spectrometry of purified RHO-1.

918   Performed by the Proteomics Facility, Biochemistry Department, University of Cambridge.

919   (C) Negative-stain transmission electron micrographs of fibrillised RHO-1. TEM of RHO-1 negatively

920   stained using 2% uranyl acetate. RHO-1 was incubated for 1 week in a ThT assay to fibrillise. Scale bar =

921   200 nm.

922   (D) Negative-stain transmission electron micrographs of *pmyo-2::RHO-1::Venus* worm lysates, day 1. TEM

923   of RHO-1 fibrils negatively stained using 2% uranyl acetate. Scale bar = 200 nm.

924   (E) Negative-stain transmission electron micrographs of RHO-1::HisAvi and KIN-19::HisAvi purified from

925   worms lysates with nickel beads, day 7. Scale bar = 200 nm.

926

927   **Figure supplement 6 (related to Figure 2): Structured illumination microscopy reveals that RHO-1**

928   **forms ThT positive fibril structures _in vivo_**

929   (A) Left image: SIM image of RHO-1 fibrils isolated by affinity purification from transgenics expressing

930   RHO-1::HisAvi at day 7 and labelled using the amyloid dye ThT. Right image: Zoomed in image of left

931   image highlighting the fibril bundles formed by RHO-1 _in vivo_. Circled in red, single fibrils; in green, fibril

932   bundles and in blue, fibril clusters. Scale bars: 5 and 1 µm, respectively. (B) Two colour SIM images of

933   RHO-1 fibrils extracted from transgenics expressing RHO-1::tagRFP (day 2). Fibrils were stained with ThT

934   prior to imaging. Left image: SIM image of ThT positive RHO-1::tagRFP fibrils. Central image: SIM image

935   of the same fibril as shown in left but upon tagRFP excitation. Right image: Overlay of ThT and tagRFP

936   channels displays co-localization of ThT and RFP of extracted RHO-1::tagRFP fibrils. Lower panel: imaging

937   of control extracts from young animals (sterile _fem-1(-)_ mutants). Scale bar: 1 µm.

938

939   **Figure 3: Age-dependent protein aggregation impairs pharyngeal and body-wall muscle function.**

940   (A) Aged animals with high levels of pharyngeal KIN-19 aggregation have reduced pharyngeal pumping.

941   N= 23-28 animals analyzed per group. T-test: $p < 0.0001$.

942   (B) Aged animals with high levels of KIN-19 aggregation in the body-wall muscle display reduced

943   thrashing. Mean body bends per seconds are set to 100% in animals with low aggregation. Mann-

944   Whitney test: $p < 0.0001$.

945   (C) Young animals with RHO-1 aggregation in pharynx have impaired pharyngeal pumping. N= 26-27

946   animals analyzed per group. T-test: $p < 0.0001$.

947   (D) Overexpression of RHO-1 without aggregation in the body-wall muscles does not influence thrashing.

948   Mean body bends per seconds are set to 100% in *Punc-54::yfp* transgenic animals. Mann-Whitney test:

949   non-significant.

950   SEM represented, independent biological repeats in supplementary file 2 and source data in

951   supplementary file 1.

952

953   **Figure supplement 7 (related to Figure 3): Markers for age-dependent protein aggregation**

954   (A) Increased pharyngeal KIN-19 aggregation with age in animals expressing *Pkin-19::KIN-19::tagRFP*.

955   (B) Increased body-wall muscle KIN-19 aggregation with age in animals expressing *Pmyo-3::KIN-

956   19::tagRFP*.

957   (C) Increased pharyngeal RHO-1 aggregation with age in animals expressing *Pmyo-2::RHO-1::tagRFP*.

958   Already young adults display abundant RHO-1 aggregation.

959   (D) Absence of RHO-1 aggregation in the majority of animals expressing *Punc-54::RHO-1::Venus*.

960   Numbers of worms indicated in the bars. Significance calculated low + medium versus high aggregation

961   levels compared to day 2 of adulthood; Fisher's exact test: **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. See

962   source data in supplementary file 1.

963   (E) RHO-1::tagRFP aggregates abundantly at day 2 compared to KIN-19::tagRFP. Fluorescent micrograph

964   of the upper body region of animals expressing *Pkin-19::KIN-19::tagRFP* or *Pmyo-2::RHO-1::tagRFP*. Days

965   of adulthood and exposure times displayed under images. Arrow heads highlight animals with high levels

966   of KIN-19 aggregation in anterior bulb. Scale bar: 100 µm

967   (F) KIN-19::tagRFP but not RHO-1::Venus aggregates in body-wall muscle (small puncta). Fluorescent

968   micrograph of whole animals expressing *Punc-54::RHO-1::Venus* or *Pmyo-3::KIN-19::tagRFP*. Head region

969   on the left. Days of adulthood and exposure times displayed under images. Scale bar: 200 μm.

970

971   **Figure supplement 8 (related to Figure 3): KIN-19 aggregation in body-wall muscles impairs thrashing**

972   (A) KIN-19 aggregation in body-wall muscles impairs thrashing. Mean body bends per seconds are set to

973   100% in *Pmyo-3::tagRFP* transgenics. Mann-Whitney test: ****$p < 0.0001$

974   (B) No effect on pumping detected in animals with mixed levels of KIN-19::tagRFP aggregation. N= 29-39

975   animals analyzed per group. T-test: non-significant.

976   (C) Fluorescent tagRFP does not affect pharyngeal pumping in young animals. N= 26-27 animals analyzed

977   per group. T-test: non-significant.

978   (D) Fluorescent tagRFP does not affect pharyngeal pumping in aged animals. N= 23-26 animals analyzed

979   per group. T-test: non-significant.

980   SEM represented, independent biological repeats in supplementary file 2 and source data in

981   supplementary file 1.

982

983   **Figure supplement 9 (related to Figure 3): KIN-19 and RHO-1 aggregation impacts muscle structure**

984   (A, B) Animals with KIN-19 aggregates show defective body-wall muscle structure at day 4. (A)

985   Representative single-plane image showing wrinkled, thinner and faded actin filaments in the presence

986   of KIN-19 aggregates. Top panel: F-actin staining with phalloidin (green). Bottom panel: Merged image

987   with KIN-19 aggregates (magenta). Scale bar 30 μm. (B) Quantification of the decline in body-wall muscle

988   structure with KIN-19 aggregation. Fisher's exact test: ****p< 0.0001. See source data in supplementary

989   file 1.

990   (C, D) Animals with RHO-1 aggregates show defective pharyngeal muscle structure at day 2. (C)

991   Representative single-plane image showing holes in the actin filaments in the presence of RHO-1

992   aggregates. Top: merged view of pharynx (RHO-1::tagRFP, tagRFP in magenta and F-actin staining with

993   phalloidin in green); Bottom: zoom into boxed area. Scale bar: 15 μm, zoom: 3 μm. (D) Quantification of

994   defective pharyngeal muscle structure with RHO-1 aggregation. Fisher's exact test: ****p< 0.0001. See

995   source data in supplementary file 1.

996

997   Supplementary file 1: source data

998   Supplementary file 2: Pharyngeal pumping and thrashing repeats

999   Supplementary file 3: parameters for thrashing analysis

1000

Figure 1

A Body-wall muscle KIN-19::mEOS2 aggregation

B Pharyngeal KIN-19::mEOS2 aggregation

Aggregation level
(green emitting):

☐ Low ☐ Medium ☐ High

Aggregation level
(photoconverted red):

☐ Low ☐ Medium ☐ High
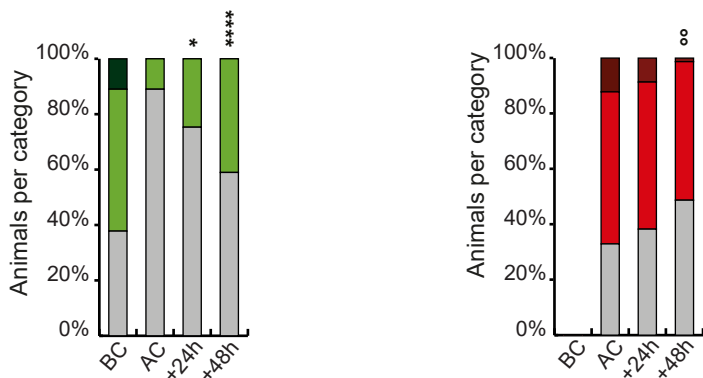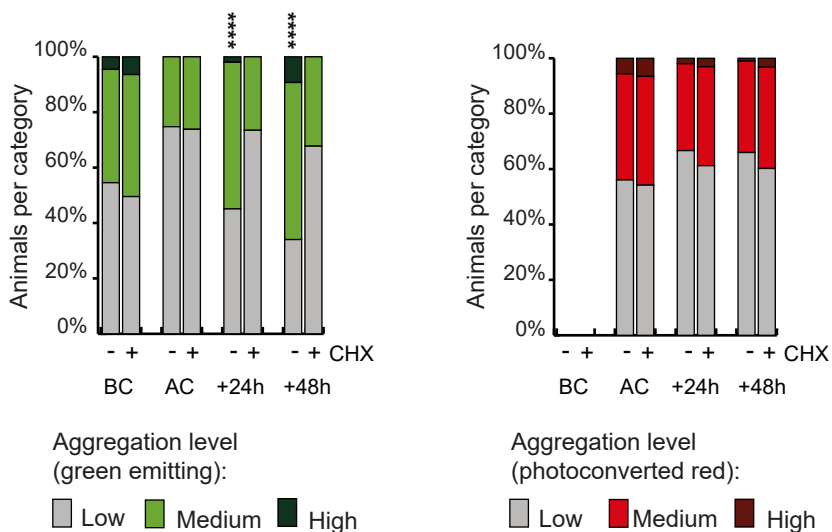
C Pharyngeal KIN-19::mEOS2
Day 7, 24h after conversion

Newly synthesized
KIN-19

Converted KIN-19

Merged
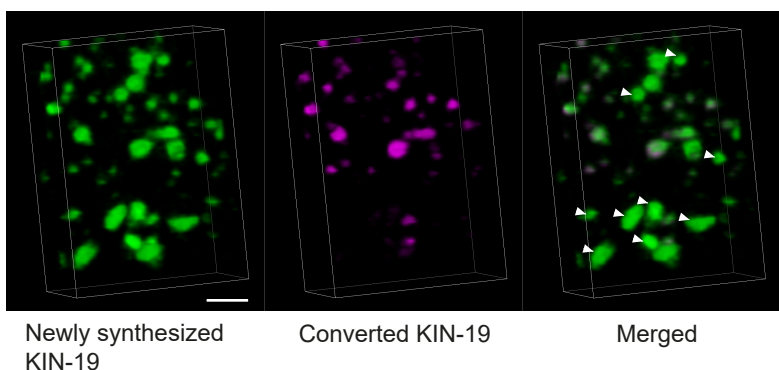
Figure supplement 1

A KIN-19::mEOS2 expressed in the body wall muscle

Before FRAP   6 sec after bleaching   5 min after bleaching

C

- ● Body-wall muscle KIN-19::mEOS2 (day 11)
- □ Pharyngeal KIN-19::mEOS2 (day 9)

B KIN-19::mEOS2 expressed in the pharynx

Before FRAP   6 sec after bleaching   3 min after bleaching   8 min after bleaching

D

- ◆ Pharyngeal mEOS2 (Day 9)
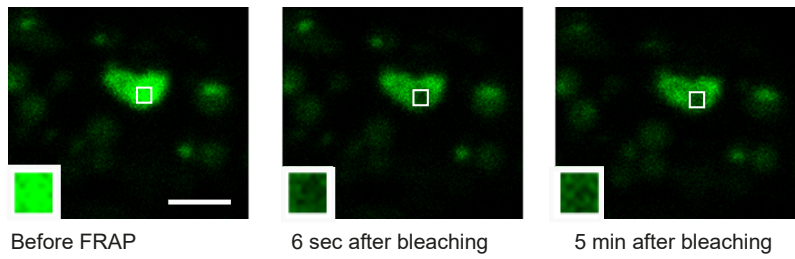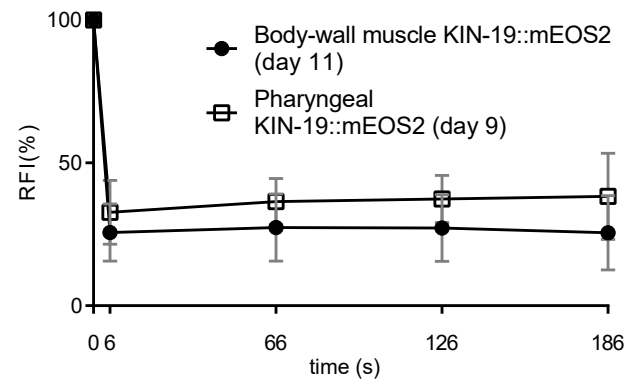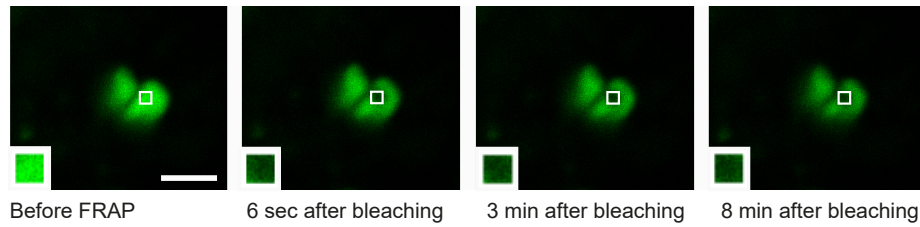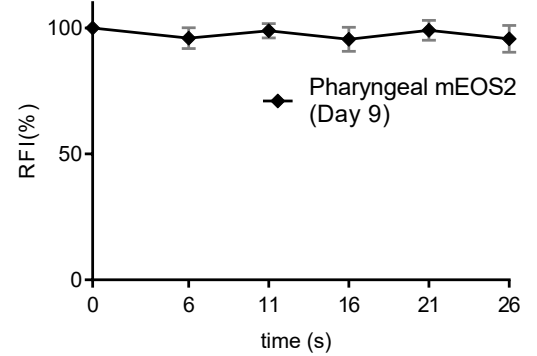
E Pharyngeal KIN-19::mEOS2 aggregation, photoconversion at day 2

Aggregation level (green emitting): Low Medium High

Aggregation level (photoconverted red): Low Medium High
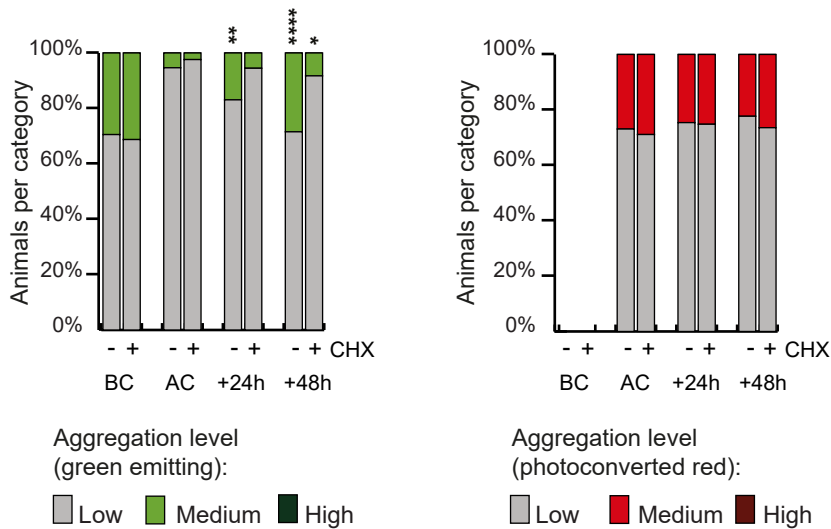
F RHO-1::mEOS2 expressed in the pharynx at day 1, 24h after photoconversion

Red   Green   Merge

Zoom
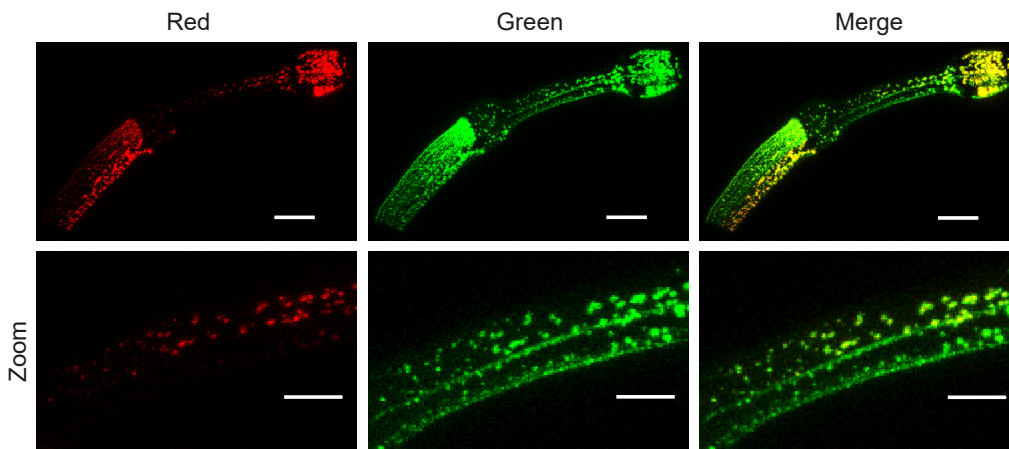
Figure 2

A Venus: FLIM images
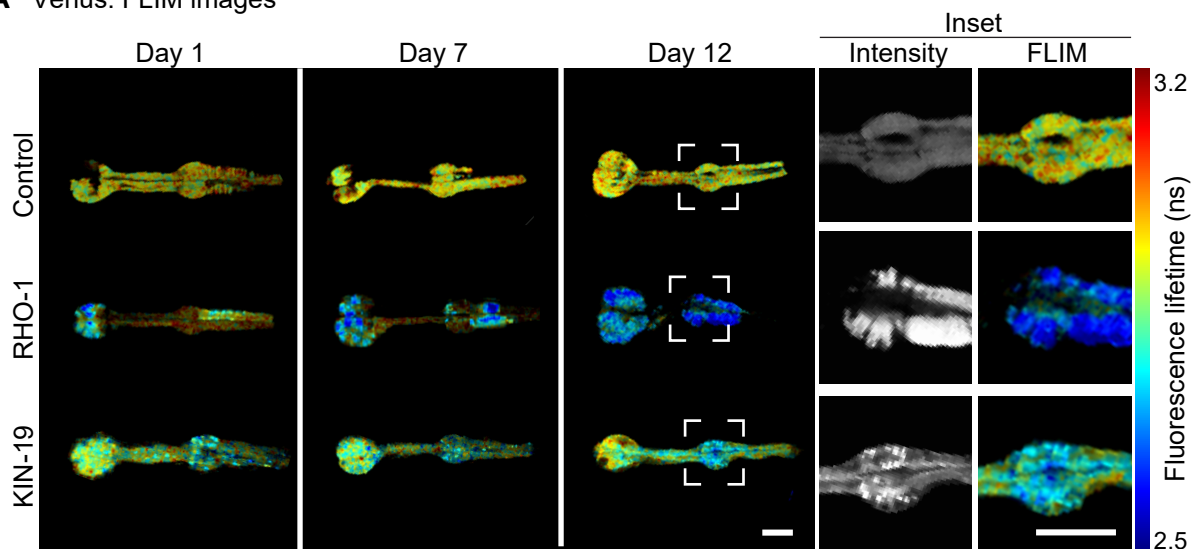
B Venus: fluorescence lifetimes
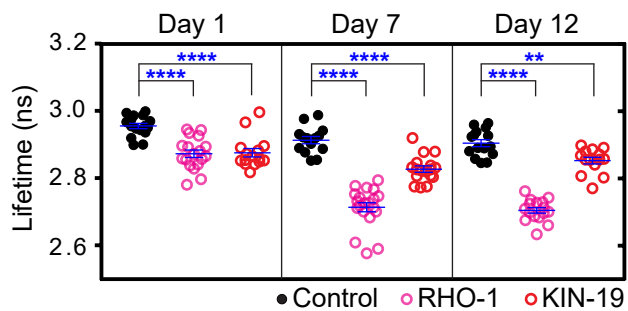
C Venus: Day 12 histograms
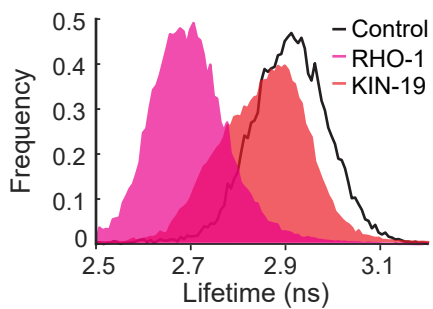
Figure supplement 2

A   Fibrillation Propensity profile for KIN-19

Position	Sequence	C-Score
31	YVSINV	-46.235
188	SINAHL	-45.82
41	EVAIKL	-45.76
109	MKTVLM	-45.75
110	KTVLML	-45.65
187	ASINAH	-45.595
9	DFIVAT	-45.435
305	QQSQSS	-45.21
42	VAIKLE	-45.02

B   Fibrillation Propensity profile for RHO-1

Position	Sequence	C-Score
18	KTCLLI	-46.14
146	AIAEQI	-46.117
78	DVILMC	-46.05
20	CLLIVF	-45.955
79	VILMCF	-45.74
187	SKCMIL	-45.376
150	QIGAFA	-45.268
21	LLIVFS	-45.165
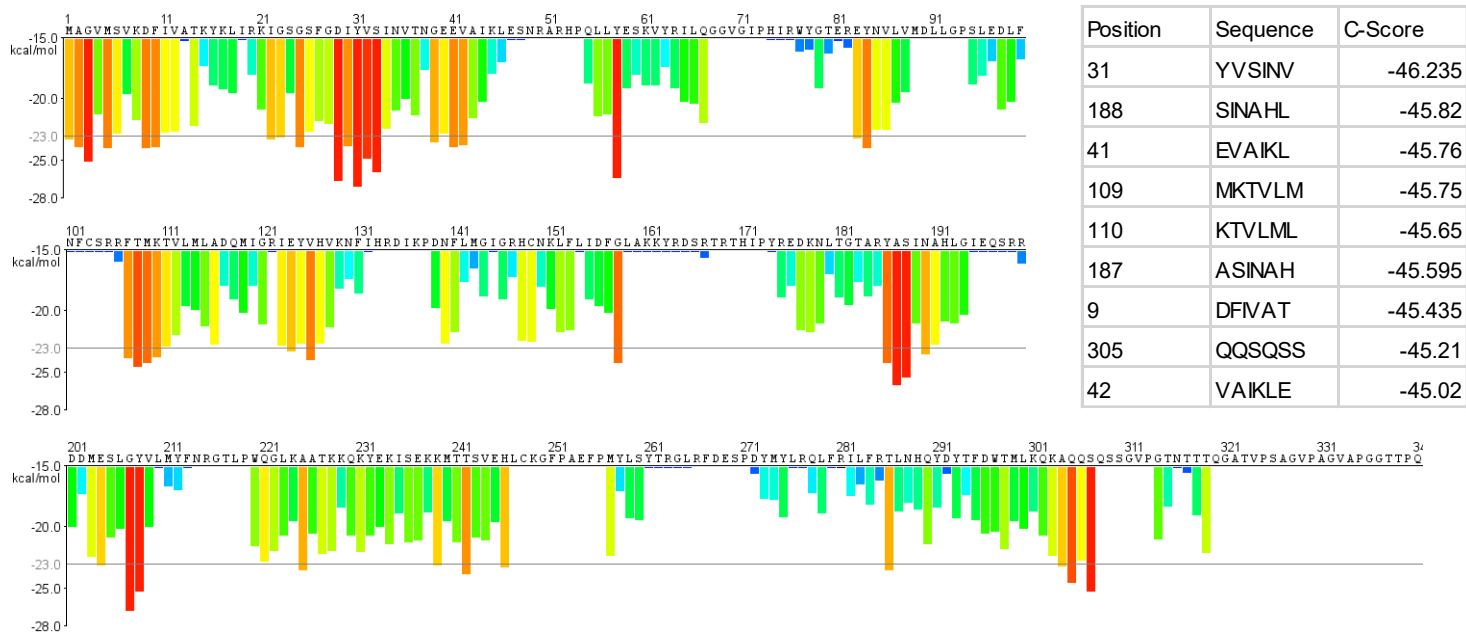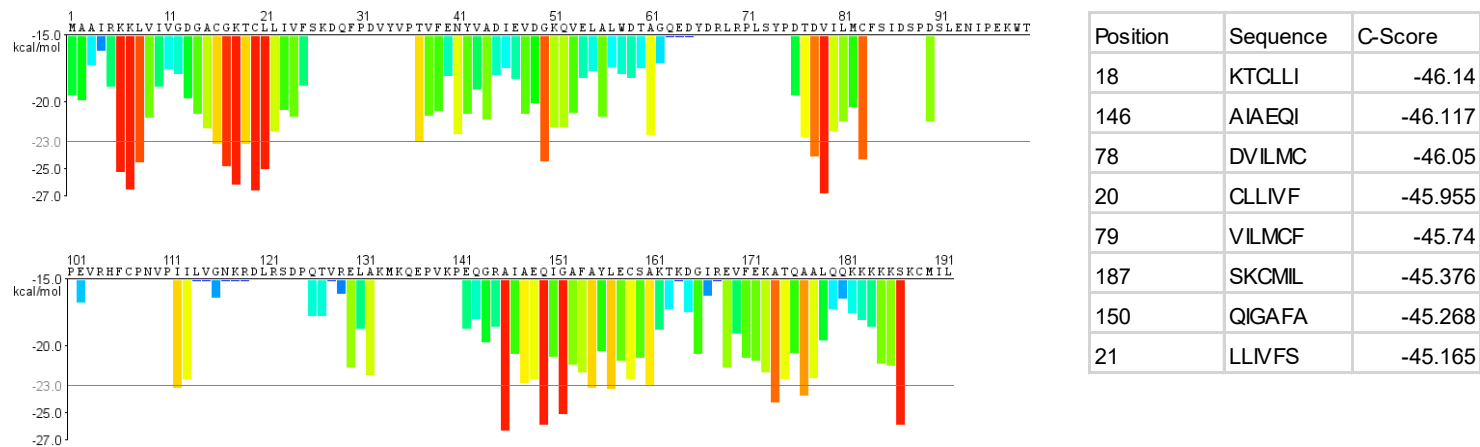
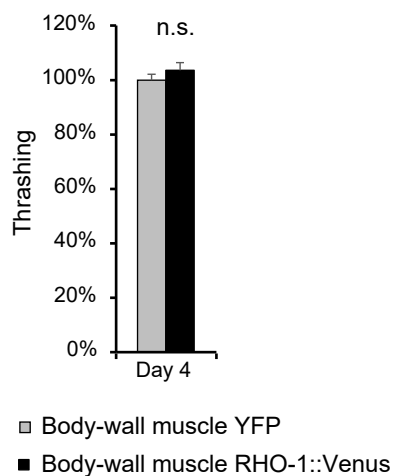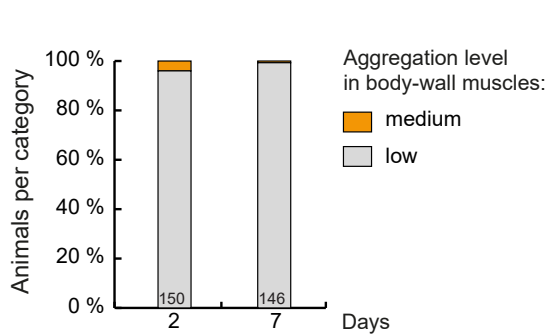# Figure supplement 3

A Pharyngeal KIN-19::Venus aggregation

Aggregation level
in anterior pharyngeal bulb:
- High
- Medium
- Low
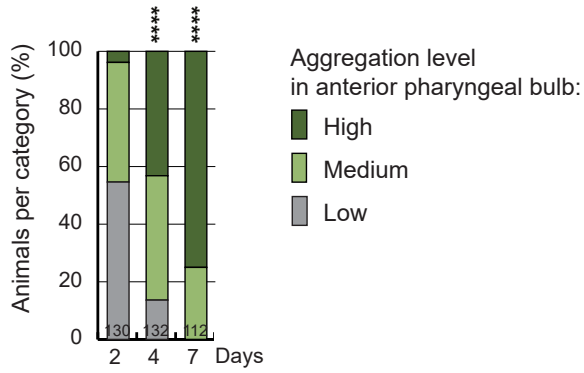
B Pharyngeal RHO-1::Venus aggregation

Aggregation level
in pharynx:
- High
- Medium
- Low
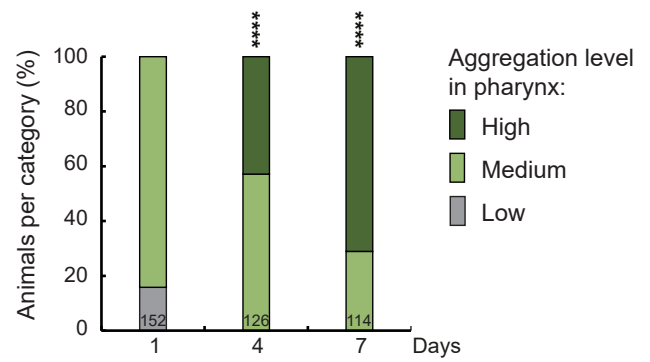
C RHO-1::Venus expressed in the pharynx, day 7

Before FRAP   6 sec after bleaching   4 min after bleaching
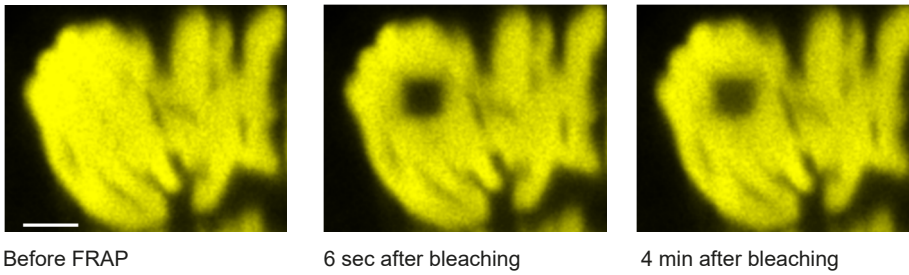
D

RHO-1::Venus				
Days of adulthood	Number of animals	Puncta evaluated	Immobile (no recovery)	Mobile (recovery)
1	5	11	11	0
7	5	10	10	0
11	6	11	11	0
14	5	10	10	0

E

Figure supplement 4
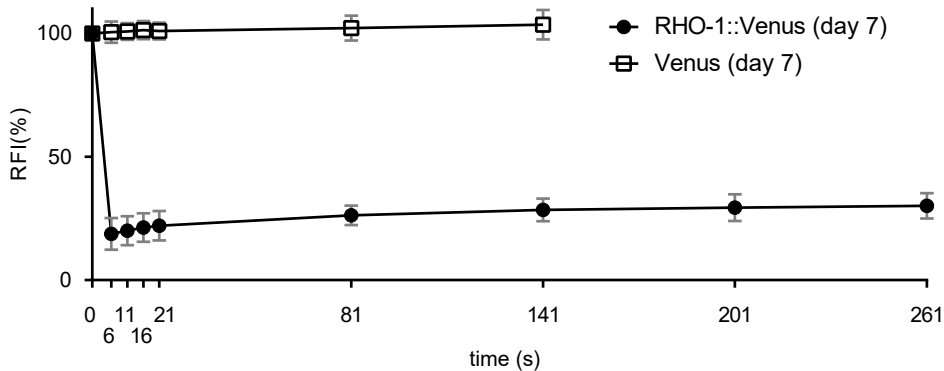
A Venus: fluorescence lifetime fits
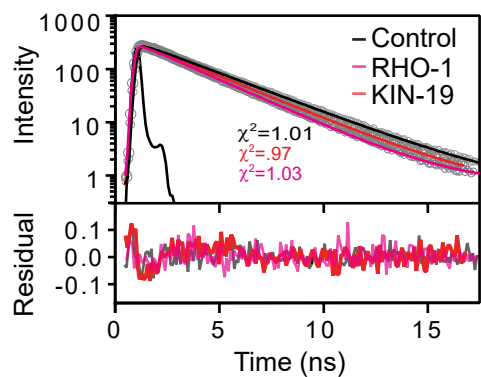
B TagRFP: fluorescence lifetimes

C Pharyngeal RHO-1::tagRFP with X34 staining

Merge   X34

Zoom

Merge   X34
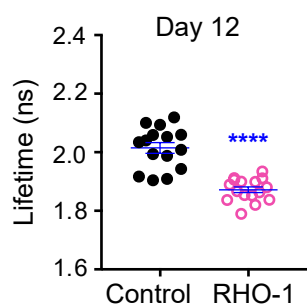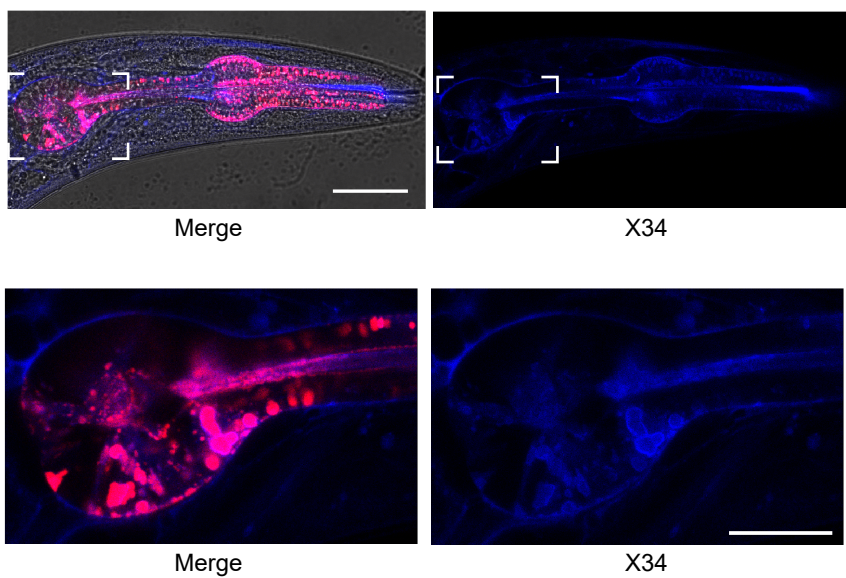
D Wild-type with X34 staining

Merge

Zoom

X34

E Pharyngeal RHO-1::tagRFP with X34 staining

Pre-bleach   Post-bleach

Merge

X34

Figure supplement 5

A

Lanes: 1 2 3 4

- 260kDa
- 140kDa
- 100kDa
- 70kDa
- 50kDa
- 40kDa
- 35kDa
- 25kDa
- 15kDa
- 10kDa

◄ Fusion protein 40kDa
◄ TEV protease 27kDa
◄ RHO-1 21kDa

B

Rho 1 sample
MALDI MS

Protein MW = 21503 Da
MALDI MW = 21500 Da

MALDI analysis

Rho 1 sample		
Charge	m/z	Mass [Da]
1	21501.4	21500.4
2	10750.7	21499.4
	21500.0	Average Mass

C

RHO-1 fibrils

RHO-1 fibrils

D

RHO-1::Venus fibrils, day 1

RHO-1::Venus fibrils, day 1

E

RHO-1::HisAvi, day 7

KIN-19::HisAvi, day 7

Figure supplement 6

A ThT staining of fibrils (RHO-1::HisAvi, day 7)
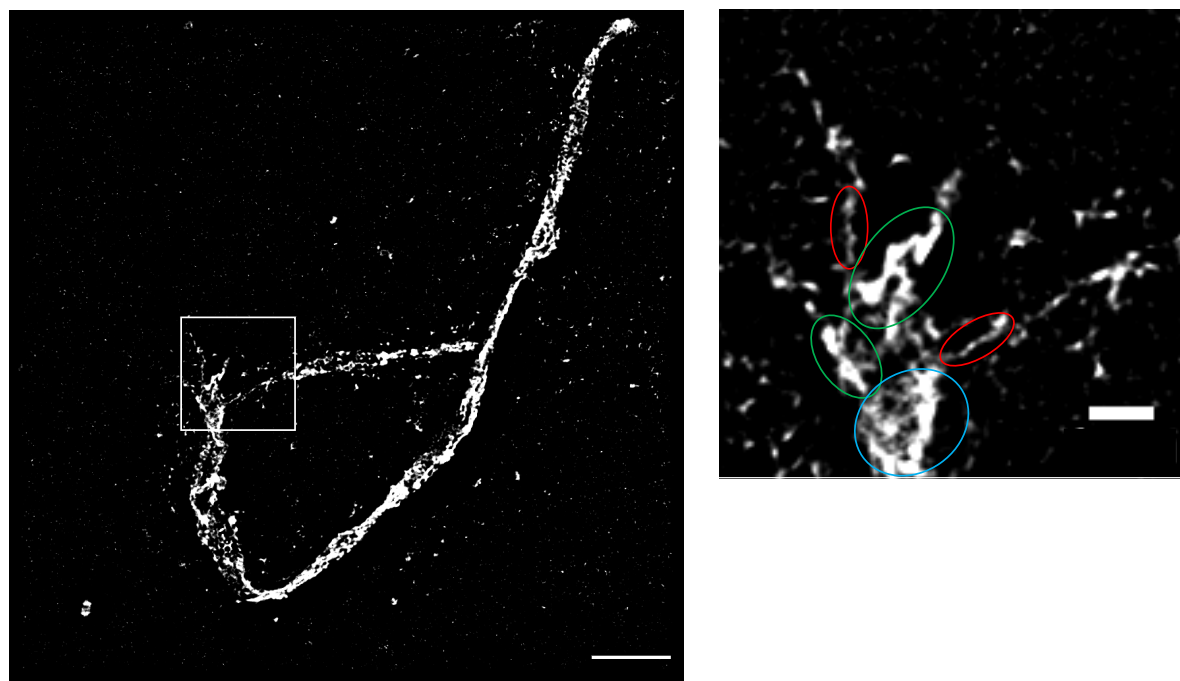
B ThT staining of fibrils
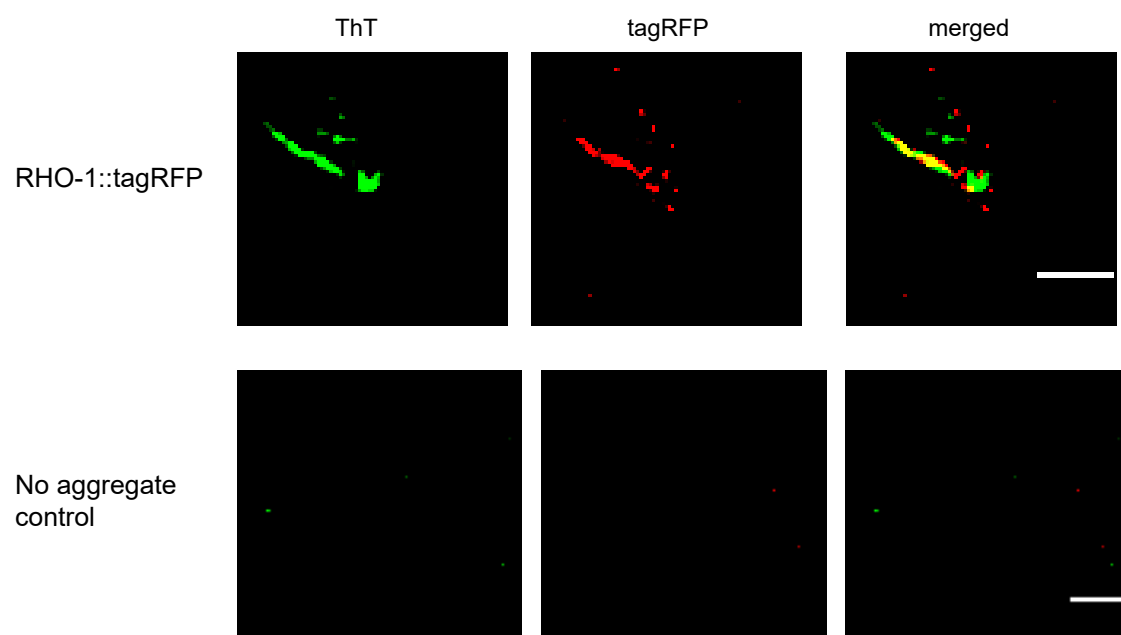
Figure 3

A Pumping
Pharyngeal KIN-19::tagRFP

Mean Frequency (Hz)

****

Day 7

Aggregation level:
□ Low ■ High

B Thrashing
Body-wall muscle KIN-19::tagRFP

Thrashing

****

Day 4

Aggregation level:
□ Low ■ High

C Pumping
Pharyngeal RHO-1::tagRFP

Mean Frequency (Hz)

****

Day 2
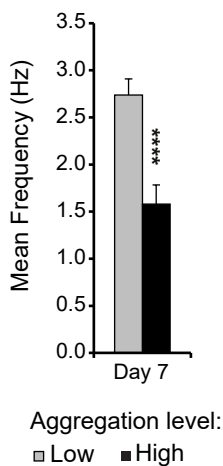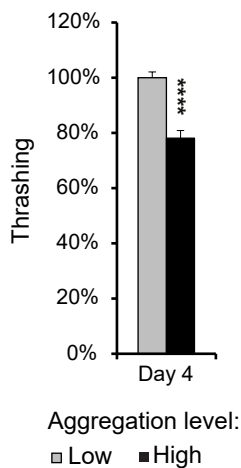
□ Wild-type (N2)
■ Pharyngeal RHO-1::tagRFP
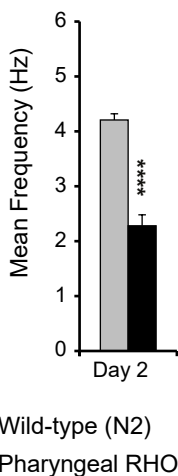
D Thrashing
Body-wall muscle RHO-1::Venus
(no aggregation)

Thrashing

n.s.

Day 4

□ Body-wall muscle YFP
■ Body-wall muscle RHO-1::Venus

Figure supplement 7

A Pharyngeal KIN-19::tagRFP aggregation
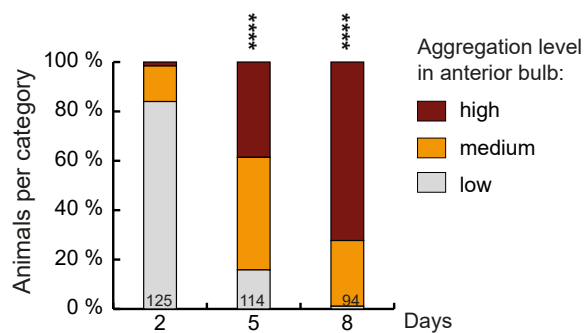
B Body-wall muscle KIN-19::tagRFP aggregation
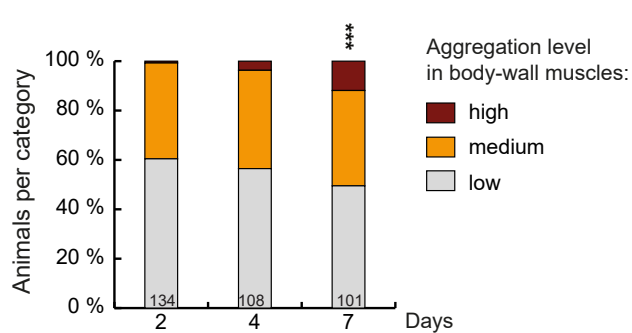
C Pharyngeal RHO-1::tagRFP aggregation
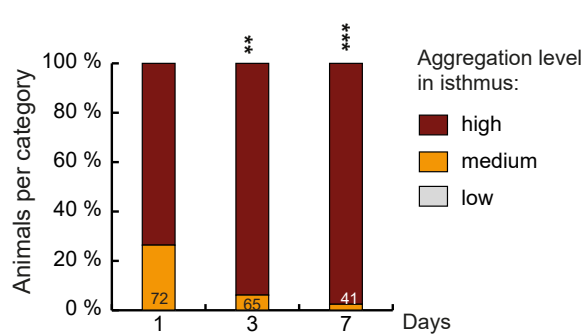
D Body-wall muscle RHO-1::venus aggregation

E Pharyngeal KIN-19::tagRFP
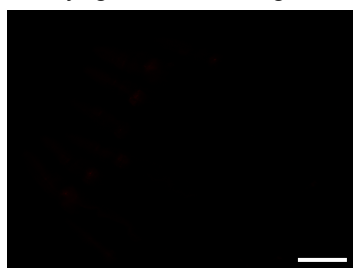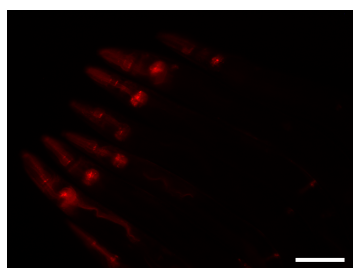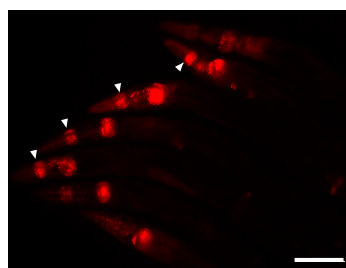
Day 2; 50ms   Day 2; 500ms   Day 7; 500ms

Pharyngeal RHO-1::tagRFP
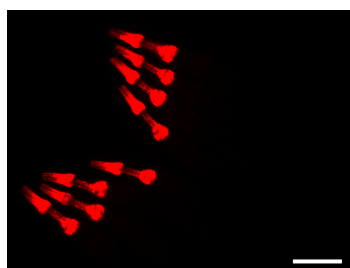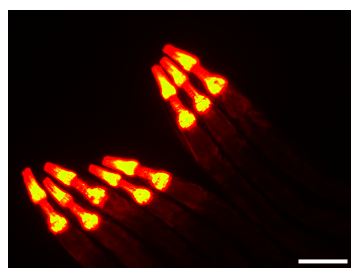
Day 2; 50ms   Day 2; 500ms
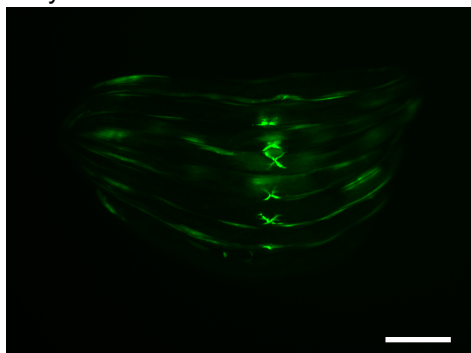
F Body-wall muscle RHO-1::venus   Body-wall muscle KIN-19::tagRFP
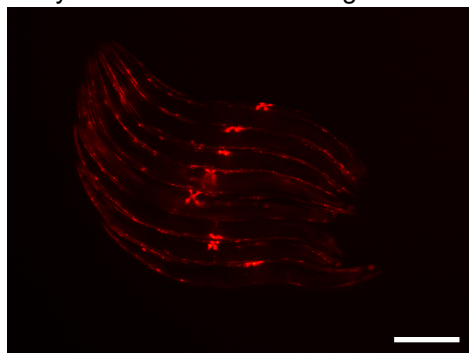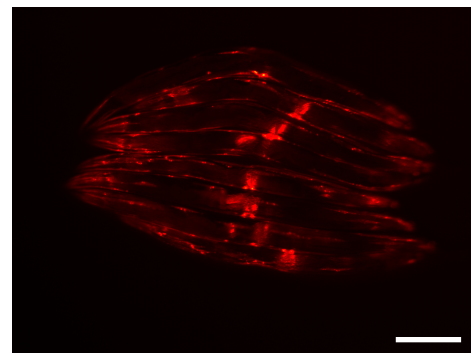
Day 2; exposure: 1s   Day 2; exposure: 1s   Day 4; exposure: 1s

Figure supplement 8

A Thrashing with body-wall muscle KIN-19::tagRFP

□ Body-wall muscle tagRFP
□ Wild-type (N2)
■ Body-wall muscle KIN-19::tagRFP
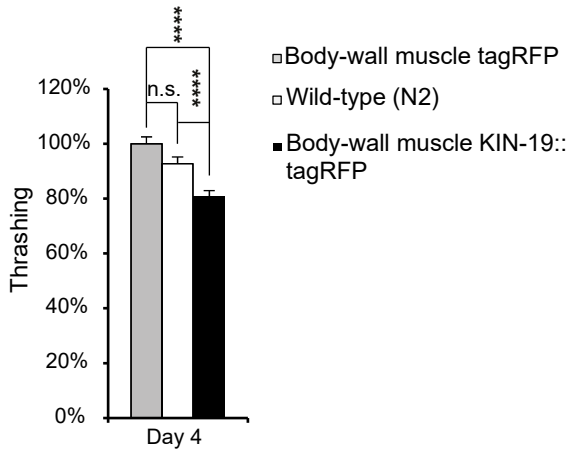
B Pumping with pharyngeal KIN-19::tagRFP

□ Pharyngeal tagRFP
■ Pharyngeal KIN-19::tagRFP

C Pumping with pharyngeal tagRFP
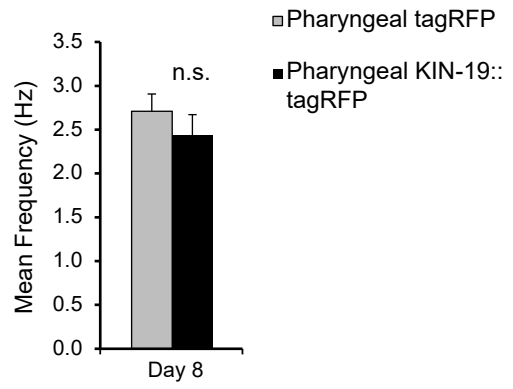
□ Wild-type (N2)
■ Pharyngeal tagRFP

D Pumping with pharyngeal tagRFP

□ Wild-type (N2)
■ Pharyngeal tagRFP

Figure supplement 9
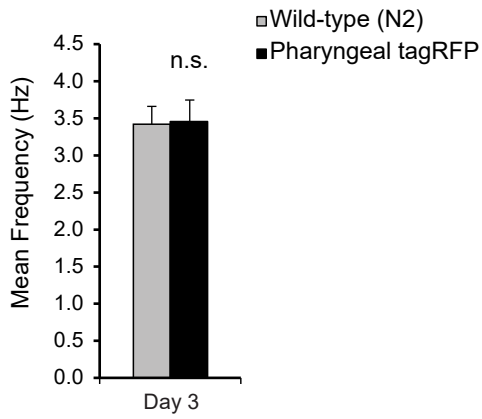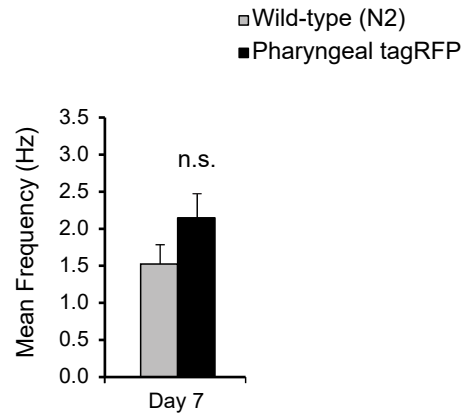
A F-actin staining in body-wall muscles

KIN-19::tagRFP   tagRFP (control)
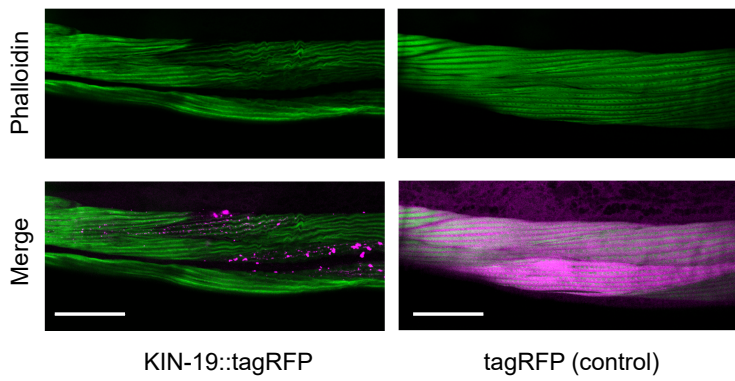
B Quantitive analysis of F-actin in body-wall muscles

Muscle structure:
- Normal
- Mild defect
- Severe defect
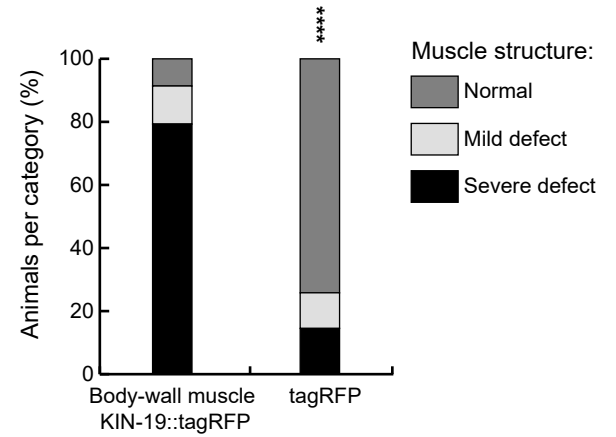
C F-actin staining in pharyngeal muscles

RHO-1::tagRFP   tagRFP

RHO-1::tagRFP   tagRFP (control)
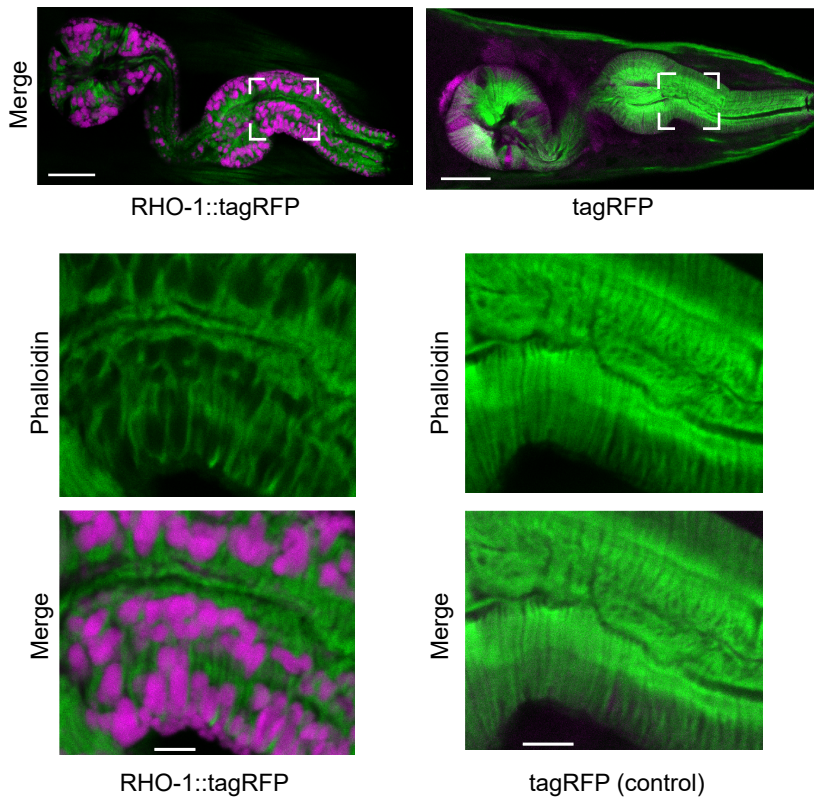
D Quantitive analysis of F-actin in pharyngeal muscles

Muscle structure:
- Normal
- Severe defect