

1 The UBAP2L ortholog PQN-59 contributes to stress granule
2 assembly and development in *C. elegans*

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13 **Abstract**

14 When exposed to stressful conditions, eukaryotic cells respond by inducing the formation of
15 cytoplasmic ribonucleoprotein complexes called stress granules. Stress granules are thought to have
16 a protective function but their exact role is still unclear. Here we use *C. elegans* to study two proteins
17 that have been shown to be important for stress granule assembly in human cells: PQN-59, the
18 ortholog of human UBAP2L, and GTBP-1, the ortholog of the human G3BP1 and G3BP2 proteins.
19 Both proteins fall into stress granules in the embryo and in the germline when *C. elegans* is exposed
20 to stressful conditions. None of the two proteins is essential for the assembly of stress induced
21 granules, but the granules formed in absence of PQN-59 or GTBP-1 are less numerous and dissolve
22 faster than the ones formed in control embryos. Despite these differences, *pqn-59* or *gtbp-1* mutant
23 embryos do not show a higher sensitivity to stress than control embryos. *pqn-59* mutants display
24 reduced progeny and a high percentage of embryonic lethality, phenotypes that are not dependent on
25 stress exposure and that are not shared with *gtbp-1* mutants. Our data indicate that both GTBP-1 and
26 PQN-59 contribute to stress granule formation but that PQN-59 is, in addition, required for *C. elegans*
27 development.

28

29 **Author summary**

30 The formation of so-called stress granules is an adaptive response that cells and organisms put into
31 action to cope with changes in internal and environmental conditions and thus to survive to stressful
32 conditions. Although it is generally thought that stress granule formation protects cells from stress-
33 related damage, the exact role of stress granules in cells and organisms is not well understood.
34 Moreover, the mechanisms governing stress granule assembly, and if and how the ability to form
35 stress granules is important for *C. elegans* development is still unclear.

36 Our work focuses on two conserved proteins, known to be involved in stress granule assembly in
37 mammalian cells, and investigates their role in *C. elegans* embryos. We find that these proteins are
38 important but not essential to assemble stress-induced granules in *C. elegans*. We moreover did not
39 observe a different sensitivity to stress exposure between wild-type and mutant developing embryos,
40 suggesting that at least in these conditions these proteins do not exert a protective role.

41

42 **Introduction**

43 Eukaryotic cells are sensitive to changes in internal or environmental parameters, including variations
44 in oxygen supply, salt concentration, pH, temperature or viral infection. Each one of these conditions
45 might be sensed as a stressful stimulus by the cell. In return, cells activate the integrated stress
46 response pathway which leads to translation inhibition of most mRNAs and to the assembly of stress
47 granules (1). Stress granules are membraneless organelles formed by the condensation of proteins
48 and RNA molecules into liquid droplets through a mechanism of liquid-liquid phase separation (2).
49 Different protein entities and RNA molecules are recruited into stress granules and their composition
50 varies according to the cell type and the triggering stress (3,4).

51 Formation of stress-induced granules is a reversible process, hence removal of the stress stimulus
52 results in dissolution of the granules. The current model describing the pathway through which cells
53 assemble stress granules involves disassembly of the polysomes with consequent translation
54 inhibition either via phosphorylation of the translation initiation factor eIF2 α (Eukaryotic Initiation
55 Factor 2 alpha) (5) or via the inhibition of eIF4G (Eukaryotic Initiation Factor 2 G) (6). The mRNAs
56 released from the polysomes are then bound to RNA binding proteins and recruited into the stress
57 granules (7,8). In mammalian cells, together with the translation initiation factor eIF2 α , other proteins
58 are important nucleators of stress granules. These include G3BP1 and G3BP2 (Ras GTPase-activating
59 protein-binding protein 1 and 2) and UBAP2L (Ubiquitin Associated Protein 2 Like), which are
60 crucial to drive stress granule assembly in many stress conditions (9–12), and the protein TIA-1 (T-
61 cell-restricted intracellular antigen protein) (5,13).

62 Although the exact function of stress granules and their importance for cell survival and organismal
63 development have not yet been established, stress granules may exert a protective role on cells when
64 they are exposed to stress (14).

65 Stress granule assembly and function has been mainly studied in unicellular organisms and cells in
66 culture. The nematode *C. elegans* provides an excellent model to study stress granules and to address
67 their role in organismal viability. The proteins involved in stress granule formation in mammalian
68 cells are conserved and the formation of granules molecularly similar to the mammalian stress
69 granules has been observed in the somatic and germ cells (15–18).

70 *C. elegans* contains one ortholog of the mammalian G3BP1 and 2, called GTBP-1 (19) and two TIA-
71 1/TIAR orthologs (20), named TIAR-1 and TIAR-2. GTBP-1 has been only recently shown to
72 contribute to stress granule formation in *C. elegans* adult worms (18). TIAR-1 protects germ cells
73 from heat-shock (17) and TIAR-2 granules inhibit axon regeneration (21). The *C. elegans* potential

74 ortholog of UBAP2L is a protein called PQN-59 (Prion-like (glutamine/asparagine-rich) domain
75 bearing protein) (22,23). The similarity between PQN-59 and UBAP2L at the sequence level is only
76 30% (source: BlastP) but PQN-59 and UBAP2L share a very similar domain organization (Fig 1A).
77 As GTBP-1, PQN-59 is an abundant protein of the entire *C. elegans* proteome ([https://pax-
db.org/protein/1033201](https://pax-
78 db.org/protein/1033201)) (24), but its role in *C. elegans* has not been characterized.

79 Here we show that different stress stimuli trigger the formation of granules containing both PQN-59
80 and GTBP-1 in *C. elegans* embryos and germlines. We find that neither of the two proteins is essential
81 for stress granule assembly, but both contribute to this process. However, PQN-59 depletion or
82 deletion results in embryonic lethality and reduced progeny in normal growth condition, phenotypes
83 that are not observed following the depletion or deletion of GTBP-1. This suggests that PQN-59 plays
84 additional roles in the development of worms.

85

86

87 **Results**

88 **PQN-59 is a component of stress granules**

89 The UBAP2L protein is important for stress granule assembly in many stress conditions and acts
90 upstream of the stress granule components G3BP1 and 2 in this process (4,10,12,25). We set out to
91 investigate whether the *C. elegans* ortholog of UBAP2L, called PQN-59 (Fig 1A), is also a
92 component of stress granules.

93 We used a CRISPR/Cas9 generated strain expressing an endogenous C-terminal fusion of PQN-59
94 with GFP and of GTBP-1, the ortholog of human G3BP1 and 2 (Fig 1B), with RFP (see Strain List
95 table in Materials and Methods). Both PQN-59 and GTBP-1 are expressed throughout development,
96 and are widely expressed in adult *C. elegans* animals, including the germline and the embryo (Fig
97 1C, (26)).

98 Observation of untreated *pqn-59::GFP;gtbp-1::RFP* embryos revealed that both proteins are
99 cytoplasmic (in the embryos and in the germline, Figs 1D and 1E). When embryos were exposed to
100 heat-shock (30°C, 5 minutes) using a temperature-controlled stage, PQN-59 fell into granules in both
101 the anterior and posterior blastomere (Fig 1D). These granules colocalized with GTBP-1 granules
102 (Fig 1D). Similar to stress granules (27), lowering the temperature to 20°C following heat-shock
103 exposure resulted in dissolution of the PQN-59/GTBP-1 granules after about 15 minutes of recovery
104 (S1A Fig and S1 Movie). Staining of untreated wild-type embryos with PQN-59 antibodies confirmed
105 the cytoplasmic localization observed with the GFP CRISPR strain (S1B Fig). The immunostaining
106 signal was abolished after PQN-59 depletion by RNA interference (S1B Fig), confirming the
107 specificity of the antibody. Staining of embryos exposed to heat shock revealed the accumulation of
108 PQN-59 into cytoplasmic granules (S1C Fig), similar to what we observed with the *pqn-59::GFP*
109 strain.

110

111 We then asked whether following high temperature exposure PQN-59 and GTBP-1 also fall into
112 granules in the *C. elegans* germline. We found that in *pqn-59::GFP;gtbp-1::RFP* worms exposed to
113 35°C for 10 minutes, PQN-59 fell into granules in both the distal and proximal germline (Fig 1E).
114 These granules colocalized with GTBP-1 granules (Fig 1E) and dissolved after 10 minutes of
115 incubation at 20°C (S2A Fig), confirming that their formation depends on stress exposure and is

116 reversible. Therefore, heat-stress induces the formation of PQN-59/GTBP-1 containing granules also
117 in the *C. elegans* germline.

118 We then asked whether PQN-59 falls into granules when worms are exposed to other stresses. Sodium
119 Arsenite induces oxidative stress triggering the formation of stress granules (16). Adult worms
120 incubated in a solution containing 20 mM Arsenite for 5 hours displayed granules containing both
121 PQN-59 and GTBP-1 (S2B Fig). The granules were observed both in the proximal and distal
122 germline.

123 The translation inhibitor Puromycin promotes polysome disassembly and stress granule formation
124 (17). We therefore asked whether incubation with Puromycin would induce PQN-59 and GTBP-1
125 granule formation. As shown in S2B Fig, worms incubated for 4 hours in a solution containing 10
126 mg/ml of Puromycin showed the appearance of PQN-59 granules that colocalized with GTBP-1 in
127 the distal and the proximal germline.

128 To conclude, the exposure of *C. elegans* animals to heat-shock, Arsenite and Puromycin, results in
129 the formation of PQN-59 cytoplasmic granules that colocalize with the known stress granule
130 component GTBP-1. The granules are reversible as they dissolve when the stress is removed. These
131 data indicate that PQN-59 is a stress granule component.

132

133 **PQN-59 is important for the formation of stress-induced GTBP-1 granules.**

134 We next asked whether PQN-59 is required to form stress granules in the embryo and in the germline.

135 In *ctrl(RNAi)* embryos exposed to 34°C prior to fixation, both PQN-59 and GTBP-1 fell into granules
136 (Fig 2A). When heat-shock was applied to *pqn-59(RNAi)* embryos, GTBP-1 fell into granule in both
137 the anterior and posterior cells. However, whereas in heat-shocked *ctrl(RNAi)* embryos GTBP-1
138 granules appeared like small, spherical and defined speckles, in heat-shocked PQN-59-depleted
139 embryos, GTBP-1 formed larger and more diffuse granules (Fig 2A). Quantifications of the GTBP-1
140 signal revealed that in PQN-59-depleted embryos the number and the intensity of GTBP-1 granules
141 are reduced compared to control embryos (Fig 2B). The depletion of PQN-59 did not result in a
142 change of GTBP-1 levels (S3A and S3B Figs). However, in *pqn-59(RNAi)* embryos that were not
143 exposed to heat-shock, GTBP-1 fell into granules in the posterior P1 blastomere that colocalized with
144 a P body marker (S3A and S3C Figs).

145 To exclude that a residual pool of PQN-59 after RNAi depletion could account for GTBP-1 granule
146 formation after stress exposure, we inserted a stop codon in the second exon of PQN-59 in a strain
147 expressing GTBP-1::GFP (S3D Fig). As revealed through western blot analysis, expression of PQN-
148 59 was absent in this strain (S3E Fig). Similarly to what we observed with the depletion of PQN-59
149 in the *pqn-59::GFP;gtbp-1::RFP* strain, GTBP-1 fell into granules in the posterior P1 blastomere in
150 the *pqn-59(cz4)* embryos (S3F Fig). In embryos exposed to heat-shock, GTBP-1 formed large and
151 diffuse aggregates.

152 As shown above (S1A Fig), heat-induced PQN-59/GTBP-1 granules dissolved when the temperature
153 was shifted back to 20°C. Images of wild-type embryos fixed after heat-shock and after 5, 10 and 20
154 minutes of recovery at 20°C, confirmed that PQN-59/GTBP-1 stress-induced granules are still present
155 after 5 minutes of recovery, and were not detected after 10 minutes (Fig 2C). In *pqn-59(cz4)* embryos,
156 however, the GTBP-1 stress-induced granules were already dissolved after 5 minutes of recovery in
157 the anterior blastomere, therefore showing a faster dissolution timing compared to the parental strain
158 (Fig 2C). The granules in the posterior blastomere did not dissolve, consistent with the fact that their
159 formation is not dependent on heat-shock exposure (S3F Fig).

160 The observation that after PQN-59 depletion GTBP-1 localization is affected and that GTBP-1 stress-
161 induced granules are reduced in number and less intense, suggests an interdependence in stress
162 granule formation between these two proteins. We therefore tested whether PQN-59 and GTBP-1
163 interact. In agreement with data in other model systems (28), PQN-59 interacted with GTBP-1 in
164 Two Hybrid assays, as shown by growth on selective medium of yeast colonies expressing GTBP-1
165 and PQN-59 (S3G Fig).

166 We then asked whether GTBP-1 granules can form in the germline when PQN-59 is depleted. As
167 shown in S4A Fig, depletion of PQN-59 abolished the formation of GTBP-1 granules in the oocytes
168 (proximal germline). However, GTBP-1 granules were still observed around the nuclei of the
169 syncytial germline (distal germline), indicating that, similar to the situation in the embryo, GTBP-1
170 granules can still form, although not throughout the entire germline.

171 The RGG domain of UBAP2L is crucial to nucleate stress granules in human cells (12,25). We deleted
172 this domain in the *pqn-59::GFP;gtbp-1::RFP* strain (S4B Fig) and tested whether PQN-59 Δ RGG
173 could still form granules after heat-shock. As shown in Fig S4C, PQN-59 Δ RGG was nucleating
174 granules that colocalized with GTBP-1, similar to the granules formed in the wild-type strain.
175 Quantification of both PQN-59 and GTBP-1 granule number and intensity revealed similar values
176 between the wild-type parental strain and the *pqn-59:: Δ RGG::GFP;gtbp-1::RFP* strain (S4D Fig).

177 Our data show that when PQN-59 is absent, GTBP-1 can still form granules after heat-shock but these
178 granules appear different from the stress granules assembled in the control strain and they dissolve
179 with faster dynamics. The deletion of the RGG domain of PQN-59 alone is not sufficient to impair
180 stress granule assembly, indicating that this domain is not essential in this process in *C. elegans*
181 embryos.

182

183 **GTBP-1 contributes to the assembly of stress-induced granules.**

184 In mammalian cells, the G3BP proteins are crucial to assemble stress granules in many stress
185 conditions (9,11,29,30). We therefore investigated whether GTBP-1 was required for the assembly
186 of PQN-59 granules in *C. elegans* after heat shock.

187 We depleted GTBP-1 in *pqn-59::GFP;gtbp-1::RFP* worms, and imaged embryos after heat-shock
188 and fixation. In GTBP-1 depleted embryos at 20°C, PQN-59 was diffused in the cytoplasm, as in
189 *ctrl(RNAi)* embryos (S5A Fig). In *ctrl(RNAi)* heat-shocked embryos we observed numerous granules
190 containing PQN-59 and GTBP-1, in both the anterior AB and posterior P1 cells of two-cell embryos
191 (Fig 3A). After GTBP-1 depletion, some PQN-59 granules were still observed in both AB and P1
192 cells but were smaller and less defined (Fig 3A). A significant decrease in PQN-59 number and
193 intensity could be quantified in GTBP-1-depleted embryos compared to control ones (Fig 3B). Heat-
194 shock of *gtbp-1(ax2029)* mutant embryos followed by PQN-59 staining resulted in a phenotype
195 similar to the GTBP-1 depletion (S5B Fig).

196 The absence of GTBP-1 did not affect the levels of PQN-59, as detected by immunofluorescence in
197 the PQN-59::GFP tagged strain or after anti-PQN-59 antibody staining (S5A and S5B Figs, and
198 quantifications in S5C and S5D Figs), indicating that the impaired stress granule assembly did not
199 depend on a change in protein amount. The small stress-induced PQN-59 granules formed in absence
200 of GTBP-1 also showed a faster dissolution dynamic following stress ceasing (Fig 3C). While in heat-
201 shocked wild-type embryos granules were still present after 5 minutes of recovery at 20°C and started
202 to dissolve after 10 minutes (Fig 3C), in *gtbp-1(ax2029)* mutant embryos the PQN-59 granules started
203 disappearing already after 5 minutes of recovery at 20°C (Fig 3C). This indicates that the biophysical
204 properties of the granules formed in absence of GTBP-1 are altered compared to control conditions.

205 Depletion of GTBP-1 also impaired PQN-59 granule assembly in the germline. Dim PQN-59 granules
206 were still visible around the nuclei in the distal germline. In the proximal germline, aberrant PQN-59
207 aggregates were observed (S5E Fig).

208 We conclude that when GTBP-1 is depleted, stress-exposed embryos contain less numerous and less
209 intense PQN-59 granules. In GTBP-1 depleted germlines, PQN-59 still falls into granules in the distal
210 and in aberrant aggregates in the proximal germline.

211

212 **TIAR-1 granules assemble in embryos depleted of GTBP-1 and PQN-59.**

213 Since depleting PQN-59 did not abolish formation of GTBP-1 granules and, vice-versa, depleting
214 GTBP-1 did not abolish the formation of PQN-59 granules after exposure to stress, we asked whether
215 depleting both proteins would result in a defect in the formation of stress granules. To address this
216 question we used as a marker the protein TIAR-1. In *C. elegans*, TIAR-1 accumulates in stress
217 granules in the germline (17,31) and in the intestine of the adult worms (18) following exposure to
218 different stresses.

219 In the *C. elegans* embryo, TIAR-1 is localized in the cytoplasm and it accumulates in the nuclei and
220 the P granules of the germ precursor cells (S6A Fig and (17,20)). We used a strain expressing TIAR-
221 1::GFP (17) and found that following heat shock of the embryo, TIAR-1 accumulated into stress-
222 induced granules which colocalized with PQN-59 (Fig 4A). Depletion of PQN-59 did not abolish
223 TIAR-1 granule formation after heat-stress exposure (Fig 4A). Our quantifications showed that the
224 number and the intensity of TIAR-1 granules was not significantly different compared to the control
225 (Fig 4B). However, we observed a higher variability in the PQN-59 depleted embryos, consistent
226 with the fact that some embryos appeared to have less granules. In *tiar-1::GFP;gtbp-*
227 *1(ax2029);ctrl(RNAi)* embryos that were heat-shocked, the majority of TIAR-1 granules were
228 detected in the P1 cell (Fig 4A) but the overall number and intensity of TIAR-1 granules did not
229 appear to be different from the wild-type parental strain (Fig 4B). In this condition, consistently with
230 the result showed in Fig 3B, PQN-59 formed granules in both AB and P1 cells, and these granules
231 colocalized with TIAR-1 granules (Fig 4A). When PQN-59 was depleted in the *tiar-1::GFP;gtbp-*
232 *1(ax2029)* embryos, TIAR-1 granules were still observed after heat-shock, but their number was
233 reduced compared to control *tiar-1::GFP* embryos (Fig 4B). This suggests that the depletion of both
234 PQN-59 and GTBP-1 proteins is not sufficient to abolish the assembly of TIAR-1 stress-induced
235 granules. In embryos that were not exposed to heat-shock, the localization and appearance of TIAR-
236 1 was not affected by the depletion of PQN-59, the mutation of GTBP-1 or both (S6A Fig).

237 We then asked whether formation of PQN-59 and GTBP-1 granules is abolished when TIAR-1 is
238 depleted. As shown in Fig 4C, the number of PQN-59 and GTBP-1 granules was not different
239 between *tiar-1(RNAi)* and *ctrl(RNAi)* embryos exposed to heat-shock (quantifications in Figs 4D and

240 4E). The intensity of the signal of PQN-59 and GTBP-1 in the granules was weekly reduced (Figs 4D
241 and 4E), a reduction that was not significant for GTBP-1::RFP. Immunostaining with α PQN-59
242 antibodies of embryos from the *tiar-1(tn1543)* mutant (17) exposed to heat-shock revealed a result
243 similar to the RNAi depletion (S6B Fig).

244 These results indicate that TIAR-1 is not essential for assembly of PQN-59/GTBP-1 granules and that
245 absence of both PQN-59 and GTBP-1, although associated with a reduced number of TIAR-1
246 granules, is not sufficient to abolish TIAR-1 granule assembly.

247

248 **PQN-59 is required for embryonic development and maintenance of brood size in a stress**
249 **independent manner.**

250 PQN-59 and GTBP-1 both contribute to proper granule formation following heat-shock. We next
251 investigated whether these two proteins are important for other functions in *C. elegans*, in normal
252 growing conditions and therefore independently of a stress response.

253 We first asked whether brood size is reduced by the depletion of PQN-59 or GTBP-1. We found that
254 depletion or null mutation of PQN-59 resulted in a significant reduction of progeny number whereas
255 the depletion or null mutation of GTBP-1 did not (Figs 5A and 5B). Co-depleting both PQN-59 and
256 GTBP-1 or depleting PQN-59 in the *gtbp-1(ax2029)* mutant resulted in a small but significant
257 increase in brood size compared to the PQN-59 depletion alone (Fig 5A and S7A Fig).

258 We also found that depleting PQN-59 resulted in about 50% embryonic lethality (Fig 5C) a value
259 similar to the PQN-59 mutant (Fig 5D). These results suggest that PQN-59 has an important function
260 during embryonic development. On the contrary, depletion or mutation of GTBP-1 did not result in
261 significant embryonic lethality (Figs 5C and 5D). Depleting GTBP-1 did not increase lethality of *pqn-*
262 *59(RNAi)* embryos compared to the depletion of PQN-59 alone (Fig 5C), it actually weakly rescued
263 (see discussion). This result was confirmed by the depletion of PQN-59 in the *gtbp-1(ax2029)* mutant
264 (Fig S7B).

265 We then dissected wild-type and mutant hermaphrodites, exposed embryos to 34°C for 10 minutes
266 and analysed how this treatment (Fig 5E) impacted on their viability. After 24 hours of recovery at
267 20°C, we found that embryonic lethality ranged from about 70% to 80% and we did not detect a
268 significant difference between the wild-type, able to assemble proper stress granules, and the mutant
269 embryos (Fig 5F).

270 Taken together our results suggest that PQN-59 and GTBP-1 do not help embryos to better resist to
271 exposure to heat. Our results also indicate that PQN-59 has additional roles in adult life and during
272 development that are independent of GTBP-1 and stress granule formation.

273

274

275 Discussion

276 Here we have studied the function of two conserved proteins, PQN-59, the ortholog of UBAP2L, and
277 GTBP-1, the ortholog of G3BP1/2 in assembly of stress granules in worm embryos and in worm
278 germlines.

279 Both PQN-59 and GTBP-1 are cytoplasmic proteins that condense into granules in response to stress
280 exposure. In *Drosophila melanogaster*, Lingerer/PQN-59 and Rasputin/GTBP-1 interact in Yeast
281 Two Hybrid assays (28). In human cells, G3BP-1 and UBAP2L coimmunoprecipitate and mutations
282 in UBAP2L that abolish the interaction with G3BP-1, are unable to rescue the stress granule assembly
283 defect of UBAP2L depletion (12,25). *C. elegans* GTBP-1 was isolated in pull down of PQN-59 from
284 embryos (unpublished), and we found that PQN-59 and GTBP-1 interact in a Yeast Two-Hybrid
285 assay, supporting the hypothesis that PQN-59 and GTBP-1 are in a complex in *C. elegans*. In contrast
286 with their human orthologs, the interaction of these two proteins or their presence is not essential for
287 the formation of stress-induced granules, as revealed by looking at GTBP-1 or PQN-59 and TIAR-1.
288 However, the assembly of stress-induced granules in the absence of one or the other is impaired, as
289 in this condition the granules appear less numerous, less defined in their shape, and show a faster
290 dissolution timing after stress relief. This suggests that the association between PQN-59 and GTBP-
291 1 is not essential to assemble stress-induced granules but it is important to preserve stress granule
292 properties.

293 Deletion of the RGG domain of UBAP2L results in the abolishment of all interactions with stress
294 granule components and impairs stress granule assembly (12). Here we show that deletion of the
295 RGG domain in PQN-59 does not result in defects in the number of stress granules, suggesting that
296 this domain is dispensable for stress granule nucleation in the *C. elegans* embryo.

297 Single depletion of GTBP-1 and PQN-59 did not reduce the average number of TIAR-1 granules but
298 granule number was highly variable, suggesting that PQN-59 and GTBP-1 do contribute to proper
299 TIAR-1 granule formation. Consistent with a contribution, when PQN-59 was depleted in a *gtbp-1*
300 mutant, the number of TIAR-1 granules was reduced. This indicates that PQN-59 and GTBP-1 are
301 not strictly essential for TIAR-1 stress-induced granule assembly but they facilitate their formation.
302 On the opposite, depletion of TIAR-1 did not result in a significant defect in the number of GTBP-1
303 and PQN-59 granules, suggesting that TIAR-1 may act downstream in the process of stress granule
304 formation in *C. elegans* embryos.

305 Altogether, our data show that none of these proteins is required for stress induced granule assembly.
306 So, whereas in cultured human cells G3BPs and UBAP2L are important to form stress granules in
307 many stress conditions (9–12,25,32), in *C. elegans*, stress-induced granules can form in the absence
308 of GTBP-1, PQN-59, and in the absence of both suggesting that either an essential nucleator of stress
309 granules has still to be identified in this model or that the presence of disordered proteins is sufficient
310 to assemble stress induced granules in worms. This is reminiscent of work in intestinal progenitor
311 cells in *Drosophila* where canonical nucleators are not required for stress granule formation (33).

312 Depletion and mutation of PQN-59 result in additional phenotypes such as slow growth, reduced
313 progeny and embryonic lethality, all in absence of stress. These phenotypes were not observed in
314 *gtbp-1* mutant or depleted animals. A recent paper has shown that the human orthologs, G3BP1/2
315 inhibit mTORC1 signaling by targeting mTORC1 to the lysosome (34). One possibility is that the
316 phenotypes of *pqn-59* mutant embryos are dependent on GTBP-1. For example, an excess of free
317 GTBP-1 (not in complex with PQN-59) could be deleterious for worms and embryos. Co-depletion
318 of both PQN-59 and GTBP-1 resulted in a weak rescue of the embryonic lethality and the reduced
319 progeny phenotypes of *pqn-59* mutants, indicating that these phenotypes may partially depend on an
320 excess of free GTBP-1. However, this weak rescue suggests that PQN-59 has additional important
321 functions in embryos and worms that do not depend on GTBP-1. These yet to be identified functions
322 could contribute to the regulation of the response to stress or be completely independent on the role
323 of PQN-59 in stress granule assembly. Additional studies will be required to understand the molecular
324 functions of PQN-59.

325 Stress granules have been proposed to protect cells from stress. We find that exposure to heat stress
326 kills to the same extent wild-type, *pqn-59* or *gtbp-1* mutant embryos. This indicates that during
327 embryonic development, the exposure to heat stress results in developmental failure, whether
328 embryos are able to form proper stress granules or not.

329

330 Materials and Methods

331 Strains

332 The *C. elegans* strains used in this work are listed in Table 1. Worms were maintained on NGM plates
333 seeded with OP50 bacteria, using standard methods (35). All the strains were grown at 20°C and
334 incubated at 20°C after dsRNAs injections.

335 Mutant strains were generated using CRISPR/Cas-9 technology, as described in (36). Single-guide
336 RNAs and repair templates, as well as PCR primers used to detect and sequence the mutations, are
337 listed in Table 2 and Table 3 respectively. The *pqn-59* mutant strain (generated in the N2 background
338 and in the JH3199 (*gtbp-1(ax2055[gtbp-1::GFP])IV*) background) was generated by introducing a
339 frameshift mutation leading to the appearance of a premature STOP codon. The *pqn-59ΔRGG* strain
340 was generated excising the RGG-rich region (from aminoacid position 122 to aa 189), not altering
341 the reading frame.

342 **Table 1. Strain List.** The strain used in this work are listed in this table in order of appearance in the
343 text. The genotype, source and description of the mutation are also reported.

Strain name	Genotype	Source/Reference	Description
HML713	<i>pqn-59::GFP;gtbp-1::RFP(ax5000) IV</i>	This study	Strain generated by C. Hammell using CRISPR/Cas9. GFP has been inserted at the C-terminal of PQN-59 on the genotype K08F4.2(ax5000[<i>gtbp-1::tagRFP</i>]) IV.
N2	<i>Wild type</i>	CaenorhabditisGenetics Center (CGC)	
JH3199	<i>gtbp-1(ax2055[gtbp-1::GFP]) IV</i>	CaenorhabditisGenetics Center (CGC)	
ZU291	<i>pqn-59::ollas::STOP(cz4);gtbp-1(ax2055[gtbp-1::GFP]) IV</i>	This study	Strain generated by CRISPR/Cas9, inserting a frameshift mutation generating a premature STOP. Background strain is JH3199.
ZU288	<i>pqn-59::ΔRGG::GFP;gtbp-1::RFP(ax5000) – clone 1</i>	This study	Strain generated by CRISPR/Cas9, excising the RGG region of PQN-59. Background strain is HML713.
ZU289	<i>pqn-59::ΔRGG::GFP;gtbp-1::RFP(ax5000) – clone 2</i>	This study	Strain generated by CRISPR/Cas9, excising the RGG region of PQN-59. Background strain is HML713.

JH3176	<i>gtbp-1(ax2029) IV</i>	CaenorhabditisGenetics Center (CGC) (26)	
DG3922	<i>tiar-1(tn1545[tiar-1::s::tev::GFP]) II</i>	CaenorhabditisGenetics Center (CGC) (17)	
ZU287	<i>tiar-1(tn1545[tiar-1::s::tev::GFP])II;gtbp-1::tagRFP(ax5000)</i>	This study	Strain obtained crossing the DG3922 with the genotype K08F4.2 (<i>ax5000[gtbp-1::tagRFP] IV</i>).
ZU294	<i>tiar-1(tn1545[tiar-1::s::tev::GFP])II;gtbp-1(ax2029) IV</i>	This study	Strain obtained crossing the DG3922 with the JH3176.
ZU278	<i>pqn-59::ollas::STOP(cz2)</i>	This study	Strain generated by CRISPR/Cas9, inserting a frameshift mutation generating a premature STOP. Background strain is N2.
DG3929	<i>tiar-1(tn1543)(loxP:Cbr-unc-119(+)::loxP) II</i>	CaenorhabditisGenetics Center (CGC) (17)	TIAR-1 mutant generated by CRISPR/Cas9.

344 **Table 2. CRISPR reagents.**

Strain name	sgRNA sequence (5'->3')	Repair template sequence	Description
ZU291	1 FW RV TCT TGG AAC AAA CTT TTG AGA AAG GCT CCT TTC GAG CCA TGT TCC AAA	TGG AGA TAA ACT TGA CTC GTG GAC TGA <u>ACA GAA AGG</u> <u>AGC CAA AAA AGA AAA GAA</u> <u>GAA Gtc cgg att cgc caa cGA GCT</u> Cgg acc acg tct cat ggg aaa gCC	Insertion of the <i>ollas tag</i> for screening + Nucleotide substitution generating a prematureSTOP
	2 FW RV TCT TGA AAA AAA CGC TTC AGG AAA TTC TTT TCC AGA AGA TTT TTC AGC	TGA ATA GGG CAG TTA AAA CAA CAG AGG ATT TGT AGC AAG AGG CAG AG	
ZU288	1 FW RV TCT TGG AGG AAA CCT GTT GCA GTT ATA GTT ATA ACT ACA ACA G GCC CTC C	AAGGAGCCAAAAAGGAAAAG AAGAAGCCGGAAGAGGGCAG <u>TTATAACAAC</u> <i>deletion</i>	Deletion of the RGG domain (from aa 98 to aa 166)

	2	FW TCT TG G CTC GAG AAT ATC CAC CTC	RV AAA CGA GGT GGA TAT TCT CGA GCT C	<u>TATTCTCGAGCTGTTGCTCCAT</u> CATCAGCACTTGAGCCAGATG CGTTCAC	(PAM sites are inside the deleted region, therefore not shown in the repair template sequence) (clone 1)
ZU289	1	FW TCT TGG AGG GCA GTT ATA ACA ACA G	RV AAA CCT GTT GTT ATA ACT GCC CTC C	AAGGAGCCAAAAAGGAAAAG AAGAAGCCGGAAGAGGGCAG <u>TTATAACAAC</u> <i>deletion</i> <u>TATTCTCGAGCTGTTGCTCCAT</u>	Deletion of the RGG domain (from aa 98 to aa 166) (PAM sites are inside
	2	FW TCT TG G CTC GAG AAT ATC CAC CTC	RV AAA CGA GGT GGA TAT TCT CGA GCT C	CATCAGCACTTGAGCCAGATG CGTTCAC	the deleted region, therefore not shown in the repair template sequence) (clone 2)
ZU278	1	FW TCT TGG AAC AGA AAG GAG CCA AAA	RV AAA CTT TTG GCT CCT TTC TGT TCC	TGGAGATAAACTTGACTC GTGGGACTGAACAGAAAAG <u>GAGCCAAAAAGAAAAGA</u> <i>AGAAGtccgattcgccaacG</i> <i>AGCTCggaccacgtctcatggaa</i>	G nucleotide <i>insertion</i> + frameshift + <i>ollas tag</i> for screening + prematureSTOP
	2	FW TCT TGA AAA AGG AAA AGA AGA AGC	RV AAA CGC TTC TTC TTT TCC TTT TTC	<u>agCCTGAAGAGGGCAGTT</u> ATAACAACAGAGGATTTG TAGCAAGAGGCAGAG	

345

346 **LABEL**

347 Underlined sequence is targeted by the sgRNA

348 In capital bold the silently mutated **PAM sites** (the first one originally AGG mutated in AAG and the
349 second one originally CGG mutated in CTG)

350 in italic is the *OLLAS* sequence containing a SacI restriction site (italic upper cases)

351 In bold capital italic the ***nucleotide substitution*** or ***insertion***

352 Strikethrough is the premature ~~STOP codon~~ and the ~~deletion~~

353

354 **Table 3. Oligonucleotides for PCR genotyping and sequencing.**

Strain	Gene		Oligonucleotide sequence		Description
			FW primer (5'→3')	RV primer (5'→3')	
ZU291 and ZU278	<i>pqn-59</i>	1	ttctagtctagcttgcggtg	GCACCGATCTCATT GCTG	PCR product is ~1600bp. After SacI digestion of this PCR product, fragments of ~1100bp and ~500bp are generated in the mutant.
		2	accacgtctcatgggaaag	GCACCGATCTCATT GCTG	FW primer is annealing to the ollas sequence. PCR product is ~1100bp.
ZU288 and ZU289	<i>pqn-59</i>	1	CCAAAAAGGAAAAGA AGAAGC	GGATGCTGTTGTGGA TGTCC	The primers are external to the deletion. In the mutant, the PCR product is ~1300bp.
		2	CCAAAAAGGAAAAGA AGAAGC	GGATGCTGTTGTGGA TGTCC	The FW primers is internal to the deletion. In the mutant, there is no amplification.
JH3176	<i>gtbp-1</i>		cttctgaatttcgcgcgttc	GAACCTCCTCGATT CTCC	PCR product is ~1600bp. After NheI digestion of this PCR product, fragments of ~900bp and ~700bp are generated in the mutant.

355

356 RNA interference

357 A list of the genes silenced through RNAi in this study is provided in Table 4.

358 Clones from the Ahringer feeding library (37,38) were used when available. As a control, we used
359 the clone C06A6.2, previously found in the laboratory to not affect early embryonic division and
360 development (injected worms are 100% viable). To produce *pqn-59* dsRNA, a DNA fragment was
361 amplified from genomic DNA using Gateway-compatible oligonucleotide primers (as in Table 4) for
362 Gateway-based-cloning into the pDESTL4440 plasmid. The DNA was subsequently amplified using
363 standard T7 primers. For *tiar-1*, the DNA was amplified from genomic DNA using oligos with T7
364 overhangs (see Table 4). For all genes, the dsRNA was produced with the Promega Ribomax RNA
365 production system. dsRNA was injected in L4/young adult hermaphrodites which were incubated at

366 20°C. Germlines or embryos collected from injected hermaphrodites were analyzed 24 hours after
367 injection.

368 **Table 4. dsRNA sequences.**

Target gene	Clone name from Ahringer library	Oligonucleotides sequence (5'→3')	
<i>ctrl</i>	C06A6.2	Standard T7 primers	
<i>pqn-59</i>		FW CCAAATCAAGCAT GGACCA	RV TTA GTT ACT CCA GTT GTA CG
<i>gtbp-1</i>	K08F4.2	Standard T7 primers	
<i>tiar-1</i>		FW CGTAATACGACTCACT ATAGcagGAGATGAAAGTCAAC TG	RV CGTAATACGACTCA CTATAGtacCAGTAAGTGAAGCA ATG

369

370 **Live imaging of embryos exposed to heat-shock**

371 Gravid hermaphrodites were dissected on a coverslip into a drop of Egg Buffer (118 mM NaCl, 48
372 mM KCl, 2 mM CaCl₂, 2 mM MgCl₂, and 25 mM Hepes, pH 7.5) containing 1:10 volume of
373 polystyrene beads (Polybead® Hollow Microspheres, Polysciences). The temperature controller
374 CherryTemp (Cherry Biotech, Rennes, France) with its accompanying software (Cherry Biotech TC)
375 was used to control the temperature during the live imaging process. The coverslip with dissected
376 hermaphrodites was directly mounted on the chip of the CherryTemp microfluidic temperature
377 control system. The system was mounted on a Leica DM6000 microscope, equipped with
378 epifluorescence and DIC (Differential Interference Contrast) optics and a DFC 360 FX camera
379 (Leica). Time lapse images were collected every 10 seconds using 63x/1.4 numerical aperture (NA)
380 objective and LAS AF software (Leica Biosystems). Imaging was started at 20°C. The temperature
381 was then shifted at 30°C (heat-shock) for 5 to 10 minutes while imaging. For recovery, the
382 temperature was shifted back to 20°C for 15-20 minutes.

383

384 **Immunostaining of embryos and image acquisition**

385 For *C. elegans* embryos staining, 20-25 gravid hermaphrodites were dissected in a drop of M9 (86
386 mM NaCl, 42 mM Na₂HPO₄, 22 mM KH₂PO₄, and 1 mM MgSO₄) on 22 X 40 mm coverslips.

387 Control samples were left at room temperature (20-22°C) for 10 minutes. For heat-shock exposure,
388 coverslips with dissected worms and embryos, were transferred on a metal block placed in a
389 humidified incubator at 34°C for 10 minutes.

390 After the incubation time, the coverslip was mounted crosswise on the epoxy slide square, previously
391 coated with 0.1% poly-L-lysine, for embryonic squashing. The slides were then transferred on a metal
392 block on dry ice for at least 10 min. Afterward, the coverslip was removed (freeze-cracking method)
393 before fixation. Immunostaining was performed as described in (39). Briefly, embryos were fixed for
394 20 min in methanol and placed for 20 min in a solution of PBS and 0.2% Tween (PBST) and BSA
395 1% to block the nonspecific antibody binding. The slides were incubated with primary antibodies
396 diluted in PBST with 1% BSA overnight at 4°C. The list of primary antibodies used in this study is
397 in Table 5. After two washes of 10 min each in PBST, slides were incubated for 45 min at 37°C with
398 a solution containing secondary antibodies (4 µ/ml Alexa Fluor 488– and/or 568–coupled anti-rabbit
399 or anti-mouse antibodies from Molecular Probes) and 1 µg/ml DAPI to visualize DNA in PBST.
400 Slides were then washed two times for 10 min in PBST before mounting using Mowiol (30% wt/vol
401 glycerol, 3.87 mM Mowiol [Calbiochem, 475904], 0.2 M Tris, pH 8.5, and 0.1% DABCO).

402 In the case of GFP or RFP tagged strains, the slides were briefly (10 min at room temperature)
403 incubated with 1 µg/ml DAPI in PBST to visualize DNA just after methanol fixation and blocking.
404 Slides were then washed two times for 10 min in PBST and mounted with Mowiol.

405 Images were acquired using a Nikon A1r spectral (inverted Ti Eclipse) confocal microscope equipped
406 with a 60x1.4 NA CFI Plan Apochromat Lambda oil objective and four PMTS including two highly
407 sensitive detectors (GaAsp) for green and red channels. From 5 to 7 z stacks, separated by 0.5 µm,
408 were acquired. NIS Elements AR software (v.4.20.01; Nikon) was used to set acquisition parameters.

409 **Table 5. Primary antibodies list.**

Antibody	Source/Reference	Working concentration/dilution	
		IF	WB
rabbit anti-PQN-59	This study	2ng/µl	2ng/µl
rabbit anti-DCP-1	Gift from Jayne Squirrell (40)	1:5000	

mouse anti- α tubulin (DM1A)	Sigma-Aldrich	1:1000	1:2500
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410

411 **Hermaphrodite heat-shock, drug treatment, and image acquisition procedure**

412 For heat-shock, young adult worms were transferred into a drop of M9 buffer (86 mM NaCl, 42 mM
413 Na_2HPO_4 , 22 mM KH_2PO_4 , and 1 mM MgSO_4) on a glass coverslip and transferred on a metal block
414 placed into a humidified incubator for 10 minutes at 35°C. For recovery after heat-shock, worms were
415 collected from the M9 drop and transferred onto OP50 seeded NGM plates and incubated at 20°C for
416 5 or 10 minutes.

417 For drug treatment, young adult worms were transferred into a drop of M9 buffer only (control) or
418 M9 with 10 mg/ml of Puromycin (InvivoGen) or with 20 mM Arsenite (MerckMillipore). Worms
419 were incubated in the Puromycin-containing solution for 4 hours and in the Arsenite-containing
420 solution for 5 hours before imaging. Control worms were incubated in M9 buffer for the same amount
421 of time as the Puromycin or Arsenite treated worms.

422 Control and drug-treated worms were then transferred in a drop of NaN_3 30 mM (for worm paralysis)
423 and mounted on a 3% agarose pad for imaging. Imaging was performed using the Leica DM6000
424 described above. Images were acquired using the 63x/1.4 numerical aperture (NA) objective and the
425 LAS AF software (Leica Biosystems).

426

427 **Quantification of cytoplasmic protein levels**

428 The mean intensity of a defined region of interest (ROI) ($w=2.69$, $h=2.46$, $\text{area}=6.604$), always placed
429 in the anterior blastomere (AB) of a two-cell stage *C. elegans* embryo, was measured using Fiji Image
430 J. The mean intensity of an equal ROI, placed outward of the embryo, was used for background
431 subtraction. For each experiment, the obtained mean intensity values were normalized on the highest
432 value for 0 to 100 (%) scale conversion.

433

434 **Quantification of cytoplasmic granules**

435 For the quantification of PQN-59, GTBP-1 and TIAR-1 cytoplasmic granules QuPath version 0.2.3
436 was used (41). The algorithm for granule detection was based on a pixel classifier and was trained on
437 representative pictures with dedicated annotations. For each embryo, manually delineated, the total
438 number of detected granules was obtained. The average intensity of all the detected granules in each
439 embryo was background subtracted using the average embryonic intensity of the same embryo.

440

441 **Yeast two-hybrid assay**

442 The interaction between PQN-59 and GTBP-1 was assessed in the PJ69-4a yeast strain (42) using
443 single copy GAL4-activation and GAL4-DNA-binding domain-based vectors. Full-length cDNAs
444 were cloned into these vectors using Gibson reactions and transformed into the host yeast strain using
445 previously described protocols (42). Transformants were selected on SC-leu-trp plates and
446 subsequently tested for growth (3 days) on SC-trp-leu-his plates containing 3mM 3AT.

447

448 **Protein domain identification**

449 Protein domains were identified using the meta site Motif Scan tool, a free database for protein motif
450 prediction developed by the Swiss Institute of Bioinformatics (SIB), including Prosite, Pfam, and
451 HAMAP profiles (https://myhits.isb-sib.ch/cgi-bin/motif_scan). Comparable results have also been
452 obtained interrogating other online tools, such as PROSITE at ExPASy (<https://prosite.expasy.org/>),
453 MOTIF (GenomeNet, Institute for Chemical Research, Kyoto University, Japan)
454 (<https://www.genome.jp/tools/motif/>), and InterPro (<http://www.ebi.ac.uk/interpro/>).

455 Prion domains have been identified using PLAAC (<http://plaac.wi.mit.edu>).

456

457 **Antibody production**

458 To produce antibodies to PQN-59 a C-terminal fragment (aminoacid 304-712) was cloned using the
459 Gateway technology (Invitrogen) into the pDEST15. Recombinant GST-tagged PQN-59 was
460 expressed in BL21 and purified using standard protocols. Antibody production in rabbit was
461 performed by Covalab, France. The obtained anti-PQN-59 serum was purified on membrane strip
462 carrying bacterially expressed GST-PQN-59 antigen. About 5 µg of fusion protein was loaded in each
463 lane of a 10% acrylamide gel. The protein was transferred on a nitrocellulose membrane (GE

464 Healthcare). A stripe of the membrane, containing the protein, was cut and incubated for 1 hour in
465 PBS + 3% milk for blocking. The band was then incubated overnight at 4°C in 1 ml of serum diluted
466 in 1 ml of 3% milk in PBS-Tween + 4 mg of GST (to avoid GST binding). After three washes of 5 to
467 10 minutes, the antibody was eluted using a solution of glycine 100 mM, pH 2. The pH of the elution
468 solution was equilibrated to 7.5 using TRIS 1M.

469

470 **Western Blot**

471 For western blot, 50 adult worms were manually picked from NGM plates, resuspended in Laemmli
472 sample buffer and denatured at 92°C for 2 minutes. Lysates were separated by SDS-PAGE using a
473 10% Acrylamide Gel. Proteins were then transferred onto a nitrocellulose membrane (Sigma). The
474 membrane was blocked with 3% milk in PBS. After washing with a solution of PBS and 0.1% Tween
475 (PBST), the membrane was incubated overnight at 4°C with primary antibodies diluted in a 1% BSA-
476 PBST solution. The following day the membrane was washed with PBST twice for 10 minutes and
477 incubated with secondary antibodies diluted in the same solution (1:10000 HRP-conjugated anti-
478 mouse or anti-rabbit antibodies (Biorad)) in the same solution at room temperature for 45 minutes.
479 After three washes of 10 minutes each, proteins were visualized with ECL (Millipore) using a Pxie
480 machine.

481

482 **Embryonic lethality and brood size counting**

483 To count embryonic lethality and brood size, L4/young adult worms were singled onto individual
484 OP50-seeded NGM plates and incubated at 20°C for 24 hours. After 24 hours, the adult worm was
485 removed, and plates were again incubated at 20°C for 24 hours. To assess the brood size, the total
486 number of un-hatched embryos and hatched larvae were counted under a dissecting microscope. The
487 ratio between the un-hatched embryos over the total of the F1 progeny (brood size) was used to
488 calculate the percentage of embryonic lethality.

489

490 **Embryonic lethality after heat-shock**

491 For embryonic lethality after heat-shock, gravid hermaphrodites were dissected on a coverslip into a
492 drop of Egg Buffer (118 mM NaCl, 48 mM KCl, 2 mM CaCl₂, 2 mM MgCl₂, and 25 mM Hepes, pH

493 7.5) where the embryos were released. The coverslip was then transferred on a metal block placed in
494 a humidified incubator at 34°C for 10 minutes. After the heat-shock, the embryos were transferred by
495 pipetting on OP50-seeded NGM plates, counted, and incubated at 20°C for 24 hours for recovery.
496 After recovery, the number of un-hatched embryos was counted. The ratio between un-hatched
497 embryos over the number of embryos plated was used to calculate the percentage of embryonic
498 lethality after heat-shock exposure.

499

500 **Statistical analysis**

501 Statistical analysis was performed using GraphPad Prism 8. Details on the statistical test, the sample,
502 and experiment number, as well as the meaning of error bars, are provided for each experiment in the
503 corresponding figure legend, in the results and/or in the method details. Significance was defined as,
504 ns, $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

505

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515

516

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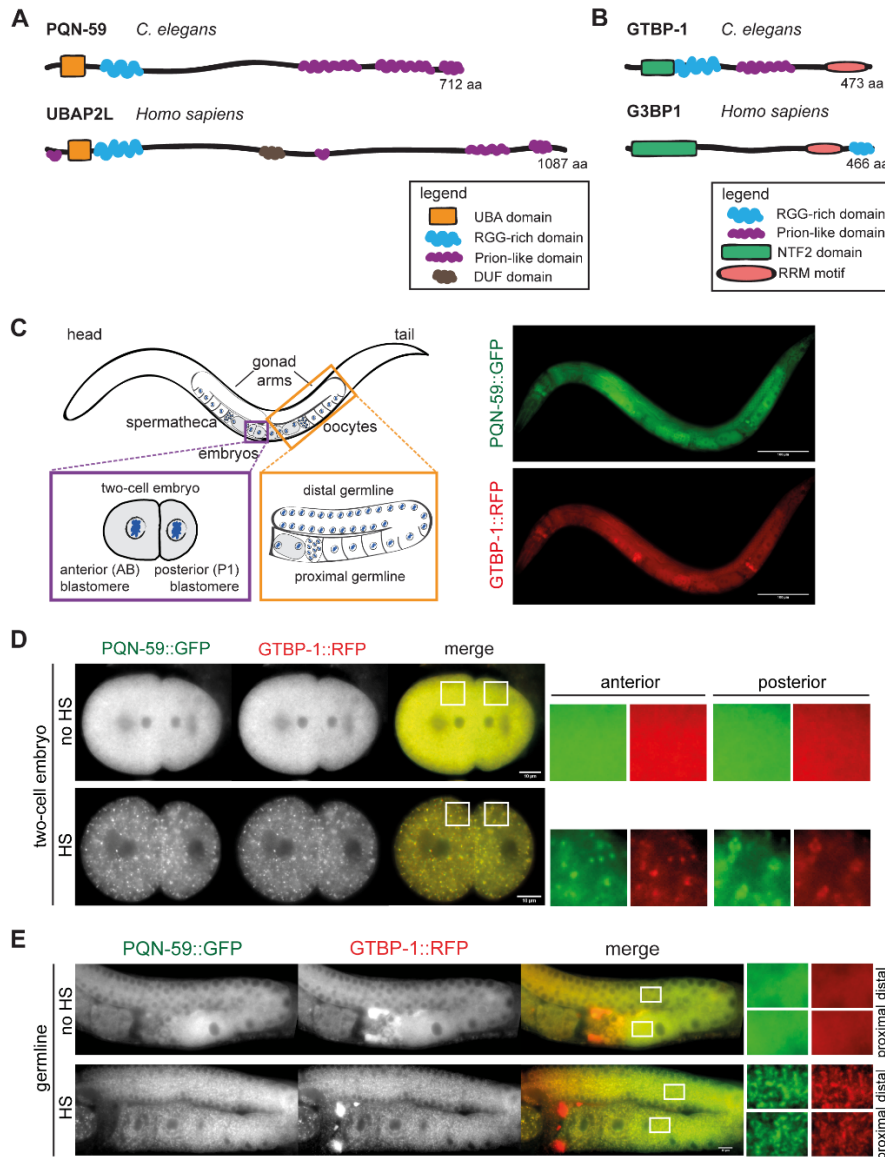
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630 **Figures**

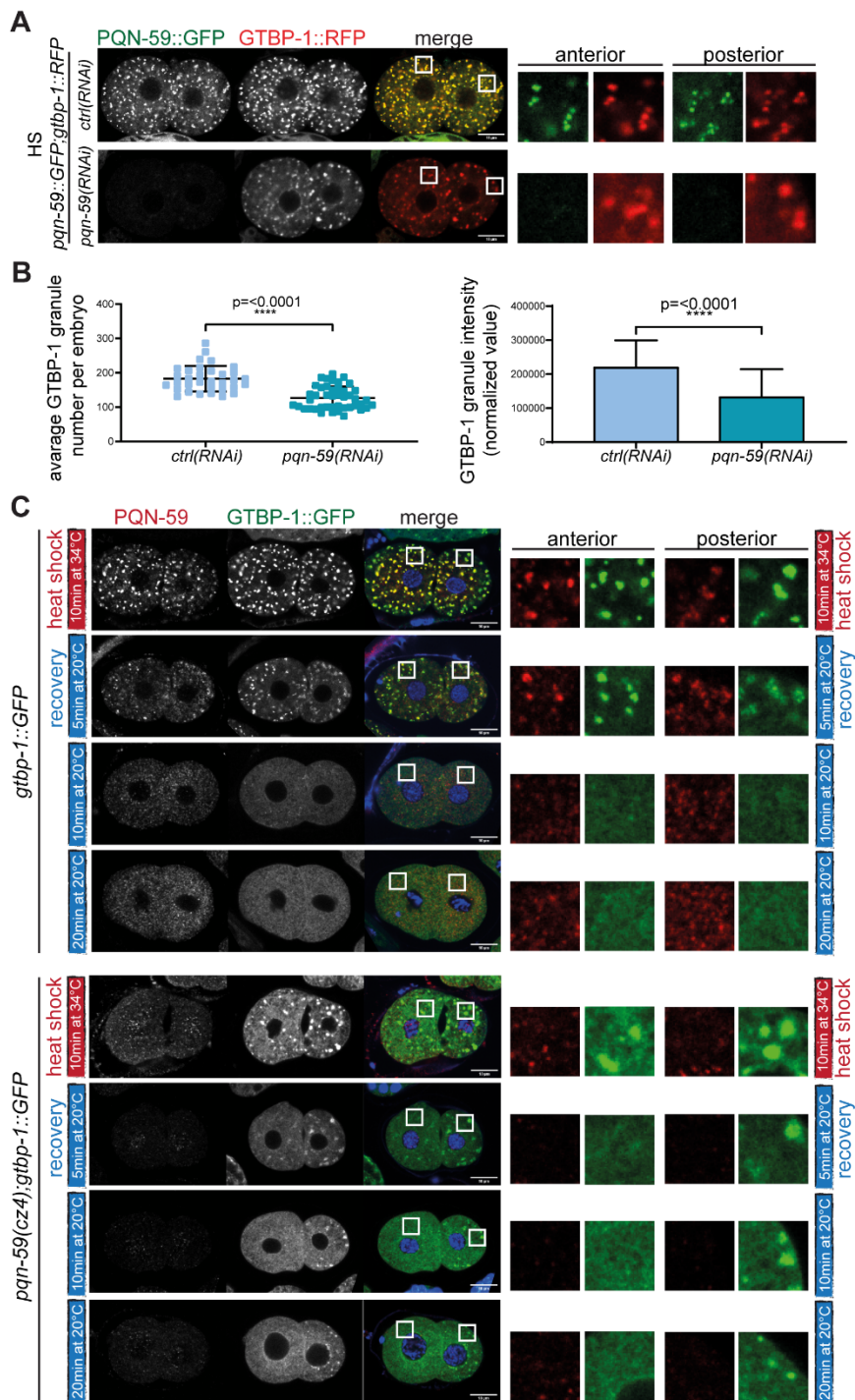


631

632 **Fig 1. PQN-59 and GTBP-1 co-localize into heat stress-induced granules in the embryo and in the**
 633 **germline.**

634 (A) and (B) Schematic representation of the protein domains of PQN-59 and its human ortholog UBAP2L (A)
 635 and of GTBP-1 and its human ortholog G3BP1 (B). (C) Schematic drawing of an adult *C. elegans* worm,
 636 with close ups of a two-cell embryo (bottom left, purple square) and of the germline (bottom right, orange square)
 637 and images of an adult animal expressing endogenous *pqn-59::GFP;gtbp-1::RFP*. Scale bars represent 100
 638 μ m. (D) Still frames from time-lapse imaging of *pqn-59::GFP;gtbp-1::RFP* embryos using a CherryTemp
 639 temperature-controlled stage. Embryos were imaged at 20°C (no heat-shock, no HS) or at 30°C for 5 min (heat-
 640 shock, HS). PQN-59/GTBP-1 granules were observed in 100% of the observed embryos (n=12, N=5). In all
 641 figures, white boxes indicate the ROI shown enlarged on the right. For embryos, ROIs are in the anterior AB
 642 cell (left) and in the posterior P1 cell (right). (E) Germlines of *pqn-59::GFP;gtbp-1::RFP* worms in control
 643 conditions (no HS, 20°C) and after heat-stress exposure (HS, 10 min at 35°C). PQN-59/GTBP-1 granules were
 644 detected in 98% of the observed gonads (n=51, N=8). In all images of germlines, white boxes in the distal (top)

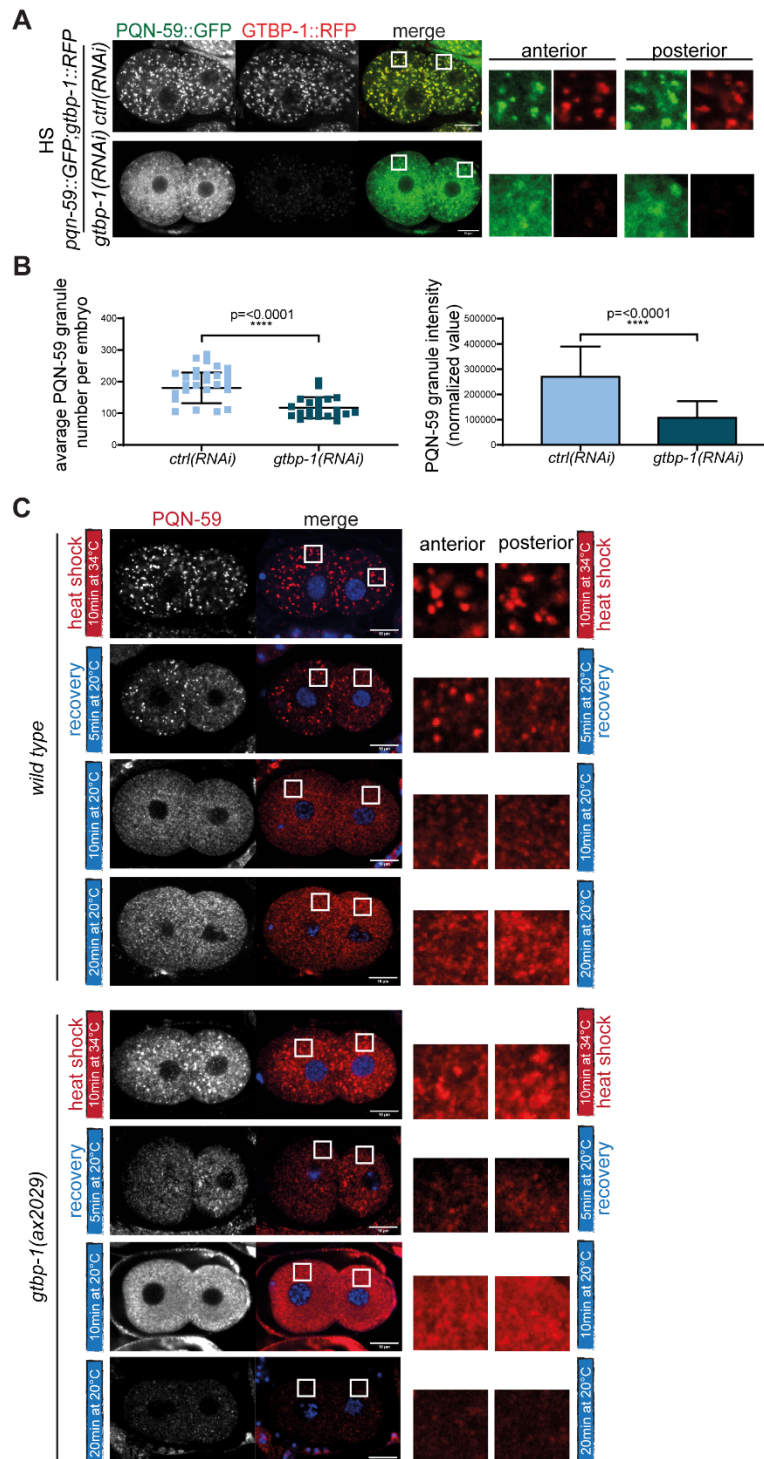
645 and proximal (bottom) germline show the ROI enlarged on the right. Scale bars represent 10 μ m. In all images,
646 ROIs are enlarged 8X (embryos) and 11.5X (germline).



647
648 **Fig 2. PQN-59 depletion impairs stress-induced GTBP-1 granule formation.**

649 (A) Single confocal planes of *pqn-59::GFP;gtbp-1::RFP* fixed two-cell embryos treated with the indicated
650 RNAi and exposed to heat-shock (HS, 34°C for 10 minutes) before fixation. (B) Quantification of the average
651 GTBP-1 granule number (left) and the average normalized GTBP-1 granule intensity (right) per embryo
652 (*ctrl(RNAi)* n=33; *pqn-59(RNAi)* n=44, N=4). Error bars indicate S.D. The P-value was determined using
653 Student's t-test. (C) Single confocal planes of *gtbp-1::GFP* and *pqn-59(cz4);gtbp-1::GFP* fixed embryos
654 immunostained with PQN-59 antibodies (red). GTBP-1 GFP signal is in green and DNA was counterstained

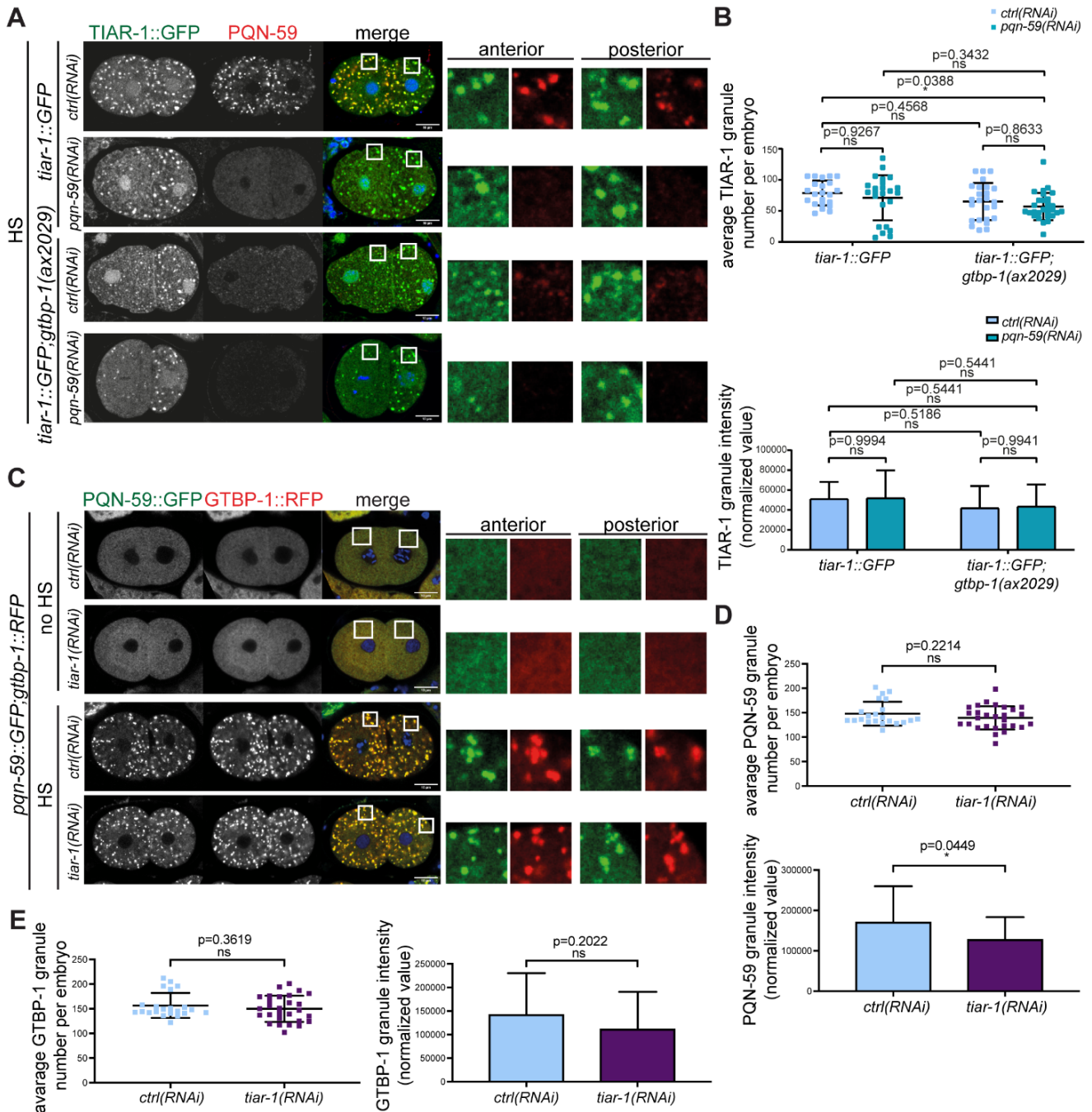
655 with DAPI (blue). Embryos were fixed at different time points: immediately after heat-shock exposure (10
 656 minutes at 34°C, red vertical line) and after recovery at 20°C for 5, 10, or 20 minutes (blue vertical line). For
 657 each time point in C, between 15 and 21 embryos were analyzed for the *gtbp-1::GFP* and between 7 and 13
 658 for the *pqn-59(cz4);gtbp-1::GFP* (N=4). Scale bars represent 10 μm. Enlarged ROIs are on the right.



659
 660 **Fig 3. GTBP-1 depletion impairs PQN-59 stress-induced granule formation.**

661 (A) Single confocal planes of *pqn-59::GFP;gtbp-1::RFP* fixed two-cell embryos treated with the indicated
 662 RNAi and exposed to heat-shock (HS, 34°C for 10 minutes) before fixation. (B) Quantification of the average
 663 PQN-59 granule number (left) and the average normalized PQN-59 granule intensity (right) per embryo

664 (*ctrl(RNAi)* n=40; *gtbp-1(RNAi)* n=22, N=4). Error bars indicate S.D. The P-value was determined using
 665 Student's t-test. (C) Single confocal planes of *wild-type* and *gtbp-1(ax2029)* fixed two-cell embryos
 666 immunostained with PQN-59 antibodies (red). DNA was counterstained with DAPI (blue). Embryos were
 667 fixed at different time points: immediately after heat-shock exposure (10 minutes at 34°C, red vertical line)
 668 and after recovery at 20°C for 5, 10, or 20 minutes (blue vertical line). Between 6 and 17 embryos were
 669 analyzed for each condition and genotype, N=3. Scale bars represent 10 μ m. ROIs are enlarged on the right.

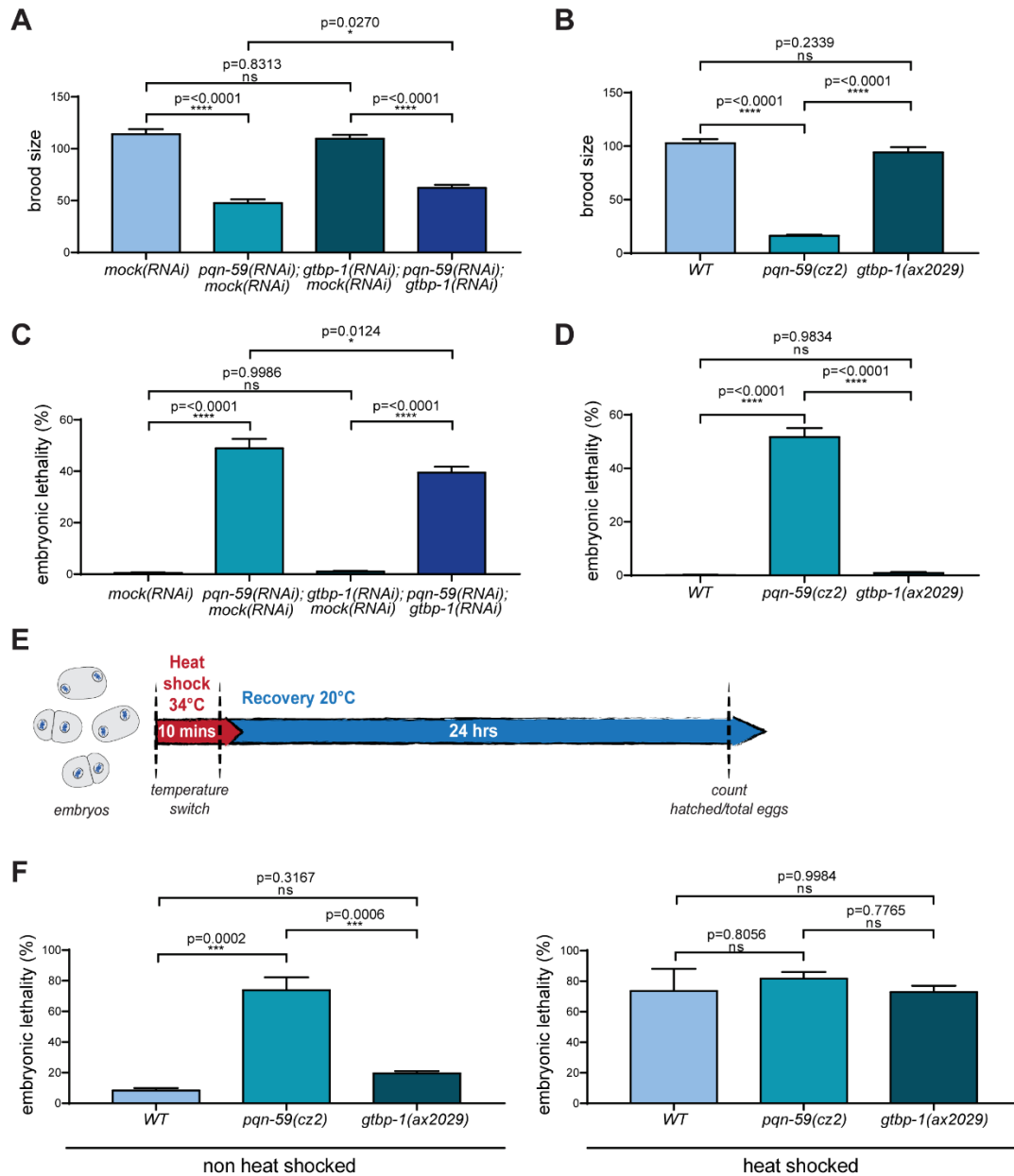


670

671 **Fig 4. The number of TIAR-1 granules is reduced in *pqn-59(RNAi)*; *gtbp-1(ax2029)* embryos.**

672 (A) Single confocal planes of *tiar-1::GFP* and *tiar-1::GFP*; *gtbp-1(ax2029)* fixed two-cell embryos treated
 673 with the indicated RNAi and immunostained with PQN-59 antibodies (red). TIAR-1 GFP signal is in green
 674 and DNA was counterstained with DAPI (blue). Embryos were exposed to heat-shock (HS, 34°C for 10
 675 minutes) before fixation. (B) Quantification of the average TIAR-1 granule number (left) and the average

676 normalized TIAR-1 granule intensity (right) per embryo. Between 22 and 29 embryos were analyzed for each
 677 condition and genotype, N=3. Error bars indicate S.D. The P-value was determined using 2way ANOVA test.
 678 (C) Single confocal planes of *pqn-59::GFP;gtbp-1::RFP* fixed two-cell embryos treated with the indicated
 679 RNAi. DNA was counterstained with DAPI (blue). Embryos were exposed to 34°C for 10 mins (HS) or left
 680 at 20°C (no HS). For all images, scale bars represent 10 μm and enlarged ROIs are on the right. (D) and (E)
 681 Quantification of the average PQN-59 in (D) and GTBP-1 in (E) granule number (top) and the average
 682 normalized PQN-59 in (D) and GTBP-1 in (E) granule intensity (bottom) per embryo (*ctrl(RNAi)* n=22; *tiar-*
 683 *1(RNAi)* n=28, N=3). Error bars indicate S.D. The P-value was determined using Student's t-test.



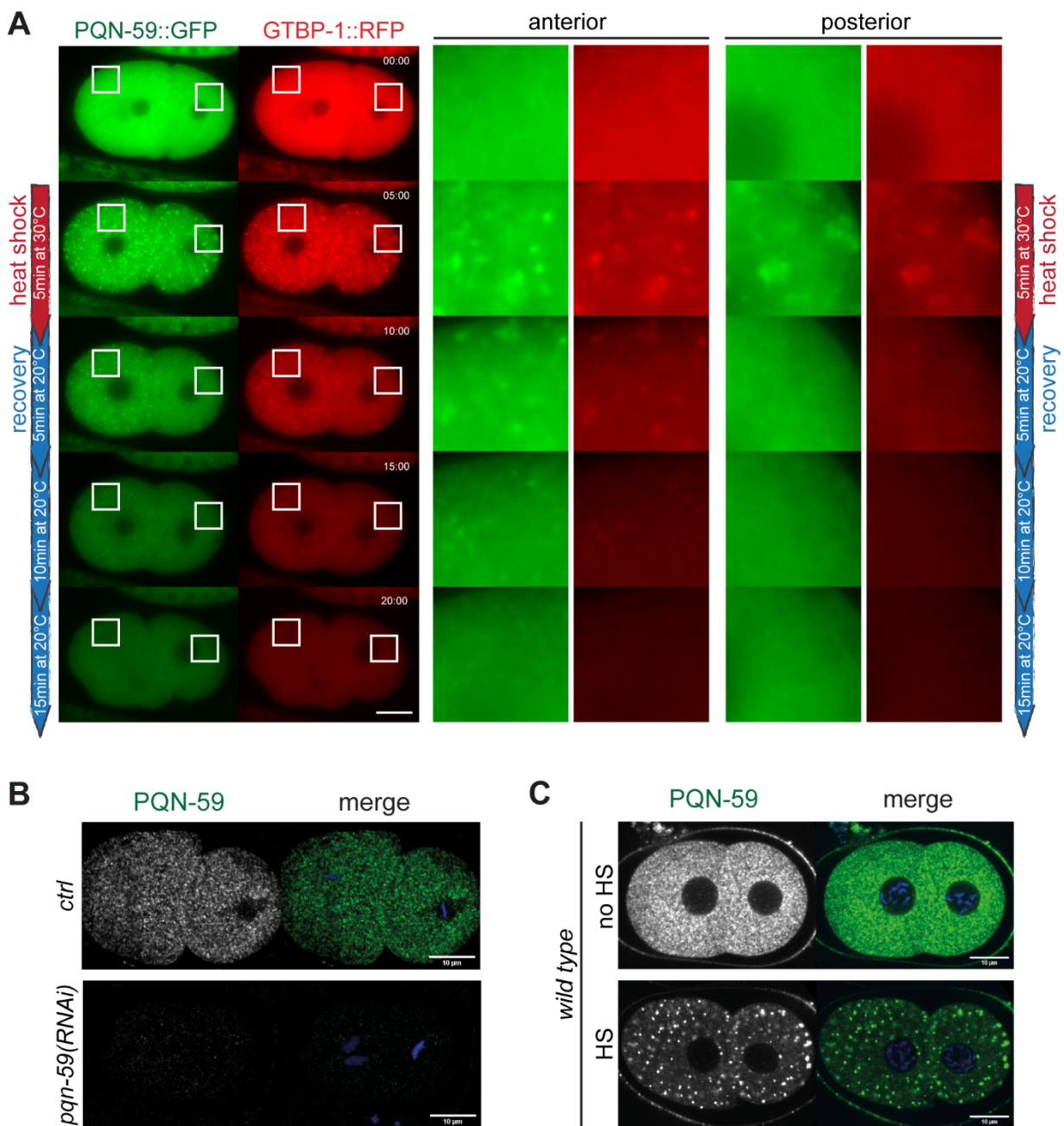
684

685 **Fig 5. PQN-59 is important for C. elegans embryonic development and brood size.**

686 (A) and (B) Brood size of wild-type embryos after RNAi depletion of the indicated genes in (A) and of wild-
 687 type, *pqn-59(cz2)* and *gtbp-1(ax2029)* strains in (B). Values correspond to the average number of eggs laid per
 688 single worm. In A the brood size of a total of 30 animals per condition was evaluated in 3 independent
 689 experiments. In B the brood size of more than 30 animals of each genotype was evaluated in 2 or more
 690 independent experiments. (C) and (D) Embryonic lethality of wild-type embryos after RNAi depletion of the

691 indicated genes in (C) and of *wild-type*, *pqn-59(cz2)* and *gtbp-1(ax2029)* strains in (D). Values correspond to
692 the percentage of un-hatched embryos over the total progeny number (un-hatched embryos and larvae). In (C)
693 embryonic lethality was assessed by counting more than 300 progeny for each condition, N=3. In (D)
694 embryonic lethality was assessed by counting more than 200 progeny for each genotype, N>2. Error bars
695 indicate S.E.M. The P-values were determined using one-way ANOVA test. (E) Timeline of the embryonic
696 survival after heat-shock (see Materials and methods for more details). (F) Lethality of embryos exposed or
697 not to heat shock was assessed as in (E) for *wild-type*, *pqn-59(cz2)* and *gtbp-1(ax2029)* strains counting the
698 un-hatched embryos over the total number of embryos. The percentage of embryonic lethality is represented
699 in F. More than 60 embryos per condition and genotype were counted in three independent experiments (N=3).
700 Error bars indicate S.E.M. The P-values were determined using one-way ANOVA test.

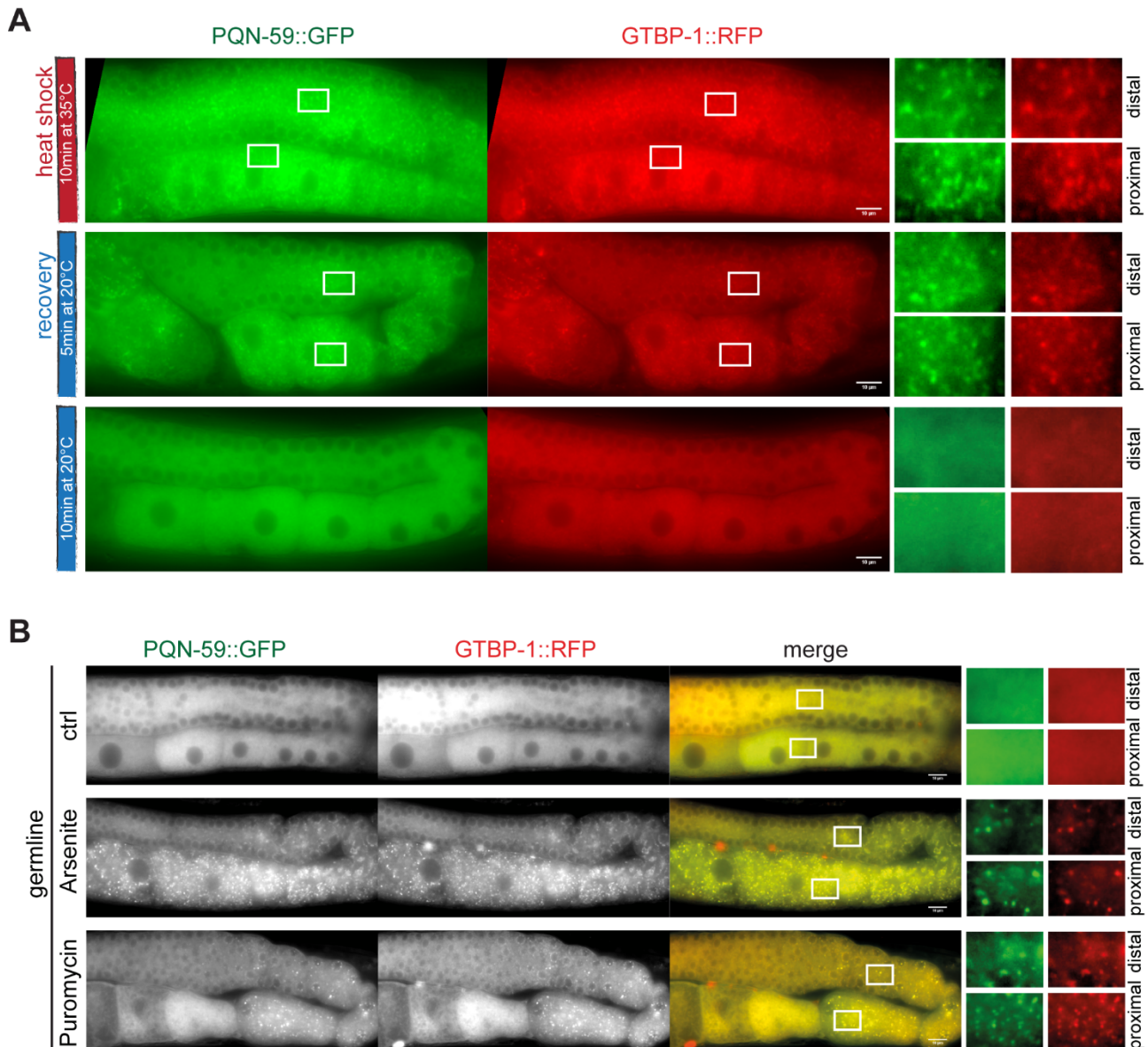
701 Supporting information captions



S1 Fig. PQN-59/GTBP-1 heat-induced embryonic granules are reversible.

704 (A) Still frames from time-lapse imaging of *pqn-59::GFP;gtbp-1::RFP* embryos using the CherryTemp
 705 temperature-controlled stage (n=12, N=3). The red vertical line on the left shows the time of exposure to HS
 706 and the blue line the time after stress release (recovery). (B) and (C) Fixed two-cell embryos immunostained
 707 with anti-PQN-59 antibodies (green). DNA was counterstained with DAPI (blue). (B) Maximum projections
 708 of confocal images of untreated (*ctrl*) or PQN-59-depleted (*pqn-59(RNAi)*) embryos, as indicated (n=25 *ctrl*
 709 and n=17 *pqn-59(RNAi)* embryos, N=2). (C) Single confocal planes of two-cell *wild-type* embryos. Embryos
 710 were left at 20°C (no HS) or exposed to 34°C for 10 mins (HS) before fixation. (N=6). Scale bars represent 10
 711 μ m. Enlarged ROIs are on the right.

712



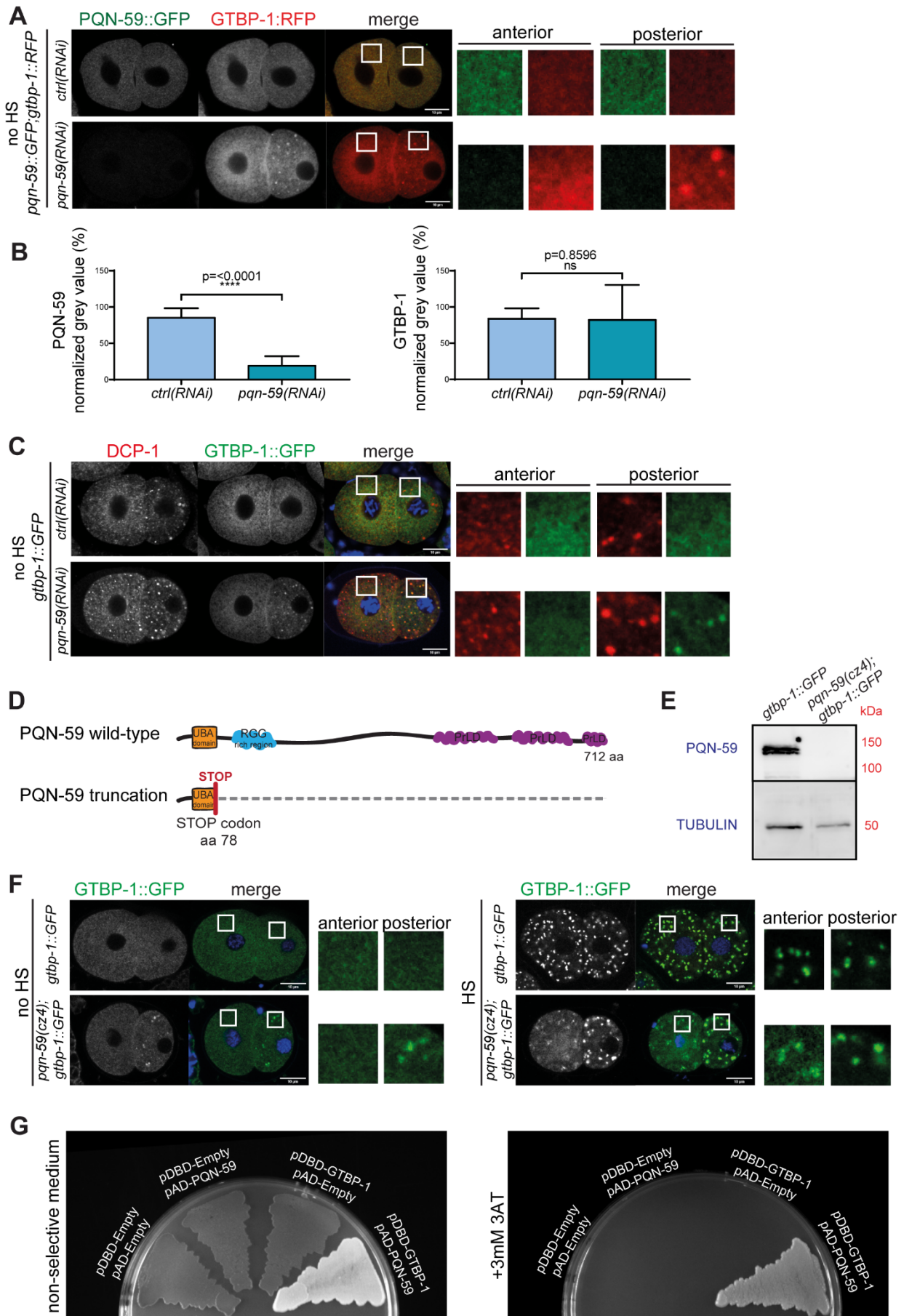
713

714 **S2 Fig. PQN-59/GTBP-1 granules form in response to several stresses in the germline and are reversible.**

715 (A) and (B) Images of germlines of *pqn-59::GFP;gtbp-1::RFP* adults. (A) PQN-59 (green) and GTBP-1 (red)
 716 form cytoplasmic granules after 5 minutes of heat exposure at 35°C (red vertical line) and dissolve after 10
 717 minutes of recovery at 20°C (blue vertical line, n=15, N=3). (B) Control (buffer) and worms treated with drugs,
 718 as indicated on the left. 85% (n=26) of the Arsenite-treated worms and 100% of the Puromycin-treated worms

719 (n=15) showed formation of PQN-59 (green) and GTBP-1 (red) granules in the germline. ROIs are shown
720 enlarged on the right. Scale bars represent 10 μ m.

721

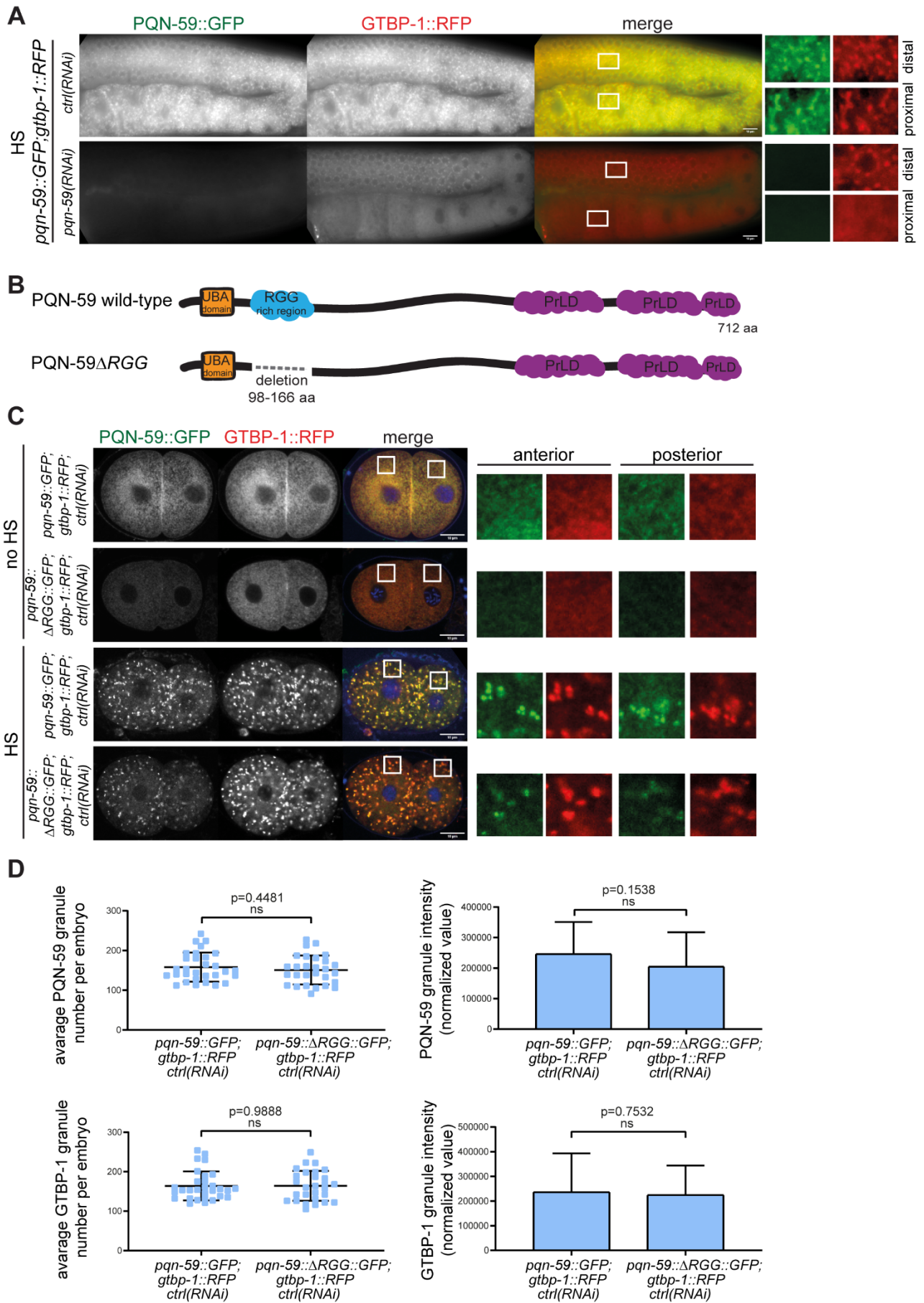


722

723 **S3 Fig.** (figure legend in the next page)

724 **S3 Fig. PQN-59 interacts with GTBP-1 and its depletion or deletion results in stress-independent GTBP-**
725 **1 clusters in the posterior embryonic blastomere.**

726 (A) Single confocal planes of *pqn-59::GFP;gtbp-1::RFP* fixed two-cell stage embryos treated with the
727 indicated RNAi in non-heat-shocked conditions (no HS). (B) Quantification of the normalized cytoplasmic
728 intensity of PQN-59 (left) and GTBP-1 (right) of *ctrl(RNAi)* and *pqn-59(RNAi)* in the anterior blastomere as
729 in (A) (*ctrl(RNAi)* n=23; *pqn-59(RNAi)* n=38, N=4). Error bars indicate S.D. The P-value was determined
730 using Student's t-test. (C) Single confocal planes of *gtbp-1::GFP* fixed two-cell stage embryos immunostained
731 with DCP-1 antibodies (red) in non-heat-shocked conditions (no HS). GTBP-1 GFP signal is in green and
732 DNA was counterstained with DAPI (blue). Embryos were treated with *ctrl* or *pqn-59(RNAi)* as indicated
733 (*ctrl(RNAi)* n=12; *pqn-59(RNAi)* n=19, N=3). (D) Illustration of the PQN-59 protein domains with the
734 indication of the STOP codon insertion in the strain *pqn-59(cz4);gtbp-1::GFP* obtained by CRISPR/Cas9. (E)
735 Western blot on worm lysate of *gtbp-1::GFP* and *pqn-59(cz4);gtbp-1::GFP* worms using PQN-59 and
736 TUBULIN (loading control) antibodies. (F) Single confocal planes of fixed two-cell stage *gtbp-1::GFP* and
737 *pqn-59(cz4);gtbp-1::GFP* embryos, at 20°C (no HS) or after exposure to 34°C for 10 mins (HS). GTBP-1
738 GFP signal is in green and DNA was counterstained with DAPI (blue). ROIs are shown enlarged on the right
739 of each set of embryos. Between 6 and 17 two-cell embryos were analyzed in each condition, N=4. Scale bars
740 represent 10 µm. (G) Yeast two-hybrid assay using the PJ69-4a yeast strain transformed with the indicated
741 plasmids. On non-selective plates, all streaks grow. Controls are in red because of lack of interaction and lack
742 of activation of the ADE-2 reporter. The streak of cells containing both PQN-59 and GTBP-1 is white,
743 indicating interaction-dependent activation of the ADE-2 reporter. On selective plates (+3mM 3AT) yeast
744 growth is observed only for the clone where both PQN-59 and GTBP-1 are expressed, indicating interaction
745 and activation of the HIS-3 reporter.



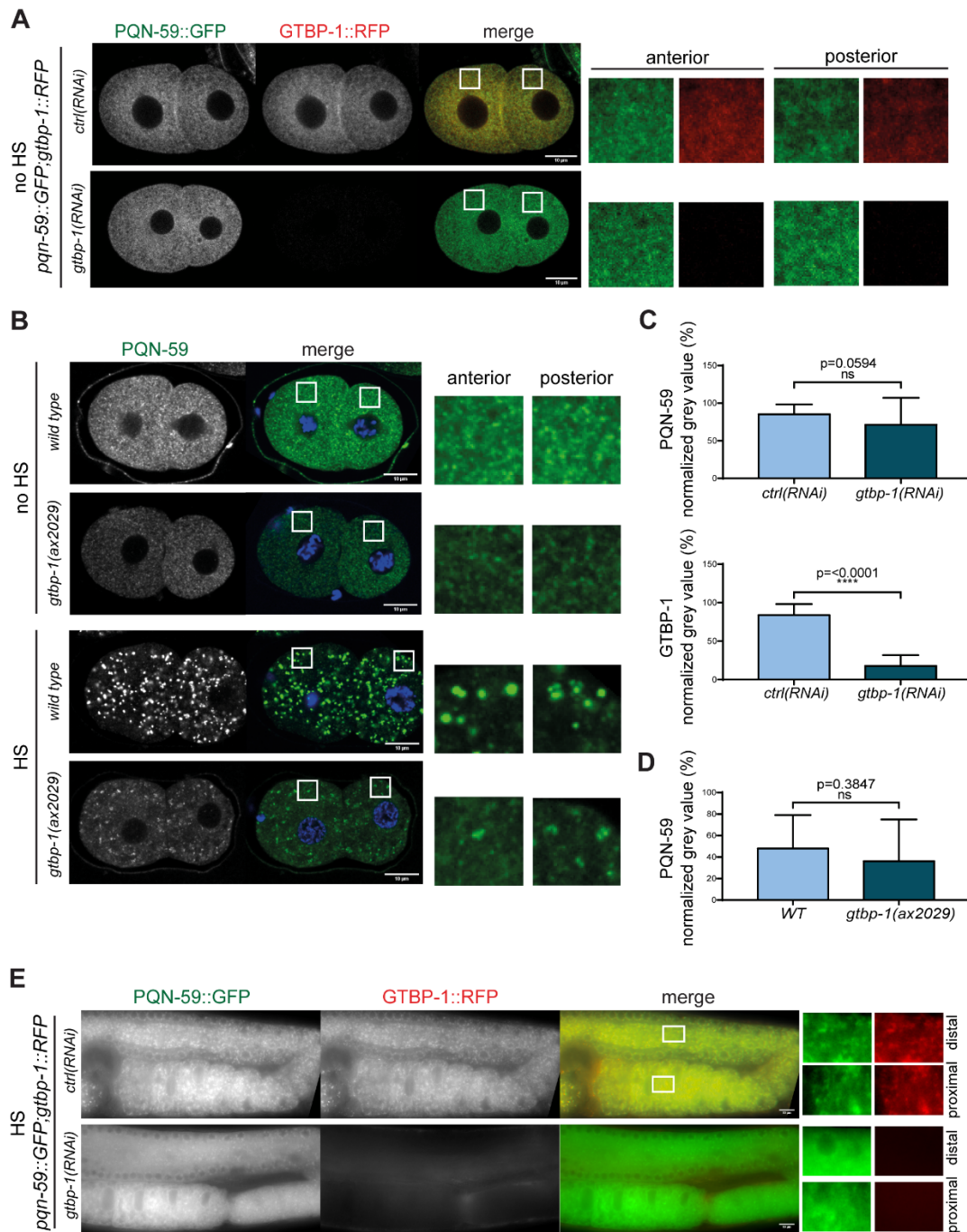
746

747 **S4 Fig.** (figure legend in the next page)

748 **S4 Fig. GTBP-1 stress-induced granule formation is impaired in the germline by depletion of PQN-59**
749 **but not in a strain in which the RGG domain has been deleted.**

750 (A) Germline pictures of *pqn-59::GFP;gtbp-1::RFP* worms treated with the indicated RNAi and exposed to
751 heat-shock (HS, *ctrl(RNAi)* n=14; *pqn-59(RNAi)* n=15, N=2). ROIs are enlarged on the right. Scale bars
752 represent 10 μ m. (B) Illustration of the PQN-59 protein with the RGG deletion introduced by CRISPR/Cas9
753 in the strain *pqn-59::GFP;gtbp-1::RFP*. (C) Single confocal planes of *pqn-59::GFP;gtbp-1::RFP* and *pqn-*
754 *59:: Δ RGG::GFP;gtbp-1::RFP* fixed two-cell stage embryos. PQN-59 GFP signal is in green, GTBP-1 RFP
755 signal is in red and DNA was counterstained in blue. On the right, ROIs are enlarged 8 X. Scale bars represent
756 10 μ m. (D) Quantification of the average PQN-59 (top left) and GTBP-1 (bottom left) granule number per
757 embryo and of the average normalized PQN-59 (top right) and GTBP-1 (bottom right) granule intensity per
758 embryo (*pqn-59::GFP;gtbp-1::RFP* n=30; *pqn-59:: Δ RGG::GFP;gtbp-1::RFP*, n=28, N=3). Error bars
759 indicate S.D. The P-value was determined using Student's t-test.

760

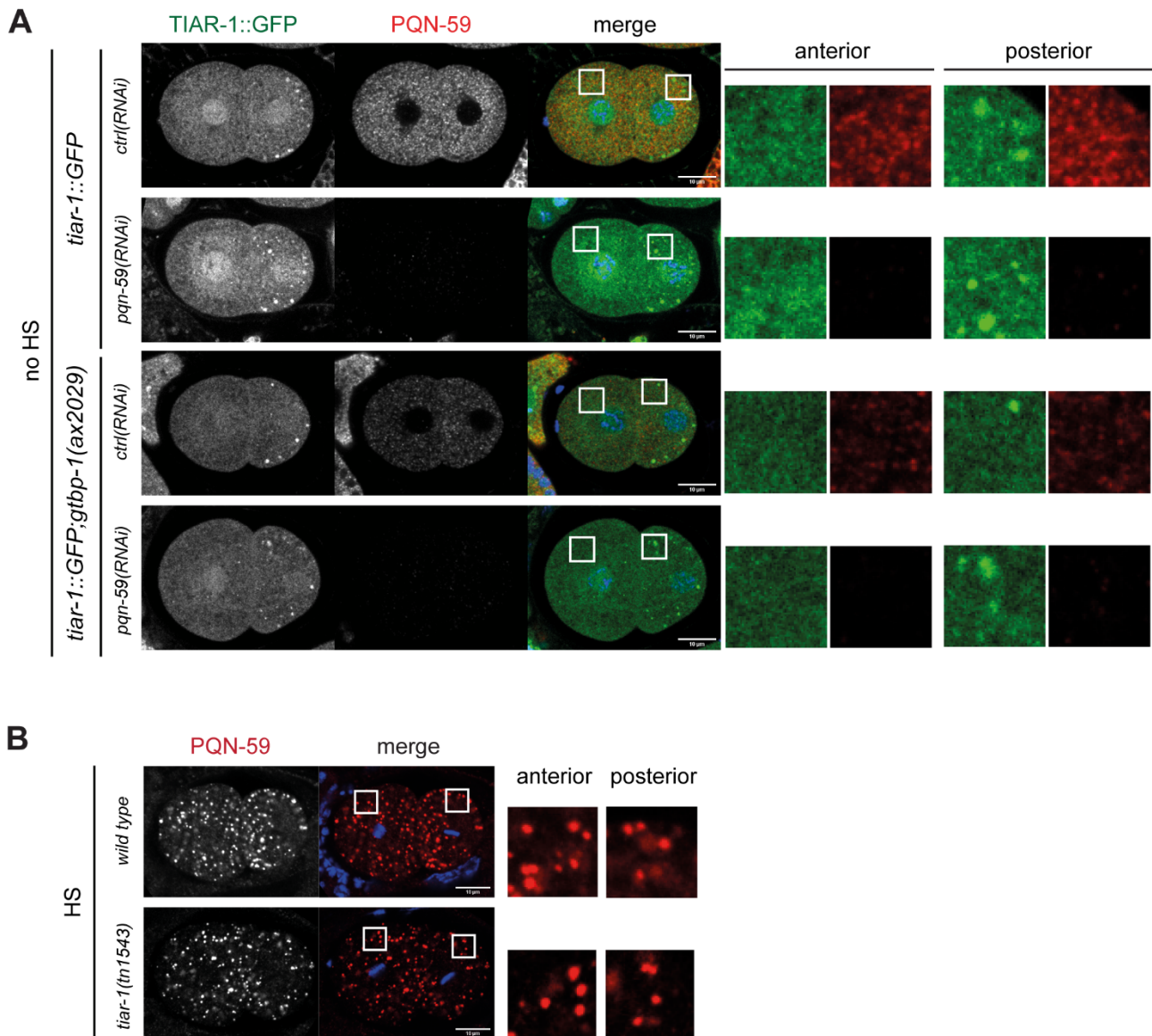


761

762 **S5 Fig. GTBP-1 null mutation impairs stress-induced PQN-59 granule formation.**

763 (A) Single confocal planes of *pqn-59::GFP;gtbp-1::RFP* fixed two-cell embryos treated with the indicated
 764 RNAi in non-heat-shocked conditions (no HS). (B) Single confocal planes of *wild-type* and *gtbp-1(ax2029)*
 765 fixed two-cell embryos immunostained with PQN-59 antibodies (green). DNA was counterstained with DAPI
 766 (blue). Embryos were exposed to 34°C for 10 mins (HS) or left at 20°C (no HS). For both (A) and (B) enlarged
 767 ROIs are shown on the right. Scale bars represent 10 μ m. (C) Quantification of the normalized cytoplasmic
 768 intensity of PQN-59 (top) and GTBP-1 (bottom) of *ctrl(RNAi)* and *pqn-59(RNAi)* embryos at 20°C as in A
 769 (*ctrl(RNAi)* n=22; *gtbp-1(RNAi)* n=31, N=4). Error bars indicate S.D. The P-value was determined using
 770 Student's t-test. (D) Quantification of the normalized cytoplasmic intensity of PQN-59 of *wild-type* and *gtbp-*
 771 *1(ax2029)* embryos at 20°C as in B (*wild type* n=14; *gtbp-1(ax2029)* n=14, N=3). Error bars indicate S.D. The
 772 P-value was determined using Student's t-test. (E) Germline pictures of *pqn-59::GFP;gtbp-1::RFP* worms

773 treated with the indicated RNAi and exposed to heat-shock (HS, *ctrl(RNAi)* n=15; *gtbp-1(RNAi)* n=16, N=2).
 774 ROIs are enlarged on the right.

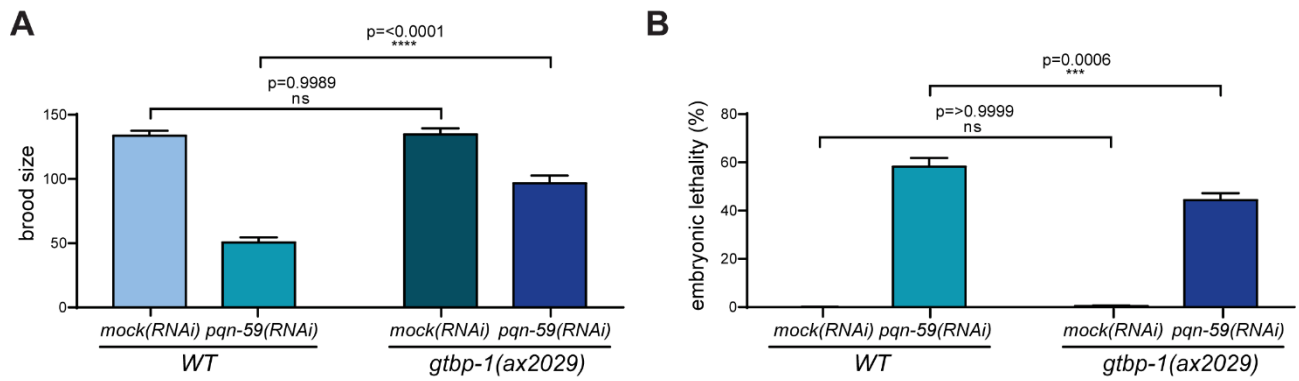


775

776 **S6 Fig. PQN-59 accumulation in stress-induced granules is not affected in *tiar-1* mutant embryos.**

777 (A) Single confocal planes of *tiar-1::GFP* and *tiar-1::GFP;gtbp-1(ax2029)* fixed two-cell embryos treated
 778 with the indicated RNAi in non-heat-shocked conditions (no HS). Embryos were immunostained with PQN-
 779 59 antibodies (red). TIAR-1 GFP signal is in red and DNA was counterstained with DAPI (blue). (N=3). (B)
 780 Single confocal planes of *wild-type* and *tiar-1(tn1543)* fixed two-cell embryos exposed to 34°C for 10 mins
 781 (HS) before fixation and immunostained with PQN-59 antibodies (red). DNA was counterstained with DAPI
 782 (blue) (*wild-type* n =15 and *tiar-1(tn1543)* n=4, N=1). For both (A) and (B) enlarged ROIs are shown on the
 783 right. Scale bars represent 10 μ m.

784



785

786 **S7 Fig. PQN-59 and GTBP-1 co-depletion does not result in an increase of the phenotype of PQN-59**
787 **depletion alone.**

788 (A) Brood size of *wild-type* and *gtbp-1(ax2029)* strains treated with the indicated RNAi. Values correspond to
789 the average number of eggs laid per single worm. The brood size of more than 15 animals of each genotype
790 was evaluated (N=2). (B) Embryonic lethality of *wild-type* and *gtbp-1(ax2029)* strains treated with the
791 indicated RNAi. Values correspond to the percentage of un-hatched embryos over the total progeny number
792 (un-hatched embryos and larvae). Embryonic lethality was assessed by counting more than 300 progeny for
793 each condition. N=2. Error bars indicate S.E.M. The P-values were determined using 2way ANOVA test.

794

795 **S1 Movie. PQN-59 and GTBP-1 form reversible cytoplasmic granules.**

796 Time lapse movie of *pqn-59::GFP;gtbp-1::RFP* embryos using the CherryTemp temperature-controlled stage.
797 Embryos are recorded at 30°C from minute 00:00 to minute 05:00. From minute 05:00 to minute 20:00 the
798 temperature is 20°C. The temperature shift is visible through the focus change at minute 05:00. Cytoplasmic
799 granules of PQN-59 (green) and GTBP-1 (red) are visible after 5 minutes of heat exposure at 30°C and dissolve
800 after 15 minutes of recovery at 20°C. Scale bar represents 10 µm.

801