

1 **PQN-59 antagonizes microRNA-mediated repression and functions in stress granule**
2 **formation during *C. elegans* development**

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17 **ABSTRACT:**

18 microRNAs (miRNAs) are potent regulators of gene expression that function in a variety of
19 developmental and physiological processes by dampening the expression of their target genes at a
20 post-transcriptional level. In many gene regulatory networks (GRNs), miRNAs function in a switch-
21 like manner whereby their expression and activity elicit a transition from one stable pattern of gene
22 expression to a distinct, equally stable pattern required to define a nascent cell fate. While the
23 importance of miRNAs that function in this capacity are clear, we have less of an understanding of
24 the cellular factors and mechanisms that ensure the robustness of this form of regulatory bistability.
25 In a screen to identify suppressors of temporal patterning phenotypes that result from ineffective
26 miRNA-mediated target repression during *C. elegans* development, we identified *pqn-59*, an ortholog
27 of human UBAP2L, as a novel factor that antagonizes the activities of multiple heterochronic miRNAs.
28 Specifically, we find that depletion of *pqn-59* can restore normal development in animals with reduced
29 miRNA activity. Importantly, inactivation of *pqn-59* is not sufficient to bypass the requirement of these
30 regulatory RNAs within the heterochronic GRN. The *pqn-59* gene encodes an abundant,
31 cytoplasmically localized and unstructured protein that harbors three essential “prion-like” domains.
32 These domains exhibit LLPS properties *in vitro* and normally function to limit PQN-59 diffusion in the
33 cytoplasm *in vivo*. Like human UBAP2L, PQN-59’s localization becomes highly dynamic during stress
34 conditions where it re-distributes to cytoplasmic stress granules and is important for their formation.
35 Proteomic analysis of PQN-59 complexes from embryonic extracts indicates that PQN-59 and human
36 UBAP2L interact with orthologous cellular components involved in RNA metabolism and promoting
37 protein translation and that PQN-59 additionally interacts with proteins involved in transcription and
38 intracellular transport. Finally, we demonstrate that *pqn-59* depletion results in the stabilization of
39 several mature miRNAs (including those involved in temporal patterning) without altering steady-state
40 pre-miRNAs levels indicating that PQN-59 may ensure the bistability of some GRNs that require
41 miRNA functions by promoting miRNA turnover and, like UBAP2L, enhancing protein translation.

42

43 **AUTHOR SUMMARY**

44 Bistability plays a central role in many gene regulatory networks (GRNs) that control developmental
45 processes where distinct and mutually exclusive cell fates are generated in a defined order. While
46 genetic analysis has identified a number of gene types that promote these transitions, we know little
47 regarding the mechanisms and players that ensure these decisions are robust. and in many cases,
48 irreversible. We leveraged the robust genetics and phenotypes associated with temporal patterning
49 mutants of *C. elegans* to identify genes whose depletion would restore normal regulation in animals
50 that express miRNA alleles that do not sufficiently down-regulate their targets. These efforts identified
51 *pqn-59*, the *C. elegans* ortholog of the human UBAP2L gene. Like UBAP2L, PQN-59 likely forms a
52 hub for a number of RNA/RNA-binding protein mediated processes in cells including translational
53 activation and in the formation of stress granules in adverse environmental conditions. Finally, we
54 also demonstrate that *pqn-59* depletion stabilizes mature miRNA levels further connecting this new
55 family of RNA-binding proteins to translation and miRNA-mediated gene regulation.

56

57 INTRODUCTION

58 Cell fate specification during animal development is tightly controlled to yield highly
59 reproducible outcomes and avoid extreme variation. While individual cell fates can be described by
60 quantifying the expression levels of RNAs that are expressed at any given time, the stable patterns
61 of gene expression for a distinct cell type are often governed by the presence and levels of a relatively
62 limited number of master regulator genes that function at the top of a gene regulation hierarchy.
63 These master regulatory genes typically function in a concentration-dependent manner whereby
64 expression levels above a critical threshold are sufficient to program downstream patterns of
65 transcription in a dominant way. Changes in cell fate specification can occur in a switch-like manner
66 when intrinsic or extrinsic changes lead to reduced expression or activity of a master regulator to
67 levels below a discrete, critical threshold [1]. What common design features of GRNs set these
68 thresholds and what cellular components ensure that the outcomes of sharp changes in the levels of
69 master regulator genes are translated into distinct cell fates have been understudied.

70 The remarkable precision of *C. elegans* post postembryonic cell fate specification relies on
71 GRNs that exhibit switch-like behavior to ensure normal temporal development. Larval development
72 proceeds through four stages (separated by molts) where the timing of individual cell divisions and
73 the patterning of temporal cell fates is invariant in wild-type animals [2]. Heterochronic genes,
74 encoding transcription factors (TFs) and RNA-binding proteins, organize the sequence of temporal
75 development through the control of stage-specific patterns of gene expression [3]. Importantly, these
76 factors function in dosage-sensitive manners and exhibit sharp temporal gradients of expression
77 (usually from high expression to low expression) that change during inter stage molts. miRNAs play
78 a central role in promoting these sharp transitions from one stage to the next by curtailing the
79 expression of discrete protein-coding genes of the heterochronic pathway that define individual
80 temporal cell fates. This process occurs in at least three separate phases of development (L1-to-L2,
81 L2-to-L3, and L4-to-adulthood) and is mediated by distinct miRNAs that regulate the translational
82 output of independent target mRNAs [4]. Importantly, heterochronic miRNAs also function in dosage-

83 sensitive manners whereby alterations in activity or the timing of their expression lead to the
84 perdurance of target gene expression; resulting in the inappropriate reiteration of earlier patterns of
85 cell division and cell fate specification at subsequent molts [4].

86 In this study, we aimed to identify candidate genes that modulate the ability of heterochronic
87 miRNAs to elicit switch-like reprogramming of temporal cell fates. Specifically, we sought to identify
88 genes whose inactivation would enable hypomorphic alleles of miRNA genes that alone are incapable
89 of dampening the expression of their mRNA targets below a required threshold to function normally.
90 These efforts identified *pqn-59*, encoding a previously uncharacterized, cytoplasmically localized
91 “prion-like” domain-containing protein, as a gene product whose inactivation suppresses loss-of-
92 function phenotypes associated with ineffective miRNA-mediated repression. Consistent with *pqn-59*
93 functioning as a modulator of heterochronic miRNA function in temporal cell fate switching, *pqn-59*
94 cannot bypass the requirement for these regulatory RNAs in the heterochronic GRN. We demonstrate
95 that the PQN-59 protein exhibits liquid-liquid phase separation (LLPS) properties *in vivo* and *in vitro*
96 and that PQN-59 is important for the assembly of RNA/RNA-binding protein assemblies called stress
97 granules during heat shock; a feature shared with its human ortholog UBAP2L. A comparison of
98 proteins associated with PQN-59 indicate that PQN-59, like UBAP2L, is likely highly integrated with
99 proteins involved in mRNA metabolism and translational activation. Finally, we show that *pqn-59*
100 depletion may suppress temporal patterning defects in miRNA mutants by increasing the steady state
101 levels of miRNAs in addition to its potential roles in translational activation. In this capacity, normal
102 PQN-59 activity functions to set and maintain gene expression levels within the range for executing
103 bistable switches in gene expression required for normal development.

104

105

106 RESULTS

107 ***pqn-59* depletion suppresses the heterochronic phenotypes of *lin-4* loss-of-function mutants**

108 In order to identify additional gene products that modulate heterochronic miRNA-dependent
109 developmental events, we performed a genome-wide suppressor screen to identify genes whose
110 inactivation via RNAi could suppress the reiterative heterochronic phenotypes of a hypomorphic allele
111 of the *lin-4* miRNA gene. The *ma161* allele of *lin-4* is defined by a single nucleotide substitution in the
112 mature *lin-4* miRNA that reduces its ability to down-regulate its target mRNA, *lin-14*, which is normally
113 turned off by the second larval stage [5]. As a consequence of these molecular defects, *lin-4(ma161)*
114 animals continually express LIN-14 throughout larval development and reiterate L1 specific patterns
115 of cell differentiation for each of the somatic blast cells at subsequent molts. Importantly, while the
116 *lin-4(ma161)* allele generates a small regulatory RNA, its developmental phenotypes are
117 indistinguishable from those completely lacking the *lin-4* gene [5, 6]. As a consequence of these
118 temporal patterning defects, *lin-4(ma161)* animals lack vulval structures required for normal egg
119 laying and also fail to induce the expression of an adult-specific *col-19::GFP* transcriptional reporter
120 after the fourth larval molt (Figure 1A and B). To identify suppressors, we exposed *lin-4(ma161)*
121 animals harboring the *col-19::GFP* reporter to individual clones of a genomic scale RNAi library and
122 identified dsRNAs that could restore normal *col-19::GFP* expression during adulthood. One of these
123 clones generated dsRNA against *pqn-59*, a highly conserved and uncharacterized *C. elegans* gene,
124 that robustly suppressed the reiterative heterochronic phenotypes of *lin-4(ma161)* mutants to a similar
125 level as other previously described suppressors (including *lin-14*, *lin-28*, and *lin-42*)(Table 1)..
126 Examination of adult *lin-4(ma161)* animals that had been exposed to *pqn-59* dsRNAs exhibited
127 normal temporal seam cell developmental division patterns and were now able to generate alae
128 production on adult cuticles, indicative of normal seam cell temporal patterning (Figure 1C).
129 Furthermore, in contrast to control RNAi animals, *pqn-59* RNAi also suppressed the vulvaless
130 phenotypes of *lin-4(ma161)* animals, enabling these animals to lay eggs (Figure 1D). Surprisingly,
131 depletion of *pqn-59* activity in wild-type animals did not induce precocious deposition of adult-specific

132 alae at the L4 molt which distinguishes it from other previously characterized *lin-4* suppressors) (Table
 133 1). Furthermore, *pqn-59* depletion in wild-type backgrounds only induced a mild, early expression of
 134 the *col-19::GFP* reporter in hypodermal cells found in the head and tail regions (H0, H1, and T cells)
 135 of approximately 17% of late L4-staged animals (L4.5 or later [7]).

Table 1. Measurement of RNAi-mediated suppression of temporal patterning phenotypes of various heterochronic mutants

#	Strain	Genotype ^a	Experimental Condition ^b	% Animals L4 alae ^c	n =	% Animals Adult alae ^c	n =	% Animals expressing adult <i>col-19::GFP</i>	n =
1	VT1367	Wild Type	control	0	80	100	100	100	100
			<i>pqn-59</i> RNAi	0	110	100	100	100	100
			<i>lin-42</i> RNAi	19	100	100	100	100	100
			<i>lin-14</i> RNAi	83	100	100	100	100	100
			<i>lin-28</i> RNAi	84	100	100	100	100	100
2	HML2	<i>lin-4(ma161)</i>	control	0	100	0	80	0	100
			<i>pqn-59</i> RNAi	0	100	56	100	62	100
			<i>lin-42</i> RNAi	0	100	46	100	77	100
			<i>lin-14</i> RNAi	4	100	81	100	73	100
3	HML6	<i>lin-4(e912)</i>	control	-	-	0	32	0	30
			<i>pqn-59</i> RNAi	-	-	0	30	5	30
			<i>lin-28</i> RNAi	16	100	89	100	89	100
4	HML11	<i>let-7(n2853)</i>	control	-	-	-	-	35 ^d	100
			<i>pqn-59</i> RNAi	-	-	-	-	60 ^d	100
			<i>lin-42</i> RNAi	-	-	-	-	61 ^d	100
			<i>lin-14</i> RNAi	-	-	-	-	58 ^d	100
			<i>lin-28</i> RNAi	-	-	-	-	84 ^d	100
5	VT2077	<i>lin-31(n1053)</i>	control	-	-	-	-	100	100
			<i>pqn-59</i> RNAi	-	-	-	-	100	100
			<i>lin-42</i> RNAi	-	-	-	-	100	100
			<i>lin-14</i> RNAi	-	-	-	-	100	100
			<i>lin-28</i> RNAi	-	-	-	-	100	100
6	VT2079	<i>lin-31(n1053); alg-1(ma192)</i>	control	-	-	-	-	7	100
			<i>pqn-59</i> RNAi	-	-	-	-	62	100
			<i>lin-42</i> RNAi	-	-	-	-	26	100
			<i>lin-14</i> RNAi	-	-	-	-	66	100
			<i>lin-28</i> RNAi	-	-	-	-	64	100

^aAnimals contain *ma105* containing an integrated *col-19::GFP* transgenic array

^bP₀ animals were exposed to bacteria expressing indicated dsRNAs and adult F1 animals were scored for the indicated phenotype

^cPresence and quality of cuticular alae structures were assayed by Normarski DIC optics. Only one side of each animal was scored.

^d*let-7(n2853)* animals exhibit some *col-19::GFP* expression in lateral seam cells and were scored positive if there was any expression in any hypodermal cells.

When animals were treated with either *pqn-59*, *lin-42*, *lin-14*, or *lin-28* dsRNAs, there was a clear difference in the expressivity of the suppressed phenotype with most positive scoring animals expressing *col-19::GFP* in seam and hyp7 cells.

136
 137 **Suppression of *lin-4* loss of function phenotypes by *pqn-59*(RNAi) requires miRNA expression**
 138 **and *lin-4* miRNA target sites in the *lin-14* mRNA**

139 Because the *lin-4(ma161)* allele generates some *lin-4* miRNA, we asked whether depleting
 140 *pqn-59* via RNAi could completely bypass the requirement for *lin-4* during post-embryonic
 141 development. To accomplish this, we repeated RNAi-based experiments in animals lacking the *lin-4*
 142 gene (e.g., *lin-4(e912)*). These experiments revealed that *pqn-59* depletion had little effect on the *col-*
 143 *19::GFP* expression in *lin-4(0)* mutants (Table 1). Furthermore, adult-specific alae formation and

144 suppression of the vulvaless phenotypes were not restored in this genetic context indicating that *pqn-*
145 *59(RNAi)* cannot bypass the normal requirement for *lin-4* in temporal patterning (Figure 1C and Table
146 1). We then aimed to determine how *pqn-59(RNAi)* modulates the temporal expression of LIN-14
147 protein, encoded by the major *lin-4* target mRNA [8]. We therefore examined the expression of a
148 functional, CRISPR-tagged *lin-14::GFP* allele in wild-type, *lin-4(ma161)* and *lin-4(e912)* genetic
149 backgrounds in experimental conditions where *pqn-59* expression is altered. In wild-type animals,
150 LIN-14::GFP expression begins during embryogenesis and is post-transcriptionally down-regulated
151 at the L1 to L2 molt by *lin-4* miRNAs [8, 9]. In contrast, LIN-14::GFP expression perdures throughout
152 development in both *lin-4(ma161)* and *lin-4(e912)* animals (Figure 1F). When *lin-4(ma161)* animals
153 were treated with *pqn-59* dsRNAs, LIN-14::GFP expression was curtailed by the early L2 stage with
154 similar kinetics as observed in wild-type animals exposed to either control or *pqn-59* dsRNAs (Figure
155 1F). In contrast, LIN-14::GFP expression is continuously maintained in *lin-4(e912)* animals in these
156 conditions indicating that *pqn-59* depletion does not bypass the requirement for this miRNA in
157 development (Figure 1F). We did note that LIN-14 expression in embryos and in L1-staged animals
158 was slightly lowered in *pqn-59(RNAi)* conditions.

159 To further this analysis, we examined alleles of *lin-14* that generate mRNAs that harbor
160 deletions in the 3'UTR of the *lin-14* transcript and have a reduced ability to be targeted by the *lin-4*
161 miRNA. Specifically, we examined whether *pqn-59(RNAi)* could suppress the reiterative, gain-of-
162 function phenotypes of *lin-14(n536)* and *lin-14(n355)* which harbor deletions of the *lin-14* 3'UTR that
163 result in the deletion of five of seven or all of the *lin-4* miRNA binding sites in the *lin-14* 3'UTR (Figure
164 1G) [8]. Each of these mutants exhibit *lin-4*-like phenotypes (with different penetrance) due to the
165 reduced ability of the *lin-14* mRNAs produced from these loci to be down-regulated the *lin-4* miRNA
166 [5]. We found that *pqn-59(RNAi)* was able to restore adult-specific alae formation in *lin-14(n536)*
167 mutant animals that retain two of the seven *lin-4* binding sites in the *lin-14* 3' UTR but not a *lin-14*
168 truncation allele, *lin-14(n355)*, that lacks all of the *lin-4* binding sites (Figure 1H). These experiments
169 suggest that the suppression of *lin-4* reiterative phenotypes mediated by reducing *pqn-59* expression

170 requires the ability of *lin-4* to both be produced and also able to physically interact with its target
171 mRNA via complementary binding sites.

172
173 ***pqn-59* depletion can suppress other heterochronic phenotypes associated with reduced**
174 **miRNA activity**

175 We next aimed to determine if the genetic interactions between *pqn-59* and *lin-4* hypomorphic
176 alleles are specific to developmental events that occur between the L1 to L2 stages of temporal
177 development or whether *pqn-59* plays a more general role in antagonizing the activities of additional
178 miRNAs that function later in the heterochronic pathway. We depleted *pqn-59* expression in two
179 strains that exhibit distinct, reiterative alterations in the post-embryonic cell lineage due to a reduction
180 in the activity of genetically separate sets of temporally regulated miRNAs. Animals containing the
181 *let-7(n2956)* allele of the *let-7* gene fail to properly down-regulate the expression of LIN-41 during late
182 larval development and as a consequence, animals reiterate the L4 patterns of hypodermal cell
183 divisions during adulthood [10, 11]. A single amino-acid substitution (S895F) in one of the two *C.*
184 *elegans* microRNA-induced silencing complex (miRISC) argonaute proteins, ALG-1, reduces the
185 efficacy of the three *let-7*-family miRNAs (mir-48, miR-241, miR-84) that are required to down-regulate
186 the expression of *hbl-1*, a transcription factor that is essential for L2-specific cell fates [12-14]. This
187 regulatory defect leads to an inappropriate reiteration of L2-stage, proliferative seam cell divisions
188 during the L3 stage [6, 15]. Both *let-7(n2853)* and *alg-1(ma192)* animals fail to express *col-19::GFP*
189 after the L4-to-adult molt (Figure 2 and Table 1). Depletion of *pqn-59* suppresses these phenotypes
190 indicating that *pqn-59* functions at multiple stages of post-embryonic development to control lateral
191 seam cell fate specification (Figure 2A and B). Examination of the adult cuticles and the numbers of
192 lateral seam of *alg-1(ma192); pqn-59(RNAi)* animals indicated that reducing *pqn-59* activity during
193 development can 1) restore the ability of these mutant animals to generate adult-specific alae and 2)
194 limit the inappropriate reiteration of L2-stage seam cell division programs that leads to excessive
195 proliferation of skin stem cells (Figure 2C).

196 *alg-1(ma192)* mutants also exhibit a synthetic phenotype with loss-of function mutations in the
197 *C. elegans* HNF-3/fork head ortholog, *lin-31*, during vulval development. LIN-31 functions
198 downstream of Ras/LET-60 signaling in vulval cell fate specification and loss-of-function alleles of *lin-*
199 *31* (e.g., *lin-31(n1052)*) result in ectopic vulval induction of additional vulval precursor cells and a
200 multi-vulval phenotype (Figure 2E and F) [16]. While *alg-1(ma192)* mutants generate a single vulval
201 structure that bursts with high penetrance at the L4-to-adult molt, *lin-31(n1053); alg-1(ma192)*
202 mutants exhibit a fully penetrant, synthetic vulvaless phenotype presumably due to alterations in the
203 timing of vulval cell fate specification (Figure 2) [6, 15]. We found that, in addition to suppressing the
204 reiterative seam cell phenotypes of *alg-1(ma192)* animals, *pqn-59* depletion almost completely
205 suppressed the vulvaless phenotypes of *lin-31(n1053); alg-1(ma192)* double mutants (Figure 2E and
206 F). This indicates that *pqn-59* also functions in the cell fate specification of vulval precursor cells.

207
208 ***pqn-59* encodes an essential protein that is localized in the cytoplasm throughout**
209 **development**

210 The *pqn-59* gene is located on the left arm of chromosome I and encodes a predicted 714
211 amino acid protein with a N-terminal ubiquitin-associated (UBA) domain implicated structurally in
212 various protein-protein interactions and in binding both ubiquitin and polyubiquitin chains (Figure 3A)
213 [17]. Additional analysis of PQN-59 protein structure indicates that it also harbors two separate
214 additional unstructured regions. Immediately after the N-terminal UBA-like domain, PQN-59 harbors
215 a stretch of arginine/glycine-rich sequences (RGG/RG) that are typically associated with RNA binding
216 proteins (Figure 3B) [18]. The C-terminal portion of PQN-59 harbors three “prion-like” domains
217 composed of amino acid stretches disproportionately enriched for glutamine and asparagine residues
218 (prion-like, Q and N, *pqn-*) [19] (Figure 3B). A comparison of PQN-59 sequence to the proteomes of
219 other model systems indicate that PQN-59 is evolutionarily related to a single *Drosophila* protein
220 called Lingerer (Lig) and two human proteins named UBAP2 and UBAP2L (Figure 4C). Lig functions
221 as a growth suppressor that associates with several RNA-binding proteins implicated in the regulation

222 of protein translation (including Rasputin(Rin)/G3BP1 a RasGAP SH3 binding protein (RasGAP-BP),
223 Caprin(Capr), and FMR1 an ortholog of the Fragile X mental retardation protein 1) [20]. Human
224 UBAP2 and UBAP2L proteins have been demonstrated to promote translation [21] and are implicated
225 in the formation of stress granules, liquid-liquid phase separating RNA-dependent condensates that
226 form in the cytoplasm in a variety of adverse cellular conditions [22-24].

227 To determine how PQN-59 activity contributes to normal animal development, we obtained a
228 deletion allele of the *pqn-59* gene, *tm2960*, that removes 420nt of the *pqn-59* locus (Figure 3A). This
229 lesion deletes the fifth exon of *pqn-59*; creating a premature stop codon upstream of the regions of
230 the gene coding for the “prion-like” domains. This mutation suppresses *col-19::GFP* mis-expression
231 phenotypes in *lin-4(ma161)* animals *pqn-59(tm2960); lin-4(ma161)* (60% adult n = 40) (Figure S1).
232 Western blots from homozygous *pqn-59(tm2960)* animals suggest that this mutation is a null allele of
233 *pqn-59* that generates no detectible PQN-59 protein and protein products of the predicted size for
234 *PQN-59(tm2960)* are not seen in *pqn-59(0); csh1s38[PQN-59::GFP]* (Figure S1). Homozygous *pqn-*
235 *59(tm2650)* animals that segregate from a balancer strain develop very slowly and exhibit a severe
236 reduction in brood size (Figures 3D and E). The reduction in fecundity includes a reduced capacity to
237 fertilize oocytes as well as a reduction in embryonic and early larval viability. Surprisingly, we did not
238 observe any appreciable alterations in post-embryonic cell lineage in *pqn-59* mutants or precocious
239 expression of adult-specific transcriptional reporters (Table I). The slow growth, sterility and larval
240 lethality phenotypes of *pqn-59(0)* mutations can be rescued with a single copy *PQN-59::GFP*
241 translational fusion targeted to chromosome II but not by related transgenes that encode *PQN-*
242 *59::GFP* alleles that lack the UBA-like or “prion-like” domains suggesting that both of these domains
243 are required for PQN-59 functions (Figure 3E). While animals harboring *PQN-59(ΔUBA)::GFP* or
244 *PQN-59(ΔPrD1-3)::GFP* transgenes as the sole *pqn-59* gene failed to exhibit normal brood sizes,
245 they exhibited no alterations in adult-specific alae formation (Table S1)

246 Proteomic analysis of whole worm lysates indicate that PQN-59 is a relatively abundant
247 protein ranking in the top 5% of proteins expressed in embryos and larva (Figure 3F) [25]. In order to

248 examine PQN-59 expression, we generated single-copy, C-terminal GFP-tagged version of *pqn-59*
249 at the endogenous locus. Animals expressing this allele exhibited wild-type development and
250 fecundity. Examination of PQN-59::GFP indicates that it is expressed throughout development and
251 in all somatic and germline cells (Figure 3 G and H) (see below). At the subcellular level, PQN-
252 59::GFP is localized exclusively in the cytoplasm with a marbled distribution. We also noted that,
253 during post-embryonic development, there is a transient increase in PQN-59 expression in all somatic
254 blast cells during and immediately after cell divisions and that PQN-59::GFP expression is maintained
255 throughout adulthood.

256

257 **The “prion-like” domains of PQN-59 exhibit LLPS properties**

258 Aside from the structured amino terminal UBA-like domain, a majority of the PQN-59 protein
259 is predicted to contain intrinsically disordered regions (IDRs) and share features with IDRs of several
260 proteins known to facilitate protein condensate formation (Figure 4A) [26-28]. Specifically, the
261 carboxy-terminal ~300 amino acids of PQN-59 are predicted to harbor three “prion-like” domains
262 (PLDs) that are characterized by stretches of amino acids that are disproportionately enriched in
263 glutamine (Q) and asparagine (N) residues when compared to most proteins in the *C. elegans*
264 proteome (Figure 4A and S3) [19]. “Prion-like” domain containing proteins have also been
265 demonstrated to exhibit natural “amyloid-like” properties which enable them to form fibril structures *in*
266 *vivo* [29]. As a consequence, proteins harboring these IDR and or “PrD-like” domains exhibit a number
267 of interesting biochemical properties including the ability to be co-precipitated by biotinylated-
268 isoxazole (b-isox), which forms crystals in a temperature-dependent manner in aqueous solution and
269 co-precipitates diverse proteins harboring low complexity domains (LCDs) from cell lysates [30, 31].
270 We tested if PQN-59 is precipitated by b-isox by using two approaches. First, we demonstrated that
271 b-isox precipitates PQN-59 from whole worm lysates (Figure 4B). Because PQN-59 could be co-
272 precipitated with other endogenous *C. elegans* proteins that are directly precipitated by the b-isox
273 compound, we purified a fragment of PQN-59 that includes the “prion-like” domains as a GFP fusion

274 protein and demonstrated that these domains are also precipitated by b-isox compound (Figure 4C).
275 The b-isox does not precipitate soluble GFP [31] or proteins from whole-worm extracts that cross
276 react with anti-PQN-59 antibodies or the minor *E. coli* proteins that co-purify with GFP-PQN-59(PrD1-
277 3) indicating the specificity of b-isox for proteins harboring LCD and PrDs.

278 .
279 To determine if the “prion-like” domains of PQN-59 also exhibit LLPS properties, purified
280 solutions of recombinant GFP-PQN-59(PrD1-3) were mixed with Ficoll or dextran sulfate. When these
281 crowding reagents were added, the solution became opaque and turbulent with an increased A_{600}
282 absorbance (Figure 4D and E). When these samples were examined with fluorescence and
283 differential interference contrast (DIC) imaging, GFP-PQN-59(PrD1-3) solutions containing
284 concentrating reagents exhibit micron-sized spherical droplets that freely move in solution and wet
285 the surface of the glass coverslips (Figure 4F). Previous studies have used the aliphatic alcohol 1,6-
286 hexandiol to probe the material properties of proteins that exhibit LLPS [32]. 1,6-hexandiol is thought
287 to disrupt the weak hydrophobic protein-protein interactions that are required for the formation and
288 stabilization of protein condensates [33, 34]. Incubation of GFP-PQN-59(PrD1-3) condensates with
289 1,6-hexandiol dramatically lowers the turbidity and absorbance of GFP-PQN-59(PrD1-3) solutions
290 and eliminates visible condensate formation in microscopy samples (Figure 4D-F). To fully
291 demonstrate that GFP-PQN-59(PrD1-3) condensates exhibit liquid-liquid-like dynamic properties we
292 performed fluorescence recovery after photobleaching (FRAP) experiments. As demonstrated in
293 Figure 4G, GFP-PQN-59(PrD1-3) condensates formed in 10% dextran sulfate solution rapidly recover
294 fluorescence when a portion of the condensate is bleached ($n > 10$). This rapid recovery was also seen
295 in condensates formed with 150mg/mL Ficoll ($n = 20$) (Movie S1) indicating that GFP-PQN-59(PrD1-
296 3) forms phase-separated liquid droplets *in vitro* (Figure 4G). Interestingly, prolonged incubation of
297 these condensates (> 1 week) at 4°C leads to the formation of a hydrogel-like material that was
298 incapable of being re-solubilized at room temperature in aqueous buffers (Figure 4H).

299

300 **Under normal growth conditions, PQN-59::GFP exhibits reduced mobility in the cytoplasm and**
301 **this feature requires the “prion-like” domains**

302 We next sought to examine the *in vivo* properties of PQN-59::GFP by comparing its diffusibility
303 to that of a soluble, monomeric GFP. To accomplish this, we performed FRAP experiments using a
304 strain harboring a single copy PQN-59::GFP (*csh1s38*) or a transgene driving soluble GFP driven from
305 the *glh-1* promoter. We focused on measuring fluorescence recovery in developing oocytes where
306 both proteins are localized in large and accessible cytoplasmic compartment (Figure 4I). As would be
307 expected for a small, soluble protein, photobleached regions rapidly recover fluorescence signal in
308 strains harboring soluble, monomeric GFP (Figure 4I). In contrast, photobleached cytoplasmic
309 regions in oocytes expressing PQN-59::GFP recover fluorescence exceptionally slowly suggesting
310 that PQN-59::GFP normally exhibits a limited diffusibility *in vivo*. We also performed FRAP on PQN-
311 59::GFP in hypodermal cells and found that recovery was also slower than that observed for similar
312 experiments with soluble GFP (Figure S2). We then sought to determine if the “prion-like” domains
313 contributed to this feature. We integrated a PQN-59::GFP transgene that lacked the three PrDs,
314 *csh1s78[pqn-59(Δ PrD1-3)::GFP]*, at the same site where we targeted full length PQN-59::GFP above.
315 This fluorescent reporter recovered faster than the full-length PQN-59::GFP reporter indicating that
316 the PrDs of PQN-59 contribute to its reduced diffusibility in the cytoplasm (Figure 4I).

317
318 **PQN-59 exhibits LLPS *in vivo* and is required for efficient stress granule formation in**
319 **developing oocytes.**

320 When cells are exposed to a variety of averse conditions, eukaryotic cells respond by
321 dramatically reducing protein translation and re-distributing the localization of mRNAs and many
322 mRNA-binding proteins [35]. Many stresses, including heat and chemicals, induce the formation of
323 ribonucleoprotein complexes, called stress granules, that are formed from pools of untranslated
324 mRNPs. These granules are dynamic, require a number of core proteins for formation and, when
325 formed, show liquid-like behaviors [35]. The human orthologs of PQN-59, UBAP2 and UBAP2L, share

326 a similar domain structure, are predicted to harbor “prion-like” and IDR domains (Figure S3) and have
327 been demonstrated to localize to and function in the formation of stress granules in a variety of averse
328 cellular conditions [22, 23, 36]. To determine if PQN-59 also functions in stress granule formation, we
329 subjected animals expressing PQN-59::GFP to heat stress and examined PQN-59::GFP localization
330 in developing oocytes. Heat stress (33°C) lead to the robust redistribution of PQN-59::GFP from its
331 normal marbled, cytoplasmic localization to a large number of cytoplasmic puncta that vary in size
332 from approximately 0.25-3.5µM (Figure 5A). To determine if the puncta observed in heat shocked
333 oocytes were indeed stress granules, we also imaged the localization of the core stress granule
334 component and sole G3BP1/2 ortholog in *C. elegans*, GTBP-1, that directly interacts with PQN-59
335 [37]. Under these conditions, PQN-59::GFP localization completely overlapped with the localization
336 of GTBP-1::tagRED indicating that PQN-59 is a component of these structures during stress (Figure
337 5A). This same PQN-59::GFP transgene was localized to similar granules by a variety of stress
338 treatments including arsenite treatment [37]. We used FRAP analysis to demonstrate that the PQN-
339 59::GFP condensates that are induced by heat treatment exhibit LLPS properties as these
340 cytoplasmic structures regain fluorescence rapidly after bleaching. As demonstrated in Figure 5B,
341 photobleached PQN-59::GFP granules rapidly recovered fluorescence at a rate that is much faster
342 than the recovery of bleached regions of PQN-59::GFP in normal growth conditions.

343 We next sought to determine what are the genetic and structural requirements of PQN-59 and
344 GTBP-1 that are required for the localization of these proteins to heat-induced stress granules. First,
345 we compared the ability of wild-type PQN-59::GFP to localize to stress granules to the dynamics of a
346 version of PQN-59::GFP that lacked the “prion-like” domains. These experiments revealed that PQN-
347 59(ΔPrD1-3)::GFP failed to localize to stress granules suggesting that the prion-like domains we had
348 previously characterized as exhibiting LLPS properties *in vitro* are required for the LLPS properties
349 of the full-length protein *in vivo* during stress granules formation (Figure 5C). In other systems, G3BP
350 proteins are important for the assembly and stabilization of stress granules during heat stress [36,
351 38]. It is thought that G3BP1 in human cells is a central node of the RNA-protein network that triggers

352 the formation of stress granules and this property is directly regulated by RNA binding [39, 40]. We
353 tested whether PQN-59::GFP could be localized to stress granules in the absence of GTBP-1
354 expression by performing heat shock experiments in *gtbp-1(0)* animals. Animals lacking *gtbp-1*
355 express normal levels of PQN-59::GFP and PQN-59::GFP localizes to stress granules upon heat
356 treatment (Figure 5C). Since a significant portion of PQN-59::GFP is localized to stress granules in
357 the developing oocytes of *gtbp-1(ax2069)* animals, we then asked if GTBP-1::tagRED association
358 with stress granules requires PQN-59 activity. These experiments revealed that GTBP-1::tagRED
359 localization to stress granules is dramatically reduced in *pqn-59(0)* animals (Figure 5D) indicating that
360 PQN-59 is required for the efficient localization of GTBP-1::tagRED to these membranless organelles.

361 Given the close association between PQN-59 and GTBP-1 and the importance of these
362 proteins in stress granule formation, we tested whether *gtbp-1* (or other stress granule components)
363 may also function in regulating temporal patterning during larval development. First we monitored
364 adult specific alae formation in *gtbp-1(0)* mutants and found these cuticular structures were expressed
365 as in wild-type animals (n = 32). We further tested whether the *gtbp-1(0)* mutation could suppress the
366 reiterative heterochronic phenotypes in *lin-4(ma161)* mutants. Compared to the substantial
367 suppression by *pqn-59(tm2960)*, combining *gtbp-1(0)* mutations with *lin-4(ma161)* had almost no
368 detectible suppression of any alae phenotypes (adult-specific alae: *lin-4(ma161)* = 0 % alae (n = 40);
369 *lin-4(ma161); gtbp-1(ax2068)* = 5% alae (n = 38); *pqn-59(tm2960); lin-4(ma161)* (45% adult n = 40).
370 Depletion of two other stress granule components, *tiar-1* and *tiar-2* [41-43], by RNAi (n > 30 each)
371 also failed to suppress heterochronic phenotypes in *lin-4(ma161)* mutants suggesting that proper
372 stress granule formation is distinct from roles in controlling temporal patterning.

373
374 **PQN-59 and UBAP2L interact with similar cellular components that are associated with stress**
375 **granule formation, translation and post-transcriptional gene regulation**

376 To gain insight in to the developmental function of PQN-59 in gene regulation, we sought to
377 identify additional proteins that PQN-59 may function with to control developmental gene expression.

378 To accomplish this, we immunoprecipitated PQN-59 from embryos using anti-PQN-59 antibodies and
379 identified associated proteins via mass spectroscopy. Triplicate experiments identified 304 proteins
380 that were reproducibly precipitated with PQN-59 antisera (Q value <0.5) (Table S2). We then
381 performed gene ontology (GO) term enrichment analysis of PQN-59 co-precipitated proteins [44].
382 This analysis indicates that PQN-59 complexes with diverse sets of protein complexes including those
383 enriched in RNA binding (mRNA and rRNA), protein translation (ribosomal proteins, translational
384 initiation, elongation and regulatory factors), transcription (Direct DNA binding factors and co-
385 activators), and transport (nuclear pore complex, motor proteins, microtubule binding) (Figure 6A). In
386 addition to core complexes involved in translation, PQN-59 interacts with ALG-1, a core miRISC
387 component whose mutation elicits phenotypes that are suppressed by *pqn-59(RNAi)* (Figure 2), and
388 GTBP-1 which colocalizes with PQN-59 in stress granules (Figure 5).

389 The structural relationships between PQN-59 and human UBAP2 and UBAP2L (and Lingerer)
390 (Figure S3) as well as their relationship to stress granule formation suggests that these proteins may
391 function in an orthologous manner to regulate gene expression and the formation of stress induced
392 RNA condensates. To determine if PQN-59 and UBAP2L interact with similar types of cellular
393 components, we compared our list of PQN-59 interacting proteins to the list of proteins that were
394 found to physically interact with UBAP2L as measured by BioID assays [24]. This molecular approach
395 utilizes a promiscuous biotin ligase fused to a protein of interest to covalently tag proximally
396 associated proteins *in vivo* [45]. When this approach was used to identify proteins that function near
397 UBAP2L *in vivo*, a total of 830 interacting proteins were identified above background [24]. To compare
398 these two lists, we employed the Ortholist server to identify orthologous pairs of human and *C.*
399 *elegans* proteins from these lists (Table S3 and S4) [46, 47]. In addition to extensive, shared
400 interactions between PQN-59 and UBAP2L with ribosomal subunits (Large Ribosomal subunits 2-7,
401 9-17, 18-22, 24-26, 28, 30, 33 and Small Ribosomal Subunits 0-3, 7-13, 17, 20, 23, 24, 27, 30), these
402 efforts identified 127 orthologous pairs of proteins (Figure 6B); indicating that PQN-59 and UBAP2L
403 are associated with overlapping complexes *in vivo*. We also noted that a large number of PQN-59

404 interacting proteins that do not share orthologous interactions with UBAP2L are enriched in GO terms
405 associated with germline- and early embryonic-specific functions; proteins that would normally
406 perform tissues specific functions not employed in human cell lines that are derived from somatic
407 sources (Figure S4).

408 In order to both visualize PQN-59 interacting proteins into functional groups, we took further
409 advantage of the large-scale application of the BioID proteomic approach outlined by Youn et al. that
410 enabled an ultrastructural view of protein-protein interactions to be organized according to functionally
411 and spatially related RNP-associated complexes (Figure 6C) [24]. When we overlaid the 127 PQN-
412 59 interacting proteins that share orthologous protein-protein interactions with UBAP2 onto this map,
413 we found that that a majority of proteins that are co-precipitated with PQN-59 are associated with
414 other proteins that are found in two types of cytoplasmic condensates: stress granules and P-bodies
415 (Figure 6C). While micron-sized stress granules are typically only visible during averse cellular
416 conditions, multiple lines of evidence indicate that the protein-protein interactions that compose these
417 bodies pre-exists in smaller forms during non-stress conditions [23, 24]. Furthermore, core miRISC
418 components are functionally associated with both p-bodies and stress granules where they are
419 thought to function in post-transcriptional regulation of miRNA target mRNAs [48].

420
421 **Depletion of *pqn-59* via RNAi alters the steady state levels of several miRNAs involved in**
422 **temporal patterning**

423 One mechanism by which *pqn-59* depletion could suppress the loss-of-function phenotypes
424 associated with reduced miRNA activity would be to alter miRNA metabolism in a manner whereby
425 the expression levels of these regulatory molecules are increased when PQN-59 expression is
426 reduced. To test this hypothesis, we measured the levels of multiple miRNAs in animals that were
427 exposed to control or *pqn-59* dsRNAs and extracted total RNA from late L4-staged animals. We then
428 examined both miRNA processing and steady state levels using quantitative northern blots. As shown
429 in Figure 7A, RNAi-mediated depletion of *pqn-59* increases the relative abundance of multiple, fully-

430 processed miRNAs including those that regulate temporal patterning (*lin-4* and *let-7*). In addition, *pqn-*
431 *59* depletion altered the levels of other mature miRNA species that are expressed in distinct tissues
432 and temporal expression patterns [49]. Increases in mature miRNA levels during *C. elegans*
433 development have been demonstrated to arise by elevated expression of miRNAs at the
434 transcriptional level [6, 50] or by dampening mature miRNA turnover [51, 52]. If *pqn-59(RNAi)* alters
435 mature miRNA levels by increasing the transcription of affected miRNA genes without improving pre-
436 miRNA processing rates, we would expect pre-miRNA levels to increase in the absence of PQN-59
437 expression. A comparison of the pre-miRNA levels in control and *pqn-59(RNAi)* conditions indicate
438 that pre-miRNA levels are not generally increased in the absence of PQN-59 (Figure 7A). Because
439 pre-miRNAs for many miRNAs are efficiently processed in wild-type animals (precluding their relative
440 accumulation), we also measured how depleting *pqn-59* expression alters pre-miRNA and miRNA
441 levels in animals that harbor null mutations in *alg-1*, encoding one of the two *C. elegans* miRNA-
442 specific argonaute proteins required for normal processing and stabilization of mature miRNAs [53].
443 Animals lacking ALG-1 expression, *alg-1(gk214)*, exhibit mild heterochronic phenotypes and
444 accumulate the precursor mRNAs for a number of miRNA genes (Figure 7A) [53, 54]. Depletion of
445 *pqn-59* in *alg-1(gk214)* animals suppresses the previously described and relatively weak
446 heterochronic phenotypes (30% gapped alae in control RNAi conditions and 0% in animals exposed
447 to *pqn-59* dsRNAs). As demonstrated in Figure 7A, depletion of *pqn-59* in *alg-1(gk214)* animals leads
448 to an increase in mature miRNA species for a number of miRNAs without altering the levels of pre-
449 miRNAs. Changes in mature miRNA levels in *alg-1(gk214); pqn-59(RNAi)* animals were validated
450 using TaqMan assays of independent RNA samples (Figure S5A). Changes in mature miRNA levels
451 for these miRNAs are not caused by an increase in the levels of two of the main miRISC components,
452 ALG-1 and AIN-1, as levels of these proteins are not altered by PQN-59 depletion (Figure S5)B.
453 These combined results suggest that mature miRNAs are stabilized in the absence of PQN-59 and
454 this increase may enable specific miRNA targets to be more efficiently regulated in these conditions.
455

457 DISCUSSION

458 Bistability is a recurrent motif in developmental biology whereby distinct cell fates, defined by
459 coherent patterns of underlying gene expression, can be switched by the activity of a key regulatory
460 molecule within an established GRN. A fundamental property of bistable gene regulatory networks
461 centers around changes in the temporal expression levels of specific, regulatory factors and the
462 control of these levels around a critical threshold. Above this critical threshold, one cell fate is stable
463 and below it, a distinct cell fate or expression program is enforced. In this manuscript we characterize
464 the *C. elegans pqn-59* gene, encoding a conserved RNA-associated protein, as a component that
465 functions to assure that cell fate specification events mediated by miRNAs during post-embryonic
466 development exhibit bistability. We identified *pqn-59* in a reverse genetic screen aimed to identify
467 suppressors of the severe temporal patterning phenotypes associated with hypomorphic alleles of
468 two heterochronic miRNAs and a unique allele of an essential miRISC component ALG-1. While *pqn-*
469 *59* depletion via RNAi can efficiently suppress these unique alleles, we also provide genetic evidence
470 that *pqn-59* is not a bypass suppressor of *lin-4* and *let-7* (i.e., depletion offers little or no suppression
471 of phenotypes associated with null alleles of these genes); indicating that PQN-59 normally functions
472 as a modulator of miRNA activity. Consistent with this interpretation, treatment of wild-type animals
473 with dsRNAs against *pqn-59* does not elicit detectible heterochronic phenotypes.

474 In order to illuminate mechanisms by which PQN-59 may function to modulate heterochronic
475 gene expression, we analyzed phenotypes associated with a deletion allele of *pqn-59* gene and also
476 characterized the expression and molecular features of PQN-59 *in vivo* and *in vitro*. Several lines of
477 evidence suggest that *pqn-59* activities are not limited to regulating features of postembryonic
478 temporal patterning and likely reflect a general role for this protein in development. First, deletion of
479 the *pqn-59* gene results in pleiotropic phenotypes including an increase in the length of larval
480 development and a dramatic reduction in fecundity. Furthermore, in contrast to the dynamic
481 expression patterns of other heterochronic genes, PQN-59 is a very abundant and ubiquitously
482 expressed protein. Third, depletion of *pqn-59* leads to the relative stabilization of several mature

483 miRNAs species and does so without dramatically altering the levels of ~70-nt pre-miRNA precursors.
484 Because miRNAs are predicted to regulate the expression of a large fraction of expressed mRNAs
485 [55, 56], inactivation of *pqn-59* may disrupt large portions of gene expression of which some, for
486 example the heterochronic pathway, are exquisitely sensitive to perturbation.

487 The human ortholog of PQN-59, UBAP2L, is an established RNA-binding protein implicated
488 in multiple biological processes. Large scale proteomic analysis of UBAP2L-associated proteins
489 indicates that UBAP2L is highly integrated with other RNA-binding protein complexes implicated in
490 stress granule formation and p-bodies, two membraneless cytoplasmic organelles formed through
491 LLPS of RNAs and a multitude of RNA-binding proteins [23, 24]. UBAP2L expression is required for
492 the formation of stress granules in adverse conditions [22-24]. Both p-bodies and stress granules are
493 highly integrated with RNA-binding proteins that play direct roles in controlling gene expression and
494 RNA turnover; including proteins directly involved in miRNA activity (e.g., miRISC components) [48].
495 Furthermore, UBAP2L appears to be directly associated with translating ribosomes and can be
496 crosslinked to ribosomal RNAs and the coding sequences of hundreds of mRNAs; indicating that it
497 may play a direct role in controlling translation [21]. Consistent with this hypothesis, artificially
498 tethering UBAP2L to target mRNAs stimulates translation and depletion of UBAP2L results in a
499 decrease in overall translational rates and a reduction in the levels of polysomes [21].

500 While the level of sequence identity between PQN-59 and UBAP2L is limited (18.6% Identity,
501 28.0% Similarity (EBLOSUM62)) (Figure S3), several lines of evidence suggest that these proteins
502 perform orthologous functions. First, PQN-59, like UBAP2L, is predicted to lack overall strong
503 secondary structure and is precipitated by biotinylated-isoxazole, a characteristic of proteins that
504 harbor exhibit LLPS features *in vivo* [31]. We also demonstrate that the prion-like domains of PQN-
505 59 themselves exhibit LLPS properties *in vitro* and that during heat stress, PQN-59 localizes to stress
506 granules and exhibits LLPS properties *in vivo*. Structure-function analysis of PQN-59 domains
507 indicate that the prion-like sequences in the C-terminus of PQN-59 are required for stress granule
508 localization. Second, like UBAP2L, PQN-59 expression is required for the efficient recruitment of

509 GTBP-1, the sole *C. elegans* G3BP ortholog, to stress granules [23, 24, 36]. Third, condensation of
510 PQN-59 into stress granules is not dependent on GTBP-1; further mirroring the epistatic relationship
511 between these proteins in human cells [22]. Finally, biochemical characterization of PQN-59
512 associated proteins indicate that UBAP2L and PQN-59 are physically associated with similar protein
513 complexes in vivo; suggesting that they both may integrate aspects of mRNA metabolism, regulation
514 and expression through these interactions.

515 Our genetic characterization of *pqn-59* during *C. elegans* development and the potential
516 conservation of functions between PQN-59 and UBAP2L enable us to propose at least two non-
517 mutually exclusive models for the function of PQN-59 in temporal patterning. A cornerstone of miRNA
518 function in the heterochronic pathway centers on the rapid downregulation of their target mRNAs
519 below a critical threshold (Figure 7B). In contrast to what happens in wild-type animals, animals
520 lacking full activity of heterochronic miRNAs (as exemplified for *lin-4(ma161)* mutants) are unable to
521 dampen the expression of their target mRNA below critical threshold required for the bistable
522 transition in temporal cell fate (Figure 7B). As a consequence, *lin-4(ma161)* animals exhibit
523 heterochronic phenotypes that are indistinguishable from those in animals that completely lack *lin-4*
524 (as exemplified for *lin-4(e912)* mutants) [6]. As demonstrated in Figure 7A, *pqn-59* depletion results
525 in the stabilization of many mature miRNAs. We hypothesize that the potentially generalized
526 stabilization of mature miRNAs elicited by depleting *pqn-59* expression may enable the levels of
527 critical miRNAs to increase to a level where they can now effectively dampen *lin-14* expression
528 (Figure 7C model 1). In addition to this potential mechanism, PQN-59, like UBAP2L, may promote
529 general protein translation. In this context, depletion of *pqn-59* reduces the normal expression levels
530 of miRNA target genes (like *lin-14*) to a level that is closer to the threshold that defines the bistable
531 switch between cell fate specification (Figure 7C model 2). Therefore, *pqn-59* may function to
532 normally assure that this bistable switch that defines the L1 to L2 transition in *C. elegans* larval
533 development is not inappropriately crossed unless a sufficient miRNA is expressed. Both of these
534 models are consistent with *pqn-59* functioning outside of the normal heterochronic GRN and

535 furthermore explain the observation that *pqn-59(RNAi)* is an efficacious suppressor of multiple miRNA
536 loss-of-function phenotypes but that *pqn-59* depletion is incapable of bypassing the activities of these
537 genes.

538 Overall, our study demonstrates that *pqn-59* functions to modulate gene expression and cell
539 fate specification during *C. elegans* development. Prior studies have indicated that UBAP2L and its
540 functions with other stress granule and RNA-binding protein partners may play a complex role in a
541 variety of human diseases. These include a role for UBAP2L overexpression in various types of
542 cancer [57-62] and in pathologies that are related to protein aggregation in neurodegeneration [63-
543 66]. We imagine that the genetic and experimental tractability of the *C. elegans* model will be
544 instrumental in discovering the underlying mechanisms by which this conserved family of proteins
545 functions.

546

547 **FIGURE LEGENDS**

548 **Figure 1. *pqn-59*(RNAi) suppresses reiterative heterochronic phenotypes associated with**
549 **reduced *lin-4*-mediated repression of *lin-14*. (A)** Genetic screen used to identify suppressors of
550 *lin-4*(*ma161*) heterochronic phenotypes. Wild-type animals express the adult-specific *col-19::GFP*
551 transcriptional reporter at the end of the L4 molt and throughout adulthood. In contrast, *lin-4*(*ma161*)
552 animals fail to express *col-19::GFP* and are unable to lay eggs due to the inappropriate reiteration of
553 L1-specific developmental programs. Depletion of *pqn-59* by RNAi suppresses both *lin-4*(*ma161*)
554 phenotypes. **(B)** Pictographs of adult wild-type and *lin-4*(*ma161*) animals exposed to control or *pqn-*
555 *59* dsRNAs. See Table 1 for details. **(C and D)** Pictographs depicting the comparison of adult-specific
556 alae and vulval structures of wild-type, *lin-4*(*ma161*), and *lin-4*(*e912*) animals exposed to control or
557 *pqn-59* dsRNAs. In panel C, solid line indicates continuous adult alae while a dashed line depicts the
558 regions of the cuticle that lack these cuticular structures. In panel D, asterisks (*) indicate the location
559 of a normal vulval structures in RNAi treated animals at the L4 to adult molt. **(E)** Quantification of the
560 wild-type, protruding vulva (*pvl*), and vulvaless (*vul*) phenotypes of the indicated animals treated with
561 control or *pqn-59* dsRNAs. **(F)** Images depicting the temporal expression patterns of an endogenous,
562 GFP-tagged allele of the protein product of the major *lin-4* target, LIN-14. **(G)** Diagram indicating the
563 genetic lesions that alter the *lin-14* 3' UTR regulatory sequences. Black bars indicate the approximate
564 locations of the complementary *lin-4* binding sites in the *lin-14* 3'UTR. **(H)** Quantification of the levels
565 of suppression mediated by depleting *pqn-59* during the development of animals the express one of
566 two gain-of-function alleles of *lin-14*. Animals were scored positive if any visible alae structures were
567 present on the adult-stage cuticle.

568
569 **Table 1 Measurement of RNAi-mediated suppression of temporal patterning phenotypes of**
570 **various heterochronic mutants.** This table contains the quantification of alae and *col-19::GFP*
571 expression phenotypes of various heterochronic mutants with and without *pqn-59*(RNAi).

572 **Figure 2. *pqn-59(RNAi)* suppresses additional miRNA-dependent reiterative heterochronic**
573 **phenotypes. (A and B)** Depletion of *pqn-59* via RNAi suppresses the *col-19::GFP* phenotypes of *let-*
574 *7* hypomorphic alleles (*let-7(n2853)*) and antimorphic alleles of genes that encode core components
575 of miRISC (e.g., *ALG-1*, *alg-1(ma192)*). See Table 1 for details on expressivity and penetrance of
576 suppression. **(C)** *alg-1(ma192)* mutants reiterate the L2-stage or larval development one additional
577 time and as a consequence, over-proliferate lateral seam cells and also fail to form alae structures
578 on the adult cuticle [6]. These *alg-1(ma192)*-dependent cell lineage phenotypes and defects in
579 generating adult-specific alae are suppressed by depleting PQN-59 expression using dsRNAs
580 against the *pqn-59* gene. Continuous yellow lines indicate regions of the cuticle where adult alae are
581 present. Dashed lines indicate regions of the cuticle where alae are abnormally absent after the L4
582 molt. For images depicting the hypodermal cells of adult animals of indicated genotypes, arrows
583 indicate the location of lateral seam cells. **(D)** Proposed seam cell lineages of wild-type, *let-7(n2853)*
584 and *alg-1(ma192)* animals after treatment with control or *pqn-59* dsRNAs. **(E and F)** *alg-1(ma192)*
585 animals exhibit a 100% penetrant synthetic vulvaless phenotype when combined with *lin-31* loss-of-
586 function alleles, *lin-31(n1053)* [6, 15]. Depletion of *pqn-59* in *lin-31(n1053); alg-1(ma192)* double
587 mutants completely suppresses these phenotypes to induce ectopic vulva in a *lin-31*-dependent
588 manner.

589
590 **Figure 3. The *pqn-59* gene encodes an abundant, ubiquitously-expressed protein that is**
591 **essential for normal fecundity. (A and B)** Diagrams of the *pqn-59* gene and protein product. In
592 panel A, the location of the *tm2960* deletion and the site of CRISPR-mediated GFP insertion that
593 generates a functionally tagged allele of *pqn-59*. Panel B indicates the three discernable protein
594 domains of PQN-59. **(C)** Evolutionary relationship between PQN-59 and orthologs in other systems.
595 **(D)** Images of 24hr, adult wild-type and *pqn-59(tm2960)* animals demonstrating the differences in
596 fertility. Arrowheads indicate developing oocytes and arrows indicate fertilized embryos. Dashed lines
597 indicate regions of the germline where sperm are present. **(E)** Graph depicting the range of offspring

598 with wild-type, *pqn-59(tm2960)* and *pqn-59(tm2960)* animals that express a functional, full-length
599 PQN-59::GFP transgene (including upstream and downstream regulatory regions) targeted to
600 chromosome II or variants of this transgene that lack the UBA or the three C-terminal prion-like
601 domains. **(F)** Proteomic data from Version 4.0 of PaxDb: Protein abundance data indicating that PQN-
602 59 is a very abundant protein. **(G)** Localization of the endogenously GFP-tagged allele of PQN-59 in
603 lateral seam cells demonstrating that PQN-59 is a cytoplasmically localized. **(H)** Images of the same
604 PQN-59::GFP transgene demonstrating that PQN-59 is expressed in all cell types throughout
605 embryonic and post-embryonic development.

606
607 **Figure 4. The “prion-like” domains of PQN-59 exhibit unique biophysical properties *in vitro***
608 **and *in vivo*.** **(A)** A majority of the PQN-59 protein is predicted to be unfolded using the Predictors of
609 Natural Disorder Regions (PONDR VSL2) algorithm [67]. **(B and C)** Endogenous, full-length PQN-
610 59 in whole worm lysates and a GFP-tagged, C-terminal fragment of PQN-59 containing the three
611 “prion-like” domains (PrDs) can be differentially co-precipitated with biotinylated-isoxazole (b-isox).
612 **(D-F)** Solutions of purified GFP-PQN-59(PrD1-3) exhibit properties of LLPS when crowding reagents
613 are added. Phase separation of GFP-PQN-59(PrD1-3) and increase absorbance phase separated
614 samples are reversed upon addition of the aliphatic alcohol 1,6-hexandiol. **(G)** Phase separated
615 droplets of GFP-PQN-59(PrD1-3) rapidly recover when photobleached. Graph depicts the rate of
616 fluorescence recovery for GFP-PQN-59(PrD1-3) droplets that were formed in either dextran sulfate
617 or ficoll concentrating agents. Graphs depict the average recovery rate of the GFP-PQN-59(PrD1-3)
618 droplets in indicated concentrating agent (n = or greater than 10). Grey regions indicate standard
619 error of measurement (SEM). **(H)** Prolonged concentration of GFP-PQN-59(PrD1-3) droplets results
620 in the formation of a GFP-PQN-59(PrD1-3) hydrogel like material. **(I)** *In vivo*, full-length PQN-59::GFP
621 exhibits slower diffusion rates than soluble GFP or GFP-PQN-59 fusion proteins lacking the three
622 prion domains. These FRAP experiments were performed in developing oocytes. Graph indicates the
623 recovery rates for the three proteins. PQN-59::GFP also exhibited slow diffusion rates in hypodermal

624 cells (Figure S3). Quantification of the average recovery rates for each GFP protein depicted in I.
625 Grey regions indicate that standard error of measurements (SEM) for 10-15 photobleaching events
626 in separate animals.

627
628 **Figure 5. The “prion-like” domains of PQN-59 are essential for LLPS properties during heat**
629 **stress and PQN-59 is important for the efficient localization of GTBP-1 to stress granules. (A)**

630 In developing oocytes, PQN-59::GFP is localized to the cytoplasm. At elevated temperatures, PQN-
631 59::GFP re-distributes to cytoplasmically localized stress granules that co-localize with the core stress
632 granule component, GTBP-1::RFP. **(B)** FRAP analysis of heat stress-induced PQN-59::GFP indicate
633 that it is likely in a liquid-liquid phase separated state as PQN-59::GFP droplets recover fluorescence
634 very rapidly when compared to PQN-59::GFP recovery at normal physiological temperatures (Figure
635 5I). **(C)** Condensation of PQN-59::GFP in heat stress conditions does not require GTBP-1 expression
636 but does require the C-terminal “prion-like” domains of PQN-59. **(D)** GTBP-1 localization to stress
637 granules in heat-shocked oocytes is severely compromised in the absence of PQN-59.

638
639 **Figure 6. PQN-59 interacts with a variety of proteins involved in a variety of molecular**
640 **processes and many of these interactions are shared with UBAP2L, the human ortholog of**

641 **PQN-59. (A)** Molecular function GO terms enrichment of PQN-59 interacting proteins isolated from
642 developing embryos. **(B)** A Venn diagram indicating the overlap of orthologous proteins identified in
643 PQN-59 I.P.s and BioID experiments performed with UBAP2L in human cells outlined in Youn et al.
644 [24]. **(C)** The non-negative matrix factorization (NMF) network of protein complexes identified through
645 massively-parallel BioID assays of RNA binding proteins derived from Youn et al. (left) [24]. Each
646 group is organized by representative GO cellular compartment term as previously described [24]. The
647 right network illustrates the location of PQN-59-interacting proteins that have orthologs that map to
648 the indicated compartments.

649

650 **Figure 7. Depletion of *pqn-59* by RNAi increases the abundance of mature miRNAs. (A)** miRNA
651 northern analysis of total RNA extracted from staged, wild-type and *alg-1(0)*, *alg-1(gk214)*, mutant
652 animals. Animals were exposed to control or *pqn-59* dsRNAs from hatching to the late L4 stage of
653 larval development prior to RNA extraction. For calculation of relative expression of mature miRNA
654 species, each experimental condition was calibrated to the expression level of 20 μ g of total RNA
655 derived from wild-type animals exposed to control dsRNA. SL2 RNA levels were used as an input
656 control. Pre-miRNA to mature RNA was calculated relative to the ratio of the signal in indicated
657 sample. (B) A model of the generic bi-stable switch that incorporates a miRNA to control temporal
658 cell fates during development. Normally (left side), the miRNA target gene is expressed above a
659 critical threshold early in development and is responsible for enforcing earlier cell fate identities. At a
660 defined point in development, the induced expression and activity of a miRNA gene rapidly curtails
661 the target expression below a critical threshold enabling a clear change in cell fate at an important
662 developmental milestone (demarcated by vertical dashed line). In miRNA mutants that under-
663 accumulate functional miRNAs (e.g., *lin-4(ma161)*), the expression and activity of a mutant miRNA
664 lowers the expression of the target gene but not below the critical threshold to enable a change in the
665 bistable switch. (C) We propose two non-exclusively mutual mechanisms by which depletion of *pqn-*
666 *59* may suppress the defects associated with ineffective miRNA-mediated gene regulation in bistable
667 developmental switches. In Model 1, depletion of *pqn-59* may increase the stability of the mutant
668 miRNAs enabling the target gene to be efficiently dampened below the threshold at the
669 developmental milestone. This model is consistent with data shown in above in panel A. Alternatively
670 (Model 2), PQN-59 may function like UBAP2L and stimulate translation of some miRNA target
671 mRNAs. Depletion of *pqn-59* would lower the expression of the target gene independent of miRNA
672 expression. This reduced expression would nearer to the critical threshold needed to initiate a
673 bistable switch in cell fate. In this scenario, the residual activity of the mutant miRNA may now be
674 enough to reduce target mRNA expression further and now below the critical threshold; enabling cell
675 fate transformation in a bistable fashion.

677

678 **SUPPLEMENTAL FIGURES, TABLES and MOVIE LEGENDS**

679

680 **Figure S1. *pqn-59(tm2960)* phenocopies *pqn-59* RNAi in suppressing *lin-4(ma161)***
681 **heterochronic phenotypes and is likely a null allele. (A) *pqn-59(tm2960); lin-4(ma161)* animals**
682 **exhibit wild type *col-19::GFP* expression (and protruding vulva phenotype) while animals harboring a**
683 **single copy of the *pqn-59* deletion allele (balanced with an *hT2 myo-2::GFP* balancer) exhibit only a**
684 **very mild *col-19::GFP* expression phenotype and no vulval induction. (B) Western blots of wild-type,**
685 ***cshIs25*[PQN-59::*GFP* CRISPR allele at *pqn-59* locus], and *pqn-59(tm2960); cshIs38* [PQN-59::*GFP***
686 **single copy on Chromosome II] animals using antibodies against the PQN-59 amino terminus.**

687

688 **Table S1. Quantification of heterochronic phenotypes of homozygous *pqn-59(tm2960)* animals**
689 **expressing variations of PQN-59::*GFP* deletion alleles targeted to chromosome II. Assays**
690 **include measurement of L4 stage and adult alae formation.**

691

692 **Figure S2. FRAP of PQN-59::*GFP* in hypodermal cells indicate that PQN-59 exhibits a reduced**
693 **diffusion rate compared to soluble GFP. (A) FRAP analysis of soluble GFP (driven from the *dcap-1***
694 **promoter) and a translational fusion of PQN-59. (B) Quantification of the recovery rates for each GFP**
695 **protein depicted in A. Graphs represent the average recovery rate and error bars indicate that**
696 **standard error of measurements (SEM) for 10-15 photobleaching events in separate animals.**

697

698 **Movie S1. Liquid-Liquid Phase Separated droplets of GFP-PQN-59(PrD1-3) exhibit intra-**
699 **droplet flow when photobleached. One half of two recently fused GFP-PQN-59(PrD1-3) droplets**
700 **were subjected to FRAP and monitored for fluorescent recovery.**

701

702 **Figure S3. Comparison of the “prion-like” domain structures and predicted folding**
703 **characteristics of the *Drosophila* and human PQN-59 orthologs.** The PLAAC: Prion-Like Amino
704 Acid Comparison Server (<http://plaac.wi.mit.edu>) was used to query the primary amino acid
705 sequences of PQN-59, Lingerer isoform A, UBAP2 and UBAP2L with the following parameters: Core
706 length of 60 and a relative weighting of background probability set to the corresponding species of
707 origin. In protein sequences below each PLAAC graph, red highlighted amino acids indicate glutamine
708 (Q) and asparagine (N) rich sequences identified by this analysis as encoding “prion-like” domains.

709
710 **Table S2. Summary Table outlining various PQN-59 interacting proteins measured by Mass**
711 **Spectroscopy.** This table categorizes PQN-59 interacting proteins (peptides), gene name, and
712 molecular descriptions. This table also quantifies the enrichment, percent change, P values, and Q
713 values from these triplicate PQN-59 I.P. experiments from embryo extracts.

714
715 **Table S3. List of orthologous human gene names for proteins immunoprecipitated in PQN-59**
716 **complexes outlined in Table S1.**

717
718 **Table S4. List of orthologous *C. elegans* gene names of proteins identified as UBAP2L**
719 **interacting proteins in BioID experiments outlined in Youn et al. 2018.**

720
721 **Figure S4. PQN-59 Interacting proteins that do not map to UBAP2L-BioID interactors are**
722 **enriched for GO terms associated with germline and fertility.** Relative GO term enrichment
723 (molecular function) scores for proteins that co-precipitate with PQN-59 from early embryos.

724
725 **Figure S5. *pqn-59* depletion corrects the reduction of mature *lin-4* and *let-7* miRNA levels**
726 **observed in *alg-1(0)* mutants and has little effect *ALG-1* or *AIN-1* expression (encoding two of**
727 **the major miRISC components). A) Taqman analysis of *lin-4* and *let-7* miRNAs isolated from wild-**

728 type or *alg-1(gk215)* animals subjected to control or *pqn-59* dsRNAs. In each measurement was
729 standardized by also quantifying the expression of U18 snoRNA in each sample. Error bars represent
730 standard deviation (n=3 biological replicates, two technical replicates). P-values were calculated
731 using a Student's t-test, and corrected for multiple comparisons using a Bonferroni correction. **(B)** In
732 the *eri-1(ok2683)* RNAi hypersensitive strain *pqn-59* depletion decreases PQN-59 to 12% of its
733 abundance in control *eri-1(ok2683)* animals. In wild-type animals *pqn-59* depletion via RNAi
734 decreases PQN-59 to less than half of its abundance in animals fed control RNAi. In contrast, ALG-
735 1 and AIN-1 abundance are minimally affected by *pqn-59* depletion. Western blots were prepared
736 with LiCOR reagents and imaged with a Classic Infrared Odyssey imager. Quantification was
737 performed in ImageJ.

738

739 **Table S5. List of *C. elegans* strains used in this study.**

740 MATERIALS AND METHODS

741

742 *C. elegans* maintenance and genetics

743 *C. elegans* strains were maintained on standard media at 20°C and fed *E. coli* OP50 [68].
744 Some strains were provided by the CGC, which is funded by NIH Office of Research Infrastructure
745 Programs (P40 OD010440). *pqn-59(tm2960)* was obtained from Shohei Mitani the National
746 BioResource Project (NBRP) at the Tokyo Women's Medical University. Hypochlorite treatment
747 followed by overnight starvation was used to synchronize animals at L1. *C. elegans* strains used in
748 this study are listed in Table S5.

749

750 RNAi feeding

751 RNAi by feeding was performed using *E. coli* (strain HT115) expressing double stranded RNA
752 corresponding to endogenous *C. elegans* ORFs (SourceBioScience, United Kingdom) using standard
753 methods [69]. This library included plasmids producing dsRNA against *pqn-59* or a control dsRNA
754 expression plasmid (pPD129.36) [70] that does not contain sequence corresponding to any *C.*
755 *elegans* gene [71, 72]. Positive scoring clones were verified by replicate experiments and dsRNA-
756 targeted gene product was confirmed by Sanger sequencing. To prevent contamination by *E. coli*
757 OP50, late L4, P₀ animals were added to RNAi plates individually after removing co-transferred
758 bacteria. Unless otherwise noted, F1 progeny were analyzed for RNAi-induced phenotypes following
759 3-4 days incubation at 20°C.

760

761 Northern Blotting and miRNA Taqman Assays

762 Total RNA was isolated using TRIzol (Invitroen) from staged populations of worms that were
763 exposed to control or *pqn-59* dsRNAs until the late L4 stage. Northern blots were performed as

764 previously described [12, 73]. Taq man analysis of extracted RNAs and calculations were performed
765 as previously described [74].

766

767 **CRISPR recombineering**

768 The GFP-tagged allele of *pqn-59* at the endogenous locus (*csHls25*) was created using
769 standard CRISPR recombineering [75]. Homologous recombination was carried out with a modified
770 version of the Cas9/Guide plasmid (pCMH1299) where the *pqn-59*-specific guide sequence,
771 GTACAACTGGAGTAACTAAC, was inserted upstream of the single RNA guide backbone.
772 Homologous repair and insertion of the GFP ORF used pCMH1304 that contained the GFP ORF plus
773 approximately 1200bp 5' and 1000bp 3' flanking regions (centered on the endogenous *pqn-59* stop
774 codon). Full-length(*csH38*) and deletion alleles (Δ UBA(*csHs86*) and Δ PrD1-3(*csHs78*)) of PQN-
775 59::GFP were targeted to Chromosome II using repair templates that target the ttTi5606 site using
776 pCMH1370, pCMH1375 and pCMH1603, respectively. CRISPR editing at the ttTi5606 target site was
777 accomplished using guide sequences in pDD122 following standard approaches [75].

778

779 **Wester blotting and antibody production**

780 Antibodies Against PQN-59 were made by immunizing separate rabbits with OVA or KLB
781 conjugated peptides against amino acids 5-19 (GDKKATSDQARLARL) or 628-643
782 (PNLSSLFMQQYSPAPH) of the predicted PQN-59 protein. Western blots used the N-terminal
783 antibody (targeting amino acids 5-19 (GDKKATSDQARLARL)). For the antibody used in IP
784 experiments, a PQN-59 a C-terminal fragment (amino acids 304-712) was cloned using the Gateway
785 technology (Invitrogen) into the pDEST15 and purified as previously described [37]. Whole worm
786 lysates were prepared by dounce homogenizing staged, wild-type or transgenic animals in an equal
787 volume of Pedro's buffer (30mM HEPES, pH 7.5, 100mM KOAc, 10mM EDTA, 10% glycerol, 1 mM
788 DTT, 1x Roche complete mini protease inhibitors, Sigma phosphatase inhibitor cocktails 1 and 2

789 (each 1:100) and 1% (v/v) SuperRNase-IN). Lysates were clarified by centrifugation at 13,817 x g for
790 20 minutes at 4°C. Protein concentrations were measured using Bradford Assay (ThermoFisher, cat#
791 23200). Antibodies used in this study are as follows: anti-tubulin (Abcam, cat# EPR13478(B)), Anti-
792 ALG-1 [15], anti-AIN-1 [76], TrueBlot HRP-conjugated anti-rabbit, anti-mouse and anti-Rat secondary
793 antibodies (eBioscience).

794 **Recombinant protein purification**

795 pCMH1726, encoding the pHIS6-PQN-59(PrD1-3)_YFP protein, was constructed using
796 GIBSON cloning with a PCR fragment of the *pqn-59* cDNA (encoding the terminal 305 amino acids
797 of PQN-59) and pHIS6_Parallele_GFP vector described in Kato et al.; replacing the Fus(LC
798 domain) ORF in pHIS6-Parallel-FUS(LC)[77]. The His6::GFP::PQN-59(PrD1-3) protein induction and
799 purification was accomplished using previously described protocols[77]. Briefly, BL21(DE3) + pRIPL
800 cells were transformed with pCMH1726 (pHIS6-parallele-GFP::PQN-59(PrD1-3)) and selected on LB
801 + ampicillin + chloramphenicol plates. A single colony was grown overnight in selective media and
802 re-inoculated into 1L of LB medium and grown to an O.D.600 of 0.6. The culture was then cooled on
803 ice for 20 minutes and 0.5mL of 1M IPTG was added. Cultures were then grown overnight at 16°C.
804 Bacteria were collected by centrifugation and lysed with a sonicator in 35mL of Lysis buffer (50 mM
805 Tris-HCl pH 7.5; 500 mM NaCl; 1% Triton X-100; 20 mM β -mercaptoethanol (BME); 1 tablet of
806 protease inhibitors (Sigma S8830, 1 tablet per 50 mL)). Samples were centrifuged at 35,000 rpm for
807 30 minutes at 4°C. The supernatant was removed to a fresh 50 mL conical and further incubated with
808 2mL of Talon Beads (Qiagen) for 1 hour at 4°C. Extract slurry was then applied to a column. Beads
809 were washed with approximately 75-100 mL of lysis buffer (above) supplemented with 20mM
810 imidazole. Samples were eluted in 2mL fractions of elution buffer supplemented with 250mM
811 imidazole and quantified for concentration and purity using SDS-PAGE.

812 **Microscopy and fluorescence recovery after photobleaching (FRAP) assays**

813 For imaging of *C. elegans* larva in figures 1 and 2, mages were acquired with a Zeiss Axio
814 Observer microscope equipped with Nomarski and fluorescence optics as well as a Hamamatsu Orca
815 Flash 4.0 FL Plus camera. An LED lamp emitting at 470 nm was used for fluorophore excitation. For
816 single images, animals were immobilized on 2% agarose pads supplemented with 100mM
817 Levamisole (Sigma). Photobleaching of phase separated droplets or transgenic animals were carried
818 out using a laser on a Nikon Ti-E microscope fitted with a Perkin-Elmer UltraVIEW VoX high speed
819 spinning disk (Yokogawa® CSU-X1) laser confocal microscope with live cell imaging capability, time-
820 lapse microscopy, photokinesis, multi-position image acquisition, 6 diode laser lines (405, 440, 488,
821 514, 561 and 640nm). Imaging was performed at room temperature using spinning-disc confocal
822 microscopy system (UltraVIEW Vox; PerkinElmer) and a charged-coupled device camera (ORCA-
823 R2; Hamamatsu Photonics) fitted to an inverted microscope (Ti Eclipse; Nikon) equipped with a
824 motorized piezoelectric stage (Applied Scientific Instrumentation). Image acquisition and analysis
825 was performed using Volocity version 6.5 (Quorum Technologies). For droplets, LLPS samples were
826 spotted onto 22mm x 22mm coverslips which were rapidly placed onto the top surface of a slide.
827 After droplets had wetted to the glass surface, individual regions were photobleached. *C. elegans*
828 samples were prepared as above and subjected to photobleaching in a similar manner to LLPS
829 droplets. Appropriate ROI outside and inside samples (adjacent to bleached regions) were taken as
830 controls. The selected region of interest (ROI) was bleached with a 100 % laser power. All the
831 measurements were performed at room temperature and at least in triplicates. For *C. elegans*
832 animals FRAP, the bleaching of ROI (5-10 μm) was performed at a 100% laser power and 50
833 iterations, after acquiring 2-20 images before bleaching. The recovery was monitored for ~100-300
834 s. The images were corrected for laser bleaching by selecting a fluorescent region outside the ROI.
835 FRAP data analysis as previously described [78].

836

837 **Proteomic analysis of PQN-59 complexes**

838 N2 worms were grown on OP50-seeded NGM plates, and embryos were harvested from
839 gravid worms by bleaching (500 mM NaOH, 15% bleach). Embryos were resuspended in IP buffer
840 (100 mM KCl, 50 mM Tris pH 7.5, 1 mM MgCl₂, 1 mM DTT, 5% glycerol, 0.05% NP40, 1 mM EDTA,
841 Protease Inhibitor Cocktail (Roche)) and frozen in liquid nitrogen. For protein extraction, the embryos
842 were ground on dry ice using a mortar and pestle. The embryonic protein homogenate was thawed
843 on ice and centrifuged at 14,000 rpm for 30 minutes. Equivalent amounts of PQN-59 serum or pre-
844 serum, for control, were incubated with 10 µl of Protein G UltraLink Resin (Thermo Scientific) on ice
845 for 1 hour and 30 minutes. After three washing steps of the beads with IP buffer, approximately 2 mg
846 of embryonic protein homogenate was added and combined samples were incubated for 2 hours on
847 ice. Beads were then washed three times with IP buffer and an additional three times with a “last
848 wash” IP buffer (100 mM KCl, 50 mM Tris pH 7.5, 1 mM MgCl₂, 1 mM DTT, 1 mM EDTA). PQN-59
849 complexes were eluted in 0.15% trifluoroacetic acid (TFA). Isolated samples were then frozen on dry
850 ice and subjected to mass spectrometry analysis. Three IP samples from two separate protein
851 homogenates were used in the analysis.

852

853 **Bioinformatic analysis**

854 Prion domains of the *pqn-59* ORF were identified using PLAAC (<http://plaac.wi.mit.edu>).
855 Additional protein domain/motif prediction tools were used to identify other, conserved domains of
856 PQN-59 including PROSITE at ExPASy (<https://prosite.expasy.org/>), MOTIF (GenomeNet, Institute
857 for Chemical Research, Kyoto University, Japan) (<https://www.genome.jp/tools/motif/>), InterPro
858 (<http://www.ebi.ac.uk/interpro/>) and SMART (<http://smart.embl-heidelberg.de>) [79]. GO term analysis
859 was performed using PANTHER Tools (<http://www.pantherdb.org>) [80, 81].

860

861

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869

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882

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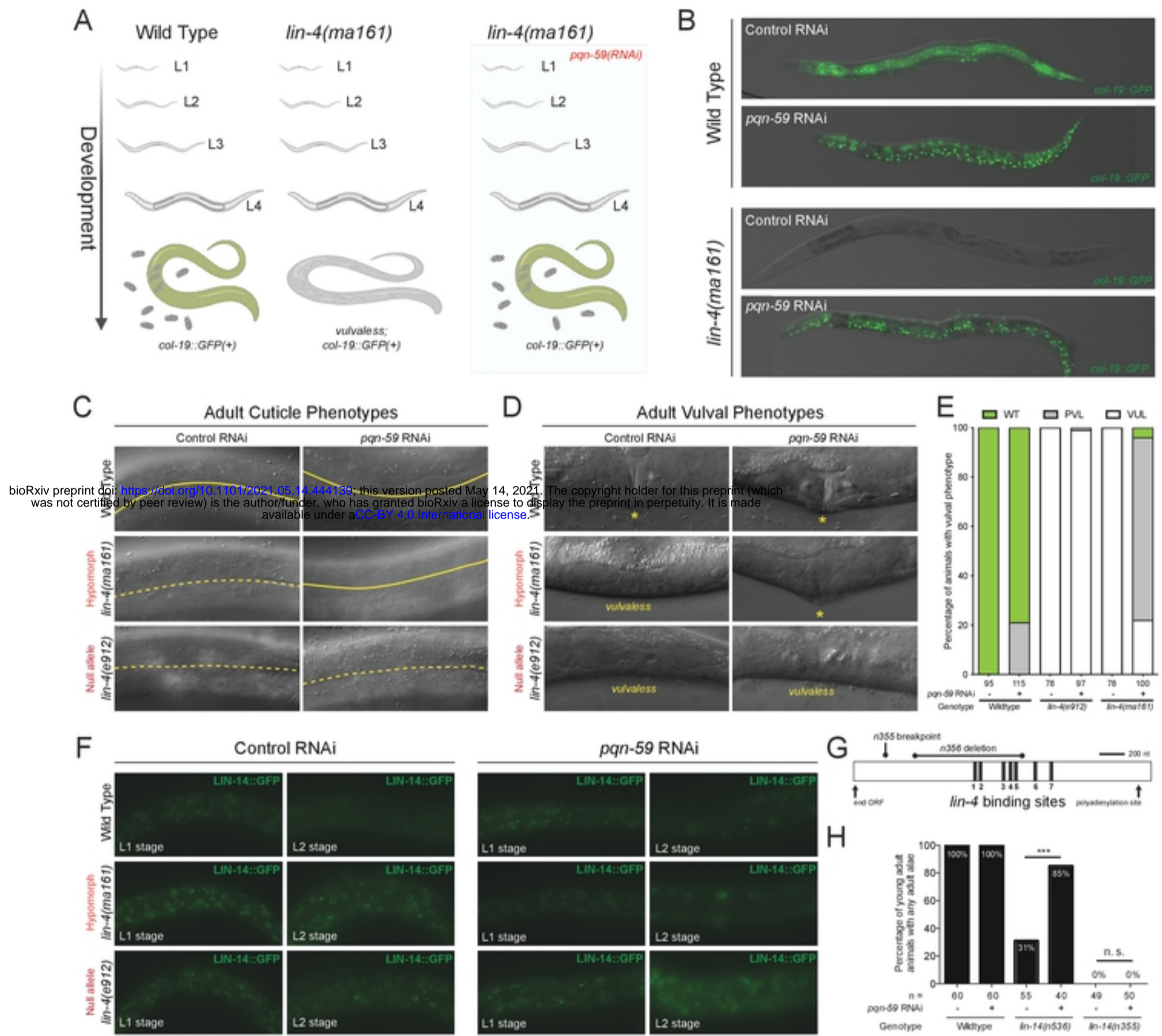


Figure 1

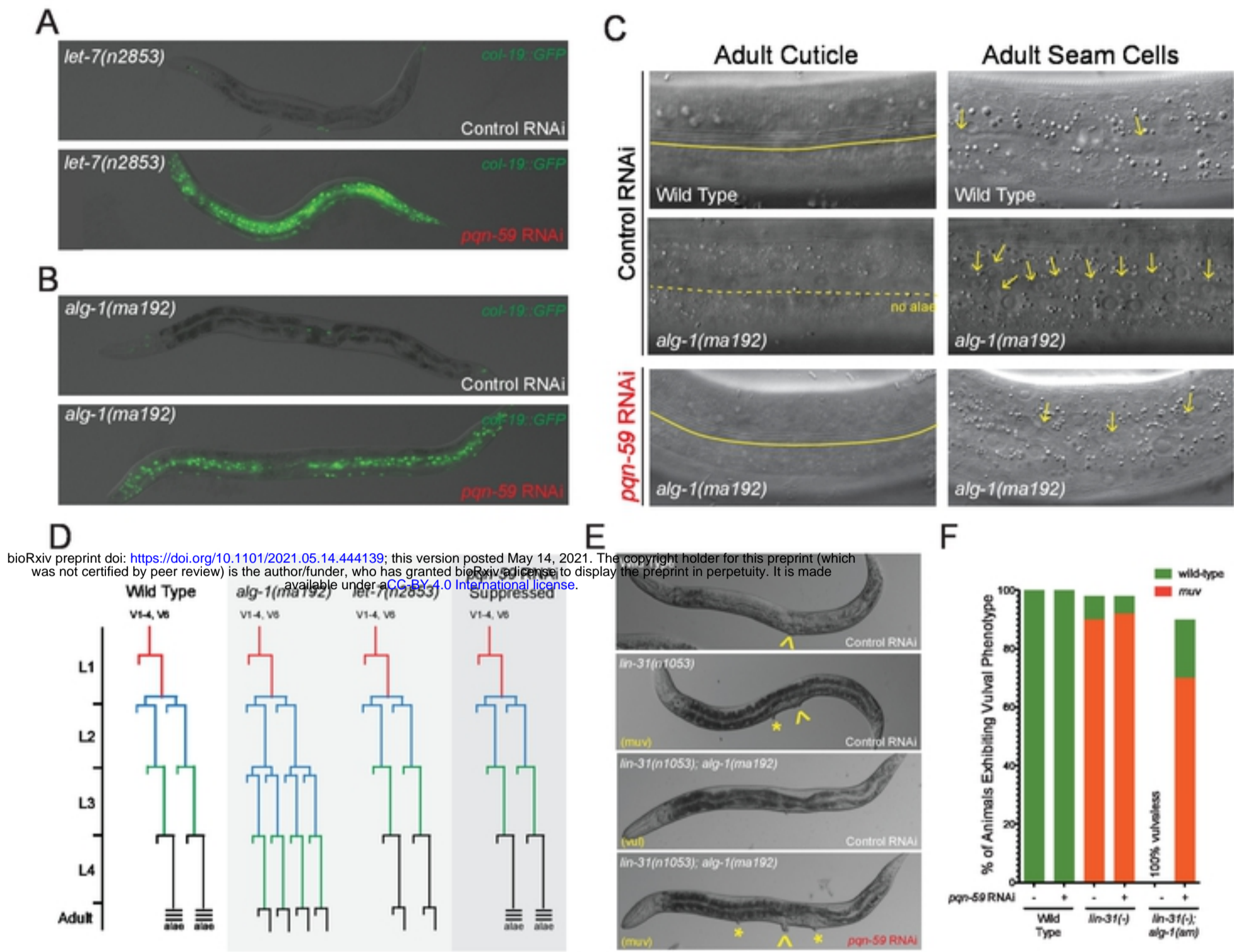


Figure 2

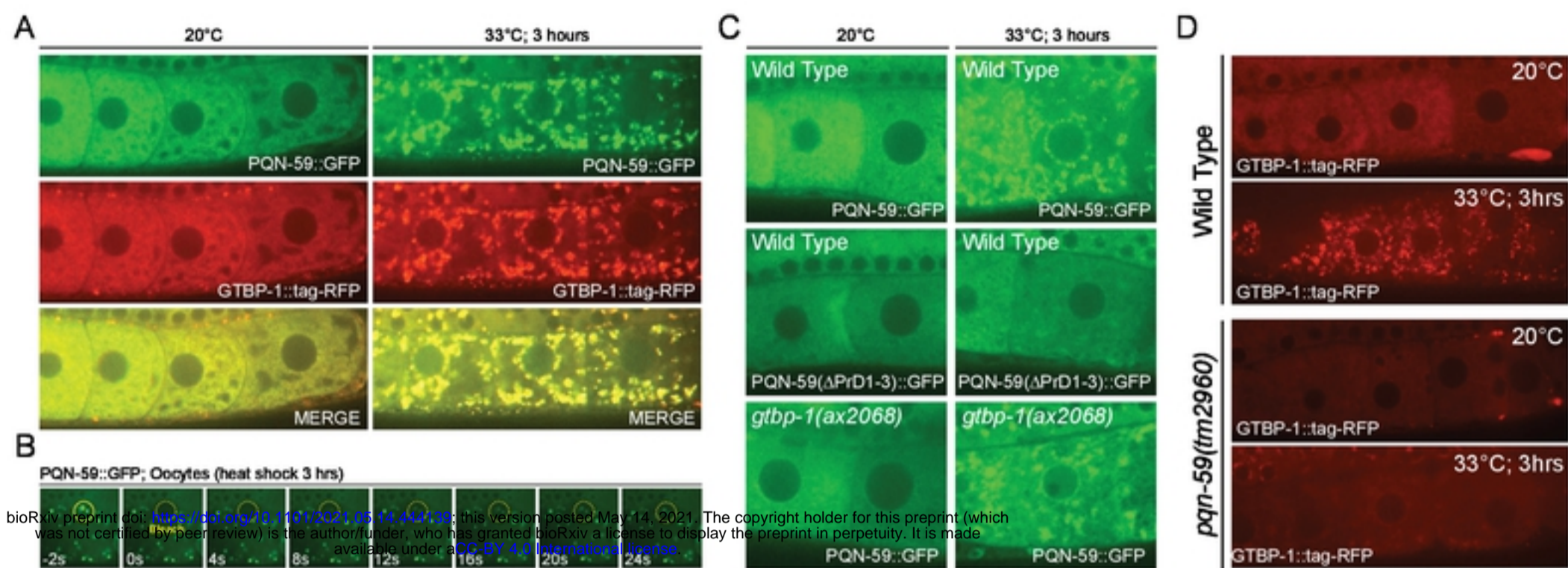


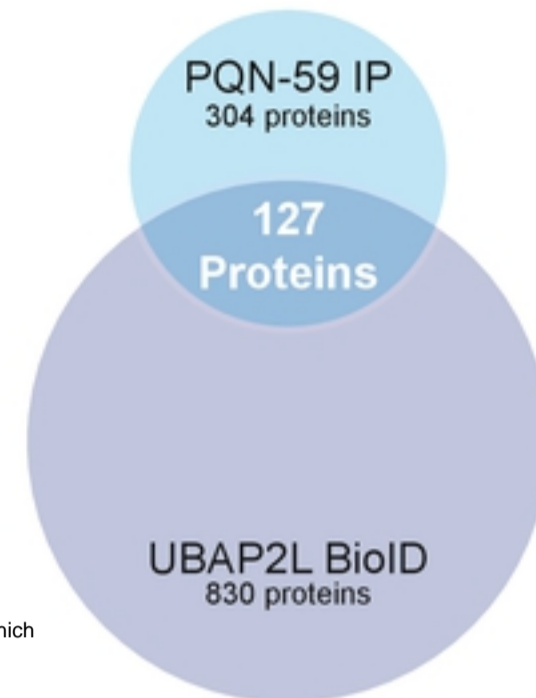
Figure 5

A

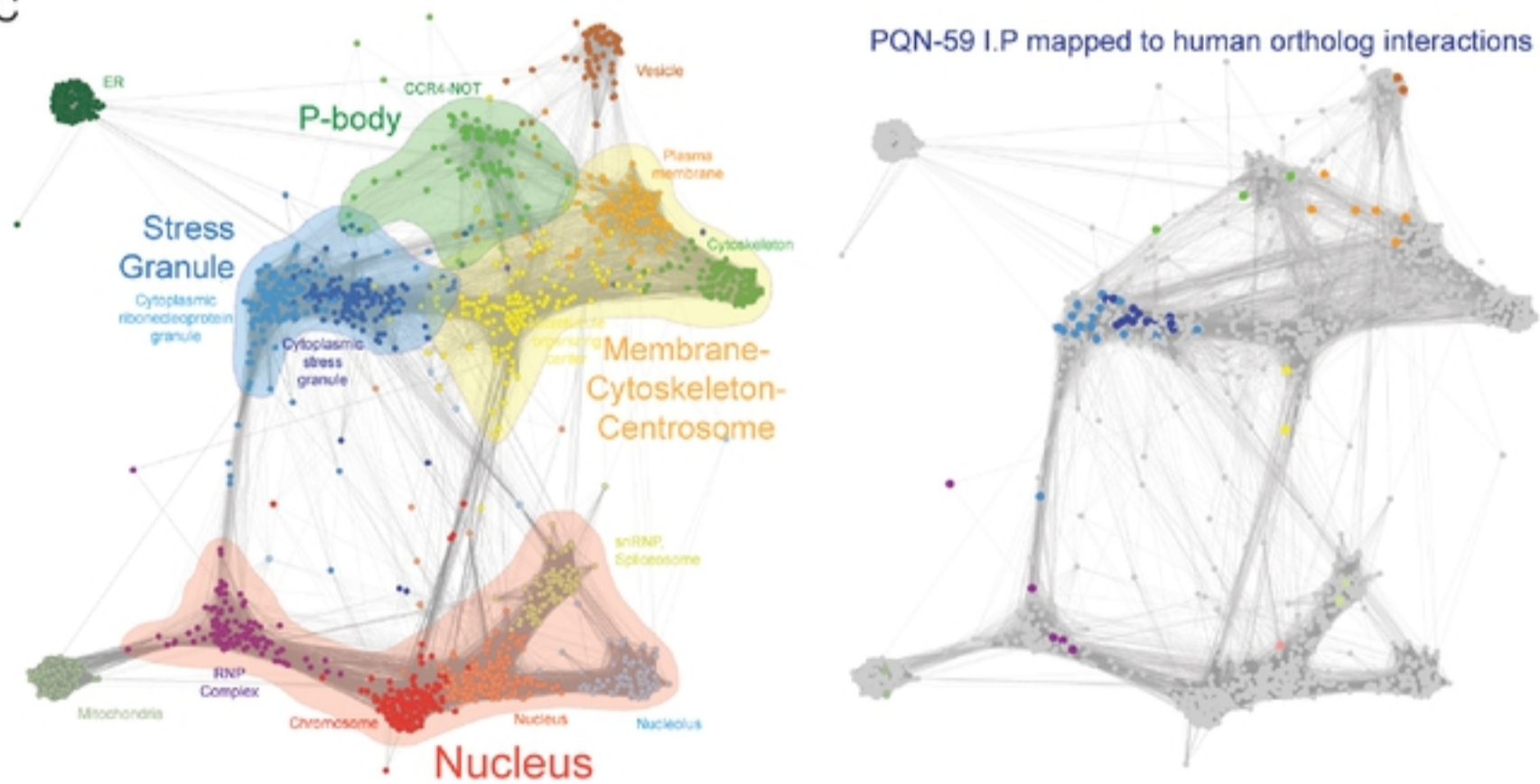
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RNA binding				
GO:0008143	poly(A) binding	35.01	2.18E-04	1.07E-02
GO:0008266	poly(U) RNA binding	30.01	3.08E-04	1.38E-02
GO:0070717	poly-purine tract binding	26.26	4.19E-04	1.82E-02
GO:0008187	poly-pyrimidine tract binding	23.34	5.53E-04	2.29E-02
GO:0003727	single-stranded RNA binding	10	2.35E-04	1.13E-02
GO:0003723	RNA binding	7.95	1.71E-41	9.07E-39
GO:0003729	mRNA binding	7.32	2.31E-09	3.83E-07
Translation				
GO:0003735	structural constituent of ribosome	22.68	5.23E-44	4.62E-41
GO:0003746	translation elongation factor activity	16.47	1.91E-04	9.72E-03
GO:0003743	translation initiation factor activity	14.9	6.30E-09	8.35E-07
GO:0043022	ribosome binding	13.34	3.86E-04	1.70E-02
GO:0090079	translation regulator activity, nucleic acid binding	12.41	4.79E-11	1.16E-08
GO:0019843	rRNA binding	10	2.35E-04	1.11E-02
Transport				
GO:0017056	structural constituent of nuclear pore	23.34	6.97E-06	5.28E-04
GO:0003774	motor activity	8.24	1.47E-04	7.79E-03
Transcription				
GO:0061629	RNA polymerase II-specific DNA-binding TF binding	16.47	1.91E-04	9.92E-03
GO:0140297	DNA-binding transcription factor binding	12.36	1.51E-07	1.82E-05
GO:0140297	DNA-binding transcription factor binding	12.36	1.51E-07	1.82E-05
GO:0001085	RNA polymerase II transcription factor binding	11.67	6.04E-04	2.46E-02
GO:0003713	transcription coactivator activity	10.21	1.16E-05	7.69E-04
Protein folding				
GO:0044183	protein folding chaperone	28.01	3.45E-05	2.03E-03
GO:0051787	misfolded protein binding	20.01	1.01E-04	5.57E-03
GO:0051082	unfolded protein binding	12.36	1.51E-07	1.91E-05

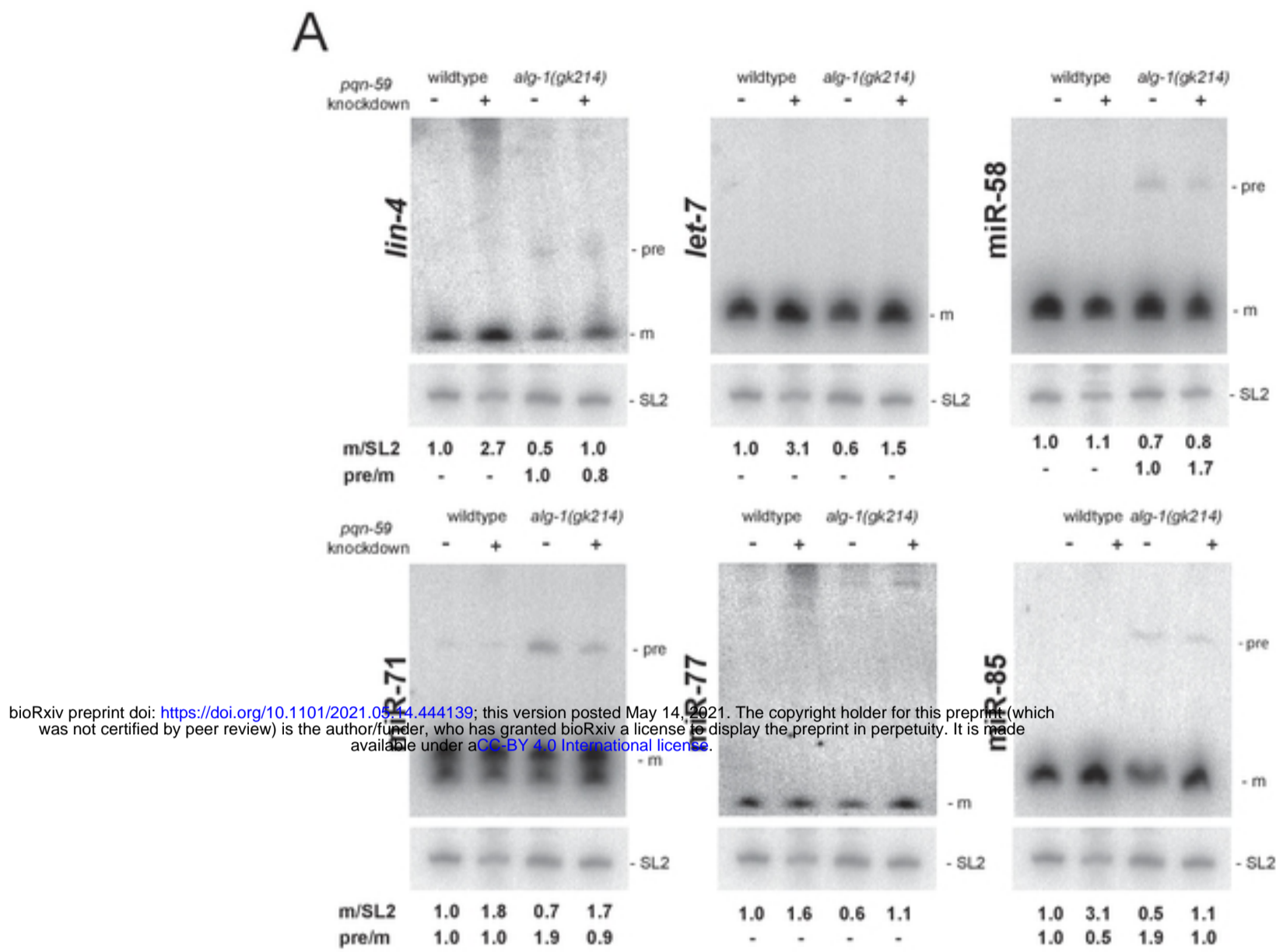
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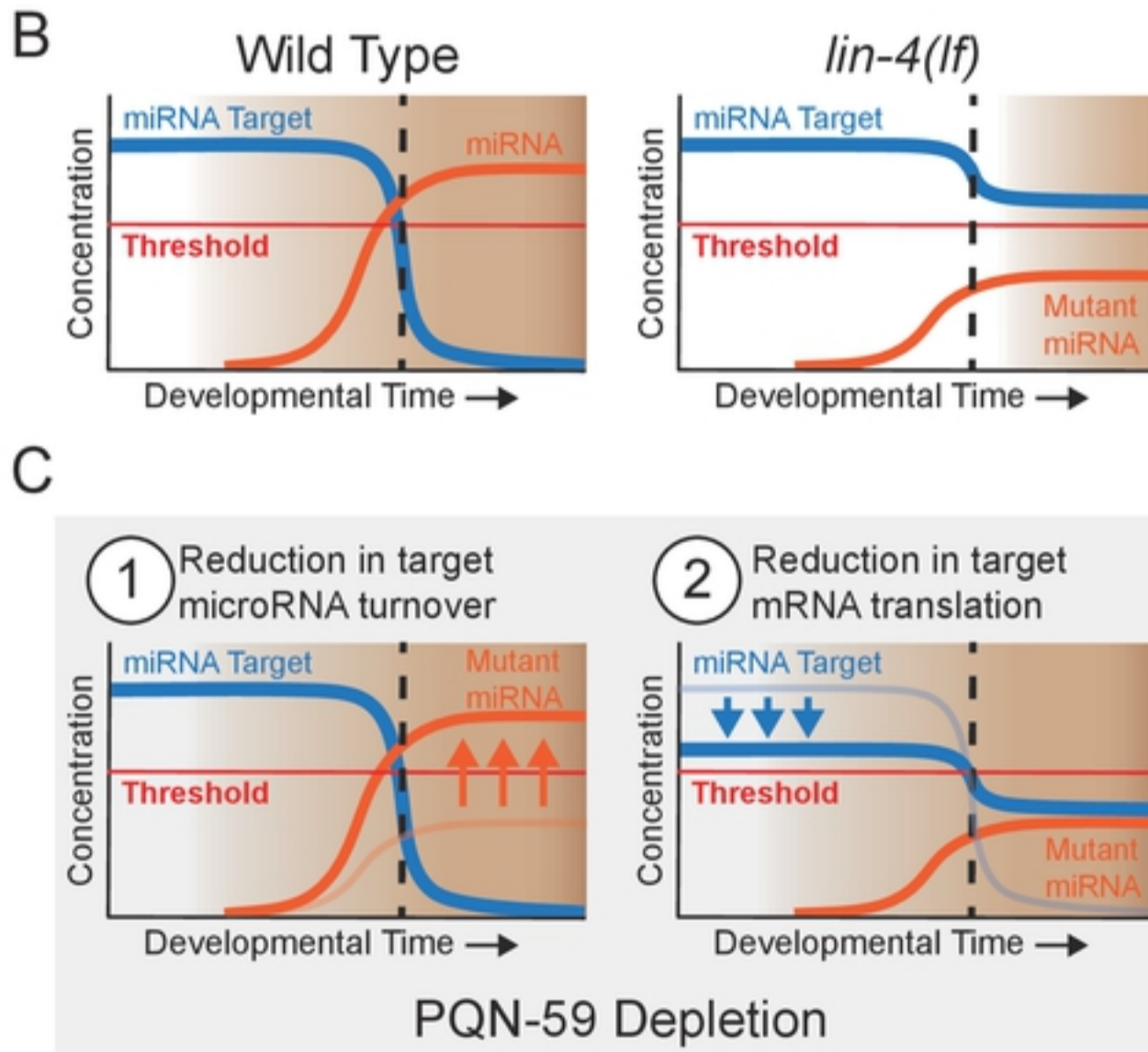


Figure 7

