Somatic aging pathways regulate reproductive plasticity resulting from early life starvation in *Caenorhabditis elegans*

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Abstract

Adverse experiences early in the life of an animal often result in changes in gene expression that are followed by changes in their adult phenotype. In the nematode *C. elegans*, starvation during the early larval stage promotes the entry into a non-feeding, stress-resistant stage where they remain as dauer larvae until environmental conditions improve. *C. elegans* adults that have experienced dauer (postdauers) retain a cellular memory of early life starvation that results in global changes in gene expression and decreased fertility. Here we show that the endocrine pathways attributed to the germline signaling regulation of somatic aging in *C. elegans* adults lacking a functional germline also regulate the reproductive phenotypes of adults that have experienced dauer as a result of early life starvation. This is in part due to the modulation of fatty acid metabolic pathways that serve to re-allocate fat reserves in postdauer adults in a manner benefitting the progeny at the expense of the parental somatic fat reservoir. Our results also show that the metabolic plasticity in postdauer animals and the inheritance of ancestral starvation memory is a result of crosstalk between somatic and reproductive tissues mediated by the HRDE-1 nuclear RNAi Argonaute.

38 **Introduction**

39 Evidence indicating that experiences during early development affect behavior
40 and physiology in a stress-specific manner later in life is abundant throughout the
41 animal kingdom (Telang and Wells, 2004; Weaver et al. 2004; Binder et al. 2008;
42 Pellegroms et al. 2009; van Abeelen et al. 2012; Zhao et al. 2014; Canario et al. 2017;
43 Dantzer et al. 2019; Vitikainen et al. 2019). In humans, individuals that experienced
44 trauma early in infancy or childhood are adversely affected by stress-related psychiatric
45 or chronic illness later in their lifetime (Danese and McEwen, 2012; Ehlert 2013;
46 Vaiserman 2015; Teicher and Samson, 2016). In addition, epidemiological studies and
47 studies using mammalian animal models have supported the “thrifty” phenotype
48 hypothesis which proposes that fetal or postnatal malnutrition results in the increased
49 risk for metabolic disorders in the offspring (Neel, 1962; Hales and Barker, 1992; Vaag
50 et al. 2012; Smith and Ryckman, 2015). For instance, individuals exposed to the WWII
51 Dutch Winter Hunger during gestation had lower glucose tolerance and increased risk of
52 obesity, diabetes, and cardiovascular diseases in adulthood compared to siblings born
53 before the famine (Painter et al. 2008; Lumey et al. 2011; Veenendaal et al. 2013).
54 Genome-scale analyses have revealed that individuals exposed pre-natally to the Dutch
55 famine had differential DNA methylation signatures in genes with functions related to
56 growth and lipid metabolism (Tobi et al. 2014). While some studies have been
57 conducted to understand the cellular mechanism by which stress early in life affect adult
58 phenotypes in animals (Weaver et al. 2004, Klengel et al. 2013), the process by which
59 stress perception is translated into tissue specific changes in gene expression remains
60 largely unknown. Thus, understanding the relationship between fetal malnutrition and

61 the increased susceptibility to metabolic disorders in adulthood is critical for
62 development of intervention and therapeutic strategies.

63 The nematode *C. elegans* makes a critical decision regarding its developmental
64 trajectory based on the environmental conditions experienced during its first larval stage
65 (L1). If conditions are poor (e.g. low food availability, crowding, or high temperatures),
66 decreased insulin and TGF-β signaling in L1 larvae promote entry into an alternative,
67 stress-resistant, non-aging, diapause stage named dauer where they can survive for
68 months. Once conditions improve, dauer larvae resume development as postdauer L4
69 larvae and continue through reproductive adulthood as postdauer adults (Cassada and
70 Russell, 1975). Alternatively, if conditions are favorable, L1 larvae proceed through
71 additional larval stages (L2-L4) until reaching reproductive adulthood (control adults)
72 (Sulston and Horvitz, 1977). Although postdauer adults are morphologically similar to
73 control adults, we previously showed that postdauer adults retained a cellular memory
74 of their early life experience that resulted in genome-wide changes in their chromatin,
75 transcriptome, and life history traits (Hall et al. 2010, 2013; Ow et al. 2018).
76 Remarkably, postdauer adults also encoded the nature of their L1 environmental
77 experience and gauged their adult reproductive phenotypes and genome-wide gene
78 expression based on this memory (Ow et al. 2018). Postdauer adults that experienced
79 crowding or high pheromone conditions (PD_{Phe}) exhibited increased fertility and
80 upregulated expression of genes involved in reproduction relative to control adults that
81 never experienced crowding (CON_{Phe}). In contrast, postdauer adults that experienced
82 starvation (PD_{Stv}) exhibited decreased fertility and an enrichment in somatic gene
83 expression compared to control adults that never experienced starvation (CON_{Stv}) (Ow

84 et al. 2018). Moreover, the reproductive and gene expression plasticity observed in
85 PD_{Phe} and PD_{Stv} with respect to their control adults is dependent on signaling between
86 somatic and reproductive tissues by distinct mechanisms: systemic RNAi signals (for
87 PD_{Phe}) or a functional germ line (for PD_{Stv}) (Ow et al. 2018).

88 The requirement of crosstalk between somatic and reproductive tissues for the
89 reproductive phenotypes is also a key regulatory feature governing adult lifespan and
90 stress response (Kenyon 2010, 2010b). In *C. elegans*, endocrine signaling has emerged
91 as one of the principal pathways extending the lifespan of animals lacking a germ line
92 due to either ablation of germ line precursor cells or a mutation in the *glp-1*/Notch
93 receptor gene. The two main effectors of endocrine signaling, the FOXO transcription
94 factor DAF-16 and the nuclear hormone receptor (NHR) DAF-12, are required for the
95 increased lifespan of germline-less animals (Hsin and Kenyon, 1999; Kenyon 2010,
96 2010b; Murphy and Hu, 2013). The nuclear localization of DAF-16 is governed by its
97 phosphorylation state in response to the physiological state of the animal. When an
98 animal experiences reproductive stress, including sterility, DAF-16 is dephosphorylated
99 and translocated to the nucleus where it can effect changes in target gene expression to
100 promote the extended longevity of germ line-less animals (Kenyon 2010, 2010b;
101 Murphy and Hu, 2013). The activity of KRI-1 (an ortholog of the human intestinal
102 ankyrin-repeat protein KRIT1/CCM1) and TCER-1 (a homolog of the human
103 transcription elongation factor TCERG1) are also required for DAF-16 nuclear
104 localization and the increased lifespan attributable to germ line loss (Berman and
105 Kenyon, 2006; Ghazi et al. 2009).

106 Nuclear hormone receptors are ligand bound receptors that recognize lipid
107 moieties and hormone ligands to mediate transcription of their target genes. In *C.*
108 *elegans*, 284 members of the NHR family have been identified. One of the best
109 characterized NHRs in *C. elegans* is DAF-12, a homolog of the mammalian vitamin D
110 receptor. Under favorable environmental conditions, DAF-12 binds to bile acid-like
111 steroid ligands (dafachronic acids or DA) to promote the expression of genes involved in
112 reproduction and growth. Under unfavorable conditions lacking DA, DAF-12 binds to its
113 co-repressor, DIN-1, to promote the expression of genes for dauer entry (Antebi 2014).
114 Additional NHRs contribute to the increased adult lifespan in germ line-less animals by
115 modulating the fatty acid metabolic status of an animal. Most of *C. elegans* NHRs are
116 derived from a common ancestor, the hepatocyte nuclear factor 4 alpha (HNF4α) that
117 acts as a lipid sensor in mammals (Antebi 2014). Of this large family of *C. elegans*
118 NHRs, NHR-49 and NHR-80 function as regulators of fatty acid metabolism by
119 promoting fatty acid desaturation in long-lived germline-less animals (Van Gilst et al.
120 2005a; Goudeau et al. 2011; Pathare et al. 2012; Ratnappan et al. 2014). Three genes
121 whose expression are promoted by NHR-49 and NHR-80 are the *fat-5*, *fat*-6, and *fat-7*
122 delta-9 (Δ9) desaturase genes, which carry out the first catalytic step in the production
123 of polyunsaturated fatty acids (PUFAs) that serve as energy reserves, membrane
124 building blocks, and signaling molecules (Brock et al. 2006; Watts and Ristow, 2017).
125 In this study, we show that steroid hormone signaling, reproductive longevity
126 signaling, and nuclear hormone receptors contribute to the decreased fertility of
127 postdauer animals that experienced early life starvation by regulating fatty acid
128 metabolism. The reproductive plasticity of starvation-induced postdauers is a result of

129 crosstalk between somatic and reproductive tissues, the effect of which is an increase in
130 lipid metabolic pathway function in an animal that has experienced dauer, resulting in
131 decreased fertility but endowing metabolic robustness to their progeny. We also show
132 that the signals between the soma and the germ line are dependent on HRDE-1, a
133 germline-specific nuclear RNAi Argonaute. Given the principal role of HRDE-1 in RNAi-
134 mediated transgenerational inheritance, this pathway may transmit the ancestral
135 starvation memory to an ensuing generation and provide the necessary hardiness to
136 survive future famine.
137

138 **Results**

139 *GLP-4 is required globally to affect reproductive plasticity resulting from early life*
140 *starvation*

141 We have previously shown that postdauer adults exhibit gene expression and
142 reproductive phenotypes that reflect their environmental and developmental history. In
143 adults that experienced starvation-induced dauer formation (PD_{Stv}), we showed that
144 gene expression changes of somatically-expressed seesaw genes and their
145 characteristic reduced brood size relative to control adults (CON_{Stv}) required a functional
146 *glp-4* gene (Ow et al. 2018). The *glp-4* gene encodes a valyl aminoacyl tRNA
147 synthetase that is expressed in the intestine, somatic gonad, and the germ line (Rastogi
148 et al. 2015). The temperature-sensitive *bn2* allele is a partial loss-of-function lesion that
149 likely results in decreased translation in both somatic and germline tissues (Beanan and
150 Strome 1992; Rastogi et al. 2015). *glp-4(bn2)* mutants exhibit increased adult lifespan

151 and stress resistance that require crosstalk between the germ line and the soma via
152 endocrine signaling pathways (Arantes-Oliveira et al. 2002).
153 To address whether the expression of *glp-4* in the intestine, somatic gonad, or
154 the germ line affect reproductive plasticity between control and PD_{Stv} adults, we
155 constructed single copy rescuing transgenes with tissue specific promoters using Mos1-
156 mediated single copy insertion (MosSCI). We performed brood size assays of control
157 and PD_{Stv} adults at 15ºC, the permissive temperature at which *glp-4(bn2)* is fertile.
158 While the brood size of PD_{Stv} wild-type N2 adults was decreased to near significance (p
159 = 0.0525) at the low temperature, the brood size of *glp-4(bn2)* PD_{Stv} adults increased
160 significantly, consistent with our previous observation (Supplemental Fig. S1;
161 Supplemental Table S11) (Ow et al. 2018). Expression of *glp-4* under its endogenous
162 promoter partially rescued the increased brood size of PD_{Stv} *glp-4* adults (Supplemental
163 Fig. S1; Supplemental Table S11). However, limited expression of *glp-4* in the intestine,
164 somatic gonad, or the germ line resulted in a brood size phenotype similar to that of *glp-*
165 *4(bn2)* mutants (Supplemental Fig. S1; Supplemental Table S11). These results
166 suggest that the contribution of GLP-4 to the reproductive plasticity of PD_{Stv} adults is not
167 due to its function in a singular tissue (germ line, somatic gonad or intestine) but rather
168 from multiple locations to promote inter-tissue crosstalk.
169
170 *Dafachronic acid-dependent DAF-12 signaling is required for decreased fecundity after*
171 *starvation-induced dauer formation*
172 Given that endocrine signaling across tissues is a prominent feature of reproductive
173 longevity, we examined whether wild-type PD_{Stv} adults shared any gene expression

174 signatures with animals lacking a functional germ line. In *glp-1* mutants, increased
175 longevity is dependent on TOR (target of rapamycin) signaling, DAF-16/FOXO gene
176 regulation, steroid hormone signaling, and fatty acid metabolism regulation (Lapierre
177 and Hansen, 2012). With the exception of TOR signaling, we found significant gene
178 expression changes in PD_{Stv} adults of key genes in each of these regulatory pathways.
179 In the steroid signaling pathway, dafachronic acid (DA) biosynthesis requires the
180 cytochrome P450 DAF-9, the Reiske-like oxygenase DAF-36, and the hydroxysteroid
181 dehydrogenase HSD-1 (Gerisch et al. 2004; Rottiers et al. 2006; Patel et al. 2008;
182 Mahanti et al. 2014). In animals lacking a functional germ line, the levels of *daf-36*
183 mRNA and the Δ^7 form of DA (Δ^7-DA) are significantly increased compared to wild-type
184 (Shen et al. 2012). Interestingly, we found that in wild-type PD_{Stv} adults with a germ line,
185 *daf-36* mRNA is also increased 3-fold (p = 3.25e-04; FDR = 0.01) compared control
186 adults (Ow et al. 2018). To investigate whether DA signaling plays a role in mediating
187 reproductive plasticity as a result of early life experience, we asked whether mutations
188 in DA biosynthesis genes would affect the reduced brood size observed in PD_{Stv} adults.
189 We found that *daf-9(rh50)* and *daf-36(k114),* but not *hsd-1(mg433),* exhibited a
190 significant increase in brood size in PD_{Stv} adults, opposite of what we observed in wild-
191 type animals (Fig. 1A; Supplemental Table S1).
192 We asked whether the steroid signaling that contributed to the reduced fertility of
193 PD_{Stv} adults acted through related NHRs, DAF-12 and NHR-8. Since null mutants of
194 *daf-12* are dauer defective, we used two *daf-12* alleles, *rh284* (Class 5 mutant) and
195 *rh285* (Class 4 mutant), with lesions in the ligand binding domain (LBD) that affect
196 steroid signaling activity but otherwise have nearly normal dauer phenotype (Antebi et

197 al. 2000). Surprisingly, *daf-12(rh284)* and *daf-12(rh285)* exhibited a significant increase
198 in brood size, similar to that observed for the DA biosynthesis mutants (Fig. 1A;
199 Supplemental Table S1). These results suggest that DA-dependent activity of DAF-12 is
200 required for reduced fertility in PD_{Stv} adults. The gene *nhr-8* is also up-regulated 2.5-fold
201 in PD_{Stv} adults (p = 7.28e-03; FDR = 0.15); thus, we asked whether a mutant disrupting
202 the LBD of NHR-8, would similarly affect the fecundity of PD_{Stv} adults (Lindblom et al.
203 2001; Magner et al. 2013; Ow et al. 2018). However, *nhr-8(ok186)* exhibited a
204 significant brood size reduction between control and PD_{Stv} animals at levels comparable
205 to wild-type N2 (Fig. 1A; Supplemental Table S1). These results indicate that the
206 reduced fertility of PD_{Stv} adults is dependent on DA biosynthesis through DAF-9 and
207 DAF-36 and the subsequent DA-binding activity of DAF-12.
208 We next asked whether the exogenous addition of DA was sufficient to reduce
209 the brood size of PD_{Stv} adults. To test this hypothesis, we induced larva into dauer by
210 starvation with or without exogenously added 40 nM of Δ^7-DA and measured their brood
211 size. The addition of Δ^7-DA did not affect the fertility of wild-type and *daf-36(k114)* (Fig.
212 1B; Supplemental Table S2). However, *daf-9(rh50)* PD_{Stv} adults exhibited a significant
213 decrease in brood size in the presence of exogenous Δ^7-DA (Fig. 1B; Supplemental
214 Table S2). DAF-9 is reported to promote a feedback loop of DA production between the
215 neuroendocrine XXX cells and the hypodermis; thus, *daf-9* mutants may be more
216 sensitive to small changes in DA concentration (Antebi 2014). Taken together, these
217 results suggest that increased DA-dependent DAF-12 signaling is necessary, but
218 perhaps not sufficient, for the reduced brood size phenotype of PD_{Stv} adults.
219

220 *The DAF-16 reproductive longevity pathway mediates reproductive plasticity*

221 DAF-16 acts as a major effector of reduced insulin/IGF-1-like signaling (IIS) by
222 promoting the expression of a group of stress response genes (Class I targets) (Tepper
223 et al. 2013). PQM-1, a C2H2 zinc finger transcription factor, acts in a mutually
224 antagonistic manner to DAF-16 by up-regulating genes associated with growth and
225 reproduction (Class II targets), as its cellular localization in the nucleus is anti-correlated
226 to that of DAF-16 (Tepper et al. 2013). Interestingly, we previously found that the
227 expression of *pqm-1* is up-regulated 3-fold (p = 1.14e-04; FDR = 5.75e-03) in PD_{Stv}
228 adults compared to control adults (Ow et al. 2018). In addition, we observed that
229 18.82% (313 genes; p < 2.52e-91, hypergeometric test) of the reported 1,663 DAF-16
230 Class I target genes and 13.21% (229 genes; p < 1.96e-37) of the 1,733 PQM-1 Class II
231 target genes significantly overlapped with the 1,121 somatically-enriched genes that we
232 previously reported to be up-regulated in PD_{Stv} adults. We also observed significant
233 overlaps between our previously reported 551 down-regulated genes in PD_{Stv} adults
234 with DAF-16 Class I genes (0.54%; 9 genes; p < 1.03e-11, hypergeometric test) and
235 PQM-1 Class II genes (4.79%; 83 genes; p < 2.83e-07) (Tepper et al. 2013; Ow et al.
236 2018) (Fig. 2A; Supplemental Table S3). In addition, *daf-18*, the functional ortholog of
237 the human PTEN tumor suppressor gene that promotes the nuclear localization of DAF-
238 16 (Ogg and Ruvkun, 1998; Gil et al. 1999; Mihaylova et al. 1999; Solari et al. 2005),
239 was down-regulated 2.8-fold (p = 6.6e-06; FDR = 5.72e-04) in PD_{Stv} adults compared to
240 control adults (Ow et al. 2018). The overlap in gene targets and the significant changes
241 in the expression of *pqm-1* and *daf-18* prompted us to ask whether PQM-1 and DAF-18
242 contribute to the reduced fertility in PD_{Stv} adults by altering the regulation of genes

243 involved in reproduction and sequestering DAF-16 in the cytoplasm. We investigated
244 this hypothesis by performing brood assays on adults carrying mutations in *pqm-1* and
245 *daf-18*. Surprisingly, the brood size of control and PD_{Stv} adults in *pqm-1(ok485)* and *daf-*
246 *18(e1375)* strains was similar to wild type, indicating that gene regulation by PQM-1 is
247 unlikely to contribute to the PD_{Stv} decreased brood size phenotype (Fig. 2B;
248 Supplemental Table S4). Since *daf-18(e1375)* is a hypomorph, we next tested the
249 possibility that DAF-16 localization may play a role in regulating postdauer reproduction.
250 *daf-16* null mutants are dauer defective; thus, we asked whether a *daf-16(mu86)* null
251 mutant expressing a rescuing transgene (*daf-16a^{AM}::gfp*) that constitutively localizes
252 DAF-16 to the nucleus would show a brood size plasticity phenotype (Lin et al. 2001).
253 Similar to what was observed for *daf-18(e1375)*, the *daf-16(mu86); daf-16a^{AM}::gfp*
254 transgenic strain displayed a reduced brood size in PD_{Stv} similar to wild-type N2 (Fig.
255 2B; Supplemental Table S4). These results suggest that the nuclear localization of DAF-
256 16, and not PQM-1, may be contributing to the diminished fertility phenotype of PD_{Stv}
257 adults.

258 To further investigate whether the nuclear localization of DAF-16 is required for
259 the reproductive plasticity between control and PD_{Stv} animals, we measured the brood
260 size of animals with mutations in genes that regulate the cellular localization of DAF-16.
261 First, we tested SMK-1/PPP4R3A, which promotes the nuclear localization of DAF-16
262 when animals are exposed to pathogenic bacteria, ultraviolet irradiation, and oxidative
263 stress, but does not affect the dauer development and reproductive timing activities of
264 DAF-16 (Wolff et al. 2006). We found that *smk-1(mn156)* mutants continued to exhibit a
265 decreased PD_{Stv} fertility compared to controls. Secondly, we examined two genes, *kri-1*

266 and *tcer-1,* that are required for DAF-16 nuclear localization and extend adult lifespan in
267 germ line-less animals, but do not regulate dauer formation (Berman and Kenyon, 2006;
268 Ghazi et al. 2009). We found that decreased brood size in PD_{Stv} was abrogated in the
269 *kri-1(ok1251)* and *tcer-1(tm1452)* mutants (Fig. 2B; Supplemental Table S4). Because
270 TCER-1 regulates the transcription of its target genes in a DAF-16-dependent and
271 independent manner (Armit et al. 2016), these results indicate that KRI-1, TCER-1, and
272 possibly DAF-16 are required for the reproductive plasticity in PD_{Stv} animals.
273
274 *Increased fatty acid metabolism promotes PD_{Stv} fertility*
275 In animals lacking a germ line, DAF-16 and TCER-1 are required to bolster the
276 expression of lipid biosynthesis, storage, and hydrolysis genes to promote adult
277 longevity (Armit et al. 2016). Similar to germ line-less *glp-1* mutants, PD_{Stv} adults
278 exhibited a significant increase in the expression of fatty acid metabolic genes targeted
279 by DAF-16 and TCER-1 (Fig. 2C; Supplemental Table S5). Specifically, 47% (7 out of
280 15) of DAF-16 and TCER-1 target genes predicted to regulate lipid synthesis and
281 storage are up-regulated in PD_{Stv} adults ($p < 6.86e-06$, hypergeometric test). Similarly,
282 50% (12 out of 24) of lipid hydrolysis genes targeted by DAF-16 and TCER-1 are up-
283 regulated in PD_{Stv} adults ($p < 1.16e-09$) (Amrit et al. 2016; Ow et al. 2018) (Fig. 2C;
284 Supplemental Table S5). Additionally, 27% (4 out of 15) of lipid catabolism genes ($p <$
285 0.008) and 12% (9 out of 74) of lipid anabolism genes ($p < 0.021$) that were not
286 identified as targets of DAF-16 or TCER-1 were up-regulated in PD_{Stv} adults (Amrit et al.
287 2016; Ow et al. 2018). Notably, none of the genes down-regulated in PD_{Stv} adults were
288 represented by the DAF-16 and TCER-1 lipid metabolic target genes (Amrit et al. 2016;

289 Ow et al. 2018) (Fig. 2C). This observation was also true for lipid anabolism and
290 catabolism genes that are not targets of DAF-16 or TCER-1. These results suggest that
291 DAF-16 and TCER-1 play a role in regulating reproductive plasticity between controls
292 and PD_{Stv} by up-regulating fatty acid metabolism.

293 To investigate this hypothesis, we asked if mutations in known regulators of lipid
294 metabolism would exhibit changes in brood size in PD_{Stv} adults when compared to
295 controls. One of the genes jointly up-regulated by DAF-16 and TCER-1 is *nhr-49*, a
296 nuclear hormone receptor homologous to the mammalian HNF4α lipid sensing nuclear
297 receptor involved in the regulation of fatty acid metabolism and the oxidative stress
298 response (Ratnappan et al. 2014; Amrit et al. 2016; Moreno-Arriola et al. 2016; Goh et
299 al. 2018; Hu et al. 2018). Additional nuclear hormone receptors, NHR-80, NHR-13, and
300 NHR-66, and the Mediator complex subunit, MDT-15, partner with NHR-49 and co-
301 regulate the expression of genes involved in various aspects of lipid metabolism such
302 as fatty acid β-oxidation, transport, remodeling, and desaturation (Van Gilst et al. 2005a,
303 2005b; Taubert et al. 2006; Nomura et al. 2010; Pathare et al. 2012; Ratnappan et al.
304 2014; Folick et al. 2015; Amrit et al. 2016). In addition, SBP-1 (homolog of mammalian
305 SREBP) and NHR-49 are co-regulated by MDT-15 as part of a transcriptional network
306 coordinating the expression of delta-9 (Δ9) fatty acid desaturase genes (Yang et al.
307 2006; Taubert et al. 2006).

308 The reduced fertility of PD_{Stv} characteristic of wild-type animals was also
309 observed in *nhr-80(tm1011)*, *nhr-13(gk796)*, *nhr-66(ok940)*, and *mdt-15(tm2182)*
310 strains, but not in *sbp-1(ep79)* (Fig. 3A; Supplemental Table S6). In addition, two *nhr-49*
311 *loss-of-function* (*lof*) mutants, *nhr-49(nr2014)* and *nhr-49(gk405)*, failed to exhibit the

312 decreased fertility in PD_{Stv} adults compared to CON_{Stv}, while a third *nhr-49(ok2165) lof*
313 allele continued to display a PD_{Stv} reduction in brood size (Fig. 3A; Supplemental Table
314 S6). Because the three *nhr-49* mutant alleles, *nr2014*, *gk405*, and *ok2165*, differ in the
315 nature and the location of their lesions (Lee et al. 2016), their biological function may
316 vary and hence result in phenotypic differences (*nr2041* and *gk405* vs. *ok2165*).
317 Animals expressing *nhr-49* gain-of-function alleles (*et7*, *et8*, and *et13*) with missense
318 lesions located at or near the LBD may augment NHR-49 activity resulting in a
319 reproductive plasticity phenotype comparable to that of wild-type N2 (Svensk et al.
320 2013; Lee et al. 2016) (Fig. 3A; Supplemental Table S6). Because of the interaction
321 between NHR-49, NHR-80, NHR-13, and NHR-66, we also examined if double and
322 triple mutants of these NHRs would have any reproductive plasticity phenotypes.
323 Double and triple mutant combinations of alleles exhibited an abolishment of the PD_{Stv}
324 decreased brood size phenotype only if they expressed the *nhr-49(nr2041)* mutation
325 (Fig. 3B; Supplemental Table S7). Thus far, our results indicate that NHR-49 and SBP-1
326 are important in the reproduction program of PD_{Stv} adults, likely by up-regulating fat
327 metabolism genes.

328 NHR-49 and SBP-1 up-regulate the expression of genes involved in fatty acid
329 biosynthesis, including the Δ9 desaturases, *fat-5*, *fat-6*, and *fat-7*, and the delta-12 (Δ12)
330 desaturase *fat-2* (Nomura et al. 2010; Han et al. 2017). FAT-5, FAT-6, and FAT-7
331 convert saturated fatty acids (SFAs) to mono-unsaturated fatty acids (MUFAs), while
332 FAT-2 catalyzes the conversion of MUFAs to poly-unsaturated fatty acids (PUFAs)
333 (Watts and Ristow, 2017). Our previous mRNA-Seq results showed that the expression
334 of *fat-5*, *fat-6*, *fat-7*, and *fat-2* increased significantly between 3.8- and 26.6-fold in wild-

335 type PD_{Stv} adults compared to controls (Ow et al. 2018). While strains expressing a
336 single *fat-5(tm331)*, *fat-6(tm420)*, *fat-7(wa36)*, or *fat-2(wa17)* mutation continued to
337 exhibit the reduced brood size phenotype in PD_{Stv} adults, double mutants expressing a
338 mutation in *fat-6* (*fat-6(tm420); fat-5(tm331)* and *fat-6(tm420); fat-7(wa36))* no longer
339 displayed the reduced PD_{Stv} phenotype. The double mutant *fat-7(wa36); fat-5(tm331)*
340 continued to display a significant fertility difference between controls and PD_{Stv} (Fig. 3C;
341 Supplemental Table S8). These results suggest: 1) a functional redundancy between
342 the Δ9 fatty acid desaturases in modulating lipid homeostasis of PD_{Stv} adults, with FAT-
343 6 playing a more principal role than FAT-5 and FAT-7; and 2) MUFAs are required for
344 the decreased fertility phenotype in PD_{Stv} adults compared to controls.

345 In *C. elegans*, MUFAs are essential for viability as a triple *fat-5; fat-6; fat-7*
346 mutant is lethal (Brock et al. 2006). MUFAs, such as oleic acid, can be remodeled to
347 become PUFAs, phospholipids, and neutral lipids such as triacylglycerols (TAGs), which
348 serve as energy storage molecules in the intestine, hypodermis, and germ line (Watts
349 and Ristow, 2017). In addition to acting as key regulators of fat metabolism, FAT-5,
350 FAT-6, and FAT-7 are also essential in promoting the long lifespan of adult worms
351 lacking a germ line (Van Gilst et al. 2005a; Brock et al. 2006; Goudeau et al. 2011;
352 Ratnappan et al. 2014). Given that MUFAs are required for the reduced fertility of PD_{Stv}
353 adults compared to controls, we asked whether the dietary addition of oleic acid to PD_{Stv}
354 animals would further reduce their brood size such that they would produce even fewer
355 progeny compared to PD_{Stv} animals not supplemented with oleic acid. To test this
356 hypothesis, we compared the brood size of PD_{Stv} adults fed *E. coli* OP50 grown with
357 oleic acid and PD_{Stv} adults whose bacterial diet was not pre-loaded with oleic acid. We

358 tested the N2 wild-type, *nhr-49(nr2041)*, *nhr-49(gk405)*, and *nhr-49(nr2041); nhr-*
359 *80(tm1011)* strains, and found that for all genotypes, the supplementation of oleic acid
360 significantly increased the progeny number produced by PD_{Stv} compared to PD_{Stv} adults
361 on the control diet (Fig. 3D; Supplemental Table S9). These results indicate that oleic
362 acid is a limiting factor for reproduction, even for wild-type animals, after passage
363 through the starvation-induced dauer stage. Altogether, these results suggest that NHR-
364 49 and SBP-1 are critical for the decreased fertility in PD_{Stv} adults by mediating the up-
365 regulation of fatty acid desaturases and promoting the synthesis of sufficient levels of
366 oleic acid needed for reproduction after the animals experienced starvation-induced
367 dauer formation.
368
369 *Starvation-induced postdauer adults have reduced fat stores*
370 In long-lived *glp-1* mutants lacking a germ line, fat stores are increased relative to wild-
371 type (Steinbaugh et al. 2015; Amrit et al. 2016). Given that PD_{Stv} adults also have an
372 increased expression of lipid metabolism genes similar to *glp-1* adults, but have limited
373 quantities of oleic acid for reproduction, we questioned what the status of fat stores
374 would be in wild-type adults with an intact germ line that had passed through dauer due
375 to starvation. Using Oil Red O (ORO) staining, we compared the amounts of neutral
376 triglycerides and lipids (O'Rourke et al. 2009) in PD_{Stv} one-day-old adults and age-
377 matched control adults. Interestingly, PD_{Stv} adults have a significantly reduced amount
378 of stored lipids relative to control adults in their intestine, but still displayed ORO
379 staining in the embryos within the uterus (Fig. 4A, 4B). These results are consistent with

380 a model that PD_{Stv} adults have increased expression of lipid metabolism genes in order
381 to produce sufficient levels of MUFAs needed for reproduction rather than lipid storage.
382 Next, we investigated whether the decreased lipid stores in PD_{Stv} adults was
383 dependent upon DAF-12 and TCER-1. Both *daf-12(rh284)* and *daf-12(rh285)* mutants
384 displayed a significant increase in lipid storage in PD_{Stv} adults relative to control adults,
385 opposite to what was observed in wild-type, indicating that DA-mediated DAF-12 activity
386 is required for regulation of fat stores in PD_{Stv} adults (Fig. 4A, 4B). Interestingly, the
387 ORO staining positively correlated with brood sizes in wild-type N2 and the *daf-12*
388 mutants: fat stores and fertility decreased in wild-type PD_{Stv} adults, while fat stores and
389 fertility increased in *daf-12* PD_{Stv} adults compared to *daf-12* CON_{Stv} adults (Fig. 1A;
390 Supplemental Table S1; Fig. 4A, 4B). In *tcer-1(tm1452*) adults, lipid staining was
391 diminished in PD_{Stv} compared to controls, similar to what is seen in wild-type (Fig. 4A,
392 4B). However, since both *tcer-1* control and PD_{Stv} adults have reduced fat stores
393 compared to their wild-type counterparts, and TCER-1 is known to positively regulate
394 NHR-49 and fatty acid metabolism, this result is likely due to the inability of these
395 animals to store fat and not because the decreased fat stores in wild-type PD_{Stv} adults is
396 TCER-1 independent. Furthermore, the brood size of *tcer-1(tm1452)* did not positively
397 correlate with their ORO staining: a very modest increase (p = 0.0546) in PD_{Stv} brood
398 size and a significant decrease in PD_{Stv} ORO staining (Fig. 4A, 4B). In addition to
399 regulation of fat metabolism genes in the intestine, TCER-1 also functions redundantly
400 with PUF-8, a member of an evolutionarily conserved stem cell proliferation modulatory
401 family, to potentiate germ cell proliferation in animals undergoing continuous
402 development (Pushpa et al. 2013). Thus, our *tcer-1(tm1452)* results suggest that TCER-

403 1 activity may also be necessary in regulating germline mechanisms that curtail fertility
404 following exit from dauer diapause.

405 During *C. elegans* reproduction, intestinal fat stores are reallocated into low-
406 density lipoproteins (LDL)-like particles (yolk lipoproteins or vitellogenins) that are
407 incorporated into oocytes through receptor-mediated endocytosis in a process called
408 vitellogenesis, and serve as the only supply of nutrients to the developing embryos
409 (Kimble and Sharrock, 1983; Grant and Hirsch, 1999). Six vitellogenins homologous to
410 the human ApoB proteins are encoded in the *C. elegans* genome, *vit-1* through *vit-6*,
411 and concomitant multiple RNAi knockdown of the *vit* genes increases adult lifespan in a
412 process that requires NHR-49 and NHR-80 (Spieth et al. 1991; Seah et al. 2016). The
413 transition from dauer to reproductive adulthood is an energetically costly process that
414 likely drains the already reduced fat reservoirs in dauers that experienced early-life
415 starvation. Because vitellogenesis mobilizes intestinal fat resources for reproduction
416 and depletes somatic lipid stores (Kimble and Sharrock, 1983), we hypothesized that
417 PD_{Stv} adults have reduced fat reservoirs because they prioritize vitellogenesis as a
418 reproductive investment over intestinal storage. To test this hypothesis, we examined
419 the fat stores in control and PD_{Stv} adults following RNAi knockdown of *vit-1,* which also
420 results in the knockdown of *vit-2*, *vit-3*, *vit-4*, and *vit-5* due to the high sequence
421 homology amongst the *vit* genes (Seah et al. 2016) (Supplemental Fig. S3A). In the
422 RNAi treated animals, PD_{Stv} adults continued to exhibit decreased fat stores compared
423 to control adults, similar to the empty vector negative controls. However, PD_{Stv} adults
424 treated with *vit* RNAi have significantly greater intestinal fat deposits than PD_{Stv} negative
425 controls ($p < 0.0001$), indicating that increased vitellogenesis in PD_{Stv} adults may be one

426 contributing factor to the lack of stored intestinal fat (Fig. 4C, 4D; Supplemental Fig.
427 S3A). These results are consistent with our previous finding that oleic acid is a limiting
428 resource for reproduction in PD_{Stv} animals, and suggests a model where PD_{Stv} adults
429 utilize fat accumulated after diapause for reproduction and not storage.
430
431 *Steroid signaling, reproductive longevity, and fatty acid metabolic pathways act*
432 *synergistically at different developmental time points to regulate reproductive plasticity*
433 Thus far, our results indicate that both DA-dependent DAF-12 activity and TCER-1 are
434 required for the reduced fertility phenotype in PD_{Stv} adults. However, the *daf-12* and
435 *tcer-1* PD_{Stv} mutant phenotypes are distinct, suggesting that they act in different
436 pathways, tissues, or developmental time points to regulate PD_{Stv} fertility. Furthermore,
437 the brood sizes of *kri-1(ok1251)* or *tcer-1(tm1425)* PD_{Stv} adults supplemented with
438 exogenous Δ^7-DA were unaffected, indicating that the effect of DA on PD_{Stv} brood size
439 is independent of the reproductive longevity pathway (Supplemental Fig. S2;
440 Supplemental Table S12). To further examine the genetic interactions of DAF-12 and
441 TCER-1, we performed epistasis analysis by measuring the brood sizes of control and
442 PD_{Stv} adults in *kri-1(ok1251); daf-12(rh284)* and *tcer-1(tm1425); daf-12(rh284)* double
443 mutants, and in *kri-1(ok1251); daf-12(rh285)* and *tcer-1(tm1425); daf-12(rh285)* double
444 mutants. For all four double mutants, we continued to observe a significantly increased
445 brood size in PD_{Stv} adults compared to controls, similar to the *daf-12(rh284)* and *daf-*
446 *12(rh285)* single mutants (Fig. 1A; Supplemental Table S1; Fig. 5A; Supplemental Table
447 S10). The combination of mutations between *kri-1(ok1251)* and *tcer-1(tm1425)* with the
448 *daf-12(rh284)* allele synergistically exacerbated the fertility phenotype of PD_{Stv} adults to

449 a more than 600-fold average compared to each of the single mutants, suggesting that
450 these pathways act in parallel (Fig. 1A; Supplemental Table S1; Fig. 5A; Supplemental
451 Table S10). However, the brood sizes of the *kri-1(ok1251); daf-12(rh285*) and *tcer-*
452 *1(tm1425); daf-12(rh285)* double mutants were statistically indistinguishable from the
453 *daf-12(rh285)* single mutant, instead suggesting that DAF-12 acts downstream of
454 TCER-1 and KRI-1 in the same pathway (Fig. 1A, Supplemental Table S1; Fig. 5A;
455 Supplemental Table S10). Since the *daf-12* and *tcer-1* mutant strains used in our
456 experiment are not null alleles, we must interpret these epistasis results cautiously.
457 However, we can make some conclusions with respect to the phenotypes of *daf-*
458 *12(rh284)* and *daf-12(rh285)*, which have previously been characterized for
459 developmental timing and dauer formation defects (Antebi et al. 1998; 2000). The *daf-*
460 *12(rh284)* mutant (P746S lesion in helix 12 of the LBD) displays delayed gonadal
461 development while the *daf-12(rh285)* mutant (Q707stop mutation in the LBD after helix
462 9) has penetrant heterochronic phenotypes that include delayed gonadal and
463 extragonadal developmental events (Antebi et al. 1998; 2000). In addition, our brood
464 assay results show that the *rh285* allele has a more severe phenotype than *rh284* in
465 terms of reproduction (Fig. 1A; Supplemental Table S1). These phenotypes are perhaps
466 due to the differences in the nature of LBD disruption that have not been characterized.
467 Although the epistasis results could be interpreted as DAF-12 acting in parallel to KRI-1
468 and TCER-1, we favor a model in which DAF-12 acts downstream of KRI-1 and TCER-1
469 due to the greater severity of the *daf-12(rh285)* allele over the *daf-12(rh284)* allele.
470 To further investigate the developmental mechanism of steroid signaling,
471 reproductive longevity, and fatty acid metabolism pathways in the regulation of

472 reproductive plasticity, we examined if DAF-12, TCER-1, and NHR-49 play a direct role
473 in the timing of germline proliferation in postdauer larvae. We previously demonstrated
474 that wild-type PD_{Stv} animals delay the onset of germline proliferation compared to
475 control animals, resulting in a reduced brood size (Ow et al. 2018). *C. elegans* begins
476 germ cell proliferation after hatching and continues throughout larval development.
477 Germ cells at the most distal ends of the gonad undergo mitotic self-renewal while germ
478 cells most proximal to the gonad undergo meiosis (Kimble and Crittenden, 2005). In *C.*
479 *elegans* hermaphrodites, undifferentiated germ cells initiate spermatogenesis during the
480 L4 larval stage, followed by a transition to oogenesis at the adult stage (L'Hernault,
481 2006). Therefore, reproduction in the hermaphroditic *C. elegans* is sperm-limited (Byerly
482 et al. 1976; Ward and Carrel, 1979; Kimble and Ward, 1988). In previous results, when
483 control and PD_{Stv} somatic development was synchronized using vulva morphology, we
484 observed significantly fewer germ cell rows in PD_{Stv} larvae compared to control larvae,
485 correlating with fewer sperm available for self-fertilization in adulthood (Ow et al. 2018).
486 To determine if DAF-12, TCER-1, and NHR-49 play a direct role in germline
487 proliferation as a mechanism to regulate reproductive plasticity, we counted the number
488 of germ cell rows in control and PD_{Stv} mutant larvae that were developmentally
489 synchronized by their somatic morphology. Because *daf-12(rh284)* and *daf-12(rh285)*
490 have altered germline morphologies that prevent accurate synchronization, we used the
491 *daf-36(k114)* mutant as a representative of the steroid signaling pathway. First, we
492 recapitulated our previous results showing that wild-type PD_{Stv} larvae have significantly
493 fewer total germ cell rows compared to control larvae due to reduced cell rows in the
494 meiotic transition zone (Fig. 5B; Supplemental Fig. S4A). In contrast, *daf-36(k114)*

495 control and PD_{Stv} larvae did not exhibit a significant difference in total (mitotic and
496 meiotic) cell rows, indicating that DAF-36-dependent DA is required during early
497 germline development for the delay in PD_{Stv} germ cell proliferation (Fig. 5B;
498 Supplemental Fig. S4). This result is consistent with the increased brood size of PD_{Stv}
499 adults in *daf-12(rh284)* and *daf-12(rh285)* mutants, which express DAF-12 proteins
500 unable to bind to DA.

501 In contrast to the *daf-36* mutant, the *nhr-49(nr2041)* mutant exhibited a significant
502 decrease in the number of total germ cell rows in PD_{Stv} larvae relative to control larvae,
503 similar to wild-type animals (Fig. 5B). Since NHR-49 is required for the reduced fertility
504 in PD_{Stv} adults, these results are consistent with the role of NHR-49 in regulating fatty
505 acid metabolism in the intestine to support vitellogenesis. Like NHR-49, TCER-1 plays a
506 role in the intestine acting to upregulate fatty acid metabolism genes in animals lacking
507 germ cells, but also plays a role in the regulation of germ cell proliferation (Pushpa et al.
508 2013; Amrit et al. 2016). We found that a mutation in *tcer-1* resulted in a similar number
509 of total germ cell rows in control and PD_{Stv} larvae, indicating that TCER-1 plays a role in
510 PD_{Stv} reproductive plasticity by regulating germ cell proliferation in addition to its
511 intestinal role (Fig. 5B). Interestingly, *tcer-1* PD_{Stv} larvae also appear to have a defect in
512 the onset of meiosis, since PD_{Stv} larvae have significantly great number of mitotic germ
513 cell rows compared to controls (Supplemental Fig. S4). Together, these results indicate
514 that DAF-12 steroid signaling and TCER-1 act early in germline development to delay
515 the onset of germ cell proliferation in PD_{Stv} animals, while NHR-49 functions later in
516 development to promote the reduced fertility of PD_{Stv} adults.
517

518 *Generational transmission of early life starvation memory*

519 Our results suggest that PD_{Stv} upregulate their fatty acid metabolism to increase fat
520 transport to embryos. In humans, nutritional stress *in utero* not only promotes metabolic
521 syndrome in adulthood of the affected individuals, but also promotes obesity in
522 subsequent generations (Painter et al. 2008; Veenendaal et al. 2013). Therefore, to
523 examine whether the starvation-induced physiology of PD_{Stv} adults is inherited, we used
524 ORO staining to quantitate the fat storage in adult F_1 progeny of control and PD_{Stv}
525 adults that did not experience starvation themselves. Surprisingly, the adult F_1 progeny
526 of PD_{Stv} adults had an increased level of stored fat compared to F_1 progeny of control
527 adults (Fig. 6), but the difference was abolished by the F_2 generation. These results
528 indicate that the F_1 progeny of PD_{Stv} adults inherit a starvation memory that results in
529 metabolic reprogramming to increase their stored fat reserves.

530 In *C. elegans*, inheritance of gene expression states is often mediated by small
531 RNA pathways (Feng and Guang, 2013; Rechavi and Lev, 2017). The germline-specific,
532 nuclear Argonaute HRDE-1/WAGO-9 has been shown to promote transgenerational
533 silencing via the formation of heterochromatin at targeted genomic loci (Buckley et al.
534 2012; Feng and Guang, 2013; Rechavi and Lev, 2017). In particular, starvation-induced
535 L1 diapause has been shown to alter the physiology and small RNA populations of
536 subsequent generations, some of which is dependent upon HRDE-1 (Rechavi et al.
537 2014). We examined whether HRDE-1 was required for the generational inheritance of
538 starvation memory by quantifying fat storage in *hrde-1(tm1200)* mutant control and
539 PD_{Stv} adults and their F_1 and F_2 progeny. First, we observed that the stored fat levels
540 between *hrde-1* control and PD_{Stv} adults were similar, suggesting that the germline-

541 specific Argonaute is required for decreased intestinal fat storage of PD_{Stv} adults (Fig.
542 6). In addition, the ORO staining of the F_1 progeny of control and PD_{Stv} adults was also
543 similar, indicating that HRDE-1 is required for the generational inheritance of starvation
544 memory (Fig. 6). Remarkably, in the F_2 generation, the grand-progeny of *hrde*-1 PD_{Stv}
545 mutants displayed increased ORO staining like that observed for the F_1 generation of
546 wild-type adults, suggestive of a generational delay in the physiological response to
547 ancestral early-life starvation upon the loss of HRDE-1 activity (Fig. 6). Our genetic
548 evidence suggests that the germline nuclear RNAi pathway plays two roles: it
549 contributes to the metabolic programming of individuals that experienced early life
550 starvation as well as promote the generational inheritance of starvation memory in the
551 progeny. Our results show that *C. elegans*, like humans, inherit the disposition for
552 increased adiposity from parents that experienced early life starvation.
553

554 **Discussion**

555 The trade-off between reproduction and longevity has long been associated with
556 the notion that in the absence of reproduction, the fat stores of an animal would be
557 redistributed to promote somatic maintenance (Williams 1966; Kirkwood 1977;
558 Westendorp and Kirkwood, 1998; Partridge et al. 2005). As such, reducing or
559 suppressing reproduction in animals often extends lifespan, in part due to enhanced
560 lipogenic processes (Fowler and Partridge 1989; Tatar et al. 2001; Kenyon 2010,
561 2010b; Judd et al. 2011). What remains unclear is how reproduction and somatic
562 maintenance is balanced by developmental signals. In this study, we show that
563 starvation early in the life of a *C. elegans* animal with an intact reproductive system

564 programs its transcriptome to promote reproduction. Because dauers are non-feeding,
565 starvation-induced dauers have limited stored fat to survive the diapause stage and
566 remodel out of dauer. We propose a model whereby steroid signaling, reproductive
567 longevity, and fatty acid metabolic pathways are reprogrammed in animals that
568 experienced starvation-induced dauer in order to delay the onset of germline
569 proliferation and redistribute intestinal fat to developing oocytes (Fig. 7). These changes
570 allow PD_{Stv} adults to delay reproduction until energy thresholds are met to provide
571 adequate levels of nutrition to fewer but albeit viable embryos at the expense of somatic
572 survival and maintenance. In the absence of a functional germ line, the fat that would
573 normally be allocated to the progeny, is channeled to nurture somatic tissues and
574 consequently extend adult lifespan. Thus, the mechanisms that prolong lifespan in the
575 absence of a functional germ line, are tantamount to the cellular programs deployed to
576 respond to developmental signals triggered by starvation early in life history in an
577 animal capable of reproduction.

578 The extended lifespan associated with animals missing a functional germ line is
579 specifically dependent on the lack of proliferating germ cells and not due to sterility
580 resulting from sperm, oocyte, or meiotic precursor cell deficiency (Hsin and Kenyon,
581 1999; Arantes-Oliveira et al. 2002). Animals without a functional germ line have up-
582 regulated fat metabolism pathways and exhibit increased levels of intestinal fat stores
583 that are associated with a longer lifespan (Kenyon 2010, 2010b; Amrit et al. 2016).
584 Postdauer adults that experienced early life starvation have a delay in the onset of germ
585 cell proliferation after dauer exit and similarly have upregulated fat metabolism
586 pathways in adulthood. These results indicate that the cellular mechanisms that

587 promote germline proliferation during a critical period in development also negatively
588 regulate lipid metabolism, leading to altered somatic fat accumulation and longevity
589 (Wang et al. 2008). While a number of genes and cellular components affecting
590 germline proliferation have been extensively investigated (Kimble and Crittenden,
591 2005), what might the actual signals communicating the state of proliferating germ cells
592 be that arbitrate lipid levels and the aging process? Because upregulation of DAF-36
593 and Δ^7-DA is crucial for aging extension in germline-less animals and reproductive
594 plasticity in PD_{Stv} adults, it is likely that the signal communicating the state of germ cell
595 proliferation may include dafachronic acids. Dafachronic acids mediating increased
596 longevity are produced in the somatic gonad, which includes the stem cell niche site of
597 the germ line (Yamawaki et al. 2010). Based on our results, DA is required for the delay
598 in germline proliferation (Fig. 5B), but DA-dependent DAF-12 activity is not necessary
599 for the increase in fatty acid metabolism observed in PD_{Stv} adults (Fig. 4A, 4B),
600 suggesting the possibility that dafachronic acids act in a DAF-12-independent manner to
601 regulate germ cell proliferation and fat metabolism. In addition, our results showing that
602 *hrde-1* is required for the altered fat metabolism in PD_{Stv} adults (Fig. 6) raise the
603 possibility that small RNAs could be another type of signaling molecule that
604 communicates the state of the germ line to the soma. Small RNA pathways are potent
605 surveillance mechanisms that guard against the threat of foreign genetic invaders such
606 as viruses and transposons that can compromise germline integrity (Almeida et al.
607 2019). Because germ line proliferation occurs throughout the lifetime of *C. elegans*, it is
608 likely that small RNA pathways survey the proliferative nature of the germ line at all

609 stages of animal development and potentially act as signaling molecules to regulate
610 somatic processes dependent upon the germ line.

611 One of the most intriguing findings of this study is that the starvation memory of
612 the parent is “bequeathed” to the F_1 progeny, triggering elevated levels of fat stores,
613 presumably as a physiological defense against ancestral famine (Fig. 6). Interestingly,
614 the increased intestinal fat inherited by the F_1 progeny of wild-type postdauer parents
615 bypassed the F_1 generation in the *hrde-1* mutant and was only evident in the F_2
616 generation (Fig. 6). One potential explanation is that small RNA signals are transmitted
617 to the F_1 generation via the HRDE-1 nuclear RNAi pathway to effect somatic
618 phenotypes. However, with the exception of DAF-16, none of the germline longevity
619 pathway genes or the vitellogenesis genes examined in this study are categorized as
620 direct HRDE-1 targets (Buckley et al. 2012). Given that the life stage (adulthood) at
621 which the HRDE-1 targets were identified is the same life stage that were used in this
622 study, we speculate that HRDE-1 may be indirectly targeting endocrine and
623 vitellogenins genes by: 1) targeting germ line genes that then affect somatic gene
624 expression; or 2) indirectly regulating the function of the endocrine and vitellogenin
625 genes by targeting a different repertoire of somatic targets. Interestingly, we find that
626 62% of small RNAs associated with HRDE-1 are target genes (984 out of 1587)
627 expressed in somatic tissues (neuronal, intestine, hypodermis, and muscle), while 31%
628 of HRDE-1 targets (486 out of 1587) are germline genes; and of these, 17% uniquely
629 overlap between HRDE-1 targets (272 out of 1587) and germline genes only (Ortiz et al.
630 2014; Kaletsky et al. 2016). While silencing somatic genes in the germ line is consistent
631 with the described function of HRDE-1, our results raise the possibility that HRDE-1

632 may also directly, or indirectly, target somatic genes. Accordingly, HRDE-1 is known to
633 contribute to the heritability of a cohort of small RNAs targeting nutrition and lipid
634 transporter genes that was inherited for at least three generations from populations that
635 experienced L1 larval arrest (Rechavi et al. 2014). In addition, HRDE-1 is required for
636 the repression of a group of genes activated upon multi-generational high temperature
637 stress that is inherited for at least two generations in the absence of the stress (Ni et al.
638 2016). Thus, it is not unlikely that HRDE-1 and the nuclear RNAi pathway could serve
639 as a signaling referees between the soma and the germ line to effect changes due to
640 environmental and developmental signals to perdure ancestral starvation memory.

641 Our study showed that PD_{Stv} adults have upregulated expression of lipid
642 metabolism genes as a means to load embryos with increased fat and potentially
643 protect progeny against future starvation conditions. Not only are fatty acids critical for
644 cell and organelle maintenance and act as efficient energy storage molecules but they
645 can regulate aging by acting as signaling molecules (e.g. as nuclear hormone receptor
646 ligands). Indeed, the lipid profiles in a number of species change as animals age
647 resulting in the acceleration of cell and tissue damage such that levels of certain lipids
648 can serve as aging biomarkers (Papsdorf and Brunet, 2019). Also, studies have
649 reported that fatty acids can alter animal physiology through the modification of
650 chromatin states either by the direct addition of lipid moieties to chromatin or by
651 activating signaling pathways that affect chromatin landscapes (Hansen et al. 2013;
652 Papsdorf and Brunet, 2019). Furthermore, MUFAs have been shown to be the link
653 between H3K4me3 modifiers and lifespan in *C. elegans*, while the inheritance of
654 epigenetic memory through soma-germline crosstalk in response to cellular stresses are

655 associated with the H3K4me3 modification complex (Tauffenberger and Parker, 2014;
656 Kishimoto et al. 2016; Han et al. 2017). Altogether, these studies provide an exciting
657 groundwork to examine how the chromatin states in PD_{Stv} animals correlate with
658 metabolic plasticity due to early life starvation across generations.

659 During the course of its natural history, *C. elegans* occupies ephemeral
660 environments such as rotting fruit or decomposing vegetation, where conditions and
661 food availability are highly unpredictable. The dauer larvae affords *C. elegans* a survival
662 and dispersal strategy to escape harsh environmental conditions by often associating
663 with passing invertebrate carriers. Accordingly, *C. elegans* is often found in the wild as
664 dauers. Once a food source is found, dauers resume reproductive development to
665 colonize the new habitat. Upon exhaustion of resources and population expansion,
666 young larvae enter dauer and thereby repeating the boom and bust life cycle
667 (Schulenburg and Félix, 2017). Because of frequent environmental perturbations, an
668 adopted phenotypic plasticity strategy would ensure an advantage in species survival.
669 The generation following a bust period would inherit the cellular programs for increased
670 somatic lipid stores. It is thus remarkable that the cellular mechanisms to ensure
671 survival of the species are fundamentally similar between humans and nematodes, two
672 species that have diverged hundreds of millions of years ago, once again underscoring
673 the relevance of a simple roundworm in understanding basic animal physiology.
674

675 **Materials and methods**

676 C. elegans *strains and husbandry*

677 N2 Bristol wild-type strain was used as the reference strain. Worms were grown in
678 Nematode Growth Medium (NGM) seeded with *Escherichia coli* OP50 using standard
679 methods at 20°C unless otherwise indicated (Brenner et al. 1974; Stiernagle 2006).
680 Mutants that were not previously backcrossed were backcrossed at least four times to
681 our laboratory N2 wild-type before use. Strains used in this study are listed under
682 Supplemental Material and Methods (Supplemental Table S14).
683
684 *Transgenic strains*
685 Single copy insertions, *pdrSi1* (*Pglp-4::glp-4 cDNA::gfp::glp-4 3'UTR*), *pdrSi2* (*Pnhx-*
686 *2::glp-4 cDNA::gfp::glp-4 3'UTR*), *pdrSi3* (*Pzfp-2 ::glp-4 cDNA::gfp::glp-4 3'UTR*), and
687 *pdrSi4* (*Ppgl-1::glp-4 cDNA::gfp::glp-4 3'UTR*), were generated by Mos1-mediated
688 single copy insertion (MosSCI) (Frokjaer-Jensen et al. 2008). Information on plasmid
689 construction are provided in Supplemental Material and Methods. Primers sequences
690 are listed in Supplemental Table S15. Following integration into *unc-119(ed3)* animals
691 segregated from EG6699 [*ttTi5605 II; unc-119(ed3) III; oxEx1578* (*eft-3p::gfp* + *Cbr-unc-*
692 *119*)] that have lost the *oxEx1578* array, MosSCI insertions were genetically crossed
693 into the *glp-4(bn2)* background.
694
695 *Brood assays*
696 Brood assays were done as previously described using at least three biological
697 independent replicates (Ow et al. 2018). Statistical significance was determined using
698 Prism v.8 (GraphPad Software; La Jolla, California, USA).
699

700 *Oil Red O staining*

701 Fat stores were stained using Oil Red O (ORO) dye as previously described (O'Rourke
702 et al. 2009). Images were captured with either a Nikon Eclipse Ci with Spot 5.2 software
703 or with an iPhone through iDu Optics® equipped with a LabCam® adapter (New York,
704 NY, USA). Color images were separated into their RGB channel components and the
705 intensity measured on the green channel (Yen et al. 2010) using ImageJ (NIH).

706

707 *RNA interference*

708 Gravid adults were treated with hypochlorite to obtain embryos using standard methods
709 (Stiernagle 2006). Embryos were placed on NGM plates supplemented with 1mM IPTG
710 and 50 μg/ml carbenicillin seeded with a 10x concentrated bacterial culture expressing
711 the *k09f5.2* (*vit-1*) RNAi clone obtained from the Ahringer library (Kamath et al. 2001).
712 Embryos were allowed to grow until adulthood at which time they were treated again
713 with hypochlorite to obtain embryos. The recovered embryos were grown until day one
714 of adulthood and collected for ORO staining.

715

716 *Dafachronic acid supplementation*

717 The Δ^7 form of dafachronic acid (Δ^7-DA) (a kind gift from Frank Schroeder) was added
718 to a freshly grown culture of *E. coli* OP50 at a concentration of 40 nM and immediately
719 seeded onto NGM plates. NGM plates supplemented with an equivalent volume of
720 ethanol (the Δ^7-DA solvent) to those of the Δ^7-DA-supplemented NGM plates were used
721 as the control plates. Seeded plates were allowed to dry overnight before use.

722

723 *Oleic acid supplementation*

724 Animals were induced into dauer by starvation on peptone-less NGM plates seeded
725 with *E. coli* OP50 pre-loaded with oleic acid (NuChek Prep, Inc.; Elysian, Minnesota,
726 USA) as described by Devkota et al. 2017. Briefly, OP50 was grown overnight at 37°C
727 in liquid LB supplemented with 600 μM of oleic acid or with an equivalent volume of
728 ethanol (the oleic acid solvent) to serve as the control. Cultures were pelleted and
729 washed several times with M9 buffer (Stiernagle, 2006) and resuspended at a 10x
730 concentration. The 10x OP50 was seeded onto peptone-less NGM plates and allowed
731 to dry overnight before use. Brood assays were performed as described before (Ow et
732 al. 2018).

733

734 *Germ cell rows*

735 All worm strains used for germ cell row counting have the integrated transgene *gld-*
736 *1*::*gfp*::*3xFLAG* from the BS1080 strain in their genetic background. Germ cell row
737 assays were done as previously described (Ow et al. 2018). Worms with L3 vulva
738 morphology were identified by using Nomarski DIC microscopy at 630x (Seydoux et al.
739 1993) and DAPI stained using the standard whole worm DAPI staining protocol. The
740 stained worms were imaged using a Leica DM5500B microscope with a Hamamatsu
741 camera controller C10600 ORCA-R2. When performing the germ cell row counts, the
742 start of the transition was identified when at least two cells in a row exhibited the
743 crescent-shaped nuclei morphology (Shakes et al. 2009).

744

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Competing interests

The authors declare no competing interests.

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1058 **Figure legends**

1059 **Figure 1.** Adult reproductive plasticity is dependent on steroid signaling. (*A*) Brood
1060 size comparison of PD_{Stv} relative to CON_{Stv} in wild-type N2, *daf-12(rh284)*, *daf-*
1061 *12(rh285)*, *daf-9(rh50)*, *daf-36(k114)*, *hsd-1(mg433)*, and *nhr-8(ok186)* strains. Assays
1062 were performed at 20°C from at least three biologically independent trials. Significance
1063 asterisks (** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$; Student's *t*-test) refer to the
1064 comparison of PD_{Stv} to CON_{Stv} of a given genotype. Significance ampersands ($^{\&\&\&}$ $p <$
1065 0.001, $^{\&\&\&\&}$ $p < 0.0001$; one-way ANOVA with Fisher's LSD test) refer to the comparison
1066 of PD_{Stv}/CON_{Stv} brood size change between a given mutant genotype to wild-type N2.
1067 Additional brood size data is provided in Supplemental Table S1. (*B*) Brood size
1068 comparison of PD_{Stv} grown in the presence of 40 nM Δ^7-dafachronic acid (Δ^7-DA)
1069 relative to the brood size of PD_{Stv} grown in the absence of Δ^7-DA of wild-type N2, *daf-*
1070 *9(rh50)*, and *daf-36(k114)* strains. Assays were conducted at 20°C from four biologically
1071 independent trials. **** $p < 0.0001$ (Student's *t*-test) indicates the comparison between
1072 PD_{Stv} with Δ^7-DA to PD_{Stv} without Δ^7-DA for *daf-9(rh50)* adults. $^{\&}$ $p < 0.05$ (one-way
1073 ANOVA with Fisher's LSD test) refers to the comparison of brood size fold change
1074 (PD_{Stv} with Δ^7-DA to PD_{Stv} without Δ^7-DA) between *daf-9(rh50)* relative to wild-type N2.
1075 Additional brood size data can be found in Supplemental Table S2.

1076

1077 **Figure 2.** DAF-16 and TCER-1 modulation of fatty acid metabolism is important for
1078 adult reproductive plasticity. (*A*) Venn diagram depicting overlap of DAF-16 and PQM-1
1079 targets (Tepper et al. 2013) with differentially expressed N2 PD_{Stv} genes compared to
1080 CON_{Stv} adults (Ow et al. 2018). See Supplemental Table S3. (*B*) Brood size comparison

1081 of PD_{Stv} relative to CON_{Stv} in wild-type N2, *daf-18(e1375)*, *pqm-1(ok485)*, *daf-16(mu86);*
1082 *daf-16a^{AM}::gfp*, *smk-1(mn156)*, *kri-1(ok1251)*, and *tcer-1(tm1425*) strains. Assays were
1083 performed at 20°C from at least three biologically independent replicates. ** $p < 0.01$, ***
1084 $p < 0.001$, **** $p < 0.0001$ (Student's *t*-test) refer to the comparison of PD_{Stv} brood size
1085 relative to CON_{Stv} of a given genotype. & $p < 0.05$, &&&& $p < 0.0001$ (one-way ANOVA
1086 with Fisher's LSD test) indicate the comparison of the PD_{Stv}/CON_{Stv} brood size change
1087 between a given mutant genotype to wild-type N2. Additional brood size data is
1088 provided in Supplemental Table S4. (*C*) Venn diagram comparing DAF-16 and TCER-1
1089 targets with functions in lipid metabolism from germ line-less *glp-1(e2141ts)* animals
1090 (Amrit et al. 2016) with differentially expressed N2 PD_{Stv} genes compared to CON_{Stv}
1091 adults (Ow et al. 2018). See Supplemental Table S5.
1092
1093 **Figure 3.** NHR-49, SBP-1, and FAT-6 modulate adult reproductive plasticity. (*A*)
1094 Brood size comparison of PD_{Stv} relative to CON_{Stv} for wild-type N2, *nhr-49(nr2041)*, *nhr-*
1095 *49(ok2165)*, *nhr-49(gk405)*, *nhr-49(et8)*, *nhr-49(et7)*, *nhr-49(et13)*, *nhr-80(tm1011)*, *nhr-*
1096 *13(gk796)*, *nhr-13(gk796)*, *nhr-66(ok940)*, *mdt-15(tm2182)*, and *sbp-1(ep79)* strains.
1097 Additional brood size data is provided in Supplemental Table S6. (*B*) Brood size
1098 comparison of PD_{Stv} relative to CON_{Stv} for wild-type N2, *nhr-49(nr2041); nhr-*
1099 *80(tm1011)*, *nhr-80(tm1011)*; *nhr-13(gk796)*, *nhr-49(nr2041)*; *nhr-13(gk796)*, *nhr-*
1100 *49(nr2041); nhr-80(tm1011)*; *nhr-13(gk796)*, and *nhr-49(nr2041)*; *nhr-66(ok940)* strains.
1101 Additional brood size data is provided in Supplemental Table S7. (*C*) Brood size
1102 comparison of PD_{Stv} relative to CON_{Stv} for wild-type N2, *fat-6(tm331)*, *fat-5(tm420)*, *fat-*
1103 *7(wa36)*, *fat-2(wa17)*, *fat-6(tm331)*; *fat-5(tm420)*, *fat-7(wa36)*; *fat-5(tm420)*, and *fat-*

1104 *6(tm420)*; *fat-7(wa36)* strains. Additional brood size data is provided in Supplemental
1105 Table S8. (*D*) Brood size of PD_{Stv} wild-type N2, *nhr-49(nr2041)*, *nhr-49(gk405)*, and *nhr-*
1106 *49(nr2041); nhr-80(tm1011)* animals fed with *E. coli* OP50 pre-loaded with or without
1107 600 μM of oleic acid (OA). Additional brood size data is provided in Supplemental Table
1108 S9. All assays were performed at 20°C from at least three biologically independent
1109 replicates. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ (Student's *t*-test)
1110 indicate brood size comparison within a given genotype. $^{\&}$ $p < 0.05$, $^{\&\&\&\&}$ $p < 0.0001$ and
1111 n.s. (no significance) (one-way ANOVA with Fisher's LSD test) indicates the comparison
1112 of PD_{Stv}/CON_{Stv} brood size change between a given genotype to wild-type N2.
1113

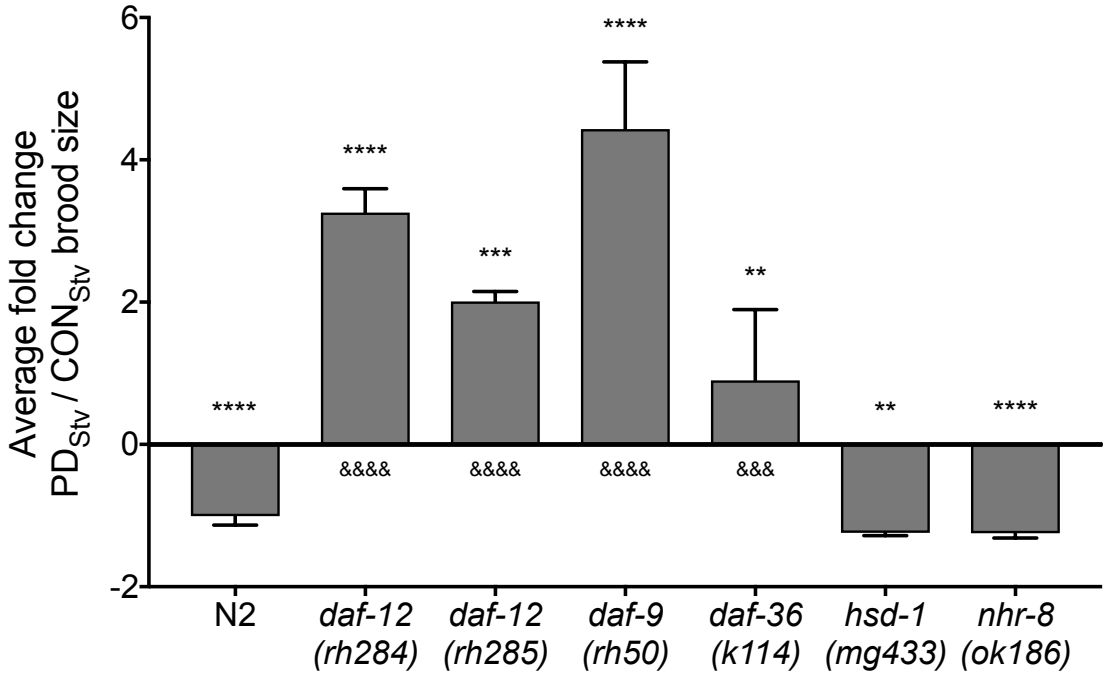
1114 **Figure 4.** Fat stores are reduced in wild-type postdauer adults that experienced
1115 starvation-induced dauer. (*A*) Neutral lipid staining with Oil Red O (ORO) in CON_{Stv} and
1116 PD_{Stv} wild-type N2, *daf-12(rh284)*, *daf-12(rh285)*, and *tcer-1(tm1452)* one-day-old adults
1117 grown at 20°C. **** $p < 0.0001$; Student's *t*-test comparison between CON_{Stv} and PD_{Stv}
1118 of the same genotype. $^{\&\&\&\&}$ $p < 0.0001$; one-way ANOVA with Fisher's LSD test
1119 between wild-type N2 CON_{Stv} and mutant CON_{Stv}. $^{\$\$\$\$}$ $p < 0.0001$; one-way ANOVA
1120 between wild-type N2 PD_{Stv} and mutant PD_{Stv}. n.s. indicates no significance between
1121 wild-type N2 PD_{Stv} and mutant PD_{Stv} (one-way ANOVA with Fisher's LSD test). Total
1122 sample size is indicated by *n* from at least 3 biologically independent trials. (*B*)
1123 Representative micrographs of one-day-old adults stained with ORO. *(C)* ORO staining
1124 of CON_{Stv} and PD_{Stv} wild-type N2 fed with empty RNAi vector or *vit-1* RNAi, which
1125 knocks down *vit-1*, *2*, *3*, *4,* and *5* (see Results). **** $p < 0.0001$; Student's *t*-test
1126 comparison between CON_{Stv} and PD_{Stv} of the same RNAi. $^{\&\&}$ $p = 0.0038$ and $^{\&\&\&\&}$ $p <$

1127 0.0001 (Student's *t*-test) denote comparison between *vit-1* RNAi to empty RNAi vector
1128 control of the same condition. *(D)* Representative micrographs of ORO staining of (C)
1129 showing whole worms and their embryos.
1130
1131 **Figure 5.** DAF-12 acts downstream of KRI-1 and TCER-1 to regulate adult
1132 reproductive plasticity. (*A*) Brood size comparison of PD_{Stv} relative to CON_{Stv} for wild-
1133 type N2, *kri-1(ok1251); daf-12(rh284)*, *tcer-1(tm1425); dat-12(rh284)*, *kri-1(ok1251); daf-*
1134 *12(rh285)*, and *tcer-1(tm1425); dat-12(rh285)*. * $p < 0.05$; *** $p < 0.001$, **** $p < 0.0001$
1135 (Student's *t*-test) indicates the brood size comparison between PD_{Stv} relative to CON_{Stv}
1136 of a given genotype. $^{\&\&\&\&}$ $p < 0.0001$ (one-way ANOVA with Fisher's LSD test) indicates
1137 the comparison of PD_{Stv}/CON_{Stv} brood size change between a given genotype to wild-
1138 type N2. Assays were performed at 20°C from at least three independent biological
1139 replicates. Additional brood size data is provided in Supplemental Table S10. (*B*) Total
1140 germ cell rows in CON_{Stv} and PD_{Stv} wild-type N2, *daf-36(k114), nhr-49(nr2041),* and *tcer-*
1141 *1(tm1452)* larva exhibiting L3 vulva morphology (see Materials and methods). ** $p <$
1142 0.01; **** $p < 0.0001$ (Student's *t*-test) indicate comparison between CON_{Stv} and PD_{Stv}
1143 within a genotype. Total sample size is indicated by *n* from at least three biologically
1144 independent trials.
1145
1146 **Figure 6.** Transgenerational inheritance of starvation memory. *(A)* ORO lipid
1147 staining of wild-type N2 and *hrde-1(tm1200)* control and postdauer P_0, F_1, and F_2
1148 generations. Only the P_0 PD_{Stv} generation experienced starvation during early
1149 development. **** $p < 0.0001$; Student's *t*-test comparison between CON_{Stv} and PD_{Stv} of

1150 the same genotype and generation. n.s. indicates no significance. Assays were
1151 performed at 20°C from three independent biological replicates. Total sample size is
1152 indicated by *n*. (*B*) Representative micrographs of N2 and *hrde-1(tm1200)* CON_{Stv} and
1153 PD_{Stv} P_0, F_1, and F_2 worms stained with ORO.
1154
1155 **Figure 7.** Model depicting genetic pathways acting as contributors to fatty acid
1156 metabolism of postdauer animals that experienced early life starvation. Signaling
1157 between the soma and the germ line establishing metabolic plasticity and the
1158 transmission of ancestral starvation memory are dependent on HRDE-1.

Ow et al. Figure 1

A



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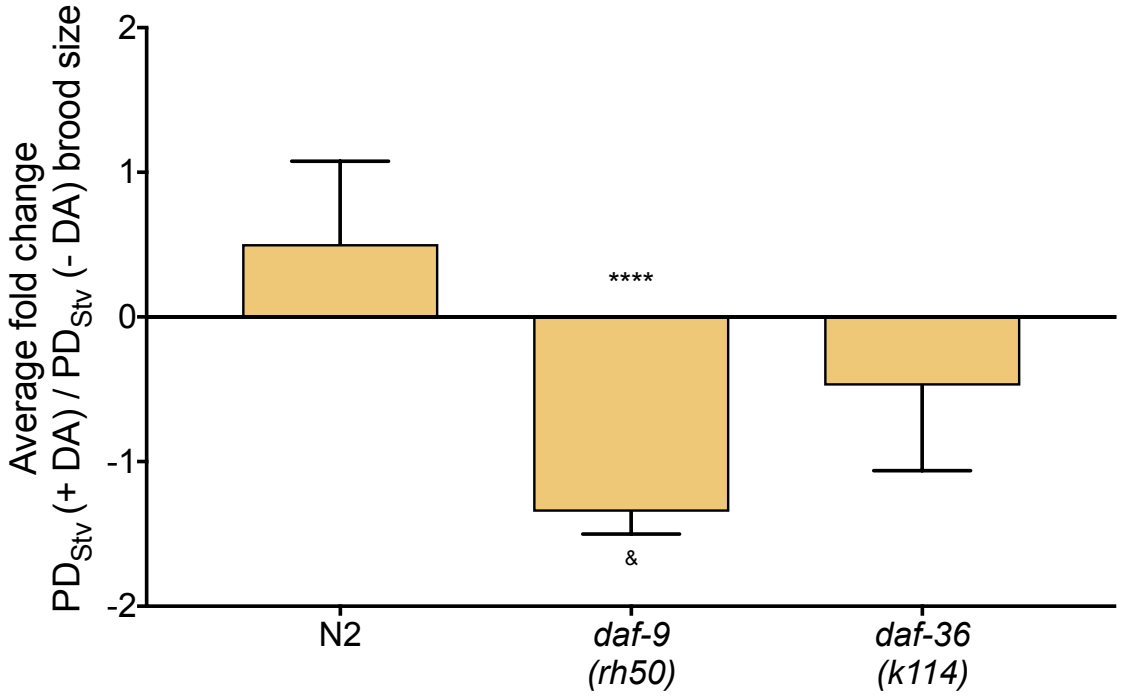

Ow et al. Figure 2

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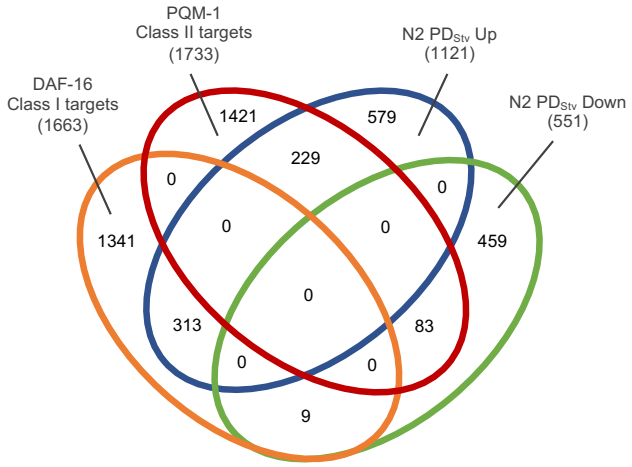

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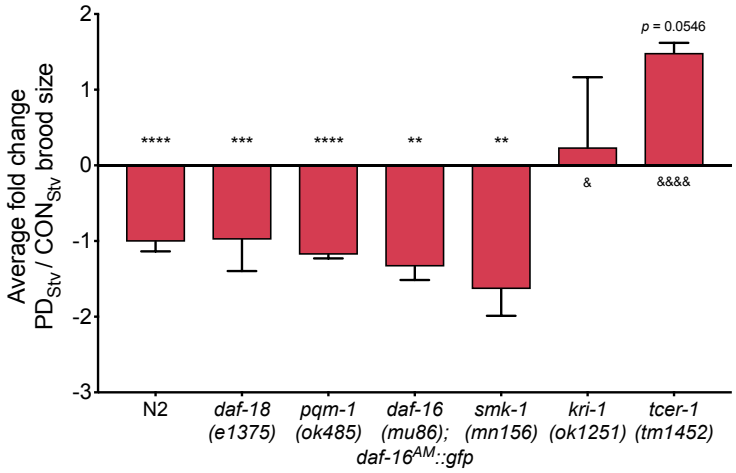

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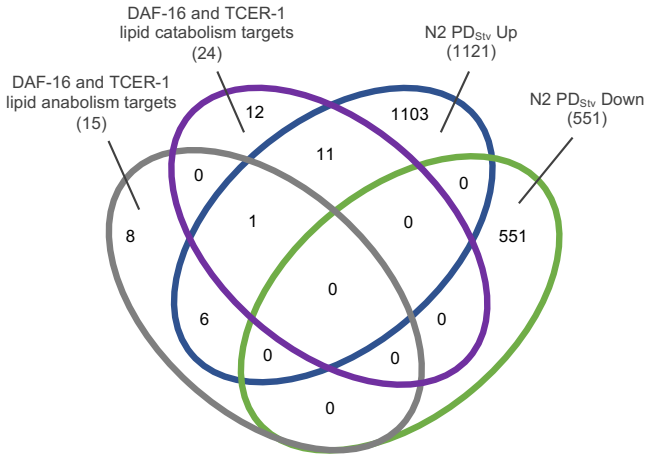

Ow et al. Figure 3

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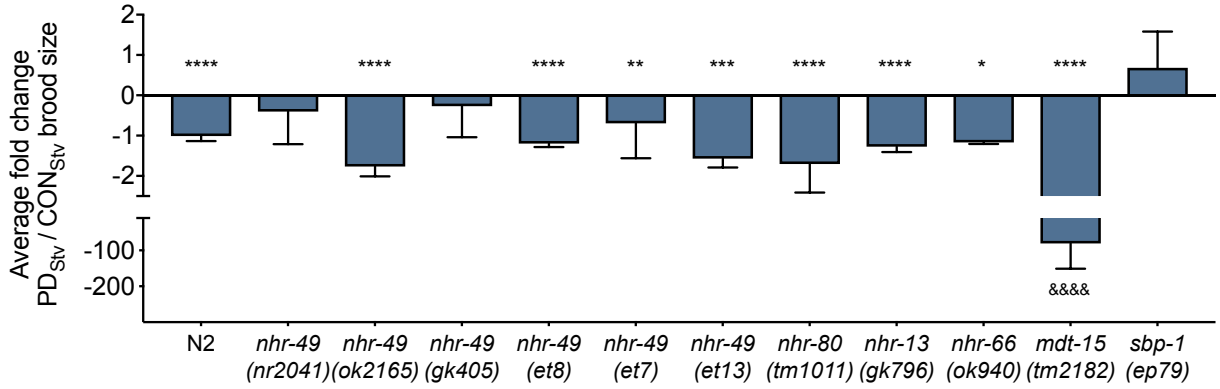

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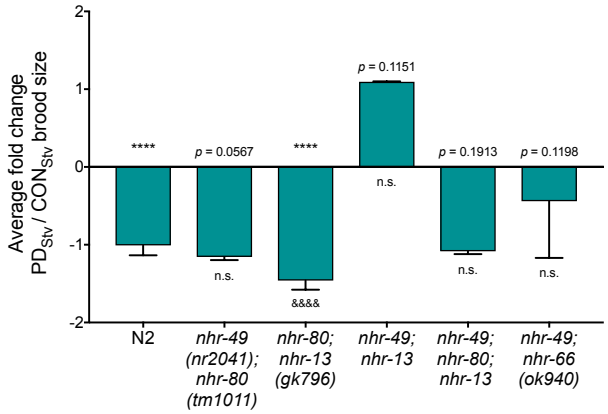

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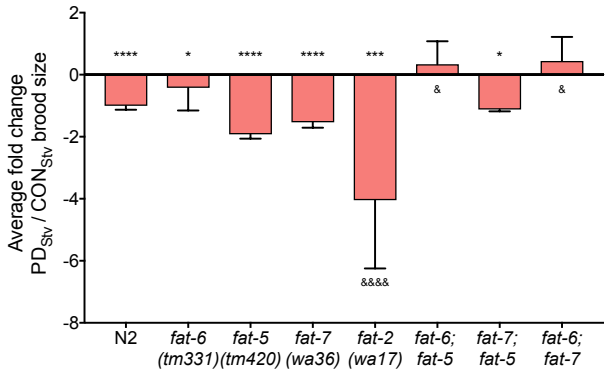

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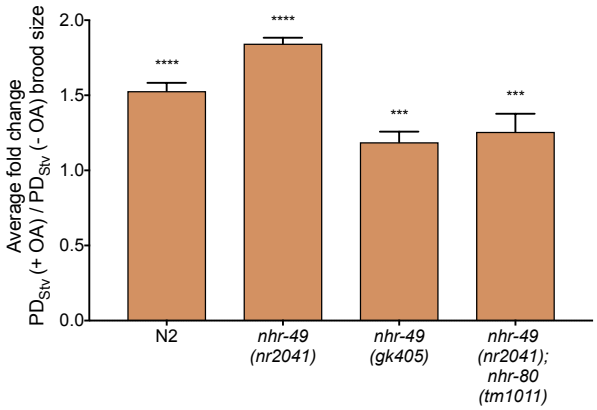

Ow et al. Figure 4

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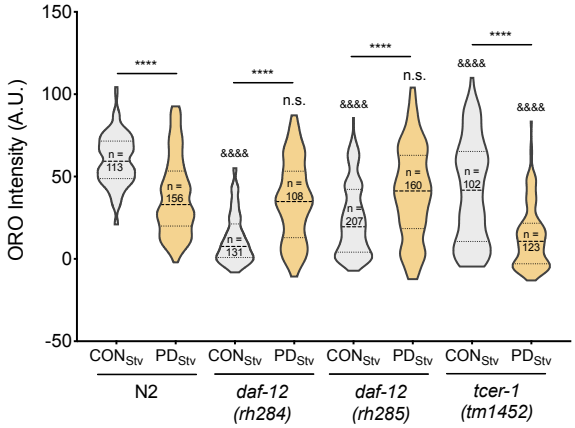

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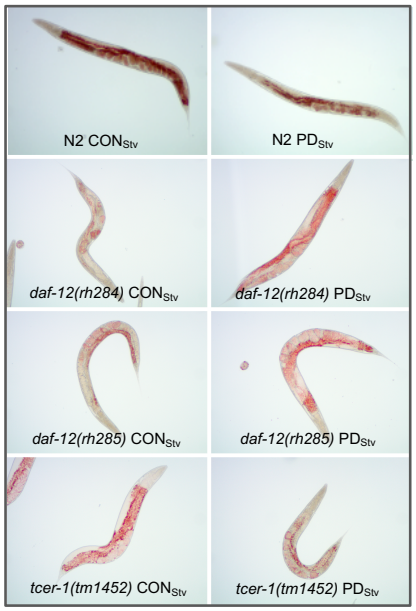

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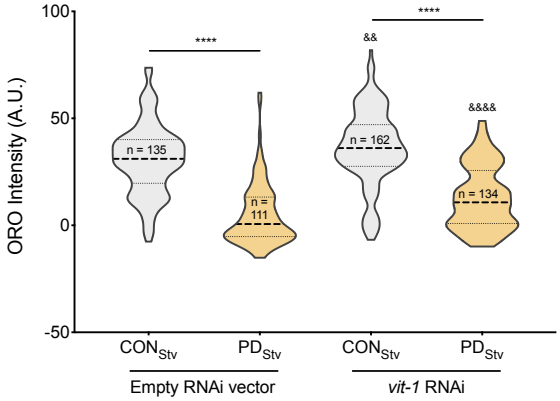

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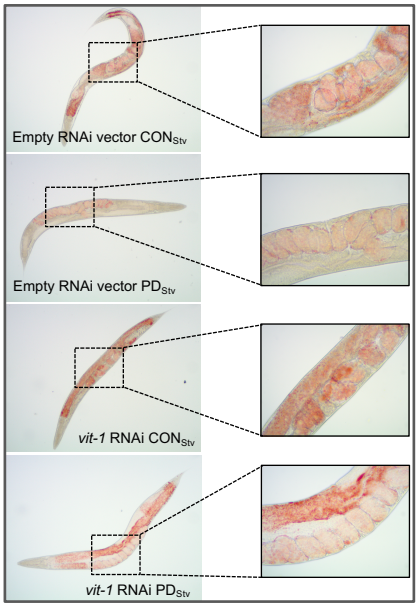

Ow et al. Figure 5

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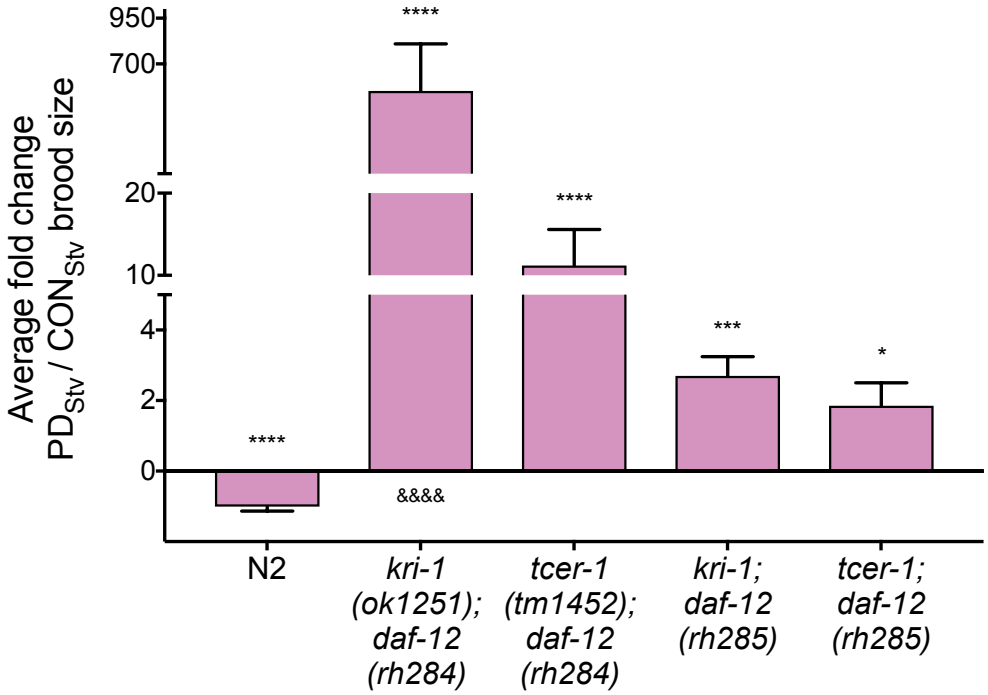

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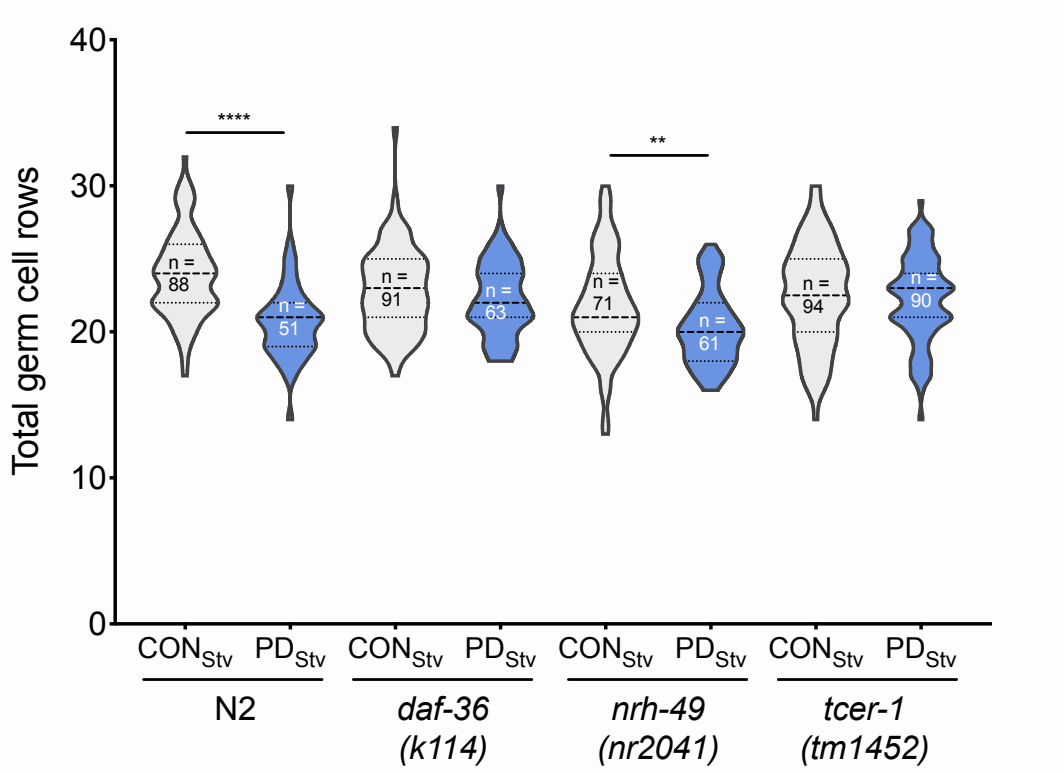

Ow et al. Figure 6

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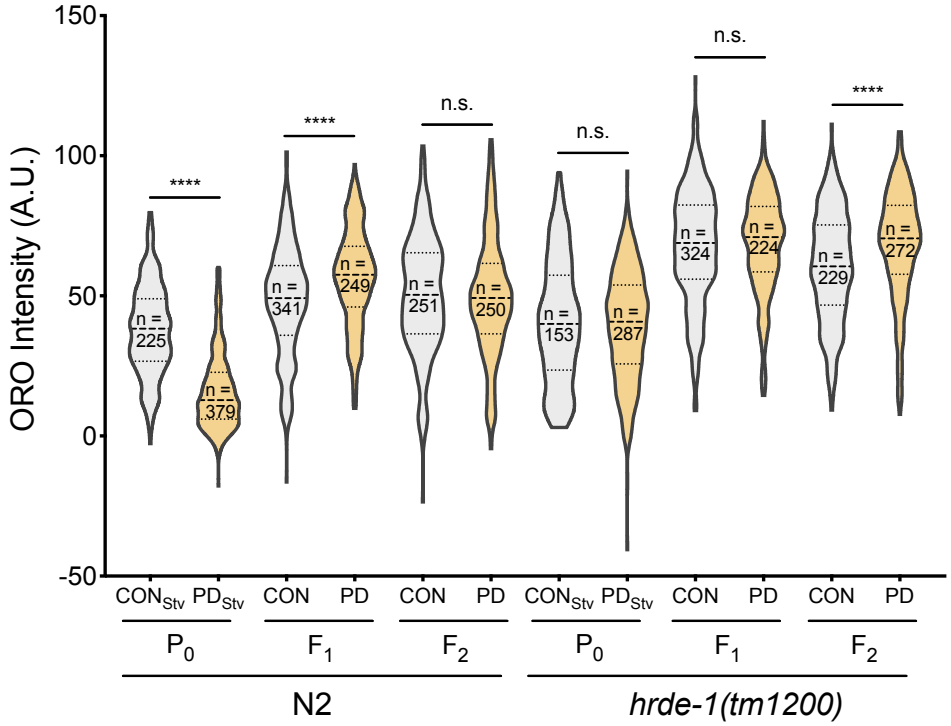

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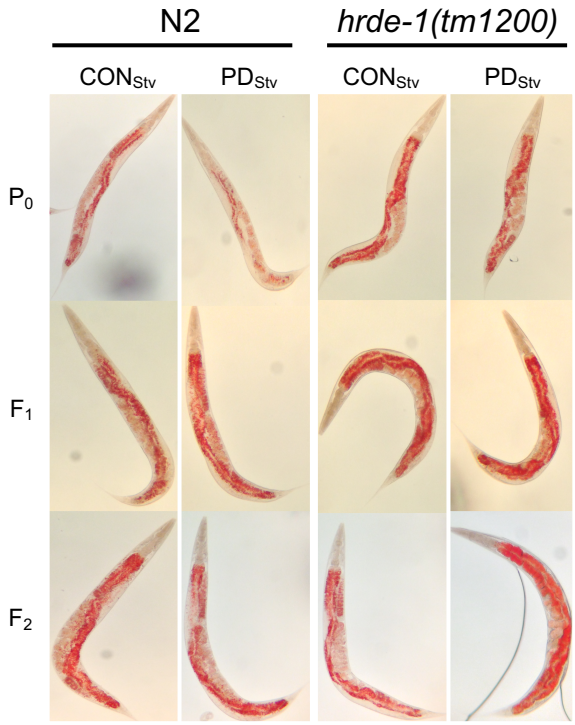
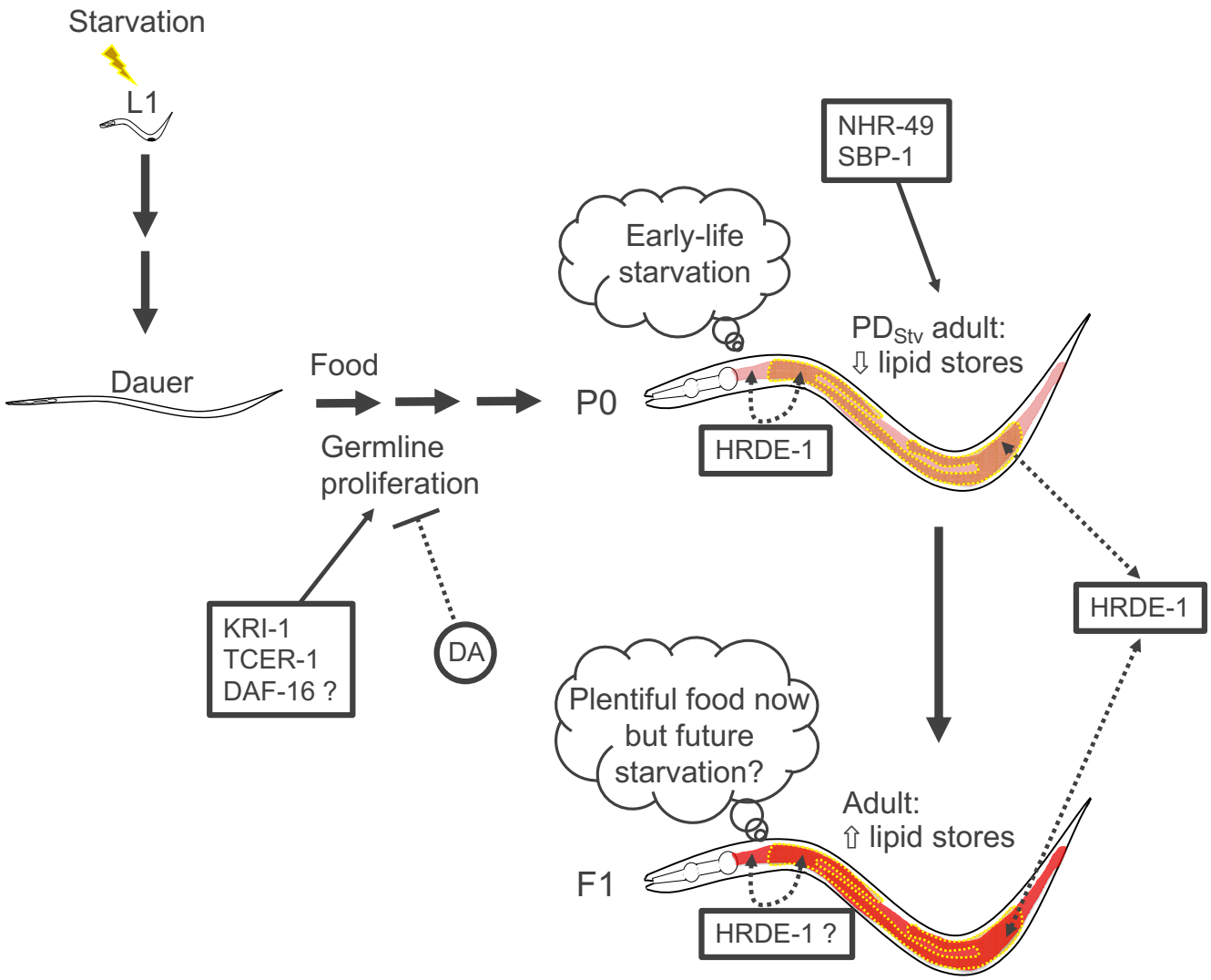
Ow et al. Figure 7