Dynein light chain DLC-1 facilitates the function of GLD-1 germline cell fate regulator in *C. elegans*

Supplemental Materials

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Including: Supplemental Tables 1 and 2, Supplemental Note, Supplemental Figures S1-S5

Supplemental Table 1. List of strains used in this study.

Genotype	Transgene description	Strain	Reference
Transgenes: GFP::H2B::3'UTR			
unc-119(ed3) axIs1649 III	*pie-1* prom::GFP::H2B::*cye-1* 3'UTR + *unc-119(+)*	JH2261	Merritt et al., 2008
unc-119(ed3) III; axIs1668	*pie-1* prom::GFP::H2B::*spn-4* 3'UTR + *unc-119(+)*	JH2311	Merritt et al., 2008
unc-119(ed3)III; axIs1713	*pie-1* prom::GFP::H2B::*mes-3* 3'UTR + *unc-119(+)*	JH2377	Merritt et al., 2008
unc-119(ed3) III; axIs1688	*pie-1* prom::GFP::H2B::*mex-3* 3'UTR + *unc-119(+)*	JH2333	Merritt et al., 2008
unc-119(ed3) III; axIs1721	*pie-1* prom::GFP::H2B::*puf-5* 3'UTR + *unc-119(+)*	JH2418	Merritt et al., 2008
unc-119(ed3) III; rrrIs1	*pie-1* prom::GFP::H2B::*cye-1* 3'UTR + *unc119(+)*	RAF1	Biedermann et al., 2009
dhc-1(js121) I/hT2 I; axIs1649/hT2 III	*pie-1* prom::GFP::H2B::*cye-1* 3'UTR + *unc-119(+)*	UMT377	this study
Transgenes: ORF+3'UTR			
gld-1(q485) I; mntSi19 [pME5.13;GLD-1 wt] II	*gld-1* prom::GLD-1^{wt}::OLLAS::*gld-1* 3'UTR	UMT327	this study
gld-1(q485) I/hT2 (I;III); mntSi18 [pME5.17; GLD-1 ndb mutant] V	*gld-1* prom::GLD-1^{ndb}::OLLAS::*gld-1* 3'UTR	UMT314	this study
gfp::3xflag::mex-3 gld-1(q495) I; mntSi19 II	*gld-1* prom::GLD-1^{wt}::OLLAS::*gld-1* 3'UTR	UMT384	this study
gfp::3xflag::mex-3 gld-1(q495) I/hT2 (I;III); mntSi18 V	*gld-1* prom::GLD-1^{ndb}::OLLAS::*gld-1* 3'UTR	UMT374	this study
CRISPR tagged strains			
spn-4(tn1699[spn-4::gfp::3xflag]) V	-	DG4185	Tsukamoto et al., 2017
dlc-1(tm3153)/qCl III; spn-4(tn1699[spn-4::gfp::3xflag]) V	-	UMT356	this study
puf-5(tn1726[gfp::3xflag::puf-5]) II	-	DG4215	Tsukamoto et al., 2017
puf-5(tn1726[gfp::3xflag::puf-5]) II; dlc-1(tm3153) III/hT2 (I;III)	-	UMT359	this study
mex-3(tn1753[gfp::3xflag::mex-3]) I	-	DG4269	Tsukamoto et al., 2017
mex-3(tn1753[gfp::3xflag::mex-3]) dhc-1(js121) I/hT2 (I;III)	-	UMT364	this study
Mutant Strains; no transgene			
dlc-1(tm3153)/qC1 III	-	UMT222	Wang et al., 2016
gld-3(q730)/mIn1 [mIs14 dpy-10(e128)] II; him-5(e1490) V	-	JK3375	Eckmann et al., 2002
nos-3(q650) II	-	JK2589	Kraemer et al., 1999
gld-2(q497)/dpy-5(e61) unc-13(e51) I	-	JK1743	Kadyk and Kimble, 1998
dhc-1(or195) I	-	EU828	Hamill et al., 2002
gld-1(q485) I/hT2 [bli-4(e937) let-?(q782) qIs48] (I;III)	-	JK3025	Francis et al., 1995a
dhc-1(js121) I/hT2 (I;III); jsIs37 V	-	NM2040	Koushika et al., 2004
gld-2(q497) I/hT2 (I;III)	-	UMT235	this study
gld-3(q730)/mT1 II; dlc-1(tm3153)/mT1 III	-	UMT317	this study
nos-3(q650)/mT1 II; dlc-1(tm3153)/mT1 III	-	UMT318	this study
gld-2(q497)/hT2 I; dlc-	-	UMT319	this study

1(tm3153)/hT2 III			
dhc-1(or195) I; gld-3(q730)/mIn1 II	-	UMT325	this study
ttTi5605(mos) II; unc-119(ed3) III; oxEx1578[eftp::GFP+*Cbr-unc-119]*	-	EG6699	Frøkjaer-Jensen et al., 2008
unc119(ed3) III; oxTi365 V	-	EG8082	Frøkjær-Jensen et al., 2014
gld-1(q485)/hT2 I; dlc-1(tm3153)/hT2 III; him-8(tm611) IV	-	UMT350	this study
gld-1(op236) I	-	TG34	Schumacher et al., 2005
gld-1(op236)/hT2 I; dlc-1(tm3153)/hT2 III	-	UMT383	this study
dlc-1(tm3153) unc-32(e189) glp-1(q46) III/hT2 (I; III)	-	UMT387	this study
gld-3(q730)/mT1 II; dlc-1(tm3153) unc-32(e189) glp-1(q46)/mT1 III	-	UMT388	this study
gld-2(q497)/hT2 I; dlc-1(tm3153) unc-32(e189) glp-1(q46)/hT2 III	-	UMT390	this study

Supplemental Table 2. Analysis of tagged protein or reporter transgene repression in meiotic pachytene region of germline.

Genotype	% derepressed	*n*
spn-4::gfp::3xflag V	0	64
dlc-1(tm3153) III; spn-4::gfp::3xflag V	0	34
gfp::3xflag::puf-5 II	0	51
gfp::3xflag::puf-5 II; dlc-1(tm3153) III	0	22
axIs1649 (pie-1 prom::GFP::H2B::*cye-1* 3'UTR) *III*	0	30
dhc-1(js121) I; *axIs1649 (pie-1* prom::GFP::H2B::*cye-1* 3'UTR) *III*	0	18
gfp::3xflag::mex-3 I	0	35
gfp::3xflag::mex-3 dhc-1(js121) I	0	22
gfp::3xflag::mex-3 gld-1(q485) I; gld-1::ollas(wt) II	0	43
gfp::3xflag::mex-3 gld-1(q485) I; gld-1::ollas(ndb) V	0	35

n = number of germlines scored for repression of gene expression in meiotic phase cells. Scoring was 1 day after L4 stage.

Supplemental Note

Dissected gonads from both single heterozygous *gld-1(-)/hT2* and double heterozygous *gld-1(-)/hT2; dlc-1(-)/hT2* animals resemble wild type germlines where proliferative cells are restricted to the distal end of the gonad (Fig. S1A and C). Homozygous *gld-1(-)* mutant germline exhibits proximal tumor phenotype where germ cells enter meiosis but exit meiotic prophase early and continue to proliferate (Francis et al., 1995b Francis et al., 1995a Jones et al., 1996) (Fig. S1B). Homozygous *gld-1(-); dlc-1(-)* double mutant germline exhibits same tumor phenotype as *gld-1(-)*, however, some germ cell nuclei appear larger which is reminiscent of *dlc-1(-)* mutant phenotype (Fig. S1D).

Supplemental Figures

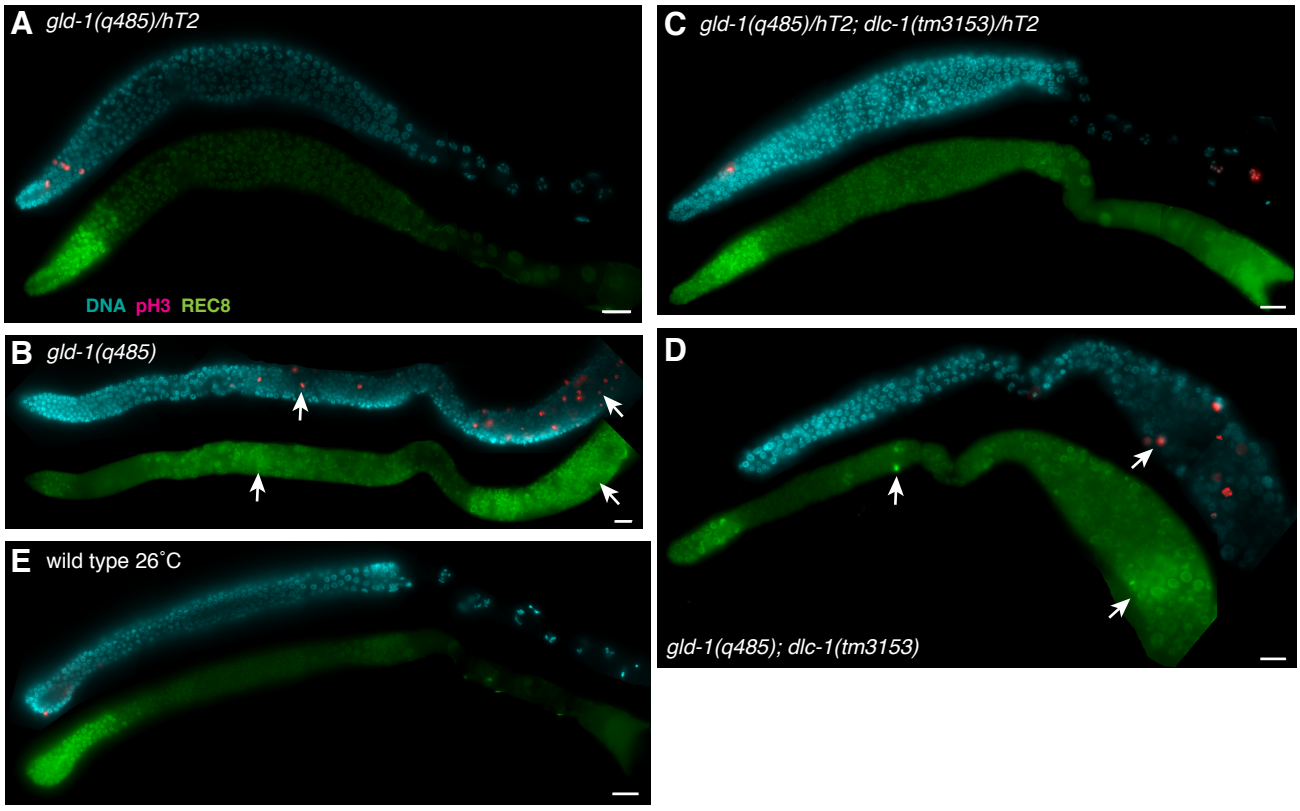
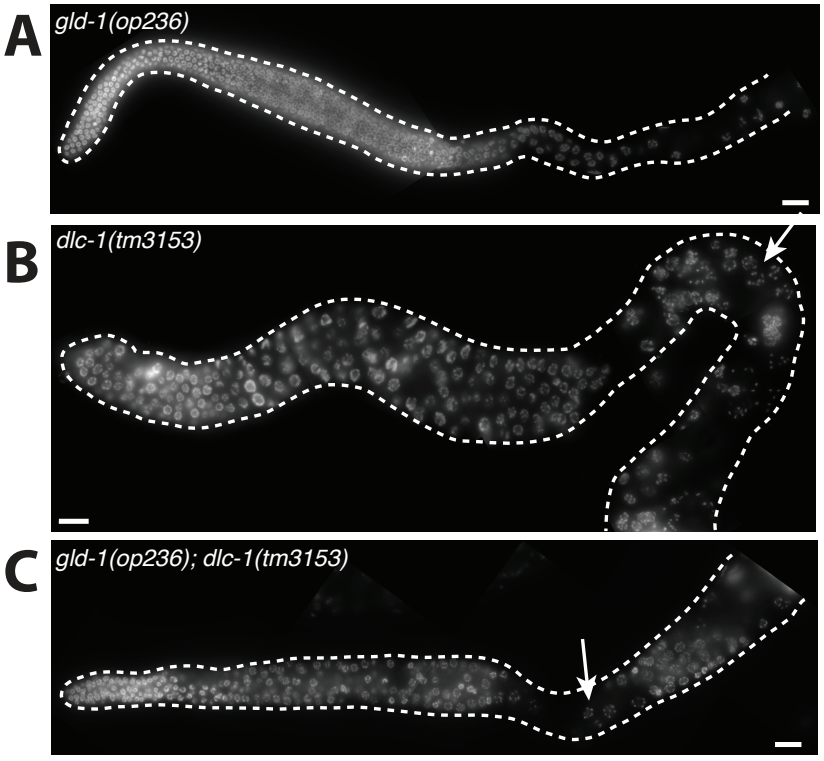


Figure S1. Germline phenotypes of heterozygous and homozygous *gld-1(-)* and *gld-1(-); dlc-1(-)* mutant alleles as well as of wild type control cultured at 25˚C. Dissected gonads were stained for the M-phase marker pH3 (pink), stem and progenitor cells marker REC-8 (green), and DNA (blue). (A) Germline from a heterozygous mutant animal *gld-1(q485)/hT2*. (B) Dissected gonad from single mutant animal *gld-1(q485)* (null allele). (C) Dissected gonad from double heterozygous animal *gld-1(q485)/hT2; dlc-1(tm3153)/hT2.* (D) Dissected gonad from double mutant animal *gld-1(q485); dlc-1(q485)*. White arrows indicate aberrant cell proliferation (tumor). (E) Wild type control cultured at 26°C. Scale bars: 10 μm.



D

Phenotype of *gld-1* and *dlc-1* mutant animals.

Genotype	% disorganized germline	*n*
gld-1(op236)	0	31
dlc-1(tm3153)	38	53
gld-1(op236); dlc-1(tm3153)	40	35

n = number of germlines scored.

Figure S2. No synthetic interactions between *dlc-1* null allele and a weak *gld-1(op236)* mutant. Germlines in A-C were dissected and chromatin was stained by DAPI. (A) *gld-1(op236)* germlines are superficially normal at 20°C. (B) A subset of *dlc-1(tm3153)* germlines at 20°C exhibit patches of pachytene cells unable to progress to diplotene (white arrow). (C) *gld-1(op236); dlc-1(tm3153)* germlines at 20°C showing disorganized germline phenotypes with diplotene cells interspersed with cells unable to progress from pachytene. (D) Quantification of disorganized germline phenotypes in the indicated genetic backgrounds at 20°C, 1 day after L4 stage.

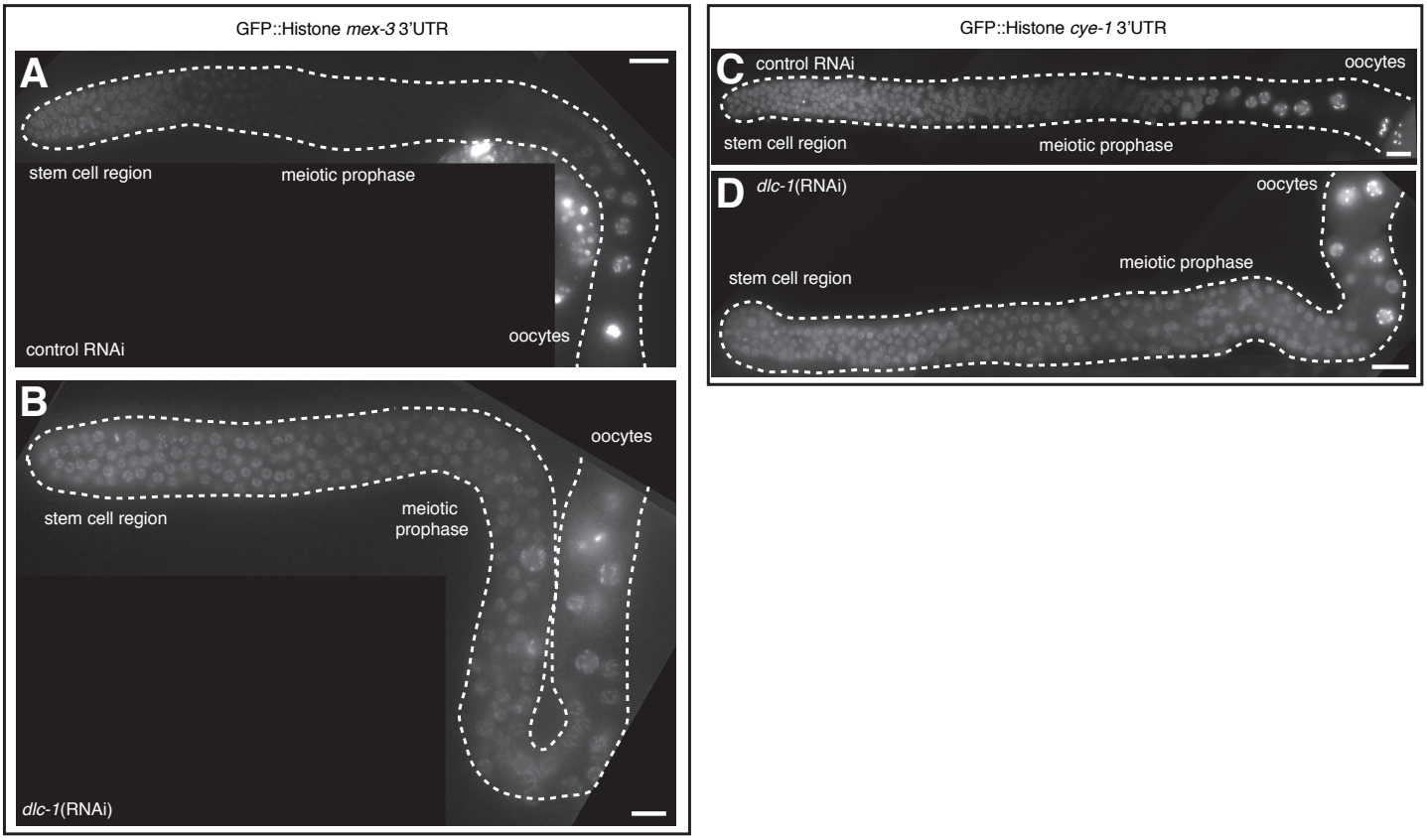


Figure S3. DLC-1 facilitates regulation of *mex-3* and *cye-1* GLD-1 target mRNAs. Fluorescent micrographs of dissected gonads expressing GFP-tagged Histone H2B under control of *mex-3* 3'UTR after either (A) control or (B) *dlc-1(RNAi)*. Fluorescent micrographs of dissected gonads expressing GFP-tagged Histone H2B under control of *cye-1* 3'UTR after either (C) control or (D) *dlc-1(RNAi)*. This *cye-1* transgenic reporter strain was generated in a different laboratory than the other transgenic reporters used in this study, however, it yielded similar expression pattern and results obtained using the *cye-1* transgenic reporter strain generated by Merritt et. al. (Biedermann et al., 2009, Merritt et al., 2008) (Fig. 5A). Scale bars: 10 μm.

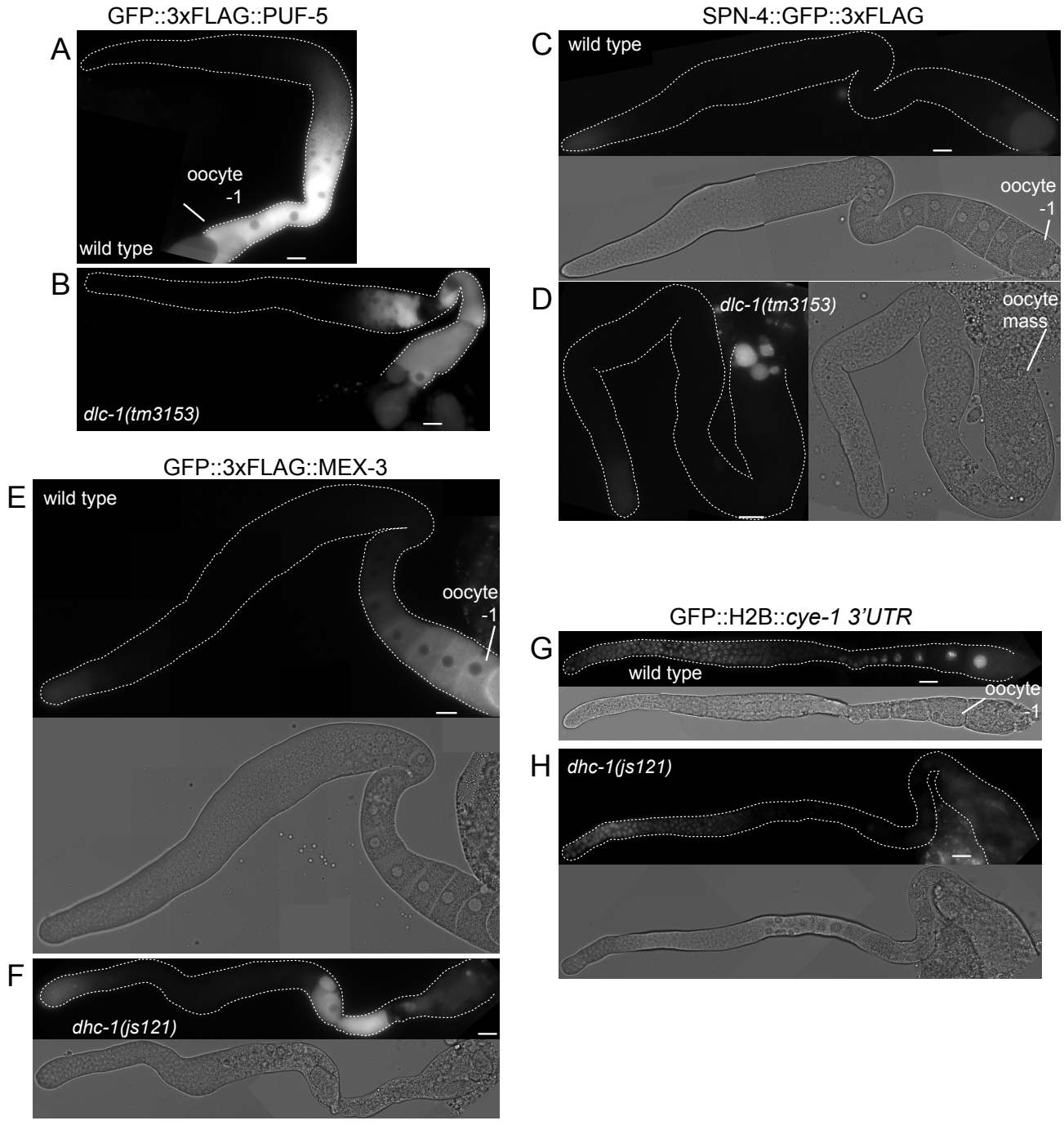


Figure S4. Regulation of *puf*-5 and *spn*-4 is independent of DLC-1, and regulation of *mex-3* and *cye-1* is independent of dynein motor. Fluorescent micrograph of dissected gonad expressing GFP::3xFLAG::PUF-5 in wild type (A) and *dlc-1(tm3153)* (B) genetic background. Fluorescent and brightfield micrographs of dissected gonads expressing SPN-4::GFP::3xFLAG in wild type (C) and *dlc-1(tm3153)* (D) genetic background. Fluorescent and brightfield micrographs of dissected gonads expressing GFP::3xFLAG::MEX-3 in wild type (E) and *dhc-1(js121)* (F) mutant background. GFP::H2B::*cye-1* 3'UTR in wild type (G) and *dhc-1(js121)* (H) mutant background. Scale bars: 10 μm.

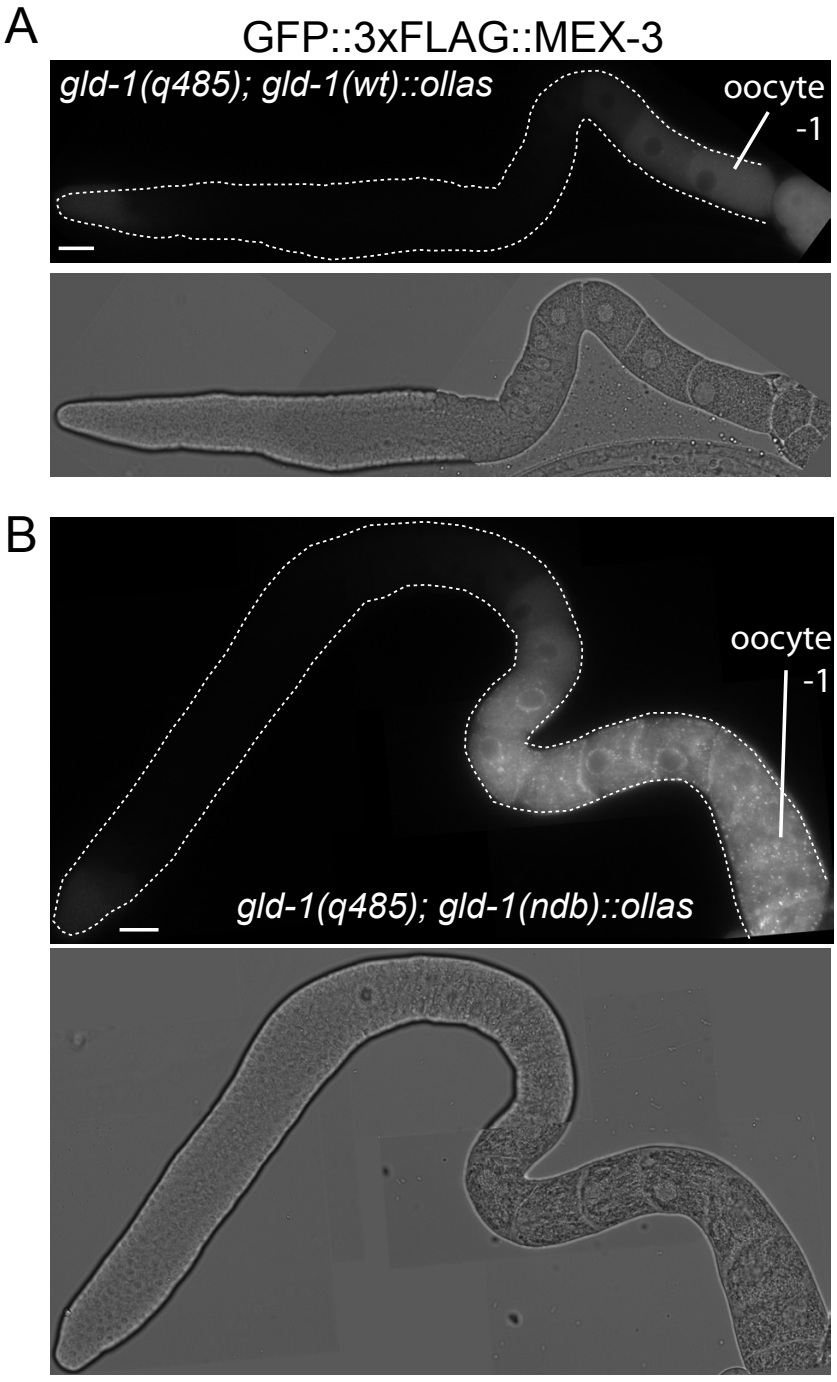


Figure S5. DLC-1 binding GLD-1 is not required to regulate MEX-3 expression *in vivo*. Fluorescent and brightfield micrographs of dissected gonads expressing GFP::3XFLAG::MEX-3 in (A) *gld-1(q485); gld-1^{wt}::ollas* and (B) *gld-1(q485); gld-1^{ndb}::ollas* genetic background. Scale bars: 10 μm.

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