Supplementary Materials for

A PUF hub drives self-renewal in *C. elegans* germline stem cells

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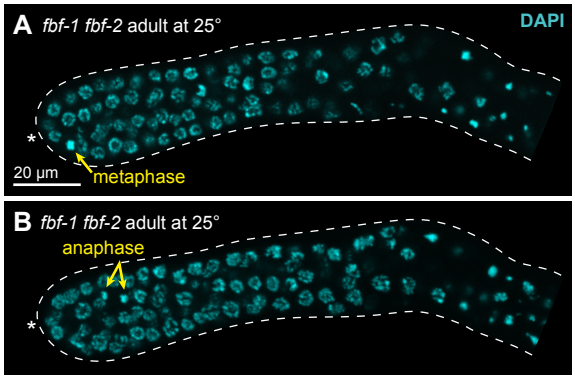


C **RNAi against other *pufs***

Genotype	RNAi	Distal germ cells, adults at 25° % PZ	% sperm	n
fbf-1 fbf-2	empty	**87**	0	12
	puf-5	**93**	0	14
	puf-6	**66**	0	22
	puf-7	**72**	0	16
	puf-8	**86**	0	18
	puf-9	**100**	0	17

Figure S1: *fbf-1 fbf-2* mutant characterization

A, B. Representative images of gonads extruded from *fbf-1 fbf-2* adult hermaphrodites that were staged to 18 hours after L4 at 25° and stained with DAPI (cyan). Morphologies consistent with metaphase (A) and anaphase (B) are annotated (yellow arrows). Other conventions as in Figure 1F,G. The images in A and B show distinct confocal Z-sections from the same gonad. The scale bar in A applies to both images. Strong loss of function alleles used here and throughout are *fbf-1(ok91)* and *fbf-2(q704)*.

C. State of distal germ cells in *fbf-1 fbf-2* adult hermaphrodites raised at 25° on either empty RNAi or RNAi against various *puf* genes. All were staged to 18 hours after L4. Germ cell states were scored as described in Figure 1H legend.

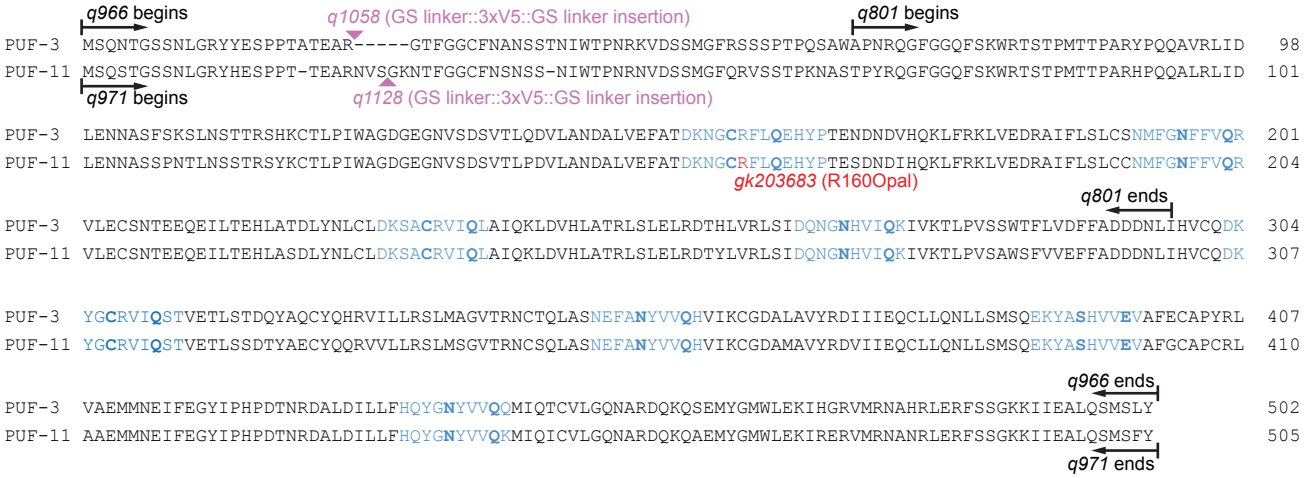


Figure S2: Annotated PUF-3 and PUF-11 protein sequences

Alignment of PUF-3 and PUF-11 amino acid sequences to show: molecular endpoints of key deletion mutants *q966, q801* and *q971* (black brackets); nonsense mutant *gk203683* (red); insertion sites for GS linker::3xV5::GS linker in *puf-3^{V5}(q1058)* and *puf-11^{V5}(q1128)* (magenta triangles); residues in PUF repeats (blue) (Zhang *et al.* 1997), with amino acids critical for RNA interaction (bold) (Wang *et al.* 2002).

	# GC/animal (mean ± sd)								
Genotype	**15°**	n	*p*-value	**20°**	n	*p*-value	**25°**	n	*p*-value
fbf-1 fbf-2	91 ± 20	16	n/a	128 ± 30	20	n/a	many & still dividing	50	n/a
fbf-1 fbf-2; puf-3(q966)	nd	nd	nd	84 ± 19	10	0.002	many & meiotic entry	43	n/a
fbf-1 fbf-2; puf-3(q801)	nd	nd	nd	102 ± 13	10	0.061	many & meiotic entry	50	n/a
fbf-1 fbf-2; puf-11(q971)	nd	nd	nd	25 ± 9	10	<0.001	11 ± 7	20	n/a
fbf-1 fbf-2; puf-11(gk203683)	nd	nd	nd	42 ± 11	20	<0.001	14 ± 8	20	n/a
fbf-1 fbf-2; puf-3(q966) puf-11(q971)	7 ± 3	20	<0.001	16 ± 7	20	<0.001	4 ± 2	20	n/a
fbf-1 fbf-2; puf-3(q801) puf-11(gk203683)	5 ± 4	23	<0.001	11 ± 8	11	<0.001	9 ± 5	8	n/a

Figure S3: Triple and quadruple mutant germ cell counts

Number of germ cells (GC) made in *fbf-1 fbf-2; puf* triple mutants and *fbf-1 fbf-2; puf-3 puf-11* quadruple mutants at 15°, 20° and 25°. Germlines were scored as described in Figure 1C, with the following modification: GC number were not counted in larger germlines with distal germ cells in meiotic prophase; these were scored as “many & meiotic entry”. nd, not done. *p*-value compared to respective *fbf-1 fbf-2* control was determined using Welch’s ANOVA and Games-Howell *post-hoc* test; n/a, not applicable.

A

Genotype	Distal germ cell state, adults at 25°			
	% PZ	% meiotic	% sperm	n (arms)
fbf-1 fbf-2	**97**	3	0	100
fbf-1 fbf-2; puf-3^{V5}(q1058)	**96**	4	0	100
fbf-1 fbf-2; puf-11^{V5}(q1128)	**99**	1	0	100

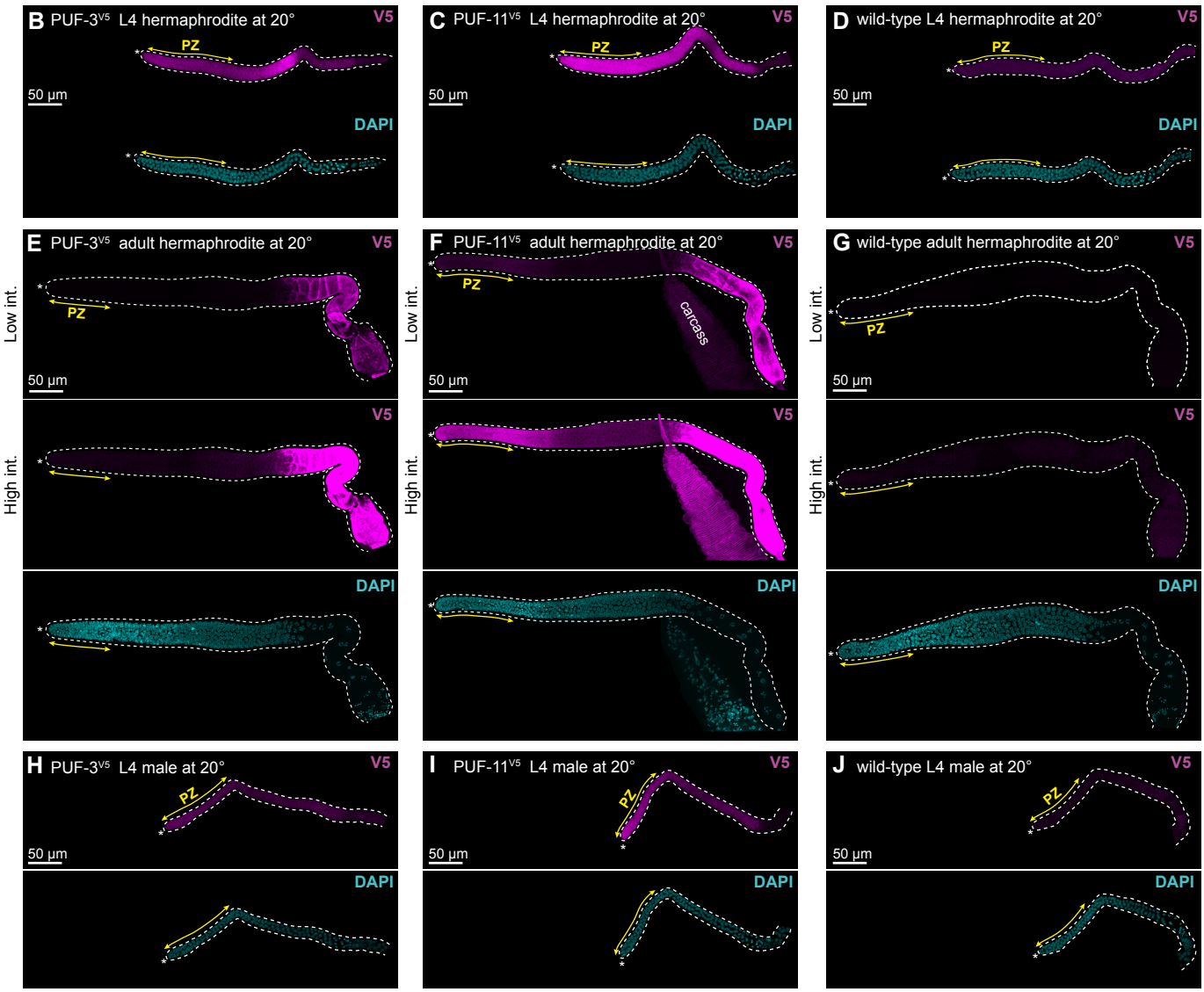


Figure S4: PUF-3^{V5} and PUF-11^{V5} function and expression in whole gonads

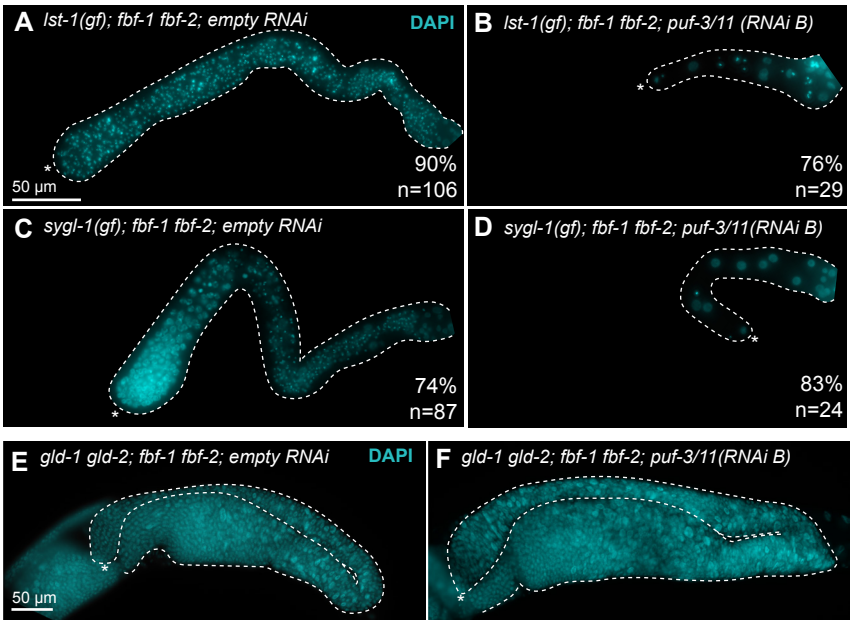
A. Epitope-tagged alleles of *puf-3* and *puf-11* did not enhance pGlp phenotype of *fbf-1 fbf-2* mutants and hence made functional protein. Strains were raised at 25° and assayed 18 hours past L4. Germ cell state was scored as described in Figure 1H and 3B.

B-J. Representative images of PUF-3^{V5} and PUF-11^{V5} expression in whole gonads at 20°. Gonads were extruded from *puf-3(q1058)* (B,E,H), *puf-11(q1128)* (C,F,I) and wild-type control (D,G,J) animals and then stained with α-V5 (magenta) and DAPI (cyan). Images are maximum intensity Z projections taken by confocal microscopy. PZ is indicated by a double headed arrow (yellow), other annotation convention as in Figure 1F,G.

B-D. Gonads from mid-L4 staged hermaphrodites.

E-G. Gonads from adult hermaphrodites staged to 24 hours past mid-L4. Intensity (int.) of the V5 signal was adjusted uniformly across images in Adobe Photoshop, with high or low intensity indicated at left. “Carcass” labels an adjacent worm carcass that slightly overlaps with the gonad of interest.

H-J. Germlines from mid-L4 staged males.



G

Genotype	RNAi	% animals with abnormal embryos *in utero*	n
gld-1 gld-2/+; fbf-1 fbf-2/+	empty	1	79
	puf-3/11	100	74

Figure S5: Epistasis results

A-D. Epistasis tests using *lst-1(gf)* and *sygl-1(gf)*. Representative compound microscopy images of gonads extruded from adults staged to 18 hours past L4 at 25°, stained with DAPI (cyan) and then scored for the Glp phenotype (4-8 germ cells differentiated as sperm, as defined in Austin and Kimble (1987). We expected undifferentiated cells in the distal gonad on the empty RNAi, typical of *fbf-1 fbf-2* mutants at this temperature (Figure 1F). However, the distal state of differentiation was variable (also noted in Shin *et al.* 2017), so penetrance of the depicted phenotype is given at bottom right. Most *sygl-1(gf); fbf-1 fbf-2* germlines on empty RNAi had undifferentiated cells at the distal end (74%, n=87) (C), but some had differentiated. For *lst-1(gf); fbf-1 fbf-2,* only 10% remained undifferentiated while 90% had differentiated (n=106) (A). The scale bar in A applies to all images, and all conventions are as in Figure 1F,G. Genotype for *lst-1(gf)* strain is *lst-1(ok814); qSi267 [Pmex-5::LST-1::3xFLAG::tbb-2 3' end] fbf-1(ok91) fbf-2(q704)* and *sygl-1(gf)* is *sygl-1(tm5040); qSi235 [Pmex-5::SYGL-1::3xFLAG::tbb-2 3' end] fbf-1(ok91) fbf-2(q704*). We utilized *puf-3/11* RNAi clone B in these experiments.

E,F. Epistasis tests using *gld-1 gld-2*. Representative compound microscopy images of adults staged to 24 hours past L4 at 20°, stained with DAPI (cyan), and scored for either a tumorous germline typical of *gld-1 gld-2* or a Glp germline (containing 4-8 germ cells differentiated as sperm, as defined in Austin and Kimble (1987). The scale bar in E applies to both images, with other conventions as in Figure 1F,G. Genotype is *gld-2(q497) gld-1(q361); fbf-1(ok91) fbf-2(q704).* We used *puf-3/11* RNAi clone B in these experiments.

G. Confirmation that *puf-3/11* RNAi worked in *gld-1 gld-2* epistasis test. Effective *puf-3/11* RNAi causes embryo lethality (Hubstenberger *et al.* 2012; this work). We therefore DAPI stained and scored for abnormal morphology of *in utero* embryos in *gld-1 gld-2/+* siblings to show that *puf-3/11* had been successfully knocked down.

Table S1. Nematode strains used in this study

Name	Genotype	Reference
N2	wild-type	Brenner, 1974
EG7866	*oxTi564 II; unc-119(ed3) III*	Frøkjær-Jensen *et al.*, 2014
JK3107	*fbf-1(ok91) fbf-2(q704)/ mIn1[mIs14 dpy-10(e128)] II*	Crittenden *et al.*, 2002
JK3108	*fbf-1(ok91) fbf-2(q704)/ mIn1[mIs14 dpy-10(e128)] II; him-5(e1490) V*	this work
JK3743	*fog-1(q785) I/ hT2[qIs48] (I;III)*	Thompson *et al.*, 2005
JK4256	*puf-3(q801) IV/ nT1[qIs51] (IV;V)*	this work
JK4862	*glp-1(q46) III/ hT2[qIs48] (I;III)*	Kershner *et al.*, 2014
JK5411	*sygl-1(tm5040) I; fbf-1(ok91) fbf-2(q704) qSi235/ mIn1[mIs14 dpy-10(e128)] II*	Shin *et al.*, 2017
JK5537	*lst-1(ok814) I; fbf-1(ok91) fbf-2(q704) qSi267/ mIn1[mIs14 dpy-10(e128)] II*	Shin *et al.*, 2017
JK5778	*gld-2(q497) gld-1(q361)/ ccIs4251 unc-15(e73) I; fbf-1(ok91) fbf-2(q704)/ mIn1[mIs14 dpy-10(e128)] II*	this work
JK5908	*puf-11(gk203683) IV*	this work
JK5915	*puf-3(q966) IV*	this work
JK5925	*fbf-1(ok91) fbf-2(q704)/ mIn1[mIs14 dpy-10(e128)] II; puf-11(gk203683) IV*	this work
JK5926	*fbf-1(ok91) fbf-2(q704)/ mIn1[mIs14 dpy-10(e128)] II; puf-3(q966) IV*	this work
JK5991	*puf-3(q801) puf-11(gk203683) IV/ nT1[qIs51] (IV;V)*	this work
JK5993	*fbf-1(ok91) fbf-2(q704)/ mIn1[mIs14 dpy-10(e128)] II; puf-3(q801) IV*	this work

JK5996	*puf-11(q971) IV*	this work
JK6051	*fbf-1(ok91) fbf-2(q704)/ mIn1[mIs14 dpy-10(e128)] II; puf-11(q971) IV*	this work
JK6080	*puf-3(q1058) IV*	this work
JK6143	*fbf-1(ok91) fbf-2(q704)/ dpy-10(q1074) oxTi564 II; puf-3(q801) puf-11(gk203683) IV/ nT1[qIs51] (IV;V)*	this work
JK6272	*fbf-1(ok91) fbf-2(q704)/ mIn1[mIs14 dpy-10(e128)] II; puf-3(q1058) IV*	this work
JK6280	*puf-11(q1128) IV*	this work
JK6282	*fbf-1(ok91) fbf-2(q704)/ mIn1[mIs14 dpy-10(e128)] II; puf-11(q1128) IV*	this work
JK6321	*puf-3(q966) puf-11(q971) IV/ nT1[qIs51] (IV;V)*	this work
JK6333	*fbf-1(ok91) fbf-2(q704)/ dpy-10(q1074) oxTi564 II; puf-3(q966) puf-11(q971) IV/ nT1[qIs51] (IV;V)*	this work
JK6401	*lst-1(q869) sygl-1(q828) I/ hT2[qIs48] (I;III)*	Haupt *et al.*, 2019

Table S2. CRISPR alleles generated in this study

Allele	Description	Guide(s) (5'–3')	Repair template (5'–3')[1]	Parent strain
q966	*puf-3(null)*	UGCUUUGACU CAUAUUGGUA and GCCUAUGUGU AUCUAGUACA	gatgtcttccaaaaaaataaattttcaggtag tctTcgtaccaatatacacataggcaattttt attcatttccatttgaatcctaaccc	wild-type
q971	*puf-11(null)*	UGCUUUGACU CAUAUUGGUA and AAUGUCCUUU UACUAGAUAU	accatttttcccaaaaaaatattttcagttagt ctAcgtaccaatatatagAcaattcatttgca ttattatcctaacccccactcacgt	wild-type
q1058	*puf-3* exon 1 insertion of GS linker:: 3xV5:: GS linker	ACGGAGGCUC GUGGCACAUU	cgagagcccgccaacggcgacggaggct cgtGGATCTGGTAAGCCTATCC CTAACCCTCTCCTCGGTCTAG ATAGTACTGGAAAGCCAATCC CAAACCCACTCCTCGGACTTG ATAGCACCGGTAAGCCTATCC CTAACCCACTCCTCGGACTTG ATAGCACCGGATCTggAacCttc ggtggttgcttcaacgccaaca	wild-type
q1074	*dpy-10* frameshift[2]	GCUACCAUAG GCACCACGAG[3]	n/a	EG7866
q1128	*puf-11* exon 1 insertion of GS linker:: 3xV5:: GS linker	UCCGGAAAAA ACACAUUCGG	tccgccaacgacggaggctcgcaatgtgtc cGGATCTGGTAAGCCTATCCC TAACCCTCTCCTCGGTCTAGA TAGTACTGGAAAGCCAATCCC AAACCCACTCCTCGGACTTGA TAGCACCGGTAAGCCTATCCC TAACCCACTCCTCGGACTTGA TAGCACCGGATCTggaaaGaaT acCttTggcggttgcttcaact	wild-type

[1] Uppercase letters denote mutations (including insertions, PAM mutations and/or seed sequence mutations)

[2] *dpy-10(wild-type):* tggaaaccgtaccgct**CG**tggtgcctatggtag
dpy-10(q1074): tggaaaccgtaccgct**GCC**tggtgcctatggtag

[3] Arribere *et al.*, 2014

Table S3. Yeast-two hybrid plasmids used in this study

Plasmid	Insert description	Cloning site	Vector backbone	Reference
pJK1580	HA::SYGL-1(aa 1-206)	NcoI	pACT2	Shin *et al.*, 2017
pJK2015	HA::LST-1(aa 1-328)	XhoI	pACT2	Shin *et al.*, 2017
pJK2033	V5::FBF-1(aa 121-614)	NdeI	pBTM116	this work
pJK2034	V5::PUF-3(aa 88-502)	NdeI	pBTM116	this work
pJK2037	V5::PUF-11(aa 91-505)	NdeI	pBTM116	this work
pJK2046	V5::FBF-2(aa 121-632)	NdeI	pBTM116	this work
pJK2056	V5::PUF-9(aa 162-704)	NdeI	pBTM116	this work

SUPPLEMENTARY REFERENCES

Arribere, J. A., R. T. Bell, B. X. Fu, K. L. Artiles, P. S. Hartman *et al.*, 2014 Efficient marker-free recovery of custom genetic modifications with CRISPR/Cas9 in *Caenorhabditis elegans*. Genetics 198**:** 837-846.

Austin, J., and J. Kimble, 1987 *glp-1* is required in the germ line for regulation of the decision between mitosis and meiosis in C. elegans. Cell 51**:** 589-599.

Brenner, S., 1974 The genetics of *Caenorhabditis elegans*. Genetics 77**:** 71-94.

Crittenden, S. L., D. S. Bernstein, J. L. Bachorik, B. E. Thompson, M. Gallegos *et al.*, 2002 A conserved RNA-binding protein controls germline stem cells in *Caenorhabditis elegans*. Nature 417**:** 660-663.

Frøkjær-Jensen, C., M. W. Davis, M. Sarov, J. Taylor, S. Flibotte *et al.*, 2014 Random and targeted transgene insertion in *Caenorhabditis elegans* using a modified *Mos1* transposon. Nat Methods 11**:** 529-534.

Haupt, K. A., A. L. Enright, A. S. Ferdous, A. M. Kershner, H. Shin *et al.*, 2019 The molecular basis of LST-1 self-renewal activity and its control of stem cell pool size. Development 146.

Hubstenberger, A., C. Cameron, R. Shtofman, S. Gutman and T. C. Evans, 2012 A network of PUF proteins and Ras signaling promote mRNA repression and oogenesis in *C. elegans*. Dev Biol 366**:** 218-231.

Kershner, A. M., H. Shin, T. J. Hansen and J. Kimble, 2014 Discovery of two GLP-1/Notch target genes that account for the role of GLP-1/Notch signaling in stem cell maintenance. Proc Natl Acad Sci U S A 111**:** 3739-3744.

Shin, H., K. A. Haupt, A. M. Kershner, P. Kroll-Conner, M. Wickens *et al.*, 2017 SYGL-1 and LST-1 link niche signaling to PUF RNA repression for stem cell maintenance in *Caenorhabditis elegans*. PLoS Genet 13**:** e1007121.

Thompson, B. E., D. S. Bernstein, J. L. Bachorik, A. G. Petcherski, M. Wickens *et al.*, 2005 Dose-dependent control of proliferation and sperm specification by FOG-1/CPEB. Development 132**:** 3471-3481.

Wang, X., J. McLachlan, P. D. Zamore and T. M. T. Hall, 2002 Modular recognition of RNA by a human Pumilio-homology domain. Cell 110**:** 501-512.

Zhang, B., M. Gallegos, A. Puoti, E. Durkin, S. Fields *et al.*, 1997 A conserved RNA-binding protein that regulates sexual fates in the *C. elegans* hermaphrodite germ line. Nature 390**:** 477-484.