UNC-6 and UNC-40 promote dendritic growth through PAR-4 in C. elegans neurons

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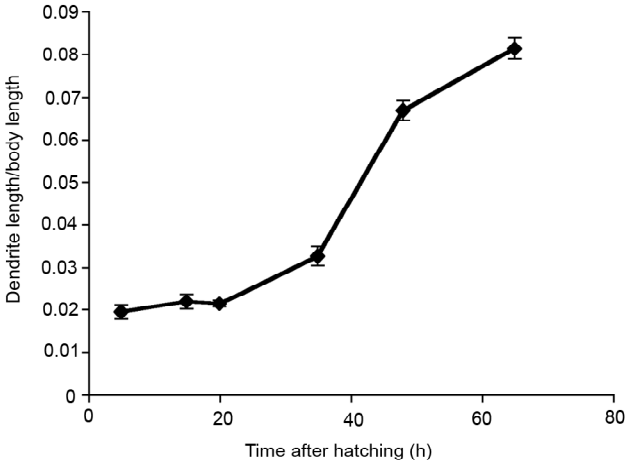
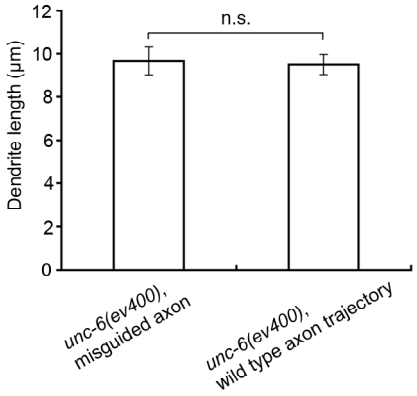
Supplementary Figure-1 (Shen).



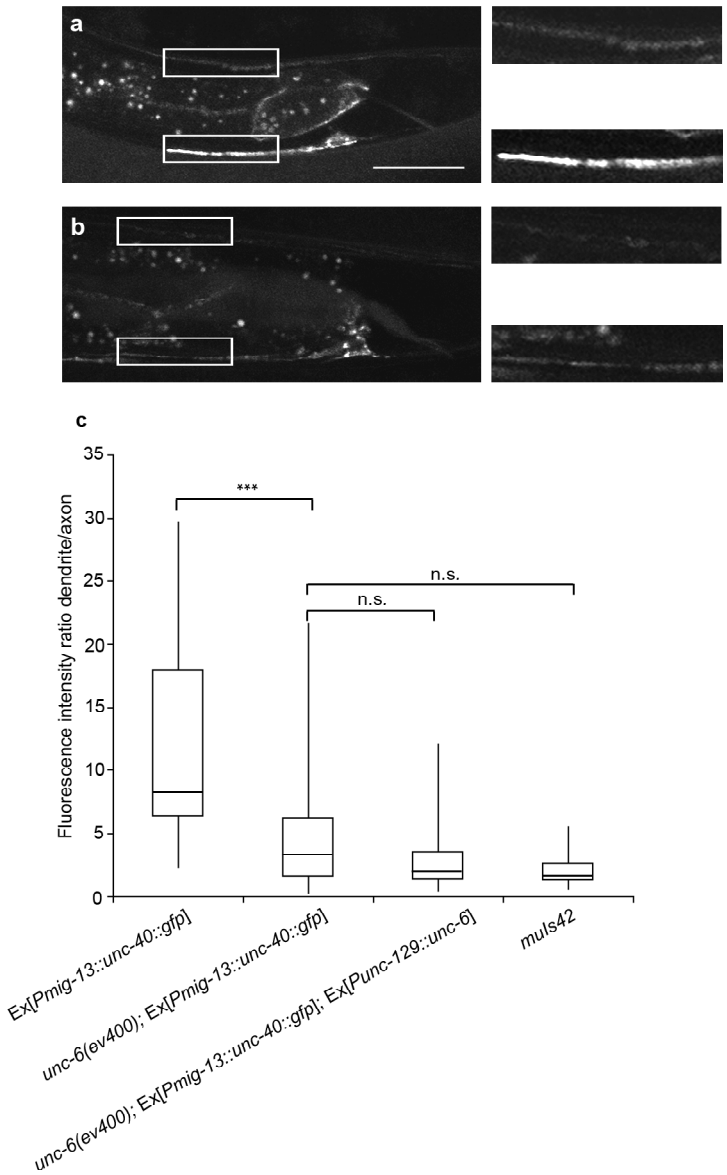
Supplementary Fig. 1. Dendrite length is not secondary to body length. Dendrite growth proceeds independently of the rate of body growth and decreases in rate in adults. n≥20 on dendrite measurement, n≥12 on body measurement. Error bars: standard error of the mean (s.e.m).

Supplementary Figure-2 (Shen).



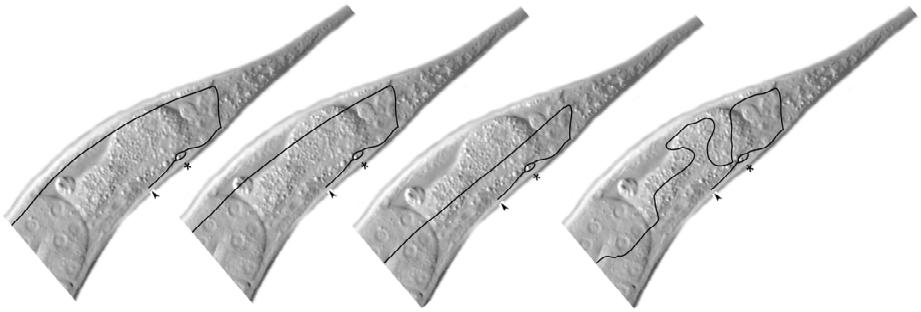
Supplementary Fig. 2. Dendrite length is not dependent on axon guidance. Dendrite length is not significantly different in unc-6 mutant animals displaying axon guidance phenotypes from unc-6 mutant animals expressing a wild-type axon trajectory. n≥35. Error bars: standard error of the mean (s.e.m).

Supplementary Figure-3 (Shen).



Supplementary Fig. 3. UNC-40 localizes to the dendrite in an unc-6-dependent manner. (a, b) Lateral views of L3 animals expression Ex[Pmig-13::unc-40::gfp]. (a) UNC-40::GFP expression in wild-type animals shows preferential localization to the dendrite. (b) UNC-40::GFP expression in unc-6 mutants suppresses preferential localization to the dendrite. (c) Intensity ratio of dendrite to axon UNC-40::GFP fluorescence in wild-type, unc-6 mutant and unc-6 mutant expressing unc-6 in dorsal muscle (Ex[Punc-129::unc-6]) compared to muIs42 (Pmig-13::mig-13::gfp). All animals are L3 stage, all pictures are lateral view with ventral down and anterior to the left, all quantification from anterior edge of soma to anterior tip of dendrite. Scale-bar 20 μm. n≥20. ***p<0.001, Mann-Whitney U test.

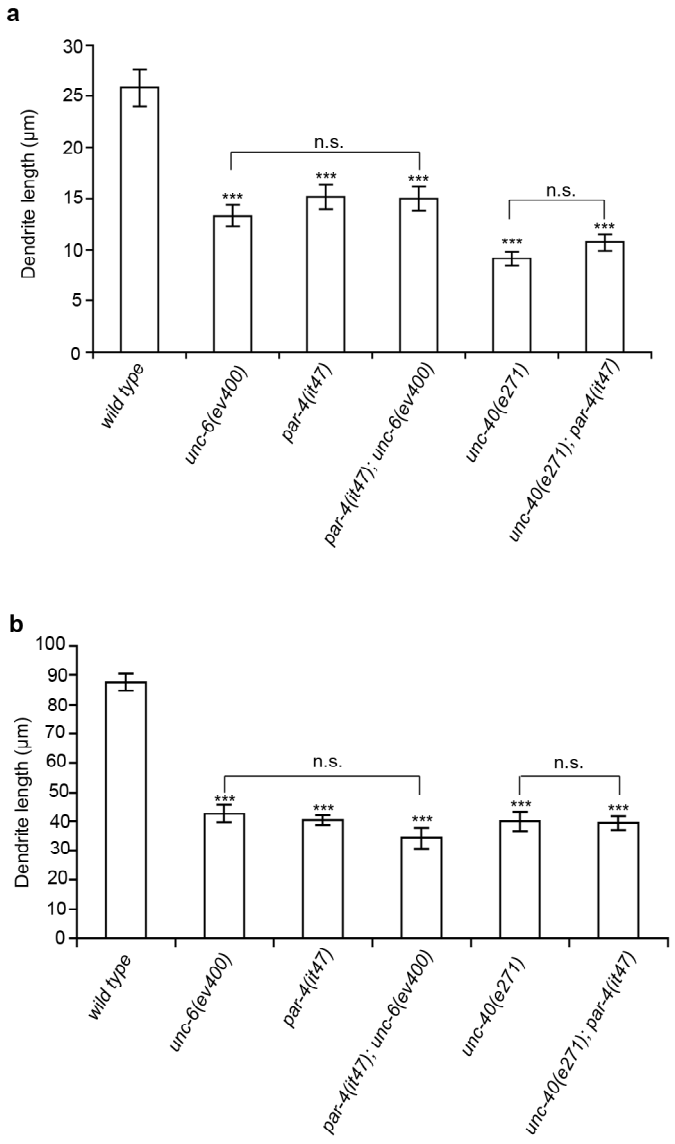
Supplementary Figure-4 (Shen).



Genotype	dorsal axon trajectory	mid-dorsal axon trajectory	mid-body axon trajectory	dorsal-ventral axon trajectory	Σ dorsal	Σ not dorsal	% dorsal	χ^2	p
wild type	76	0	0	0	76	(1)	100		
unc-6	76	12	0	12	76	24	24	wrt wild type 18.4	<0.001
unc-6; Ex*[Punc-6::unc-6::nlg-1TM::mCherry]* (membrane tethered)	56	9	2	6	56	17	23	wrt *unc-6* 0.01	>0.1
unc-6; Ex*[Punc-129::unc-6]* (dorsal muscle)	56	25	6	23	56	54	49	wrt *unc-6* 14.1	<0.001

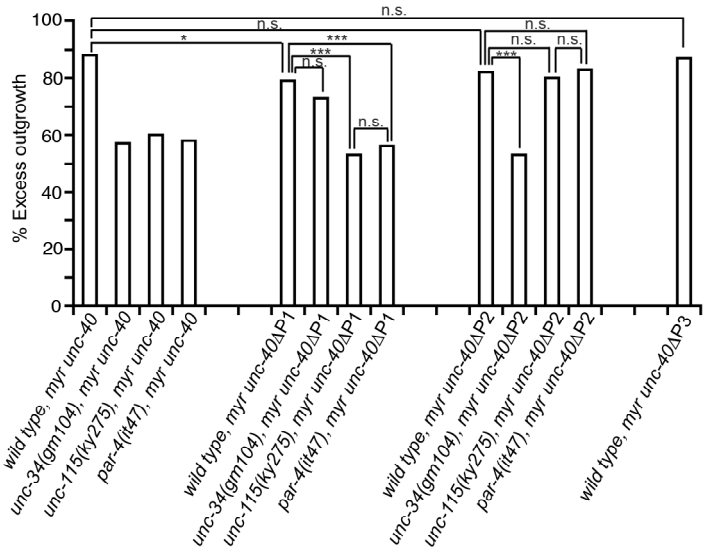
Supplementary Fig. 4. Axon guidance phenotypes in unc-6 overexpression experiments.
Compared to the penetrance of axon guidance phenotypes in unc-6 mutant animals, the expression of a membrane-tethered unc-6 construct (Ex[Punc-6::unc-6::nlg-1TM::mCherry]) had no effect, while the expression of unc-6 in dorsal body wall muscles (Ex[Punc-129::unc-6]) doubled the penetrance of dorsal axon misguidance. n≥73. Error bars: standard error of the mean (s.e.m). ***p<0.001, X^2-test.

Supplementary Figure-5 (Shen).



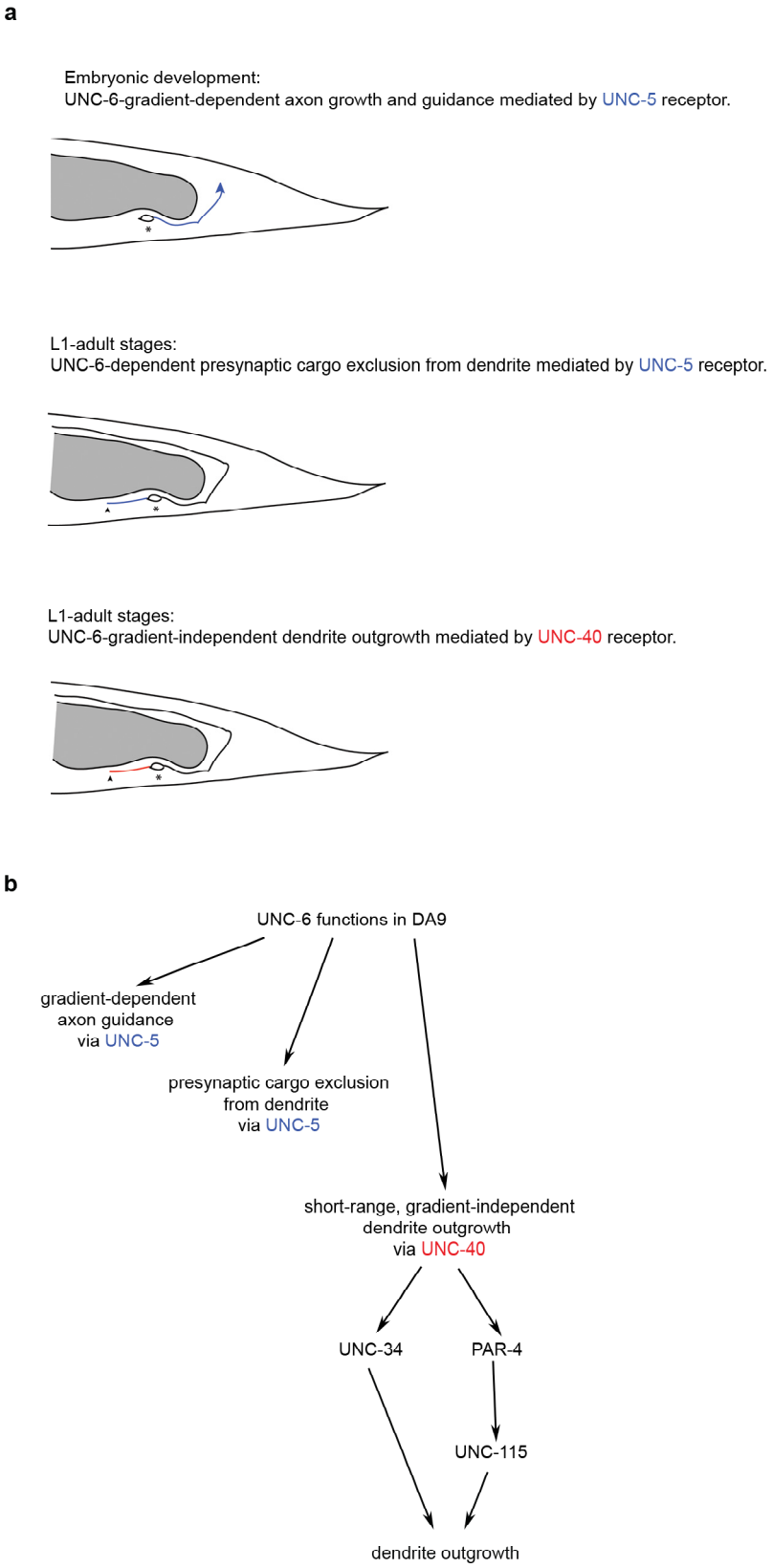
Supplementary Fig. 5. par-4 acts in the same pathway as unc-6 and unc-40. Graphs of dendrite lengths in wild-type, unc-40, par-4, unc-40; par-4, unc-6 and par-4; unc-6 mutants at L4 (a) and adult stages (b). n≥30. Error bars: standard error of the mean (s.e.m.). ***p<0.001, student's t-test.

Supplementary Figure-6 (Shen).



Supplementary Fig. 6. unc-40 regulates par-4 via the intracellular P2 motif. Quantification of gain of function phenotypes in AVM in adult worms in myr::unc-40, myr::unc-40ΔP1, myr::unc-40ΔP2 and myr::unc-40ΔP3 animals and their suppression in unc-34, unc-115 and par-4 mutants. n≥90. Error bars (not visible): standard error of proportion. *p<0.05, ***p<0.001, X^2-test.

Supplementary Figure-7 (Shen).



Supplementary Fig. 7. UNC-6 plays multiple roles in the development of one neuron. (a) During embryonic development, UNC-5 repels the DA9 axon from the graded ventral UNC-6 source; during larval development and adulthood, UNC-6 regulates dendrite outgrowth and exclusion of presynaptic cargo from the dendrite via the UNC-40 and UNC-5 receptors respectively. (b) Dendrite outgrowth is mediated by short-range UNC-6 signal acting via a novel pathway including UNC-40 and PAR-4.

Supplementary Table 1.

In all tables below, n represents the number of animals quantified in each category. Mean represents mean length of dendrite in that category in µm, s.e.m. is the standard error of the mean. Where p is indicated it represents the p-value outcome of a student's t-test or Pearson's X^2-test as specified. Unless indicated otherwise, the p-value in a category is in a comparison of that population to wild-type. If the comparison is with a different genotype, the genotype is named before the p-value. In tables in which such comparisons occur, wild-type (wt) is stated as well.

Fig. 1g

wt	L1	L2	L3	L4	YA	A
n	28	30	113	50	21	32
mean	5.75	10.82	12.82	25.83	64.18	87.58
s.e.m.	0.49	0.87	0.48	1.79	2.21	2.71

Fig. 2e

wt	L1	L2	L3	L4
n	28	30	113	50
mean	5.75	10.82	12.82	25.83
s.e.m.	0.49	0.87	0.48	1.79

unc-6	L1	L2	L3	L4
n	26	31	105	27
mean	3.48	7.44	9.18	13.34
s.e.m.	0.38	0.56	0.38	1.02

unc-5	L1	L2	L3	L4
n	53	38	105	68
mean	4.65	8.95	13.83	20.09
s.e.m.	0.42	0.77	0.47	0.77

unc-40	L1	L2	L3	L4
n	24	28	104	25
mean	0.70	4.78	5.96	9.12
s.e.m.	0.23	0.63	0.48	0.70

unc-34	L1	L2	L3	L4
n	28	36	21	22
mean	1.55	5.37	6.45	12.34
s.e.m.	0.43	0.57	0.84	1.05

unc-115	L1	L2	L3	L4
n	36	31	36	28
mean	3.0	5.8	9.1	10.5
s.e.m.	0.24	0.62	0.64	0.78

Fig. 2f.

	wt	unc-6	unc-5	unc-40	unc-34	unc-115
n	112	105	105	104	21	36
mean	12.79	9.18	13.83	5.96	6.45	9.1
s.e.m.	0.48	0.38	0.47	0.48	0.84	0.64
p		1.34x10-8	0.13	1.23x10-19	1.51x10-7	2.00x10-4

Fig. 3a

	wt	Pmig-13::unc-40	unc-40	unc-40;Pmig-13::unc-40	unc-6	unc-6;Pmig-13::unc-40
n	115	37	107	20	105	34
mean	12.82	15.59	6.08	11.47	9.18	9.74
s.e.m.	0.47	0.84	0.48	1.07	0.38	0.50
p		0.006	1.38 x10-19	0.26	6.21x10-9	2.22x10-5

Fig. 4a

	wt	unc-6	unc-6;Punc-6::unc-6::nlg-1TM::mCherry	unc-6; Punc-129::unc-6	Punc-6::unc-6::nlg-1TM::mCherry
n	115	108	27	28	30
mean	12.82	9.13	11.19	12.64	17.18
s.e.m.	0.47	0.37	0.94	0.47	0.79
p		3.06x10-9	0.13	0.78	1.70x10-5

Fig. 5c

	wt	par-4	par-4;Pitr-1::par-4::gfp	Pitr-1::par-4::gfp
n	115	108	52	30
mean	12.82	10.12	12.16	23.88
s.e.m.	0.47	0.32	0.65	1.24
p		3.47x10-6	0.40	1.68x10-12

Fig. 6a.

	wt	par-4	unc-6	par-4; unc-6	unc-40	unc-40; par-4	unc-34	unc-34, par-4	unc-115	par-4; unc-115
n	112	108	105	48	104	96	21	33	36	41
mean	12.79	10.12	9.18	8.60	5.96	6.16	6.45	3.50	9.1	9.25
s.e.m.	0.48	0.32	0.38	0.58	0.48	0.41	0.84	0.56	0.64	0.7
p		wt	wt	unc-6	wt	unc-40	wt	unc-34	wt	par-4
		6.34x10-6	1.34x10-8	0.4	1.23x10-19	0.75	1.51x10-7	5.88x10-3	2.00x10-4	0.26; unc-115 0.93

Fig. 6b.

	wt	unc-6	unc-40	par-4	unc-6;Pitr-1::par-4::gfp	unc-40;Pitr-1::par-4::gfp	par-4;Pmig-13::unc-40
n	112	105	104	108	30	21	33
mean	12.79	9.18	5.96	10.12	21.44	21.37	9.39
s.e.m.	0.48	0.38	0.48	0.32	0.85	1.31	0.78
p		wt	wt	wt	unc-6	wt	wt
		1.34x10-8	1.23x10-19	6.4x10-6	2.11x10-15	2.31 x 10-6	3.16x10-4
					wt	unc-40	par-4
					1.36x10-11	3.37x10-10	0.36

Fig. 6c.

	wt	unc-115	par-4	par-4; unc-115	Pitr-1::par-4::gfp	unc-115; Pitr-1::par-4::gfp.
n	112	36	108	41	30	2
mean	12.79	9.1	10.12	9.25	23.88	9.30
s.e.m.	0.48	0.64	0.32	0.7	1.24	0.97
p		wt	wt	par-4	wt	Pitr-1::par-4::gfp
		2.00x10-4	6.34x10-6	0.26; unc-115 0.93	1.68x10-12	4.83x10-13 unc-115 0.87

Fig.7c.

	wt	par-4	unc-34	unc-34, par-4
n	105	101	101	125
guidance	0/105	0/101	3/101	13/125
s.e.p.	-	-	0.02	0.03
p (χ2)				unc-34 0.03

Fig.7d.

	wt	par-4	unc-34	unc-34, par-4
n	105	101	101	125
guidance	0/105	0/101	0/101	8/125
s.e.p.	-	-	-	0.02
p (χ2)				unc-34 0.015

Supplementary Fig. 1.

	L1	L2	L3	L4	YA	A
n body length	16	14	13	12	17	27
Mean body length	298.38	496.32	601.15	794.54	962.26	1077.43
s.e.m. body length	8.52	15.48	11.55	17.62	8.55	16.80
Mean dendrite/ body length (Fig. 1g)	0.019	0.022	0.021	0.033	0.067	0.081
Propagated error dendrite/body length	0.002	0.002	0.001	0.002	0.002	0.003

Supplementary Fig. 2.

	Unc-6 dorsal axon	Unc-6 axon misguided
n	36	46
mean	9.66	9.49
s.e.m.	0.64	0.47
p		unc-6 dorsal axon 0.75

Supplementary Fig. 3.

	Pmig-13::unc-40::gfp	unc-6, Pmig-13::unc-40::gfp	unc-6, Pmig-13::unc-40::gfp, Punc-129::unc-6	muIs42
n	21	22	21	20
median	8.83	3.26	1.97	1.67
IQR	11.64	4.765	2.28	1.42
p (Mann-Whitney U test)		Pmig-13::unc-40::gfp 0.00077	unc-6, Pmig-13::unc-40::gfp 0.17	unc-6, Pmig-13::unc-40::gfp 0.066

Supplementary Fig. 4.- see **Supplementary Figure 4.**

Supplementary Fig. 5a.

	wt	unc-6	par-4	par-4; unc-6	unc-40	unc-40; par-4
n	52	27	28	56	25	48
mean	25.8	13.3	15.1	15.0	9.1	10.7
s.e.m.	1.8	1.01	1.2	1.1	0.7	0.8
p		5.6x10-8	4.1 x10-6	2.0 x10-6	2.5 x10-12	8. 2 x10-11
				unc-6		unc-40
				0.29		0.14

Supplementary Fig. 5b.

	wt	unc-6	par-4	par-4; unc-6	unc-40	unc-40; par-4
n	32	28	37	39	21	36
mean	87.58	42.71	40.40	34.31	40.10	39.47
s.e.m.	2.71	3.08	1.72	3.62	3.27	2.33
p		4.5 x10-15	1.9 x10-20	5.9 x10-18	2.2 x10-14	3.1 x10-20
				par-4		unc-40
				0.13		0.85

Supplementary Fig. 6.

genotype n	gain of function		s.e.p	p ((χ2)
myr::unc-40	113	100	0.03	
unc-34; myr::unc-40	94	54	0.05	myr::unc-40 <.0001
unc-115; myr::unc-40	92	55	0.05	myr::unc-40 <.0001
par-4; myr::unc-40	90	52	0.05	myr::unc-40 <.0001
myr::unc-40ΔP1	113	89	0.04	myr::unc-40 0.048
unc-34;myr::unc-40ΔP1	92	67	0.05	myr::unc-40ΔP1 0.32
unc-115; myr::unc-40ΔP1	118	62	0.05	myr::unc-40ΔP1 <.0001
par-4; myr::unc-40ΔP1	94	53	0.05	myr::unc-40ΔP1 <.0001
				unc-115;myr::unc-40ΔP1 0.58
myr::unc-40ΔP2	97	80	0.04	myr::unc-40 0.21
unc-34; myr::unc-40ΔP2	90	47	0.05	myr::unc-40ΔP2 <.0001
unc-115; myr::unc-40ΔP2	94	75	0.04	myr::unc-40ΔP2 0.63
par-4; myr::unc-40ΔP2	90	74	0.04	myr::unc-40ΔP2 1.0
				unc-115; myr::unc-40ΔP2 0.67
myr::unc-40ΔP3	101	88	0.03	myr::unc-40 0.76