

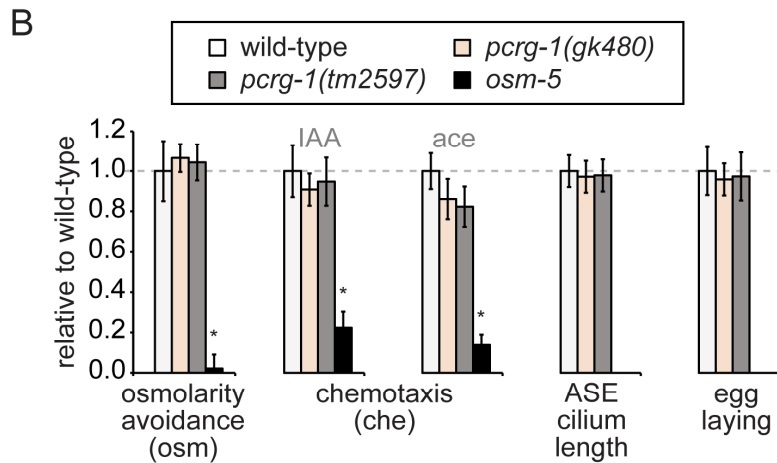
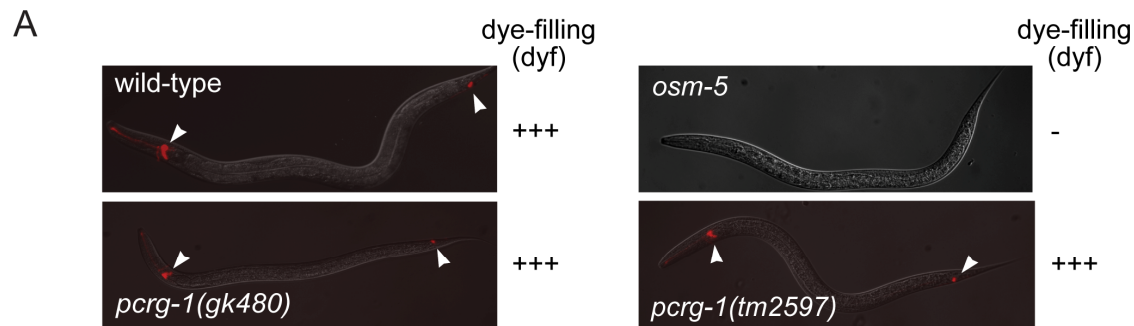
Supplemental Materials

Molecular Biology of the Cell

Loucks et al.

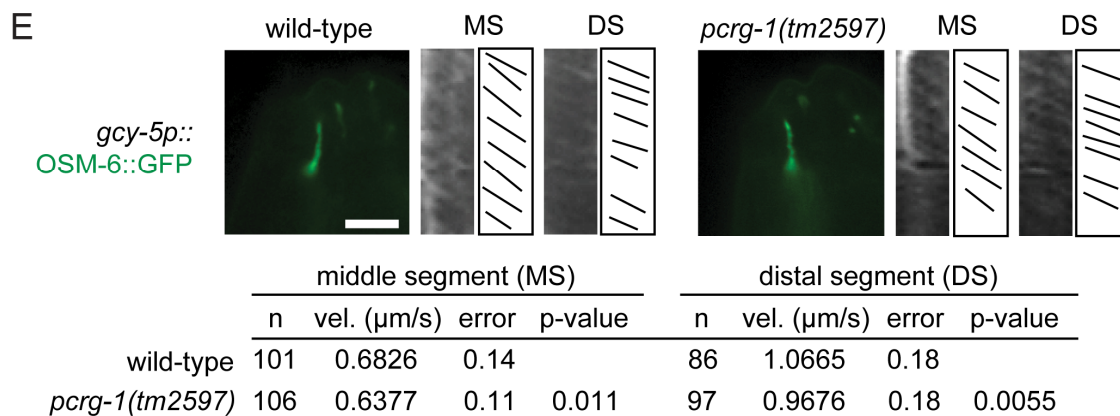
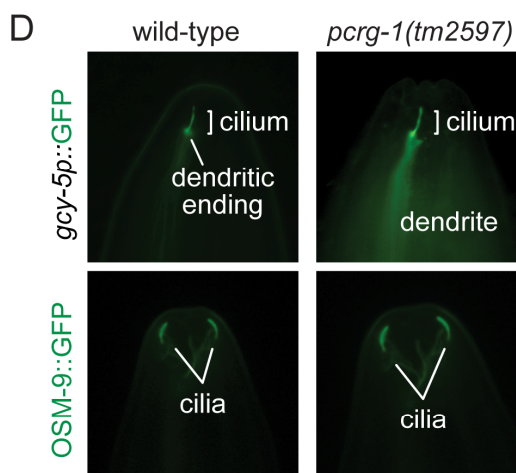
Supplementary Material

Supplementary Figures Legends

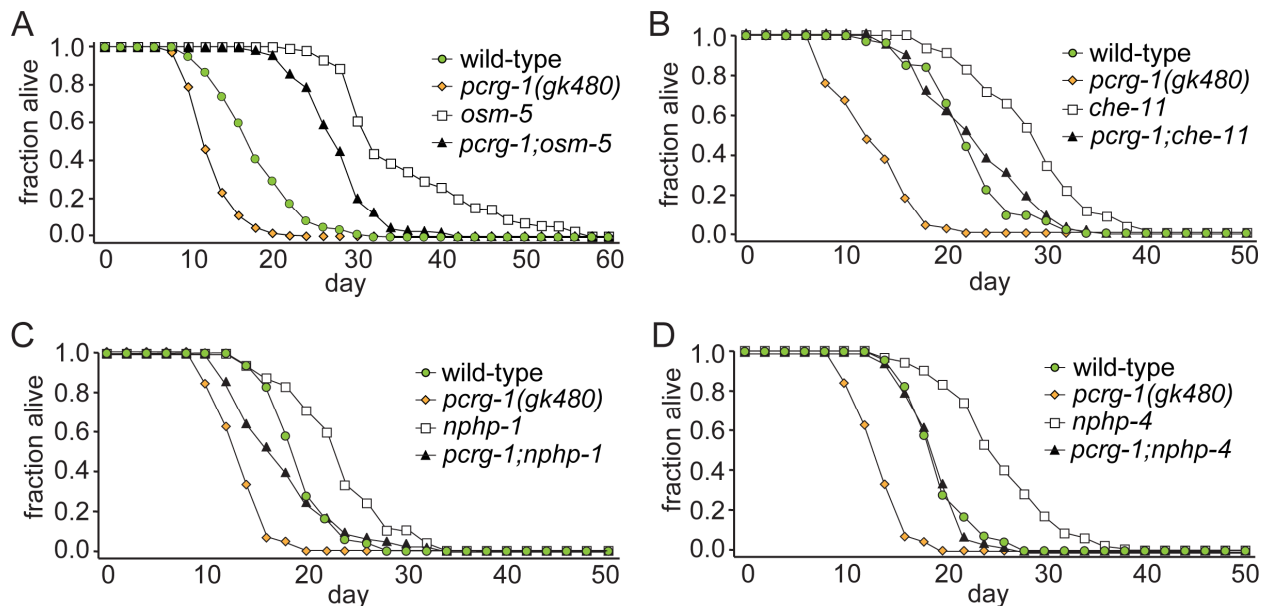


C

	post-embryonic development (h)
wild-type	45.1 +/- 1.5
<i>daf-16</i>	44.1 +/- 1.1
<i>daf-2</i>	49.4 +/- 2.6
<i>osm-5</i>	45.8 +/- 1.9
<i>pcrg-1(gk480)</i>	44.7 +/- 1.0
<i>pcrg-1(tm2597)</i>	44.8 +/- 1.2

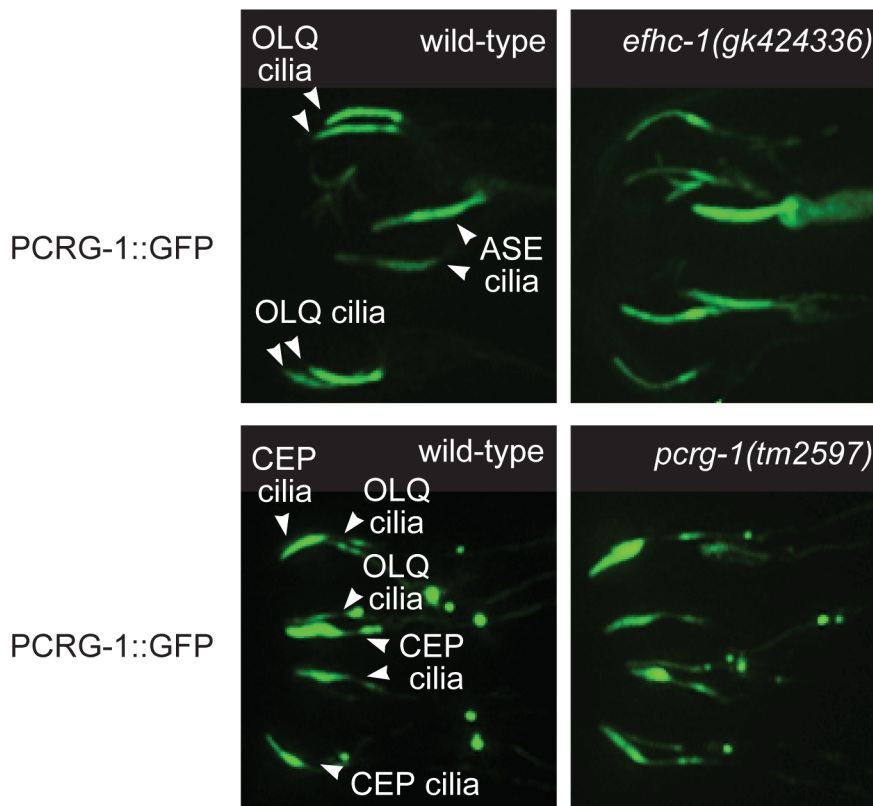


SUPPLEMENTARY FIGURE 1: Analyses of *pcrg-1* mutant alleles. (A) Dye-filling analysis of *pcrg-1* mutant worms. Dye-filling in wild-type (N2), *osm-5* (dye-filling defective), *pcrg-1(gk480)* and *pcrg-1(tm2597)* worms is shown. Both amphid (head) and phasmid (tail) cell bodies of neurons that uptake dye (marked as ‘+++’) are denoted by arrowheads. (B) Detailed characterization of the *pcrg-1* alleles. Results of several assays, including the osmotic avoidance (Osm) assay, chemotaxis (Che) to isoamyl alcohol (IAA) and acetone (Ace), lengths of ASER cilia (as visualized by *gcy-5p::GFP*), and egg-laying characteristics (Egl). Wild-type (N2) values are shown as white bars, *pcrg-1(gk480)* as light grey bars, *pcrg-1(tm2597)* as grey bars, and *osm-5* as black bars. For each assay, wild-type values are plotted as 1, and the values of all other strains are shown as fractions of wild-type values. *, statistically significantly different compared to wild-type ($P < 0.05$). (C) Results of developmental timing assays for wild-type (N2), *daf-16*, *daf-2*, *osm-5*, and both *pcrg-1* alleles (*gk480*, *tm2597*). Both *pcrg-1* alleles show normal developmental timing to the L4 stage. (D) Representative images examining ASER and OLQ cilium integrity in wild-type (N2) and *pcrg-1(tm2597)* mutant backgrounds. ASER cilium visualized by *gcy-5::GFP*; OLQ cilia visualized by *OSM-9::GFP*. Relative positions of cilia and dendritic endings are also denoted on each image. (E) Kymographic analyses of intraflagellar transport of the ASE-specific fusion protein (*gcy-5p::OSM-6::GFP*) in wild-type (N2) and *pcrg-1(tm2597)* mutant animals. Top: representative still images for each strain, with representative kymographs and associated anterograde traces. Bottom: numerical scoring of velocities in both middle segments (MS) and distal segments (DS) of cilia. Scale bar, 5 μm .

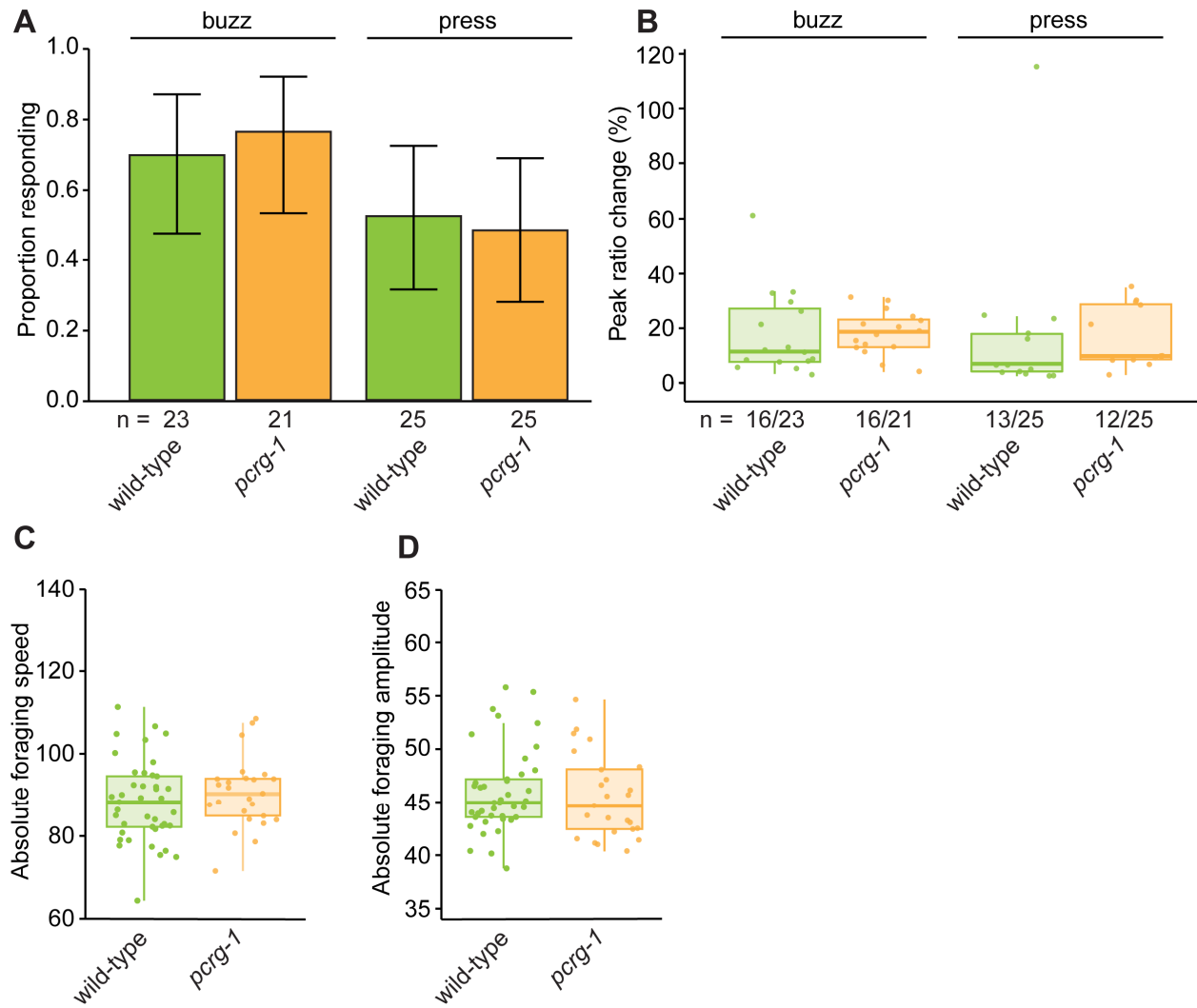


SUPPLEMENTARY FIGURE 2: A second mutant allele of *pcrg-1* similarly affects lifespan in a parallel pathway to that of other ciliary proteins. (A) The lifespan of a *pcrg-1;osm-5* double mutant is significantly different from that of either single mutant. (B) The lifespan of a *pcrg-1;che-11* double mutant is significantly different from that of either single mutant. (C) The lifespan of a *pcrg-1;nphp-1* double mutant is significantly different from that of either single mutant. (D) The lifespan of a *pcrg-1;nphp-4* double mutant is significantly different from that of either single mutant. For experimental number of animals (n) and p values comparing differences between strains in lifespan experiments see Supplementary Table 1. For experimental number of

animals (n) and p values comparing differences between strains in lifespan experiments see supplementary material Table S1.



SUPPLEMENTARY FIGURE 3:EFHC-1::GFP localization is independent of PCRG, and PCRG-1::GFP localization is independent of EFHC-1. Ciliary localization of EFHC-1 is unchanged in *pcrg-1(tm2597)* mutants compared to wild-type animals, and ciliary localization of PCRG-1 is unchanged in *efhc-1(gk424336)* mutants compared to wild-type animals.



SUPPLEMENTARY FIGURE 4: *pcrq-1* mutants show no defects in OLQ-related mechanosensory or behavioural responses. (A) Loss of PCRQ-1 has no effect on the activation of OLQ neurons upon gentle mechanical stimulation (buzz) or a stronger stimulation (press). In this panel, n represents the total number of animals assessed. Each bar represents the number of worms responding to mechanical stimulation over the total number of worms assessed per strain, and the error bars represent the 95% binomial confidence intervals. Binomial logistic regression followed by Tukey's HSD (honest significant difference) test was used to determine significance for each pair of strains. Differences between all strains are not significant. (B) The peak ratio change (%) representing the amplitude of the neuronal response, is similar between strains for animals responding to each stimulus. In this panel, n represents the number of animals responding to the stimulus over the total number of animals assessed. (C) *pcrq-1* mutants showed similar foraging speeds and (D) foraging amplitudes, two behaviours mediated by the OLQ neurons. Here the absolute values of the forward foraging speed/amplitude are depicted.

Supplementary Table

SUPPLEMENTARY TABLE S1: Statistical analysis of lifespan experiments for all Figures presented in study.

Figure	Strain	Mean Lifespan (days)	n	p-value (vs.)
3A	wild-type	19.83 +/- 0.37	81	n/a (wild-type)
	<i>pcrg-1(gk480)</i>	13.86 +/- 0.28	84	<0.0001 (wild-type)
	<i>pcrg-1(tm2597)</i>	14.53 +/- 0.35	60	<0.0001 (wild-type)
3B	wild-type	19.46 +/- 0.42	71	n/a (wild-type)
	<i>pcrg-1(gk480)</i>	14.79 +/- 0.36	68	<0.0001 (wild-type)
	<i>pcrg-1(gk480); PCRG-1::GFP (construct A)</i>	19.29 +/- 0.41	68	0.7865 (wild-type)
3C	wild-type	20.87 +/- 0.43	87	n/a (wild-type)
	<i>pcrg-1(tm2597)</i>	14.93 +/- 0.32	82	<0.0001 (wild-type)
	<i>pcrg-1(tm2597); PCRG-1::GFP (construct B)</i>	20.05 +/- 0.52	76	0.441 (wild-type)
3D	wild-type	20.87 +/- 0.43	87	n/a (wild-type)
	<i>pcrg-1(tm2597)</i>	14.93 +/- 0.32	82	<0.0001 (wild-type)
	wild-type; PCRG-1::GFP (construct B)	21.47 +/- 0.50	83	0.18 (wild-type)
4A	wild-type	18.60 +/- 0.53	67	<0.0001 (<i>gpc-1</i>), n/a (wild-type), <0.0001 (<i>pcrg-1</i>)
	<i>pcrg-1(tm2597)</i>	13.45 +/- 0.45	62	<0.0001 (<i>gpc-1</i>), <0.0001 (wild-type), n/a (<i>pcrg-1</i>)
	<i>gpc-1(pk298)</i>	22.17 +/- 0.50	69	n/a (<i>gpc-1</i>), <0.0001 (wild-type), <0.0001 (<i>pcrg-1</i>)

Figure	Strain	Mean Lifespan (days)	n	p-value (vs.)
	<i>pcrg-1(tm2597); gpc-1(pk298)</i>	21.38 +/- 0.45	64	0.1231 (<i>gpc-1</i>), 0.0012* (wild-type), <0.0001 (<i>pcrg-1</i>)
4B	wild-type	18.60 +/- 0.53	67	<0.0001 (<i>gpa-1</i>), n/a (wild-type), <0.0001 (<i>pcrg-1</i>)
	<i>pcrg-1(tm2597)</i>	13.45 +/- 0.45	62	<0.0001 (<i>gpa-1</i>), <0.0001 (wild-type), n/a (<i>pcrg-1</i>)
	<i>gpa-1(pk15)</i>	21.82 +/- 0.61	67	n/a (<i>gpa-1</i>), <0.0001 (wild-type), <0.0001 (<i>pcrg-1</i>)
	<i>pcrg-1(tm2597); gpa-1(pk15)</i>	19.97 +/- 0.62	65	0.0825 (<i>gpa-1</i>), 0.0827 (wild-type), <0.0001 (<i>pcrg-1</i>)
4C	wild-type	18.60 +/- 0.53	67	0.4275 (<i>gpa-11</i>), n/a (wild-type), <0.0001 (<i>pcrg-1</i>)
	<i>pcrg-1(tm2597)</i>	13.45 +/- 0.45	62	<0.0001 (<i>gpa-11</i>), <0.0001 (wild-type), n/a (<i>pcrg-1</i>)
	<i>gpa-11(pk349)</i>	18.33 +/- 0.43	72	n/a (<i>gpa-11</i>), 0.4275 (wild-type), <0.0001 (<i>pcrg-1</i>)
	<i>pcrg-1(tm2597); gpa-11(pk349)</i>	15.49 +/- 0.40	67	<0.0001 (<i>gpa-11</i>), <0.0001 (wild-type), 0.0060* (<i>pcrg-1</i>)
4D	wild-type	18.60 +/- 0.53	67	<0.0001 (<i>gpa-11XS</i>), n/a (wild-type), <0.0001 (<i>pcrg-1</i>)
	<i>pcrg-1(tm2597)</i>	13.45 +/- 0.45	62	<0.0001 (<i>gpa-11XS</i>), <0.0001 (wild-type), n/a (<i>pcrg-1</i>)
	<i>gpa-11XS</i>	25.68 +/- 0.79	75	n/a (<i>gpa-11XS</i>), <0.0001 (wild-type), <0.0001 (<i>pcrg-1</i>)
	<i>pcrg-1(tm2597); gpa-11XS</i>	22.39 +/- 0.82	82	0.0161* (<i>gpa-11XS</i>), <0.0001 (wild-type), <0.0001 (<i>pcrg-1</i>)
4E	wild-type	18.60 +/- 0.53	67	<0.0001 (<i>odr-3</i>), n/a (wild-type), <0.0001 (<i>pcrg-1</i>)
	<i>pcrg-1(tm2597)</i>	13.45 +/- 0.45	62	<0.0001 (<i>odr-3</i>), <0.0001 (wild-type), n/a (<i>pcrg-1</i>)

Figure	Strain	Mean Lifespan (days)	n	p-value (vs.)
	<i>odr-3(n1605)</i>	22.52 +/- 0.58	85	n/a (<i>odr-3</i>), <0.0001 (wild-type), <0.0001 (<i>pcrg-1</i>)
	<i>pcrg-1(tm2597); odr-3(n1605)</i>	15.80 +/- 0.39	61	<0.0001 (<i>odr-3</i>), <0.0001 (wild-type), 0.0025* (<i>pcrg-1</i>)
4F	wild-type	18.60 +/- 0.53	67	<0.0001 (<i>odr-3;gpa-11XS</i>), n/a (wild-type), <0.0001 (<i>pcrg-1</i>)
	<i>pcrg-1(tm2597)</i>	13.45 +/- 0.45	62	<0.0001 (<i>odr-3;gpa-11XS</i>), <0.0001 (wild-type), n/a (<i>pcrg-1</i>)
	<i>odr-3(n1605); gpa-11XS</i>	27.31 +/- 0.91	67	n/a (<i>odr-3;gpa-11XS</i>), <0.0001 (wild-type), <0.0001 (<i>pcrg-1</i>)
	<i>pcrg-1(tm2597); odr-3(n1605); gpa-11XS</i>	27.96 +/- 1.10	53	0.6048 (<i>odr-3;gpa-11XS</i>), <0.0001 (wild-type), 0.0025* (<i>pcrg-1</i>)
5A	wild-type	18.45 +/- 0.53	85	<0.0001 (<i>osm-5</i>), n/a (wild-type), <0.0001 (<i>pcrg-1</i>)
	<i>pcrg-1(tm2597)</i>	13.22 +/- 0.33	74	<0.0001 (<i>osm-5</i>), <0.0001 (wild-type), n/a (<i>pcrg-1</i>)
	<i>osm-5(p813)</i>	35.57 +/- 0.90	84	n/a (<i>osm-5</i>), <0.0001 (wild-type), <0.0001 (<i>pcrg-1</i>)
	<i>pcrg-1(tm2597); osm-5(p813)</i>	28.14 +/- 0.53	71	<0.0001 (<i>osm-5</i>), <0.0001 (wild-type), <0.0001 (<i>pcrg-1</i>)
5B	wild-type	18.45 +/- 0.53	85	<0.0001 (<i>che-11</i>), n/a (wild-type), <0.0001 (<i>pcrg-1</i>)
	<i>pcrg-1(tm2597)</i>	13.22 +/- 0.33	74	<0.0001 (<i>che-11</i>), <0.0001 (wild-type), n/a (<i>pcrg-1</i>)
	<i>che-11(e1810)</i>	27.16 +/- 0.86	71	n/a (<i>che-11</i>), <0.0001 (wild-type), <0.0001 (<i>pcrg-1</i>)
	<i>pcrg-1(tm2597); che-11(e1810)</i>	20.02 +/- 0.53	74	<0.0001 (<i>che-11</i>), 0.061 (wild-type), <0.0001 (<i>pcrg-1</i>)

Figure	Strain	Mean Lifespan (days)	n	p-value (vs.)
5C	wild-type	18.57 +/- 0.51	76	<0.0001 (<i>nphp-1</i>), n/a (wild-type), <0.0001 (<i>pcrg-1</i>)
	<i>pcrg-1(tm2597)</i>	12.19 +/- 0.28	93	<0.0001 (<i>nphp-1</i>), <0.0001 (wild-type), n/a (<i>pcrg-1</i>)
	<i>nphp-1(ok500)</i>	23.29 +/- 0.44	87	n/a (<i>nphp-1</i>), <0.0001 (wild-type), <0.0001 (<i>pcrg-1</i>)
	<i>pcrg-1(tm2597); nphp-1(ok500)</i>	16.25 +/- 0.39	73	<0.0001 (<i>nphp-1</i>), 0.061 (wild-type), <0.0001 (<i>pcrg-1</i>)
5D	wild-type	19.77 +/- 0.54	71	<0.0001 (<i>nphp-4</i>), n/a (wild-type), <0.0001 (<i>pcrg-1</i>)
	<i>pcrg-1(tm2597)</i>	13.97 +/- 0.37	71	<0.0001 (<i>nphp-4</i>), <0.0001 (wild-type), n/a (<i>pcrg-1</i>)
	<i>nphp-4(tm925)</i>	26.98 +/- 0.60	84	n/a (<i>nphp-4</i>), <0.0001 (wild-type), <0.0001 (<i>pcrg-1</i>)
	<i>pcrg-1(tm2597); nphp-4(tm925)</i>	17.09 +/- 0.56	77	<0.0001 (<i>nphp-4</i>), 0.0044* (wild-type), <0.0001 (<i>pcrg-1</i>)
6A	wild-type	19.92 +/- 0.48	74	<0.0001 (<i>daf-16</i>), n/a (wild-type), <0.0001 (<i>pcrg-1</i>)
	<i>pcrg-1(tm2597)</i>	12.46 +/- 0.37	69	0.26 (<i>daf-16</i>), <0.0001 (wild-type), n/a (<i>pcrg-1</i>)
	<i>daf-16(mu86)</i>	13.08 +/- 0.30	89	n/a (<i>daf-16</i>), <0.0001 (wild-type), <0.0001 (<i>pcrg-1</i>)
	<i>pcrg-1(tm2597), daf-16(mu86)</i>	12.68 +/- 0.27	68	0.19 (<i>daf-16</i>), <0.0001 (wild-type), <0.0001 (<i>pcrg-1</i>)
6B	wild-type	18.05 +/- 0.29	86	<0.0001 (<i>daf-2</i>), n/a (wild-type), <0.0001 (<i>pcrg-1</i>)
	<i>pcrg-1(tm2597)</i>	11.76 +/- 0.23	90	<0.0001 (<i>daf-2</i>), <0.0001 (wild-type), n/a (<i>pcrg-1</i>)
	<i>daf-2(e1370)</i>	34.07 +/- 1.01	83	n/a (<i>daf-2</i>), <0.0001 (wild-type), <0.0001 (<i>pcrg-1</i>)

Figure	Strain	Mean Lifespan (days)	n	p-value (vs.)
	<i>pcrg-1(tm2597);daf-2(e1370)</i>	33.43 +/- 0.89	74	0.33 (<i>daf-2</i>), <0.0001 (wild-type), <0.0001 (<i>pcrg-1</i>)
6C	wild-type	18.07 +/- 0.30	86	<0.0001 (<i>eat-2</i>), n/a (wild-type), <0.0001 (<i>pcrg-1</i>)
	<i>pcrg-1(tm2597)</i>	12.75 +/- 0.35	80	<0.0001 (<i>eat-2</i>), <0.0001 (wild-type), n/a (<i>pcrg-1</i>)
	<i>eat-2(ad465)</i>	22.76 +/- 0.45	84	n/a (<i>eat-2</i>), <0.0001 (wild-type), <0.0001 (<i>pcrg-1</i>)
	<i>pcrg-1(tm2597);eat-2(ad465)</i>	17.05 +/- 0.36	91	<0.0001 (<i>eat-2</i>), 0.175 (wild-type), <0.0001 (<i>pcrg-1</i>)
6D	wild-type	19.09 +/- 0.47	68	<0.0001 (<i>clk-1</i>), n/a (wild-type), <0.0001 (<i>pcrg-1</i>)
	<i>pcrg-1(tm2597)</i>	13.36 +/- 0.42	69	<0.0001 (<i>clk-1</i>), <0.0001 (wild-type), n/a (<i>pcrg-1</i>)
	<i>clk-1(e2519)</i>	21.97 +/- 0.46	74	n/a (<i>clk-1</i>), <0.0001 (wild-type), <0.0001 (<i>pcrg-1</i>)
	<i>pcrg-1;clk-1</i>	17.95 +/- 0.54	83	<0.0001 (<i>clk-1</i>), 0.3840 (wild-type), <0.0001 (<i>pcrg-1</i>)
7C	wild-type	18.18 +/- 0.36	135	<0.0001 (<i>efhc-1(gk424336)</i>), n/a (wild-type), <0.0001 (<i>efhc-1(tm6235)</i>)
	<i>efhc-1(gk424336)</i>	13.97 +/- 0.14	150	n/a (<i>efhc-1(gk424336)</i>), <0.0001 (wild-type), 0.0135* (<i>efhc-1(tm6235)</i>)
	<i>efhc-1(tm6235)</i>	14.51 +/- 0.17	145	0.0135* (<i>efhc-1(gk424336)</i>), <0.0001 (wild-type), n/a (<i>efhc-1(tm6235)</i>)
7D	wild-type	19.08 +/- 1.08	110	<0.0001 (<i>efhc-1</i>), n/a (wild-type), <0.0001 (<i>pcrg-1</i>), <0.0001 (<i>pcrg-1;efhc-1</i>)
	<i>pcrg-1(tm2597)</i>	13.04 +/- 0.64	149	0.6138 (<i>efhc-1</i>), <0.0001 (wild-type), n/a (<i>pcrg-1</i>), 0.9508 (<i>pcrg-1;efhc-1</i>)

Figure	Strain	Mean Lifespan (days)	n	p-value (vs.)
	<i>efhc-1(gk424336)</i>	13.49 +/- 0.42	136	n/a (<i>efhc-1</i>), <0.0001 (wild-type), 0.9508 (<i>pcrg-1</i>), 0.1627 (<i>pcrg-1;efhc-1</i>)
	<i>pcrg-1(tm2597);efhc-1(gk424336)</i>	12.35 +/- 0.49	154	0.1627 (<i>efhc-1</i>), <0.0001 (wild-type), 0.9508 (<i>pcrg-1</i>), n/a (<i>pcrg-1;efhc-1</i>)
8B	<i>pcrg-1(tm2597)</i>	17.22 +/- 0.45	99	<0.0001 (<i>pocr-4::PCRG::GFP</i>)
	<i>pocr-4::PCRG::GFP</i>	20.43 +/- 0.55	99	<0.0001 (<i>pcrg-1</i>)
8C	wild-type	18.01 +/- 0.47	95	<0.0001 (<i>pocr-4::egl-1</i> (1)), <0.0001 (<i>pocr-4::egl-1</i> (2))
	<i>pocr-4::egl-1</i> (1)	23.58 +/- 0.51	100	<0.0001 (<i>pocr-4::egl-1</i> (2)), <0.0001 (wild-type)
	<i>pocr-4::egl-1</i> (2)	22.98 +/- 0.68	86	<0.0001 (<i>pocr-4::egl-1</i> (1)), <0.0001 (wild-type)
Supp. 2A	wild-type	22.37 +/- 0.55	71	<0.0001 (<i>osm-5</i>), n/a (wild-type), <0.0001 (<i>pcrg-1</i>)
	<i>pcrg-1(gk480)</i>	13.00 +/- 0.46	72	<0.0001 (<i>osm-5</i>), <0.0001 (wild-type), n/a (<i>pcrg-1</i>)
	<i>osm-5(p813)</i>	35.61 +/- 0.69	71	n/a (<i>osm-5</i>), <0.0001 (wild-type), <0.0001 (<i>pcrg-1</i>)
	<i>pcrg-1(gk480);osm-5(p813)</i>	31.55 +/- 0.71	58	<0.0001 (<i>osm-5</i>), <0.0001 (wild-type), <0.0001 (<i>pcrg-1</i>)
Supp. 2B	wild-type	22.37 +/- 0.55	71	<0.0001 (<i>che-11</i>), n/a (wild-type), <0.0001 (<i>pcrg-1</i>)
	<i>pcrg-1(gk480)</i>	13.00 +/- 0.46	72	<0.0001 (<i>che-11</i>), <0.0001 (wild-type), n/a (<i>pcrg-1</i>)
	<i>che-11(e1810)</i>	28.58 +/- 0.65	79	n/a (<i>che-11</i>), <0.0001 (wild-type), <0.0001 (<i>pcrg-1</i>)

Figure	Strain	Mean Lifespan (days)	n	p-value (vs.)
	<i>pcrg-1(gk480); che-11(e1810)</i>	23.51 +/- 0.64	74	<0.0001 (<i>che-11</i>), 0.1241 (wild-type), <0.0001 (<i>pcrg-1</i>)
Supp. 2C	wild-type	19.83 +/- 0.37	81	<0.0001 (<i>nphp-1</i>), n/a (wild-type), <0.0001 (<i>pcrg-1</i>)
	<i>pcrg-1(gk480)</i>	13.86 +/- 0.28	84	<0.0001 (<i>nphp-1</i>), <0.0001 (wild-type), n/a (<i>pcrg-1</i>)
	<i>nphp-1(ok500)</i>	23.45 +/- 0.56	83	n/a (<i>nphp-1</i>), <0.0001 (wild-type), <0.0001 (<i>pcrg-1</i>)
	<i>pcrg-1(gk480); nphp-1(ok500)</i>	18.25 +/- 0.66	64	<0.0001 (<i>nphp-1</i>), 0.2159 (wild-type), <0.0001 (<i>pcrg-1</i>)
Supp. 2D	wild-type	19.83 +/- 0.37	81	<0.0001 (<i>nphp-4</i>), n/a (wild-type), <0.0001 (<i>pcrg-1</i>)
	<i>pcrg-1(gk480)</i>	13.86 +/- 0.28	84	<0.0001 (<i>nphp-4</i>), <0.0001 (wild-type), n/a (<i>pcrg-1</i>)
	<i>nphp-4(tm925)</i>	25.97 +/- 0.62	75	n/a (<i>nphp-4</i>), <0.0001 (wild-type), <0.0001 (<i>pcrg-1</i>)
	<i>pcrg-1(gk480); nphp-4(tm925)</i>	19.65 +/- 0.35	69	<0.0001 (<i>nphp-4</i>), 0.6937 (wild-type), <0.0001 (<i>pcrg-1</i>)