Table S1. Strains

A Strains used for experiments presented in main text

Strain	Genotype	Reference/Source
CB1370	*daf-2(e1370)*	(Riddle et al., 1981)
DR1567	*daf-2(m577)*	(Gems et al., 1998)
DR1572	*daf-2(e1368)*	(Gems et al., 1998)
CF1553	*muIs84 [pAD76 (sod-3::GFP)]*	(Libina et al., 2003)
CF1038	*daf-16(mu86)*	(Lin et al., 1997)
CB4037	*glp-1 (e2141)*	(Priess et al., 1987)
PD8488	*rrf-1(pk1417)*	(Sijen et al., 2001)
GC678	*tnIs6[lim-7::GFP; rol-6 (su1006)]; qIs19[lag-2::GFP; rol-6(su1006)*]	(Killian and Hubbard, 2004)
GC888	*glp-1(bn18)*	(Kodoyianni et al., 1992)
GC833	*glp-1(ar202)*	(Pepper et al., 2003a)
RB712	*daf-18(ok480)*	*C. elegans* Gene Knockout Consortium, Oklahoma City and Vancouver, Canada, and the *Caenorhabditis* Genetics Center (CGC), Minneapolis, MN, USA
CF1295	*daf-16(mu86) I; daf-2(e1370) III; muEx108[pKL99-2(DAF-16::GFP/daf16bKO) + pRF4(rol-6(su1006)]*	(Lin et al., 2001)
GC1019	*rrf-1(pk1417);daf-2(e1370)*	This work
GC854	*daf-2(e1370); glp-1(e2141)*	This work
GC1071	*ins-3(ok2488)* [a]	This work: 2x backcross of RB1915; *C. elegans* Gene Knockout Consortium and the CGC [a]
GC1039	*ins-33(tm2988)*	This work: 8x backcross of FX02988; National Bioresource Project for the Nematode *C. elegans,* Tokyo, Japan
GC865	*daf-16(mu86); muEx108[pKL99-2(daf-16::GFP/daf16bKO) + pRF4(rol-6(su1006))]*	This work: constructed from CF1038 *daf-16(mu86)* (Lin et al., 1997) and CF1295 *daf-16(mu86) I; daf-2(e1370) III; muEx108[pKL99-2(DAF-6::GFP/daf16bKO) + pRF4(rol-6(su1006)]* (Lin et al., 2001), this fusion uses isoform a of *daf-16* and contains a stop codon before isoform b
GC1079	*ins-3(ok2488); naEx187*[pGC467, pRF4] [b]	This work
GC1078	*ins-33(tm2988); naEx186*[pGC464; pRF4] [b]	This work
GC1087	*ins-33(tm2988); naEx195*[pGC464; pRF4] [b]	This work
GC1089	*ins-33(tm2988); naEx197*[pGC464; pRF4] [b]	This work
DR1309	*daf-16(m26); daf-2(e1370)*	P. Albert and D. Riddle, via CGC
CF1442	*daf-16(mu86);daf-2(e1370); muEx169[Punc-119::GFP::DAF-16* + pRF4 *rol-6 (su1006)]*	(Libina et al., 2003)
CF1514	*daf-16(mu86); daf-2(e1370); muEx211[*pNL213 *(Pges-1::GFP::DAF-16)* + pRF4 *rol-6 (su1006)]*	(Libina et al., 2003)
CF1515	*daf-16(mu86); daf-2(e1370); muEx212[*pNL212 *(Pmyo-3::GFP::DAF-16)* + pRF4 *rol-6 (su1006)]*	(Libina et al., 2003)
GC1143	*unc-119(ed3)III; naIs43[pGC492(Prpl-11.1::daf-16cDNA::GFP::nos-2 3'UTR – unc-119(+))]* [c]	This work: microparticle bombardment (Praitis et al., 2001) of DP38 worms with pGC492
GC1144	*daf-16(mu86)I;daf-2(e1370)III; naIs43[pGC492(Prpl-11.1::daf-16cDNA::GFP::nos2 3'UTR unc-119(+))]* [c]	This work: generated by crossing GC1143 with DR1309
GC1109	*daf-16(m26) I ; daf-2(e1370) II ; naEx202 [pGC461 (Plag-2::daf-16::GFP) + pRF4]*	This work: *pGC461 was injected to DR1309*
RB777	*hcf-1(ok559)*	*C. elegans* Gene Knockout Consortium, and the CGC
GC1109	*naEx202*[pGC461; pRF4] [d]	This work
GC1004	*ins-33(tm2988)* cross	This work: taken from spontaneous male from GC1039
GC585	*pro-1(na48)/mIn1[dpy-10(e128) mIs14]*	Used as a carrier of the *mIs14* balancer
GC1142	*ins-33(tm2988);ins-3(ok2488)*	This work: generated by crossing GC1004 males to GC1071; using GC585 to balance *ins-3*

B Strains used for data presented in supplementary material

Strain	Genotype	Reference/Source
CB1375	*daf-18(e1375)*	(Riddle et al., 1981)
FT224	*xnIs87* [pMP322 *(Psyn-4::GFP-syn-4::syn-4 3' UTR); unc-119 (+)]; syn-4 tag-316(ok372) IV*	Gift from Ann Wehman and Jeremy Nance; pMP322 gift from Michael Glotzer; *ok372* removes both *syn-4* and *tag-316*
GC967	*daf-16(mu86); glp-1(ar202); naEx148* [pGP30(DAF-16::GFP) + *sur-5::GFP*]	This work: injection of GC908 *daf-16(mu86); glp-1(ar202)* with pGP30 (Henderson and Johnson, 2001) at 1ng/μl; *sur-5::GFP* at 20ng/μl; and pBluescript DNA at 80ng/μl; DAF-16 sequences in pGP30 correspond to isoform a2
GC1112	*daf-16(mu86); daf-2(e1370); naEx148* [pGP30(DAF-16::GFP) + *sur-5::GFP*]	This work: generated by crossing GC967 with DR1309 males and selecting for GFP-positive dauer worms and selecting against *glp-1(ar202)*
GC1080	*smg-1(cc546); naEx188*[pGC487; pRF4] [e]	This work
GC1081	*smg-1(cc546); naEx189*[pGC487; pRF4] [e]	This work
GC1082	*smg-1(cc546); naEx190*[pGC486; pRF4] [e]	This work
GC1083	*smg-1(cc546); naEx191*[pGC486; pRF4] [e]	This work
GC1084	*smg-1(cc546); naEx192*[pGC486; pRF4] [e]	This work
GC1085	*smg-1(cc546); naEx193*[pGC486; pRF4] [e]	This work
GC1088	*naEx196*[pGC467; pRF4] [f]	This work
GC1095	*naEx198*[pGC467; pRF4] [f]	This work
GC1076	*naEx184*[pGC464; pRF4] [f]	This work
GC1077	*naEx185*[pGC464; pRF4] [f]	This work
GC1145	*daf-2(e1370);xnIs879pMP322(Psyn-4::GFP::syn-4::syn-4 3'UTR)unc-119(+)];syn-4 tag-316(ok372)*	This work: generated by crossing FT224 with CB1370

[a] Three out of 12 *ins-3(ok2488)* worms examined for the brood size analysis shown in Fig. 6 laid exclusively dead embryos, presumably due to a maternal effect lethal mutation in the background. They were not included in the analysis.

[b] Rescuing (pGC) plasmids were injected at 10 ng/μl together with 100 ng/μl of the transformation marker pRF4 [*(rol-6(su1006)*].

[c] The presence of the transgene was determined using PCR with primers to GFP. The data shown in Fig. 3C is from the line as scored shortly after it was established and expression validated by RT-PCR. However, subsequent RT-PCR indicated that expression from the transgene was becoming silenced (likely reflected in large variation of the data, see Fig. S2), and later thaws of our frozen stocks showed no expression indicating complete silencing subsequent to the experiments presented in this data collection. Measurements conducted on thawed strains (after silencing) also lost *daf-16(+)* activity.

[d] Microinjection of DR1309 was performed with 5 ng/μl of pGC461 [P*lag-2*::*daf-16*::GFP] and 100 ng/μl of pRF4 [*(rol-6(su1006)*].

[e] *ins-3* and *ins-33* rescue: (GC1080-GC1085) generated by microinjection of PD8120 *smg-1(cc546)* (A. Fire and the *Caenorhabditis* Genetics Center, Minneapolis, MN, USA) with 20 ng/μl of the (pGC) expression plasmid and 100 ng/μl of pRF4 [*(rol-6(su1006)*].

[f] *ins-3* and *ins-33* overexpression: (GC1088, GC1095 and GC1076, GC1077) microinjection of N2 was performed with 100 ng/μl of the expression plasmid and 100 ng/μl of pRF4 [*(rol-6(su1006)*].