Supporting Information

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SI Results

Isolation of Mutants with Increased Lipofuscin Levels. To identify genes that act to delay aging, we sought mutants that age prematurely, using accumulation of the autofluorescent pigment lipofuscin as our marker. We used a fluorescence-equipped dissecting microscope to screen for mutants that showed a premature increase of lipofuscin (Fig. S1). We readily detected lipofuscin autofluorescence in old wild-type adults, whereas young wild-type adults accumulated negligible amounts of autofluorescence (Fig. 1*A*). Animals with a *daf-16* loss-of-function mutation age prematurely and accumulated abnormally high levels of lipofuscin (1) (Fig. 1 *A* and *B*). We mutagenized wild-type hermaphrodites with ethyl methanesulfonate (EMS) and isolated synchronized F_2 progeny. We treated these F_2 worms with 5′ fluorodeoxyuridine (FUdR), which blocks DNA replication and prevents eggs from developing. This treatment allowed the observation of adult F_2 worms without mixing these animals with the next generation. The F_2 animals were screened for increased intestinal autofluorescence accumulation when they reached the second or third day of adulthood. Isolates with high levels of fluorescence were moved to Petri plates without FUdR to permit them to have viable progeny. Autofluorescence levels of the progeny of the original isolates were then examined. Overall, we screened ≈20,000 mutagenized haploid genomes and picked 52 candidate mutants, 16 of which did not produce viable progeny. Of the 36 fertile isolates, 25 failed to retest. We obtained 11 mutant strains that reliably exhibited premature accumulation of intestinal autofluorescence.

We outcrossed screen isolates six times to the wild-type parent strain before characterization. Three of the isolates, *n4430*, *n4434*, and *n4435*, exhibited a weakly semidominant autofluorescent phenotype; heterozygous animals showed weak accumulation of autofluorescence and were distinguishable from both the wild type and the mutant homozygote. Among the progeny of these heterozygous animals, ≈25% showed strong fluorescence accumulation, 25% had no increased fluorescence, and 50% showed weak accumulation of autofluorescence. Age-dependent accumulation of fluorescence in the homozygous mutant isolates was comparable to that of animals bearing loss-of-function mutations in *daf-16* (Fig. 1 *A* and *B*). Of the other eight isolates, *n4501* animals had a recessive increased autofluorescence phenotype, whereas the autofluorescence phenotypes of *n4427*, *n4428*, *n4429*, *n4431*, *n4432*, *n4433*, and *n4436* animals were strongly semidominant; heterozygous animals showed high levels of autofluorescence but were still distinguishable from the homozygous mutants.

We mapped *n4430*, *n4434*, *n4435*, and *n4501* to linkage group II (LGII), and *n4427*, *n4428*, *n4429*, *n4431*, *n4432*, *n4433*, and *n4436* to LGV. *n4434* and *n4435* failed to complement *n4430*, whereas *n4501* complemented *n4430* for increased lipofuscin accumulation. In short, the 11 isolates with high autofluorescence represent at least three complementation groups: two on LGII and one or more on LGV (Table 1), suggesting that at least three genes control the age-related accumulation of autofluorescence in *C. elegans*.

Mapping and Cloning. We used visible markers (*dpy-5 I*, *bli-2 II*, *unc-32 III*, *unc-5 IV*, *dpy-11 V*, and *lon-2 X*) to determine the chromosomal linkage of newly isolated mutations that caused high autofluorescence. We generated animals heterozygous for the new mutation and for three of these markers. We picked progeny with high autofluorescence and determined if these progeny segregated the above markers.

We used the polymorphic Hawaiian strain CB4856 and the *cis*-marked triple-mutant strain *bli-2 n4430 unc-4* for single nucleotide polymorphism (SNP) mapping, essentially as described (2). We crossed CB4856 males with *bli-2 n4430 unc-4* hermaphrodites, picked F_2 animals recombinant between *bli-2* and *unc-4* (i.e., showing only one marker), and obtained homozygotes for the recombinant chromosome. We tested animals in the next generation for the presence of the *n4430* mutation and identified the approximate recombination site by determining the DNA sequences of SNPs in the area.

We cloned the gene mutated in *n4430* animals using a combination of visual mapping, polymorphism mapping, and RNAi. We genetically mapped *n4430* on LGII between *dpy-10* and *unc-4* and close to *unc-104* (Fig. S4*A*). We then used polymorphism mapping to narrow the region to ≈155 kb, an interval spanned by three cosmids, C15F1, C52E12, and C06B7. We injected a pool of these three cosmids and observed rescue of the premature autofluorescence of *n4430* animals. When injected individually, C06B7, which contains 16 predicted genes, rescued the fluorescence accumulation of *n4430* animals; cosmids C15F1 and C52E12 did not. Cosmid ZK1127, which contains 12 of the 16 predicted genes on C06B7, did not rescue. We used RNAi to reduce expression of each of the genes contained on C06B7 and found that RNAi directed against *T02G5.8* resulted in high autofluorescence. We injected a PCR product containing the entire *T02G5.8* ORF and 2 kb of the upstream promoter region into *n4430* animals and observed that transgenes containing the single gene *T02G5.8* rescued the phenotype of fluorescence accumulation of *n4430* mutants. The same PCR product also restored normal lifespan to the short-lived *n4430* mutants.

We determined the DNA sequence of *T02G5.8* in *n4430* mutants and in the two allelic mutants *n4434* and *n4435* and found predicted missense changes in each (*n4430* C393Y, *n4434* G134E, *n4435* G368E; Fig. S4).

C. elegans mutants with altered intestinal autofluorescence were previously isolated (3). One mutant described, in the gene *flu-3*, defined by the single allele *e1001*, has increased gut autofluorescence similar to that we observed in *kat-1* mutants. *flu-3* maps close to *kat-1*. We showed that *flu-3(e1001)* failed to complement *kat-1(n4430)* for the accumulation of intestinal fluorescence. Using PCR and DNA sequencing we found that *flu-3 (e1001)* animals have two mutations in *T02G5.8* (A271T, A279V; Fig. S4*C*). Together these observations indicate that *flu-3* and *kat-1* are the same gene. We used PCR-amplified regions of genomic DNA as templates in determining *kat-1* sequences. We determined the sequences of the entire coding region plus 250 bases of the upstream promoter sequence. At least two independent PCR products were used to confirm the existence of an observed mutation. All sequences were determined using an automated ABI 3100 DNA sequencer (Applied Biosystems).

***kat-1* Is Not Required for the Lifespan Extension Mediated by Many Known Aging Pathways.** Several pathways are known to control *C. elegans* aging, including insulin signaling, germ-line signaling, chemosensory signaling, mitochondrial function, and a pathway activated by the gene *sir-2.1* (4). To see whether *kat-1* acts to delay aging via one of these known longevity pathways, we crossed a *kat-1* mutation into backgrounds of long-lived *C. elegans* mutants and tested whether *kat-1* is required for the extended longevity of these mutants. Specifically, we made *kat-1* double mutants with mutants defective in the insulin-like receptor gene *daf-2* (5), the mitochondrial function gene *isp-1* (6), and two genes involved in chemosensory responses that can affect aging, *osm-5* and *che-3*

(7). *kat-1* did not fully suppress the longevity caused by mutations in these genes (Table S1). To better characterize the epistatic relationships between *kat-1* and the mitochondrial ETC pathway, we used RNAi to reduce expression of mitochondrial ETC components in wild-type and *kat-1* backgrounds. RNAi of mitochondrial genes *phi-44(T02H6.11)* and *cyc-1* resulted in a significant extension of lifespan, as previously reported (8, 9). The same RNAi clones caused a similar extension of lifespan in *kat-1* mutants, indicating that *kat-1* is not required for lifespan extension by ETC abnormalities (Table S1). A recent report (10) showed that long-lived *C. elegans* that lack germ-line signaling accumulate less fat and require induction of a specific fat lipase to increase lypolysis and promote longevity. We asked whether *kat-1* can suppress the longevity caused by an absence of the germ line in animals with a loss-of-function mutation in the germ-line-specification gene *mes-1* (11, 12). This *mes-1* mutation results in a temperature-sensitive phenotype, allowing us to propagate the strain and obtain control animals with normal germ lines at the permissive temperature, whereas at the restrictive temperature generating animals that lack germ lines and are long-lived. A *kat-1* mutation partially suppressed the long lifespan of *mes-1* mutants that lacked germ lines (24 d for a *kat-1;mes-1*double-mutant vs. 31 d for *mes-1* mutants and 20 d for the wild type). Germ-lineless *kat-1;mes-1* double mutants had a significantly longer lifespan than germ-line-containing *kat-1;mes-1* double mutants (24 d vs. 17 d; Table S1), suggesting that the effect of germ-line signaling on lifespan is partially *kat-1*-dependent. This finding is consistent with the proposed requirement for enhanced lipolysis for the increased longevity of animals lacking a germ line and also with the hypothesis that *kat-1* functions redundantly with other thiolases to mediate fatty acid oxidation. Dietary restriction (DR) extends lifespan in many organisms, including *C. elegans* (13). We tested whether *kat-1* was necessary for the lifespan extension caused by DR. Lifespans of both wild-type and *kat-1* mutant animals was similarly extended by dietary restriction, suggesting that *kat-1* is not necessary for longevity induced by DR (Table S1).

SI Materials and Methods

Strains. Strains were cultured as described (14) and maintained at 20 °C unless specified otherwise. The wild-type strain was the Bristol strain N2 (14). The following mutations were used (described in ref. 15 unless otherwise noted):

LGI: *dpy-5*(*e61*), *daf-16*(*mgDf50*) (16), *che-3*(*e1124*)
LGII: *unc-85*(*n319*), *bli-2*(*e768*), *dpy-10*(*e128*), *unc-104*(*e1265*), *unc-4*(*e120*), *bli-1*(*e769*), *kat-1*(*tm1037, n4430, n4434, n4435, e1001*) (this study, and refs. 3, 17), *n4501* (this study)
LGIII: *unc-32*(*e189*), *daf-2*(*e1370*)
LGIV: *unc-5*(*e53*), *sir-2.1*(*ok434*), *isp-1*(*qm150*)
LGV: *dpy-11*(*e224*), *unc-76*(*e911*), *n4427*, *n4428*, *n4429, n4431, n4432, n4433, n4436* (this study)
LGX: *lon-2*(*e678*), *osm-5*(*p813*), *mes-1*(*bn74ts*)
sir-2.1(*pkIs1642*) was described by Viswanathan et al. (18).

Isolation of Mutants with Increased Lipofuscin Autofluorescence. We mutagenized N2 hermaphrodites with ethyl methanesulphonate (EMS) as described by Brenner (14). After recovery on food for 1 h, individual P_0 animals were transferred to 60-mm Petri plates. After 5–6 d, the F_1 animals were dissolved by bleaching in 1.5 N NaOH, 12% NaOCl solution to recover eggs, resulting in a synchronized culture of F_2 progeny. F_2 animals were transferred to 60-mm Petri plates seeded with OP50 bacteria, 100 animals per plate. When the animals reached the L4 stage, FUdR was added to plates to a final concentration of 25 μM. We screened animals equivalent to ≈20,000 mutagenized haploid genomes for high intestinal autofluorescence at day 2 or 3 of adulthood, using a dissecting microscope equipped with fluorescence optics.

Fluorescence Measurements in Protein Extracts. Worm protein lysates for fluorescence measurements were made by sonication of pelleted worms (200 animals/sample) in 0.5 mL of lysis buffer [10 mM Hepes-KOH, 250 mM NaCl, 0.5% Nonidet P-40, 10% glycerol, 5 mM EDTA, 1 mM DTT, protease inhibitors: one Complete Mini tablet (Roche Biochemical) per 10 mL IP buffer] for three 10-s bursts on ice. The lysates were then centrifuged at 10,000 rpm for 20 min at 4 °C. Fluorescence was measured in supernatants by spectrofluorimetry with excitation of 350 nm and emission spectra of 410–440 nm.

Transgenic Animals. Germ-line transformation was performed as described (19) by injecting cosmid DNA (5-20 ng/μL) or PCR products (5-50 ng/μL) into *kat-1(n4430);unc-76(e911)* mutants. The *unc-76* rescuing construct p76-16B (20) was used as a coinjection marker. Non-Unc transgenic lines were established and scored.

Behavioral Assays, Lifespans, and Mortality. Animals were grown on NGM plates (14) seeded with OP50 bacteria without FUdR and transferred every other day to separate them from their progeny. For behavioral assays, animals were transferred to fresh NGM plates (seeded with bacteria the night before) and allowed to recover from the transfer for 30 min before locomotion and/or pumping rates were counted. Locomotion rate was determined by counting body bends per min of animals moving in a fresh bacterial lawn. Pumping rate was assayed by counting the number of movements of the rear bulb of the pharynx per 30 s of animals within the bacterial lawn. At least 15 animals per genotype per time point were assayed.

For lifespan analyses we did not pool different trials together, as the lifespans of both wild-type and mutant animals varied slightly between trials (in all trials there was a consistent statistically significant difference between wild-type and *kat-1* mutant lifespans). Therefore the *P* and median lifespan values presented are from representative experiments. For each analysis, we state how many times we performed the assay with similar results, and *n* represents the total number of animals assayed in all trials. To assess mortality using our existing lifespan data, we calculated the fraction of animals that die on any given time point of the total number of animals alive at that time.

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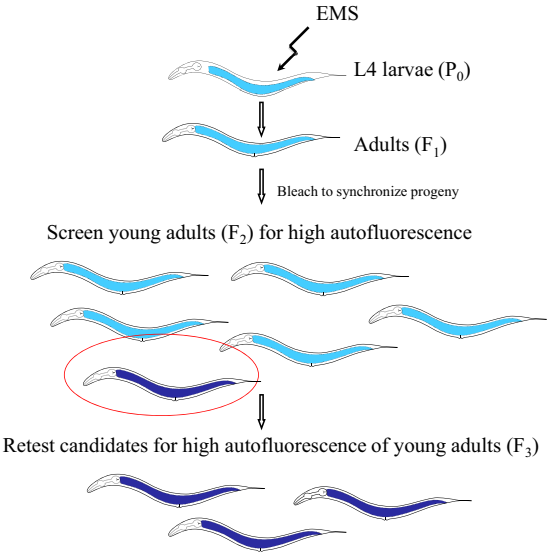


Fig. S1. A screen for mutants with high accumulation of lipofuscin-like fluorescent pigment. We screened synchronized F_2 progeny of EMS-mutagenized hermaphrodites at day 3 of adulthood for high lipofuscin-like autofluorescence. Candidates were retested in the next generation. See text for details.

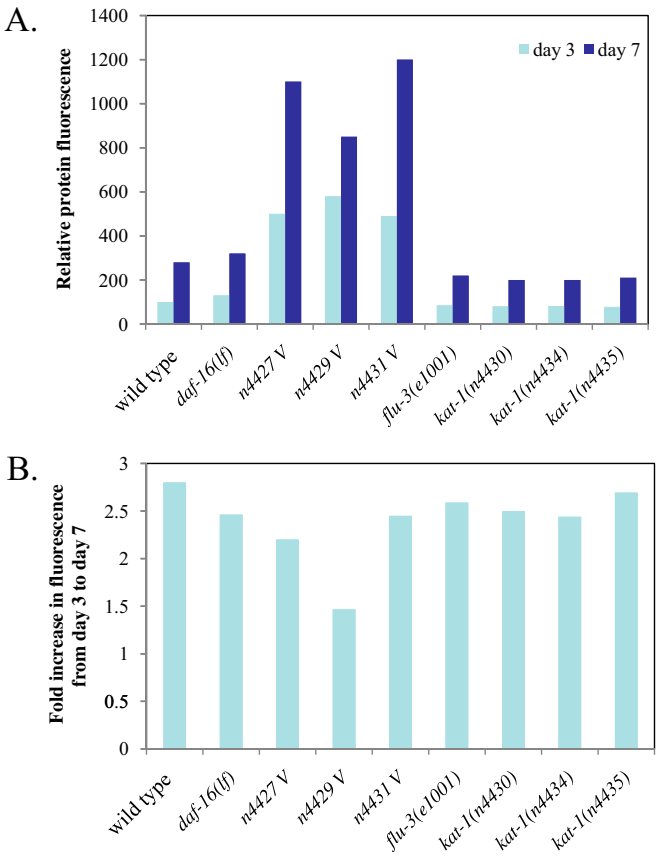


Fig. S2. *kat-1* mutants do not show increased protein fluorescence. (*A*) Fluorescence accumulation as measured by spectrofluorimetry of chromosome V isolates and *kat-1* mutants *n4430*, *n4434*, *n4435*, and *flu-3(e1001)*, compared with the wild-type and the progeroid *daf-16(mgDf50)* mutants on day 3 and day 7 of adulthood. *daf-16(lf)* mutants accumulated more fluorescence than the wild type on both days tested. Chromosome V isolates accumulated significantly more fluorescence on both days tested, whereas *kat-1(lf)* mutants accumulated slightly less fluorescence in protein extracts than the wild type on both days tested. (*B*) Increase in protein fluorescence in the above strains between day 3 and day 7 of adulthood. Both *daf-16(lf)* and *kat-1(lf)* mutants show a similar increase in protein fluorescence compared to the wild-type animals. Some of the chromosome V isolates showed a smaller increase in protein fluorescence between day 3 and day 7, possibly due to higher fluorescence on day 3. All values correspond to the emission peak at 420 nm, excitation of 350 nm.

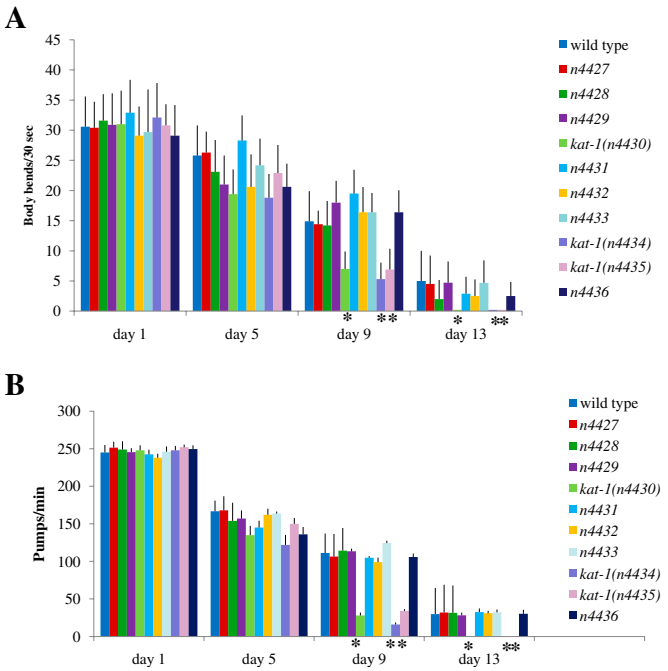


Fig. S3. Behavioral analyses of screen isolates. (*A*) Decline in locomotion rate and (*B*) pharyngeal pumping rate with age was assessed in wild-type animals (wild type, dark blue bars) and mutants isolated in the screen, as described in *Materials and Methods*. Note that only *kat-1* mutants showed premature declines in locomotion and pumping. *$P < 0.05$ vs. wild-type control.

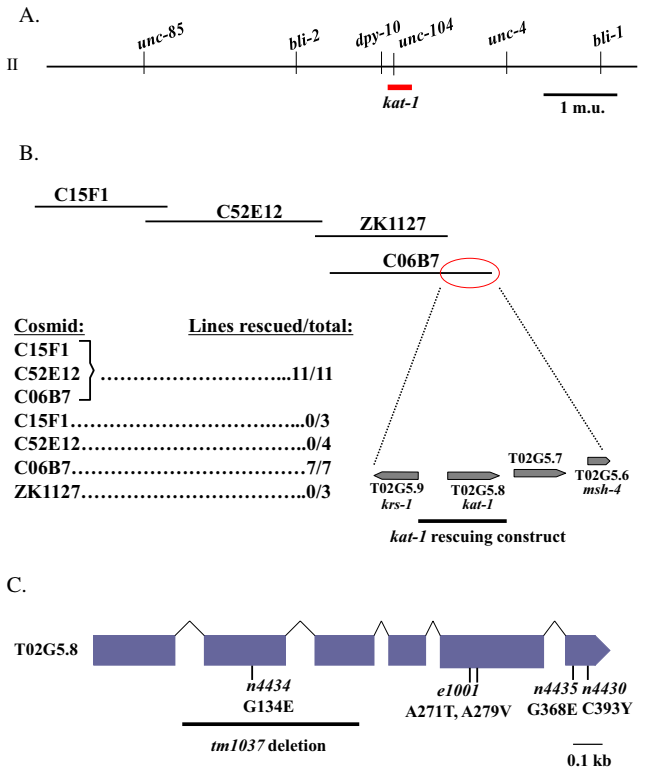


Fig. S4. Mapping and cloning of *kat-1*. (*A*) Three-point mapping placed the *n4430* mutation right of or close to *unc-104* on chromosome II. (*B*) The 155-kb *n4430*-containing region identified by polymorphism mapping is spanned by three cosmids, C15F1, C52E12, and C06B7, one of which (C06B7) rescued the fluorescence accumulation of *n4430* animals. (*C*) Mutation sites and amino acid changes of the three alleles of *T02G5.8* identified in our screen as well as of the *e1001* allele.

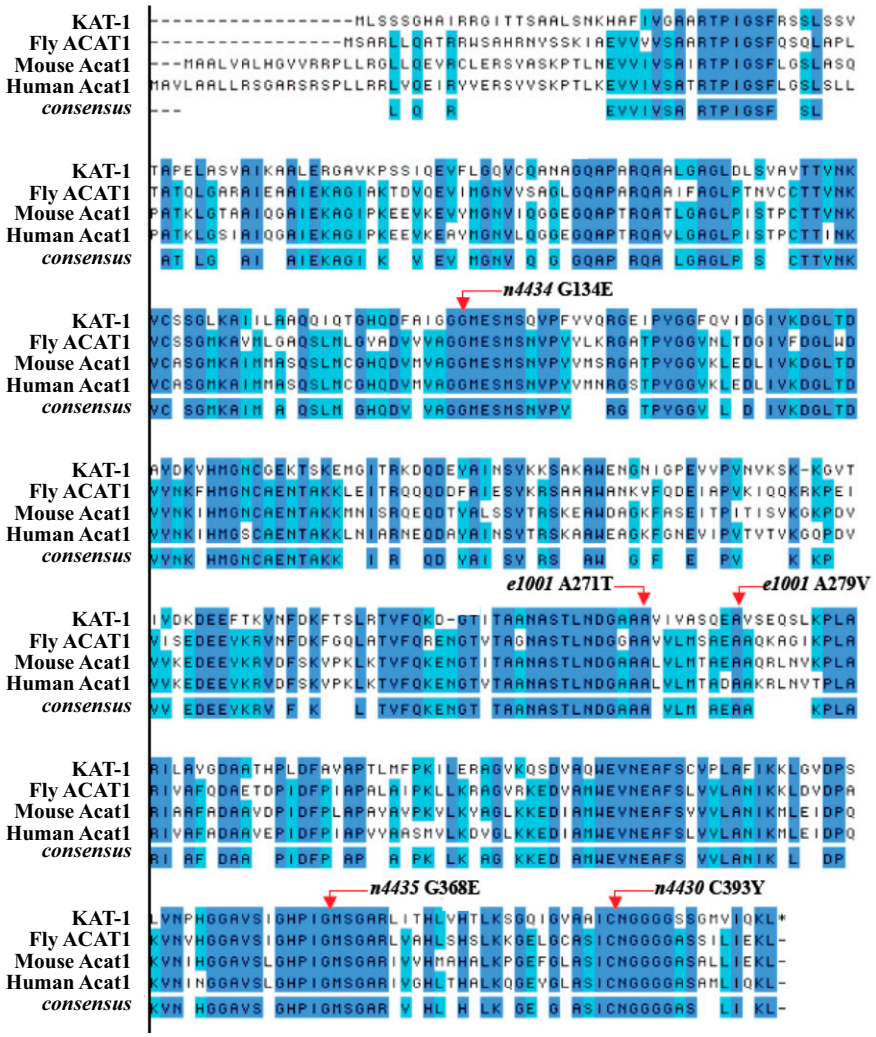
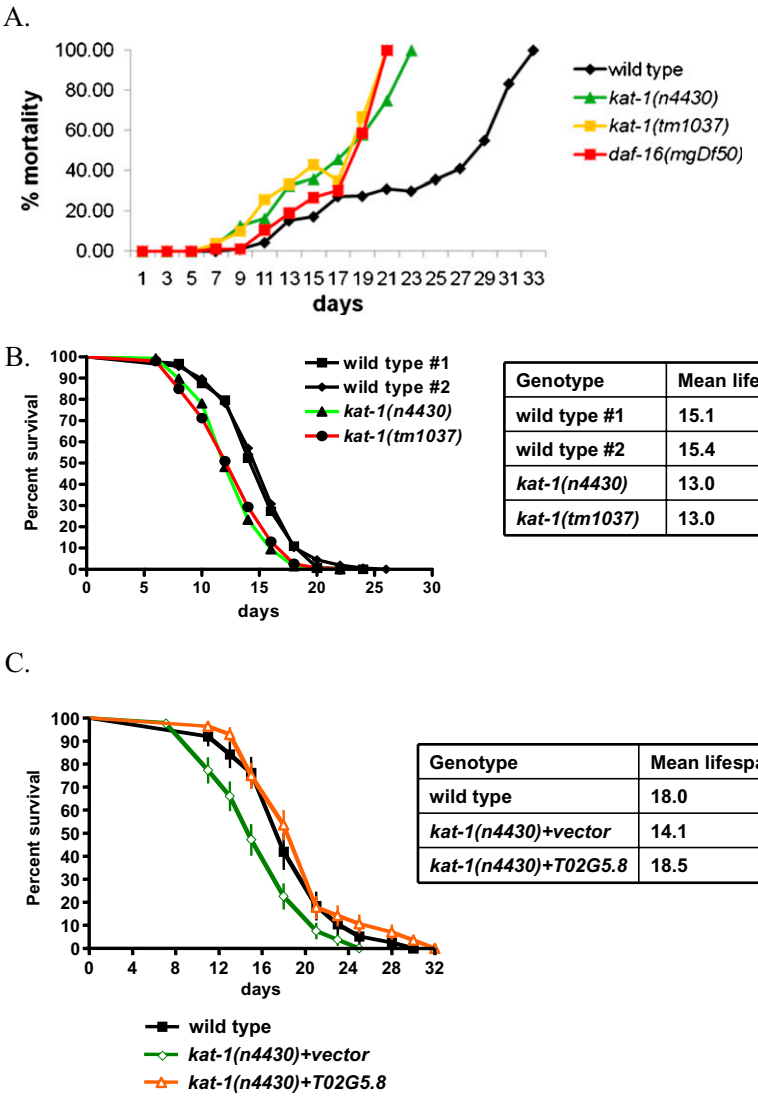


Fig. S5. *kat-1* encodes a conserved ketoacyl thiolase. Alignments of KAT-1 amino acid sequence with sequences of human, mouse, and *Drosophila* homologous proteins. Dark blue, amino acids identical in all four sequences. Light blue, sequences identical in three of the four sequences. Arrows indicate amino acids mutated in the screen isolates.



Genotype	Mean lifespan
wild type #1	15.1
wild type #2	15.4
kat-1(n4430)	13.0
kat-1(tm1037)	13.0

Genotype	Mean lifespan
wild type	18.0
kat-1(n4430)+vector	14.1
kat-1(n4430)+T02G5.8	18.5

Fig. S6. *kat-1* controls *C. elegans* lifespan. (*A*) Mortality rates of *kat-1* mutants were determined and compared with those of the wild type and the progeroid *daf-16(mgDf50)* control. Mortality rates of the *daf-16(lf)* mutants and of both *kat-1* mutants tested were prematurely increased in comparison with that of the wild type. (*B*) Lifespan analyses of *kat-1* mutants were performed on FUdR-free plates. Both *kat-1* mutants tested had significantly shorter lifespan compared with the wild type ($P < 0.0001$; wild type, $n = 135$; *kat-1(n4430)*, $n = 98$; *kat-1(tm1037)*, $n = 109$). (*C*) *kat-1* overexpression normalizes the lifespan of the *kat-1* mutants but does not result in lifespan extension beyond that of the wild type. Integrated transgenic lines overexpressing the *kat-1* gene were generated as described in *Materials and Methods*. Lifespan analyses of control lines (vector DNA injection) and *kat-1* overexpressing lines (vector + wild-type *kat-1* gene injections) are shown. Both wild-type lifespan and *kat-1* overexpressor lifespan were significantly longer than that of the *kat-1* mutant control lines ($P <$ 0.0001). The lifespan of the *kat-1* overexpressor was not significantly different from the wild-type lifespan.

Table S1. Epistasis studies of *kat-1* with genes in known aging pathways

Pathway	Genotype		Median lifespan 20 °C	Median lifespan 25 °C	*n*/no. of trials	*P* value
	Wild-type		20	12	>500/>10	NA
Fatty acid oxidation	*kat-1(n4430)*		17	10	>500/>10	$P < 0.0001$[a]
Insulin-like pathway	*daf-2(e1370)*			35	76/2	$P < 0.0001$[a]
	kat-1(n4430);daf-2(e1370)			32	92/2	$P < 0.0001$[b, c]
	daf-16(mgDf50)		12		157/2	$P < 0.0001$[a]
	daf-16(mgDf50);kat-1(n4430)		11		261/3	$P < 0.0001$[b, c]
Mitochondrial respiration	*isp-1(qm150)*			14	42/1	$P < 0.0001$[a]
	isp-1(qm150);kat-1(n4430)			12	44/1	$P < 0.0001$[b, c]
	phi-44 T02H6.11(RNAi)			18	245/2	$P < 0.0001$[a]
	phi-44 T02H6.11(RNAi);*kat-1(n4430)*			18	127/2	$P < 0.0001$[b], NS[c]
	cyc-1(RNAi)			20	195/2	$P < 0.0001$[a]
	cyc-1(RNAi);*kat-1(n4430)*			20	159/2	$P < 0.0001$[b], NS[c]
Chemosensation	*osm-5(pr813)*		41		50/2	$P < 0.0001$[a]
	kat-1(n4430);osm-5(pr813)		38		43/2	$P < 0.0001$[b, c]
	che-3(e1124)		26		50/2	$P < 0.0001$[a]
	che-3(e1124);kat-1(n4430)		25		47/2	$P < 0.0001$[b], NS[c]
Germ-line signaling	Germ line +	*mes-1(bn74)*	19		44/2	NS[a]
		kat-1(n4430);mes-1 (bn74)	17		54/2	NS[b]
	Germ line −	*mes-1(bn74)*	31		53/2	$P < 0.0001$[a]
		kat-1(n4430);mes-1 (bn74)	24		36/2	$P < 0.0001$[b, c]
Dietary restriction	DR	Wild-type	35		89/2	$P < 0.0001$[a]
		kat-1(n4430)	31		94/2	$P < 0.0001$[b, c]
sir-2.1 overexpression	*pkIs1642[sir-2.1o/e]*		31		129/3	$P < 0.0001$[a]
	kat-1(n4430);pkIs1642[sir-2.1o/e]		17		138/3	NS[b], $P < 0.0001$[c]

[a] *P* vs. wild type.
[b] *P* vs. *kat-1(n4430)*.
[c] *P* vs. the corresponding single mutant.
NA, not applicable; NS, not significant ($P > 0.05$).