Kinome-wide RNAi screen in *Caenorhabditis elegans* reveals new modulators of insulin signaling and longevity

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Abstract

The insulin/IGF-I-like signaling (IIS) pathway is a highly conserved signaling cascade that plays a crucial role in regulating longevity across species. Given its significance in aging, identifying novel kinases interacting with the IIS pathway can provide deeper insights into the mechanisms governing longevity. In this study, we performed a targeted RNAi screen of the *Caenorhabditis elegans* kinome, utilizing dauer formation as a phenotypic readout. We identified several known and novel kinase modulators of the IIS pathway. These hits were enriched with both previously documented as well as undocumented lifespan regulators. Thermotolerance assays revealed mixed trends, with some kinase inhibitions increasing while others decreasing protection. We observed a positive correlation between thermotolerance and lifespan extension, as well as between dauer formation and lifespan extension, with thermotolerance proving to be a better predictor of longevity. Our findings offer a valuable resource for researchers exploring the IIS pathway and highlight novel, unannotated kinases as potential new therapeutic targets for aging interventions.

Keywords: kinase**;** dauer; development; aging; nutrient signaling; stress response; healthspan

Introduction

The IIS pathway is a highly conserved signaling cascade that regulates longevity across species (reviewed in[1,2]). Polymorphic variants in FOXO3A, a key downstream effector of the IIS pathway, have been linked to human longevity, underscoring the pathway's significance in aging processes[3]. Reduced signaling through the IIS pathway can extend the lifespan of *C. elegans* by nearly tenfold[4]. The evolutionary conserved IIS pathway in *C. elegans* involves a kinase-regulated signaling cascade[5,6], starting with insulin-like ligands binding to the insulin/IGF-1 receptor tyrosine kinase DAF-2. This activation triggers phosphatidylinositol-3-OH kinase (PI3K) AGE-1, subsequently activating PDK-1 (3-phosphoinositide-dependent kinase-1). PDK-1 then phosphorylates and activates the serine/threonine kinases AKT-1 and AKT-2, which inhibit the transcription factor DAF-16, the *C. elegans* homolog of human FOXO, by preventing its nuclear translocation[7,8]. When IIS signaling is reduced, AKT activity decreases, allowing DAF-16 to enter the nucleus and activate genes related to stress resistance, metabolism, and longevity[5,9,10].

Given the IIS pathway's extensive influence on various biological functions, identifying novel modulators is essential for gaining a deeper understanding of the mechanisms regulating these processes. Kinases, which constitute a majority of the signaling components within the IIS pathway cascade, are of particular interest. Consequently, we focused on identifying additional kinases that potentially interact with the IIS pathway. To this end, we performed a targeted RNAi screen of the *C. elegans* kinome to identify kinases that affect phenotypes regulated by the IIS pathway. One such phenotype is dauer formation, a developmental stage induced by environmental conditions such as food availability or temperature changes[11,12]. Normally, *C. elegans* larvae pass through four larval (L1, L2, L3 and L4) stages before developing into reproductive young adults. However, under unfavorable conditions, L1 larvae enter a stress-resistant dauer stage[11,12]. The dauer stage represents a form of developmental quiescence, allowing larvae to survive extended periods of unfavorable conditions[13]. This stage is intricately controlled by IIS pathway components. Because of their unique visible appearance, dauers serve as a valuable surrogate phenotype for identifying modulators of the IIS pathway.

In this study, using the dauer phenotype as a readout, we screened the *C. elegans* kinome to identify new kinase modulators of the IIS pathway. We found that the screen hits we identified as modulators are enriched for lifespan regulators and include kinases whose knockdown extends lifespan. By performing a thermotolerance assay on these hits, we show that both dauer formation and thermotolerance positively correlate with lifespan extension, with thermotolerance being a better predictor. Our study provides a valuable resource of new kinases for researchers interested in studying the IIS pathway and novel, unannotated kinases involved in lifespan extension.

Results

Kinome-wide RNAi screen for IIS pathway modulators using dauer as the readout

Lack of food causes *C. elegans* larvae to enter a developmental quiescence stage known as dauer[14]. Loss-in-function mutations in *daf-2*, an insulin receptor can also cause larvae to enter dauer stage even in the presence of food[15,16] (**Fig. 1A**). The partial loss-of-function mutant, *daf-2(e1370)*, can develop into a young adult worm but is highly sensitive to elevated temperatures, entering 100% dauer at 25°C[15,17]. To screen the kinome and identify novel modulators of the IIS pathway, we utilized these hypersensitive mutants.

Our aim was to identify both suppressors and enhancers of the IIS pathway. Thus, our initial experiments were geared towards identifying a sub-permissive temperature that would result in ~50% dauer (**Fig. 1B**). Under our RNAi screening workflow, we observed that when grown at 22.5°C, *daf-2(e1370)* consistently formed close to 50% dauer. Hence, we decided to conduct our screen at this temperature. We reasoned that for kinases that interact with the IIS pathway, RNAi knockdown would modulate *daf-2(e1370)* dauer formation (**Fig. 1B**). For e.g., RNAi knockdown which will enhance dauer formation are likely to further lower signaling through the IIS pathway, while the one suppressing dauer formation are likely to mediate its downstream effector functions. As a controls in our assays, we used RNAi against *pdk-1* and *daf-16*. The PDK-1 (3-Phosphoinositide-dependent protein kinase-1) is a key kinase in the IIS pathway that activates AKT kinases, essential for inhibiting dauer formation in the presence of food[8]. When PDK-1 activity is reduced by RNAi, IIS signaling is lowered, promoting *daf-2(e1370)* dauer formation. DAF-16, on the other hand, is a FOXO transcription factor that acts downstream of the IIS pathway[18]. DAF-16 is normally inhibited by active IIS signaling i.e., the presence of food, preventing it from entering the nucleus. When IIS signaling is reduced, DAF-16 activates genes related to longevity and dauer formation. Knocking down *daf-16* via RNAi prevents these responses, resulting in reduced dauer formation. These RNAi controls served as positive and negative control, respectively, for the dauer phenotype in our screen (**Fig. 1C**). Our pilot assays with these controls yielded a Z'-factor of 0.79, confirming the scalability of this dauer-based phenotypic assay for kinome-wide RNAi screening.

The screening workflow (**Fig. 1D**; **see methods for detail**) that we used to conduct kinome-wide RNAi screening consisted of 96-wells liquid culture plates, allowing us to screen 31 kinases in triplicate on a single assay plate. Briefly, synchronized cultures of ~15-20 L1 larvae were dispensed into each well of a 96-well plate containing 60 µL of RNAi bacterial feed. The L1 larvae were allowed to grow for three days at 22.5°C. On the fourth day, when the worms grown on control RNAi or *pdk-1* RNAi entered dauer and *daf-16* RNAi worms became adults, we scored the number of dauers in each well under a light microscope. Dauers were identified by their dark, thin, tapered shape at both ends, aligned straight or in a comma shape with significantly reduced pharyngeal pumping. The percentage of dauers was calculated based on the total number of worms present.

Kinases identified as modulators of the IIS pathway play roles in diverse biological processes

From the Ahringer RNAi library[19], which contains over 16,000 RNAi clones, we selected and arranged a mini-96-well kinome RNAi library. Out of the 411 kinase-targeting RNAi clones available in the Ahringer collection, 357 clones produced viable cultures; the rest showed no signs of growth. We used these 357 viable clones to create a curated mini-96-well kinome library. Each plate was formatted to include an empty vector, *pdk-1*, and *daf-16* RNAi, ensuring that each plate contained its respective controls (**Fig. 2A**). Out of the 357 kinases tested, 128 RNAi clones did not exhibit any scorable phenotype. Many of these clones either failed to grow, produced sick worms, or resulted in embryonic lethality. Consequently, we were able to assess the dauer phenotype for only 229 RNAi clones (**Fig. 2B and Supplementary Table 1**). Among these, 75 RNAi clones demonstrated significant changes in dauer formation across biological repeats: RNAi against 69 enhanced, and 6 suppressed *daf-2(e1370)* dauer formation (**Table 1 and Supplementary Table 1**).

The screen yielded a broad list of kinases with diverse regulatory roles across various biological processes, emphasizing their roles in cellular signaling, development, and stress responses. The identification of several hits known to be involved in the IIS pathway and TGF-beta pathway highlights the success of the screen, which was designed to identify modulators of the IIS pathway **(Table 1 and Supplementary Table 1)**. One notable example is serine/threonine-protein kinase AKT-2, which functions downstream of PI3 kinase AGE-1 and kinase PDK-1 within the DAF-2/IIS pathway[8,20]. This kinase plays a central role in regulating developmental arrest at the dauer stage. In the context of stress response, the serine/threonine-protein kinase WNK-1 is significant for its role in osmotic stress responses, where it increases GPDH-1 translation by phosphorylating GCK-3[21,22]. Additionally, serine/threonine-protein kinase SMA-6 is a critical component of the TGF-beta signaling pathway, potentially acting as a receptor for DAF-7 and contributing to various developmental processes[23,24]. The TGF-beta signaling pathway is also a known regulator of reproductive longevity and innate immune response[24].

The screen also identified kinases known to regulate lifespan, including DAF-1 (TGF-beta receptor type I), which plays a crucial role in the TGF-beta signaling pathway affecting dauer formation[25]. HPK-1 (homeodomain-interacting protein kinase 1) is involved in stress response pathways, influencing longevity[26,27]. MNK-1 (MAP kinase-interacting serine/threonine-protein kinase 1) contributes to lifespan regulation through its roles in muscle function and MAP kinase interactions[28]. WTS-1 (Warts, also known as large tumor suppressor kinase 1), part of the Hippo signaling pathway, impacts cell proliferation and lifespan[26]. PAK-1 (p21-activated kinase 1) modulates cytoskeletal dynamics and cell signaling, affecting aging[29,30].

Cell-cycle regulation is prominently represented among the identified kinases. Notably, mitotic checkpoint serine/threonine-protein kinase BUB-1 is essential for spindle-assembly checkpoint signaling and chromosome alignment during cell division, ensuring proper genomic stability[31,32]. Additionally, Cyclin-dependent kinase 12 is crucial for transcription regulation during the cell cycle, displaying CTD kinase activity is necessary for transcription elongation[33]. Furthermore, membrane-associated tyrosine- and threonine-specific CDC2-inhibitory kinase WEE-1.3 acts as a negative regulator of the G2 to M transition by phosphorylating CDK1, thereby controlling entry into mitosis and playing critical roles in oocyte maturation, embryonic development, and chromosome structure during mitosis[34-36].

We also identified kinases with roles in immune defense mechanisms, such as NIPI-3 and Protein kinase C-like 3 (PKC-3), which play crucial roles in regulating antimicrobial peptides and enhancing resistance to fungal infections[37,38]. NIPI-3 regulates the upregulation of NLP-29 and NLP-31 in the epidermis[39], while PKC-3 is required for the expression of NLP-29 in response to fungal infection or physical injury[38].

Overall, our kinome-wide RNAi screen revealed a diverse array of kinases involved in critical biological processes, including the IIS and TGF-beta pathways, cell-cycle regulation, and immune defense.

Discovery of new kinase regulators of longevity

Given the well-established role of the IIS pathway in regulating longevity, we were keen to explore how RNAi knockdown of these kinases could influence lifespan. To identify novel longevity regulators, we performed a lifespan analysis on the hits identified from our kinome RNAi screening, utilizing a modified version of the 96-well lock-in assay for high-throughput lifespan analysis[40] (see Methods for detail). From this initial screening, we identified 31 RNAi clones as positive hits (**Supplementary Table 2 and Supplementary Table 4**). Subsequently, we tested these clones on NGM bacterial RNAi plates. Although all the hits showed a trend toward increased lifespan when knocked down post-developmentally, only 14 RNAi clones were found to significantly extend lifespan (**Fig. 3A and 3B, Table 2 and Supplementary Table 2**).

Out of the 14 RNAi treatments that significantly extended lifespan, only four have been annotated: *kin-1*, *trk-1*, *rskn-1* and *akt-2*. The other eight, namely *ZK622.1*, *C56C10.6*, *T15B12.2*, *W06F12.3*, *F52C12.2*, *T22B11.3*, *T06C10.3*, *F09A5.2*, *K06H7.1* and *T09B4.7*, remain unannotated with no available information on their functions. *kin-1*/PKA has been shown to play a role in the innate immune response and protection against cold-induced stress[41,42]. *trk-1*/TRK is a homolog of the neurotrophin receptor tyrosine kinase in *C. elegans* and a substrate for the LAR family protein tyrosine phosphatase *ptp-3*[43]. *akt-2*/AKT is involved in the IIS pathway, directly contributing to the sequestration of DAF-16 in the cytoplasm[20]. The human ortholog of unannotated kinases we identified have been reported to play role in important cellular processes and disease mechanisms. For instance, C56C10.6/ TTBK2, Tau Tubulin Kinase 2 is intriguing due to its involvement in phosphorylating tau proteins, which are implicated in neurodegenerative diseases like Alzheimer's[44,45]. Similarly, T15B12.2/ CSNK1A1 Casein Kinase 1 Alpha 1 is known for its role in regulating key signaling pathways that influence circadian rhythms and cellular responses to DNA damage[46]. Another protein of interest is the F52C12.2/TSR3, Ribosome Maturation Factor, essential for ribosome biogenesis and therefore, important in regulating protein synthesis and cellular growth[47]. The T22B11.3/LTK, Leukocyte Receptor Tyrosine Kinase, primarily expressed in hematopoietic tissues, plays a critical role in immune response modulation and development[48]. Further investigating these kinases in the context of their role in aging could provide valuable insights into how these crucial processes influence longevity and age-related diseases.

Overall, our lifespan analysis indicates that several kinases identified by the dauer phenotypic screen may regulate lifespan, showing increased effects when knocked down post-developmentally. These findings merit further investigation to elucidate their roles in aging processes.

Correlation of thermotolerance and dauer with lifespan extension in IIS pathway modulators

To assess whether the increased lifespan observed following the knockdown of specific kinases is accompanied by enhanced healthspan, we evaluated their sensitivity to an external stressor. Typically, an organism's ability to withstand noxious temperatures—a measure known as thermal stress resistance—declines with age[49]. In our experiments, we noted a significant decline in the survival rate of the wild-type sterile strain exposed to elevated temperatures (35°C) for 12 hours; survival decreased from 78% on day 6 to 38% on day 9 in aged worms (**Supplementary Fig. 1**). Hence, we decided to determine how the 31 positive primary hits identified in our study influence thermotolerance. We conducted thermotolerance assays on day 7 of adulthood, following exposure to respective RNAi from day 1. Control worms treated with an empty vector exhibited approximately 50% survival after 12 hours at 35°C. Although we observed mixed trends toward both increased and decreased survival after thermal stress, none of these changes reached statistical significance, except *T22B11.3* (**Fig. 3C, Supplementary Table 3)**. This is likely due to underlying variability in the thermotolerance assay[50]. Nonetheless, a correlation analysis examining the relationships between lifespan and thermotolerance, as well as lifespan and dauer, across each RNAi treatment, revealed significant positive correlations. We observed a Pearson correlation coefficient (r) of 0.56 (**Fig. 4A**; $R^2=0.31$, $p=0.0009$), indicating that thermotolerance is a better predictor of lifespan extension compared to dauer formation, which showed a Pearson r of 0.45 (**Fig. 4B;** $R^2=0.21$, $p=0.009$). This suggests that, despite experimental variability in thermotolerance, thermal resistance may serve as a more reliable proxy for identifying genes that extend lifespan—a finding that aligns with a recent study identifying thermotolerance assays as better predictor for screening lifespan-extending interventions[50].

Discussion

In this study, we screened for novel kinase modulators of the IIS pathway using *C. elegans* dauer formation as a phenotypic readout. Utilizing the hypersensitive *daf-2(e1370)* mutant and a feeding RNAi library, we performed RNAi knockdown of 81% of the *C. elegans* kinome. We were able to quantify dauer phenotypic scoring for approximately 64% of the kinome tested, as the remaining 36% showed no quantifiable phenotype due to larval lethality and arrest. From this, we identified 75 kinases that significantly affected dauer formation when inhibited by RNAi. Notably, 69 RNAi treatments enhanced dauer formation, while 6 suppressed.

The screen revealed a broad list of kinases with diverse regulatory roles, including those in the IIS and TGF-beta pathways, cell-cycle regulation, and immune defense. Our lifespan analysis of these hits revealed that inhibiting the activity of 14 kinases significantly extended lifespan, including *kin-1* and *trk-1*, along with ten unannotated kinases. This indicates that these kinases may play critical roles in modulating lifespan through their interaction with the IIS pathway. Furthermore, a positive correlation between thermotolerance and increased lifespan suggests that thermotolerance could serve as a reliable predictor for longevity interventions. These findings provide a foundation for further exploration of the identified kinases in aging and stress signaling, potentially leading to novel therapeutic targets.

While our lifespan screen identified only a few subsets of kinases whose knockdown significantly increased lifespan in wild type, we observed a general trend toward increased lifespan for many of the kinases tested, with ~40% of hits showing positive trends. This suggests that hits from the screen are enriched for lifespan regulators. We performed our lifespan assay at 25°C in a sterile strain background to avoid the confounding effects of fluorodeoxyuridine (FUdR)[51,52], with RNAi knockdown initiated post-developmentally. We chose to conduct post-developmental RNAi knockdown to avoid any developmental effects caused by kinase knockdown. Therefore, it is worth testing these dauer-modulating hits under different conditions where RNAi knockdown is initiated from the larval stage onwards. For instance, over the past years, we have extensively characterized one of the hits from this screen, DRL-1[53,54], and its homolog FLR-4[55]. DRL-1 kinase knockdown extends mean lifespan by nearly 60% when initiated at the L1 larval stage but fails to extend lifespan when initiated post-developmentally[53]. On the other hand, FLR-4 is unique as its knockdown extends lifespan only under specific bacterial diets[55].

An important next step will be to test these kinase hits for their specific roles in mediating phenotypes other than longevity associated with the long-lived IIS pathway mutants like *daf-2*. In this direction, our recent study with one such identified kinase hit, B0285.1/CDK-12, shows that it is crucial for maintaining DNA integrity. We used CDK-12 as a genetic tool to analyze the effects of tissue-specific DDR perturbation and DNA damage in the IIS pathway mutants[56]. We found that knocking down *cdk-12* only in the somatic uterine tissue of the IIS pathway mutants led to a DAF-16-dependent pachytene arrest of germ cells. This was achieved through the lowering of the ERK MAPK pathway below a critical threshold.

Together, our study identifies a diverse set of novel kinases that interact with the IIS pathway to regulate its functional outputs.

Methods

Caenorhabditis elegans strains and maintenance

C. elegans strains used in the study are CB1370: *daf-2(e1370) III* and TJ1060: *spe-9(hc88) I; rrf-3(b26) II*. The strains were obtained from the *Caenorhabditis* Genetics Center (CGC), University of Minnesota, MN, USA. Strains were maintained at 15 °C under standard laboratory conditions, as described previously[57]. Worm populations were maintained in 60 mm NGM agar plates (3 g/L NaCl, 17 g/L agar; 2.5 g/L peptone; 1 mM CaCl2, 5 mg/L cholesterol, 1 mM MgSO4, 25 mM KPO4) seeded with OP50 *Escherichia coli*.

Kinome-wide RNAi Screen

Day 0

Inoculation of RNAi bacterial stock

1. 500 µl of LB media containing ampicillin (100 µg/ml) was dispensed into a 2 ml 96 deep-well plate using a multichannel pipette (50–1200 µl).
2. The LB was inoculated with frozen RNAi bacterial stock using a multichannel pipette (5–100 µl).
3. The deep-well culture plates were sealed with a breathable air-sealer to allow air exchange and prevent contamination.
4. For bacterial growth, these culture plates were placed in a shaking incubator at 240 rpm, 37°C for 16 hours.

Sodium hypochlorite treatment for L1 larvae synchronization

5. The *daf-2(e1370)* mutants were propagated at 15°C until enough young adults were available.

6. Young adults were harvested by washing the culture plates with 1X M9 buffer and collected in a 15 ml centrifuge tube.
7. Worms were washed twice with 1X M9 buffer by centrifuging (using a swinging bucket rotor) the Falcon tube at 1800 rpm for 1 minute to remove any attached bacteria.
8. To the worm pellet, 5 ml of 5% sodium hypochlorite bleach solution was added and incubated for 5-7 minutes with brief vortexing every 2 minutes to break the worm bodies.
9. After 5-7 minutes of incubation, the Falcon tube was centrifuged at 1800 rpm for 1 minute to pellet eggs released from the bodies.
10. The supernatant was removed, and the egg pellet was washed thrice with 10 ml of 1X M9 buffer, each time followed by centrifugation (using a swinging bucket rotor) at 1800 rpm for 1 minute to completely remove the bleach solution.
11. Finally, the egg pellet was resuspended in 12 ml of 1X M9 buffer and incubated for 16 hours at 15°C in a rotor at a speed of 20 rpm to obtain a synchronized L1 larval population.

Day 1

Preparation of NGM RNAi feed

12. After 16 hours (step 4) of incubation, 4mM IPTG (final concentration) was added to each well for RNAi induction, using a multichannel pipette, and incubated for an additional 1 hour at 37°C, 240 rpm.
13. Bacterial cells were pelleted by centrifuging at 4000 rpm for 10 minutes in a swinging bucket rotor attached with a 96-well adaptor.
14. The supernatant was discarded by rapid inversion to avoid cross-contamination.
15. The bacterial cells were resuspended in 250 µl of NGM medium (half the concentration of the LB used) supplemented with ampicillin (100 µg/ml) and 4 mM IPTG.
16. 60 µl of the NGM RNAi feed was dispensed using a multichannel pipette into each well of flat-bottomed 96-well plates (in triplicates) as feed for the worms.

RNAi feeding of synchronized L1 larvae

17. The synchronized population of L1 larval worms obtained after 16 hours (step 11) was diluted to a concentration of 15-20 worms/10 µl drop of 1X M9 by placing worms on slides and counting under a microscope.
18. About 15-20 L1 worms were added to each well of the 96-well flat-bottomed plates containing 60 µl of NGM RNAi feed. Tubes were continuously inverted to prevent any settling of larvae in the 15-ml Falcon tube.
19. The edges of the 96-well plate were sealed with parafilm wrap to prevent the evaporation of feed during incubation.
20. The 96-well plate containing *daf-2(e1370)* L1 larvae was allowed to grow at a sub-permissive temperature of 22.5°C in an incubator shaker at 220 rpm. Note: A few tissue papers soaked in autoclaved water were placed to maintain humidity and prevent evaporation of RNAi feed from the plates.

Day 4

Scoring Dauers

21. On the day of scoring, the 96-well plates were removed from the incubator, and the lid was removed to wipe any water droplets condensed on the inner surface of the lid for easy visualization of the worms under a stereomicroscope. The lid was then placed back and dauers were scored.
22. Dauers were identified within the liquid culture as dark, thin, and tapered on the edges, aligned straight or in a comma shape in the liquid culture.
23. First, the percentage of dauer formation in each well containing control, *pdk-1*, and *daf-16* RNAi was scored. The entire plate was scored after ensuring controls had worked i.e., *pdk-1* (>90%), and *daf-16* (0%) RNAi. Percentage dauer formation was calculated by dividing the total number of dauers with the total number of worms in a well.
24. Graphs were plotted, with the percentage of dauer formation representing the average of at least two biological repeats.

Lock-in 96-well lifespan assay

To determine the effect of the positive hits from the dauer-based kinome-wide RNAi screening on lifespan extension, we performed a locked-in 96-well lifespan assay. The protocol is a modification of a previously published high-throughput lifespan screening protocol[40]. We used a synchronized population (obtained as described in step 5 above) of sterile day-1 TJ1060 strains (grown at 25°C) for lifespan analysis to avoid transfers to fresh plates due to progeny production. The 96-well lifespan assay plates were prepared by utilizing an automated 8-channel dispenser. We dispensed 0.15 mL of molten NGM agar (2mM IPTG) into each well of a 96-well plate in a laminar flow cabinet and let the plates dry overnight. Then, we seeded them with 5 mL of 5x concentrated HT115 RNAi bacteria using the automated 8-channel dispenser, as before. The plates were allowed to dry with the lid open for 3-4 hours. To these plates, a synchronized population of day-1 adult TJ1060 worms was added using an automated 8-channel dispenser. The density of the worms in 1x M9 was prepared such that approximately 10-12 worms were dispensed in a 10 µL drop. Plates were allowed to dry for approximately 45 minutes, then covered with lids, and placed in a box at 25°C. Plates were monitored once a week for the first two weeks and then daily thereafter. When the empty vector control had reached > 90% mortality, we scored all the wells for the dead and alive population. Provoked movement by continuously tapping and dropping the plate from a 7 cm height was used to differentiate live worms from dead ones. The clone was recorded as a hit if the percent mortality was <90% and it was reproducible across at least 6 trials out of a total of 10 trial plates. The hits identified from the screen were retested in a 35 mm NGM RNAi plates.

Lifespan assay in NGM RNAi plates

A synchronized population of worms was obtained from a 2-hour egg lay of day-1 adult hermaphrodites of TJ1060 grown at 15°C. Eggs were then transferred to 25°C and allowed to grow into sterile young adults. On day 1 of adulthood, 50 worms were transferred to either empty vector control or RNAi-seeded 35-mm NGM plates with HT115 bacteria. From day 1, worms were transferred to fresh RNAi-seeded plates every other day until day 7, and then twice over the next 2 weeks. Worms were scored as dead or alive every other day. Worms that failed to display touch-provoked movement were scored as dead. Worms that died from causes other than aging, such as sticking to the plate walls, internal hatching of eggs ('bagging'), or gonadal extrusion, were censored. All lifespan assays were performed at 25°C. Experiments were done in two biological replicates. The worms were scored independently by two investigators who were blinded to the study design and RNAi clone identity. Survival was plotted using GraphPad Prism and p-values determined using Mantel-Cox Log rank test in OASIS: online application for the survival analysis[58].

Thermotolerance assay

To determine the effect of each kinase knockdown on thermal stress resistance, we exposed TJ1060 worms to RNAi targeting that kinase at day-1 of adulthood. For each of the two biological replicates, we used two 35 mm NGM RNAi plates, each containing approximately 40 worms. Worms were transferred to fresh RNAi plates every other day until they reached day-7 of adulthood. At this point, they were exposed to a temperature of 35°C for 12 hours. After this period, the worms were scored for survival. Plates were scored by two independent investigators who were blinded to the study design and RNAi clone identity. Survival was plotted using GraphPad Prism and p-values determined using an unpaired Student’s t-test.

Author contributions

Conception: AM; Investigation: MC, AF, and PS; Data analysis: MC and AF; Original manuscript draft and figures: MC; Review: AM; Technical infrastructure and resources: GL and AM.

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Data availability

All data supporting the findings of this study are available in the main text, in the Supplementary Materials. Any unique materials used in the study are available from the authors or from commercially available sources. This study did not use or generate any data code.

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Figures and Tables legends

Figure 1: A kinome-wide RNAi screen to identify modulators of the IIS pathway **(A)** The IIS pathway in *C. elegans* regulates dauer formation. **(B)** Growing temperature sensitive *daf-2(e1370)* mutants at a sub-permissive temperature allows for the identification of both enhancers and suppressors of dauer. **(C)** Percentage of dauer formation (mean ± sd) in empty vector (EV) control (26.46 ± 15.82), *daf-16* (0), and *pdk-1* (95.30 ± 6.50) RNAi-treated *daf-2(e1370)* mutants grown at 22.5°C. p-values were determined using an unpaired Student's t-test. **(D)** Schematic representation of the kinome-wide RNAi screening workflow.

Figure 2: Results of the kinome-wide RNAi screen. (A) From the whole genome Ahringer RNAi library, we selected 357 viable kinase-targeting RNAi clones to create a mini-96-well kinome library plates. Each plate included controls: an empty vector control, *daf-16* and *pdk-1* RNAi. Out of 357 clones, 229 RNAi clones showed scorable dauer phenotype. **(B)** Graphical representation of all 229 kinase RNAi clones showing enhancers and suppressors of *daf-2(e1370)* dauer. Bars represent the mean, and each dot in the graph represents an individual well with 15-20 worms across two independent trials. See Table 1 and Supplementary Table 1 for more details.

Table 1: Enhancer and suppressor of *daf-2(e1370)* dauer. List of hits and their annotations from the kinome RNAi screen that, when knocked down, enhance or suppress *daf-2(e1370)* dauer formation at 22.5°C. The percentage change in dauer formation is shown relative to the empty vector control RNAi, with significance determined using an unpaired Student's t-test. See Supplementary Table 1 for the percentage of dauer formation from all the kinases tested.

Figure 3: Lifespan and thermotolerance assays of hits from the kinome-wide RNAi screen (A) Lifespan graph of 31 hits validated on NGM RNAi plates. RNAi clones that significantly increase lifespan are colored in blue, with p-values indicated as follows: *p<0.05, **p<0.01, and ****p<0.0001. p-value determined using Mantel-Cox log

rank test. See Table 2 and Supplementary Table 2 for more details on percent changes. **(B)** Relative difference in survival normalized to empty vector control RNAi of day-7 TJ1060 worms post-exposure to 35ºC for 12 hours. p-values were determined using an unpaired Student's t-test, $^*p<0.05$. See Supplementary Table 3 for more details on percent changes across trials

Table 2: List of kinases and their human orthologs, with descriptions, that significantly extend lifespan when knocked down. Strain: TJ1060. Temperature: 25°C. Post-developmental RNAi treatment. See Figure 3A, 3B, and Supplementary Table 2 for details.

Figure 4: Correlation of thermotolerance and dauer with lifespan extension. Kaplan-Meier means were used for lifespan, and means were used for thermotolerance and dauer. **(A)** Lifespan vs. Thermotolerance; Pearson correlation coefficient (r) = 0.56, $R^2 = 0.31$, $p = 0.0009$. **(B)** Dauer vs. Lifespan; Pearson $r = 0.45$, $R^2 = 0.21$, $p = 0.009$. P-values were determined using a two-tailed test.

Supplementary Figures and Tables legend

Supplementary Table 1: Percentage of *daf-2(e1370)* dauer formation in each well after treatment with RNAi clones targeting 229 kinases. Related to Figure 2B

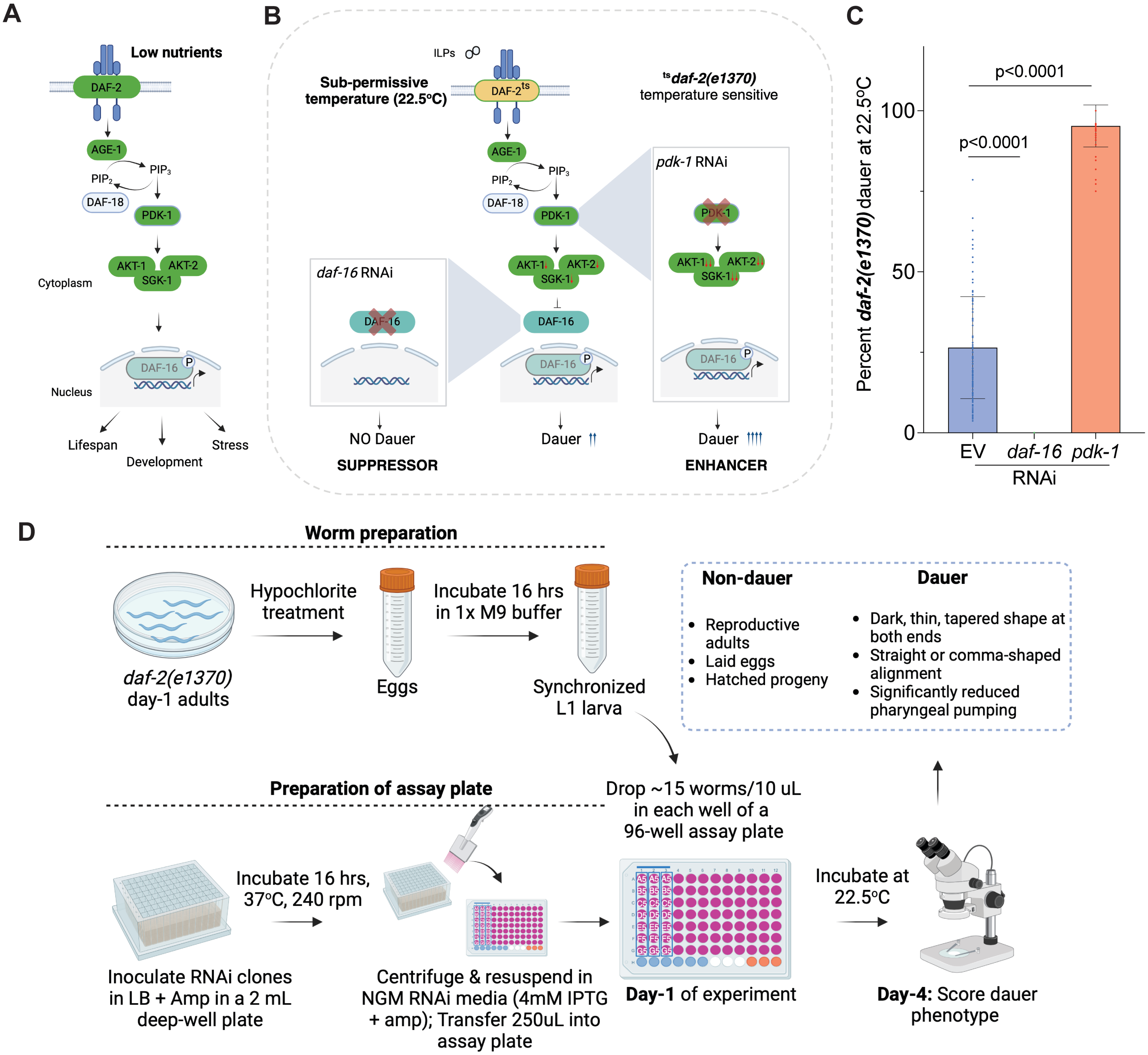
Supplementary Table 2: Percent change in the lifespan relative to empty vector control RNAi. p-value determined using Mantel-Cox log rank test. Strain: TJ1060. Temperature: 25°C. Post-developmental RNAi treatment. Related to Figure 3A and 3B.

Supplementary Figure 1: (A) Thermotolerance assay. Percent survival of TJ1060 worms after exposure to 35°C for 12 hours. The assay was performed at two different ages: day 6 and day 9 of adulthood. Values are plotted relative to age-matched non-stressed worms. Each dot represents an individual plate with approximately 35 worms across two independent trials. p-values were determined using an unpaired t-test (two-tailed).

Supplementary Table 3: Raw data and calculated percent survival of TJ1060 worms after exposure to 35°C for 12 hours across all trials. Related to Figure 3C.

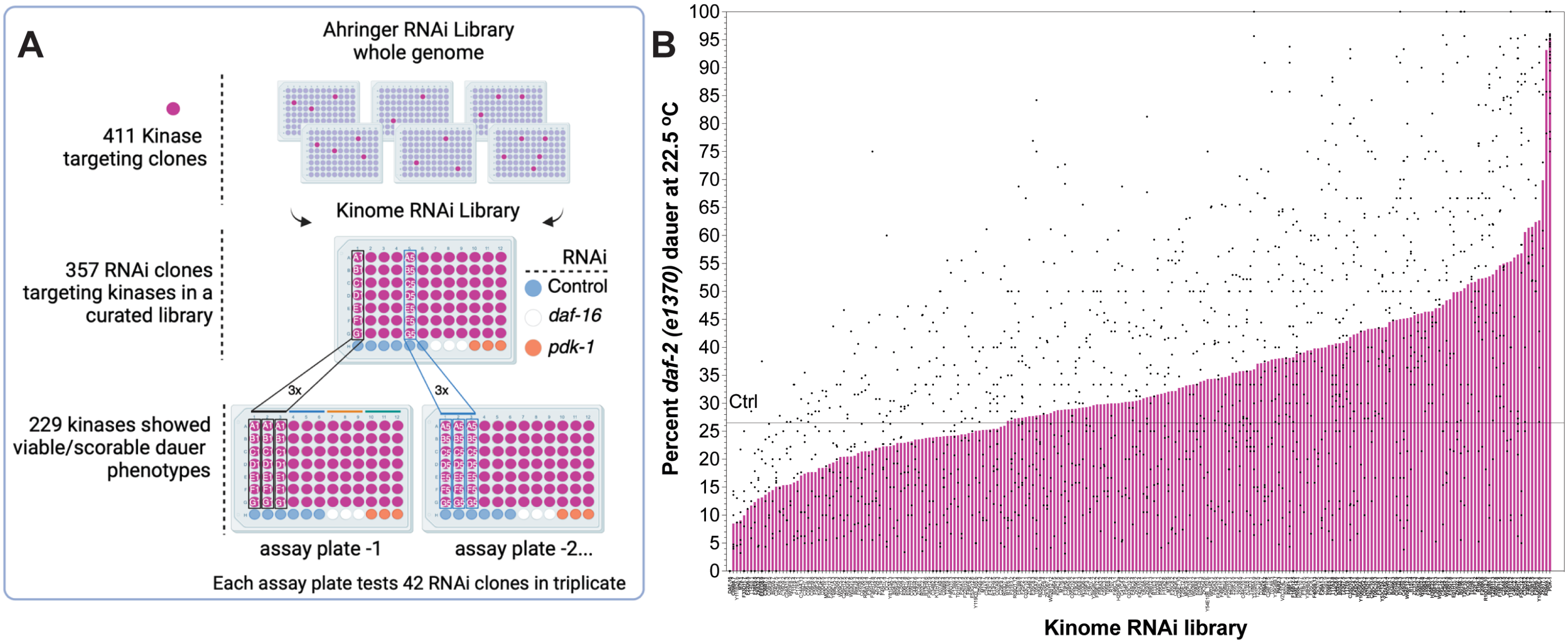
Supplementary Table 4: Raw data used in the lifespan analysis.

Figure 1



A
Low nutrients
DAF-2
AGE-1
PIP$_2$
PIP$_3$
DAF-18
PDK-1
AKT-1
AKT-2
SGK-1
Cytoplasm
DAF-16
P
Nucleus
Lifespan
Development
Stress
B
ILPs
Sub-permissive temperature (22.5°C)
DAF-2^{ts}
^{ts}daf-2(e1370) temperature sensitive
AGE-1
PIP$_2$
PIP$_3$
DAF-18
PDK-1
AKT-1
AKT-2
SGK-1
DAF-16
pdk-1 RNAi
daf-16 RNAi
NO Dauer
SUPPRESSOR
Dauer
Dauer
ENHANCER
C
Percent daf-2(e1370) dauer at 22.5°C
0
50
100
p<0.0001
p<0.0001
EV
daf-16
pdk-1
RNAi
D
Worm preparation
daf-2(e1370) day-1 adults
Hypochlorite treatment
Eggs
Incubate 16 hrs in 1x M9 buffer
Synchronized L1 larva
Non-dauer
Reproductive adults
Laid eggs
Hatched progeny
Dauer
Dark, thin, tapered shape at both ends
Straight or comma-shaped alignment
Significantly reduced pharyngeal pumping
Preparation of assay plate
Drop ~15 worms/10 uL in each well of a 96-well assay plate
Inoculate RNAi clones in LB + Amp in a 2 mL deep-well plate
Incubate 16 hrs, 37°C, 240 rpm
Centrifuge & resuspend in NGM RNAi media (4mM IPTG + amp); Transfer 250uL into assay plate
Day-1 of experiment
Incubate at 22.5°C
Day-4: Score dauer phenotype

Figure 2


A
Ahringer RNAi Library
whole genome
411 Kinase
targeting clones
Kinome RNAi Library
357 RNAi clones
targeting kinases in a
curated library
RNAi
Control
daf-16
pdk-1
3x
3x
229 kinases showed
viable/scorable dauer
phenotypes
assay plate -1
assay plate -2...
Each assay plate tests 42 RNAi clones in triplicate
B
Percent daf-2 (e1370) dauer at 22.5 °C
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
Ctrl
Kinome RNAi library

Main Table 1

Sequence	**Gene name**	**% dauer change wrt control (mean ± SEM)**	***p*-value wrt empty vector RNAi**	**Annotation**
Knockdown enhances *daf-2(e1370)* dauer				
H42K12.1	*pdk-1*	68.84 ± 2.238	<0.0001	Enables protein kinase activity. Involved in several processes, including cellular response to reactive oxygen species; dauer larval development; and regulation of synaptic assembly at neuromuscular junction. Located in axon; neuronal cell body; and nucleus. Expressed in several structures, including gonad; intestine; neurons; pharynx; and ventral nerve cord. Human ortholog(s) of this gene implicated in lung non-small cell carcinoma and prostate cancer. Is an ortholog of human PDPK1 (3-phosphoinositide dependent protein kinase 1).
K09A9.1	*nipi-3*	66.70 ± 6.532	<0.0001	Protein nipi-3; By regulating the up-regulation in the epidermis of antimicrobial peptides nlp-29 and nlp-31, plays a role in resistance to fungal infection.
ZK617.1	*unc-22*	43.41 ± 6.599	<0.0001	Twitchin; Regulator of muscle contraction and relaxation. Senses mechanical strain that occurs during muscle activity by unfolding in clearly resolvable steps at differing forces. Plays a role in the organization of sarcomeres in body wall muscles ; Belongs to the protein kinase superfamily. CAMK Ser/Thr protein kinase family.
Y106G6E.6	*csnk-1*	36.25 ± 6.988	<0.0001	Casein kinase I gamma; Involved in the asymmetric cell division of the embryo downstream of par-2 and par-3 by regulating the asymmetric cortical distribution of pkk-1, a phosphatidylinositol 4,5-bisphosphate- producing enzyme, which in turn regulates the asymmetrical distribution of grp-1, grp-2 and lin-5.
F28H6.1	*akt-2*	35.97 ± 6.606	<0.0001	Serine/threonine-protein kinase akt-2; Acts downstream of PI3 kinase age-1 and kinase pdk-1 in the daf-2/insulin receptor-like transduction pathway. Essential role in regulating developmental arrest at the dauer stage. Phosphorylates Forkhead-related daf- 16 and the longevity-promoting skn-1 transcription factors, which inhibits their entry into the nucleus and antagonizes their functions. Role in immune function and pathogen resistance. Downstream of age-1 and together with akt-2 and sgk-1, promotes cell survival during embryonic development. Belongs to the protein kinase superfamily. [...]
C03B1.5	*C03B1.5*	35.10 ± 6.599	<0.0001	Putative tyrosine-protein kinase C03B1.5.
F46F2.2	*kin-20*	34.93 ± 6.536	<0.0001	Casein kinase I isoform delta; Casein kinases are operationally defined by their preferential utilization of acidic proteins such as caseins as substrates. It can phosphorylate a large number of proteins. Participates in Wnt signaling (By similarity). Is a developmental timer that specifies temporal cell fate selection; acts to control the temporal identity of hypodermal seam cells. Required during late-larval development to prevent adult fates, particularly cell cycle exit and fusion, from being expressed too early.
C32D5.2	*sma-6*	34.19 ± 7.084	<0.0001	Serine/threonine-protein kinase sma-6; Involved in TGF-beta pathway. May be a receptor for daf-7; Belongs to the protein kinase superfamily. TKL Ser/Thr protein kinase family. TGFB receptor subfamily.
F52C12.2	*F52C12.2*	30.34 ± 5.331	<0.0001	Probable ribosome biogenesis protein CELE_F52C12.2; Probable pre-rRNA processing protein involved in ribosome biogenesis.
K06H7.1	*K06H7.1*	30.17 ± 5.612	<0.0001	Uncharacterized protein K06H7.1.

C46C2.1	*wnk-1*	29.60 ± 11.28	0.0098	Serine/threonine-protein kinase WNK; Serine/threonine-protein kinase which phosphorylates gck-3. Plays a role in osmotic stress responses during which it increases gpdh-1 translation, likely by phosphorylating gck-3. Essential for larval development and the tubular formation of the excretory canals.
T15B12.2	*T15B12.2*	28.90 ± 5.481	<0.0001	Protein kinase domain-containing protein; Belongs to the protein kinase superfamily.
ZK909.2	*kin-1*	28.76 ± 7.542	0.0002	cAMP-dependent protein kinase catalytic subunit; Essential for larval development. Controls the rhythmic contraction of enteric muscles probably by regulating G- protein coupled receptor aex-2-mediated calcium influx in GABAergic DVB neurons. Plays a role in the control of oocyte meiotic maturation by gonadal sheath cells. Belongs to the protein kinase superfamily. AGC Ser/Thr protein kinase family. cAMP subfamily.
C25A8.5	*C25A8.5*	28.50 ± 5.022	<0.0001	Tyrosine-protein kinase.
F18F11.5	*drl-1*	28.20 ± 5.174	<0.0001	Protein drl-1; Negatively regulates lifespan and health span probably by participating in nutrient sensing.
R06C7.8	*bub-1*	27.41 ± 6.801	<0.0001	Mitotic checkpoint serine/threonine-protein kinase bub-1; Serine/threonine-protein kinase essential for spindle- assembly checkpoint signaling. Plays a key role in the recruitment of the checkpoint proteins bub-3, mdf-1 and mdf-2 to unattached kinetochores. mdf-1 recruitment is independent of bub-1 kinase activity. Has a role in the correct kinetochore localization of the spindly-like protein spdl-1. In addition, during meiotic anaphase I, controls the recruitment of hcp- 1/2 and klp-19 to the ring-shaped domain formed between chromosomes. Involved in chromosome alignment, chromosome h [...]
T22B11.3		26.59 ± 4.897	<0.0001	
B0285.1	*cdk-12*	26.22 ± 5.478	<0.0001	Cyclin-dependent kinase 12; Cyclin-dependent kinase which displays CTD kinase activity: hyperphosphorylates 'Ser-2' in the C-terminal heptapeptide repeat domain (CTD) of the largest RNA polymerase II subunit, thereby acting as a key regulator of transcription elongation. Required for normal reproduction.
R10D12.10	*R10D12.10*	26.00 ± 6.750	0.0002	Protein kinase domain-containing protein.
F20B6.8	*hpk-1*	25.88 ± 6.650	0.0002	Homeodomain-interacting protein kinase 1; Serine/threonine-protein kinase required in the somatic gonadal cells to regulate germline proliferation during larval development and in adulthood. Plays a role in the development/differentiation of gonadal distal tip cells. Required for normal lifespan in a pha-4 and mxl-2- dependent manner. Also contributes to survival following heat or oxidative stress. Prevents sumoylation and inactivation of heat shock transcription factor hsf-1 which enhances hsf-1-dependent transcriptional induction of chaperones in response to heat shock. Also required [...]
R166.5	*mnk-1*	25.79 ± 5.090	<0.0001	MAP kinase-interacting serine/threonine-protein kinase mnk-1; Serine/threonine-protein kinase which is required in the germline to regulate positively lifespan. May play a role in body wall muscle contraction. May be involved in embryonic cytokinesis.
F22F1.2	*F22F1.2*	25.26 ± 7.130	0.0006	Protein kinase domain-containing protein; Belongs to the protein kinase superfamily.
T01H8.1	*rskn-1*	25.13 ± 6.561	0.0002	Putative ribosomal protein S6 kinase alpha-1; Serine/threonine kinase that may play a role in mediating the growth-factor and stress induced activation of transcription (By similarity). Suppresses germline tumor formation by preventing the dedifferentiation of secondary spermatocytes probably downstream of mpk-1 ; Belongs to the protein kinase superfamily. AGC Ser/Thr protein kinase family. S6 kinase subfamily.

C10C6.1	*kin-4*	24.82 ± 6.889	0.0005	Protein KINase.
T20F10.1	*wts-1*	24.11 ± 7.322	0.0013	Enables protein kinase activity and transcription coactivator binding activity. Involved in several processes, including apical protein localization; negative regulation of protein import into nucleus; and post-embryonic development. Located in apical plasma membrane and cytoplasm. Expressed in several structures, including excretory canal; hypodermis; intestine; spermatheca; and vulva. Human ortholog(s) of this gene implicated in colorectal cancer. Is an ortholog of human LATS1 (large tumor suppressor kinase 1).
B0198.3	*B0198.3*	23.60 ± 5.573	<0.0001	Protein kinase domain-containing protein.
ZK622.1	*ZK622.1*	23.44 ± 6.928	0.001	Tyrosine-protein kinase.
E01H11.1	*pkc-2*	23.41 ± 6.633	0.0006	Protein kinase C-like 2; PKC is activated by diacylglycerol which in turn phosphorylates a range of cellular proteins. PKC also serves as the receptor for phorbol esters, a class of tumor promoters; Belongs to the protein kinase superfamily. AGC Ser/Thr protein kinase family. PKC subfamily.
F55G1.8	*plk-3*	22.13 ± 6.669	0.0012	Serine/threonine-protein kinase plk-3; May be required for cell division and may have a role during G1 or S phase; Belongs to the protein kinase superfamily. Ser/Thr protein kinase family. CDC5/Polo subfamily.
W04B5.5	*W04B5.5*	21.95 ± 5.245	<0.0001	Protein kinase domain-containing protein; Belongs to the protein kinase superfamily.
C54G4.1	*rskn-2*	21.16 ± 5.500	0.0002	Putative ribosomal protein S6 kinase alpha-2; Serine/threonine kinase that may play a role in mediating the mitogen- and stress-induced effects on transcription. May repress transcription via phosphorylation of 'Ser-1' of histone H2A. May phosphorylate histone H3 (By similarity).
W06F12.1	*lit-1*	20.58 ± 7.115	0.0045	Serine/threonine kinase NLK; Has a role in the Wnt signaling pathway controlling the asymmetry of cell divisions during embryogenesis. Operates in the AB and EMS cell lineages influencing cell specification. Required for body wall muscle development, endoderm development, pop-1 asymmetry and T- cell division asymmetry. Lit-1/wrm-1 complex regulates pop-1 localization and is required for pop-1/par-5 interaction. Plays a role in male tail tip morphogenesis.
F39F10.3	*F39F10.3*	20.53 ± 6.795	0.0031	Protein kinase domain-containing protein; Belongs to the protein kinase superfamily.
H39E23.1	*par-1*	20.00 ± 6.673	0.0033	Serine/threonine-protein kinase par-1; Required for cytoplasmic partitioning and asymmetric cell division in early embryogenesis. Phosphorylates and restricts the asymmetry effector mex-5 (and possibly also mex-6) to the anterior cytoplasm of the zygote. Regulates mes-1 expression during early embryogenesis. Critical role in postembryonic vulval morphogenesis. Involved in the establishment of neuronal polarity.
F22B3.8	*F22B3.8*	19.93 ± 6.631	0.0032	Tyrosine-protein kinase.
T09B4.7	*T09B4.7*	19.89 ± 6.621	0.0032	Protein kinase domain-containing protein.
W08D2.8	*kin-21*	19.62 ± 7.035	0.0062	Tyrosine-protein kinase.
C50H2.7	*C50H2.7*	19.53 ± 6.541	0.0034	Uncharacterized protein.
ZC373.3	*ZC373.3*	19.29 ± 6.564	0.0039	Protein kinase domain-containing protein.
R151.4		18.89 ± 4.738	0.0001	Predicted to enable non-membrane spanning protein tyrosine kinase activity and signaling receptor binding activity. Predicted to be involved in cell differentiation; innate immune response; and transmembrane receptor protein tyrosine kinase signaling pathway. Predicted to be extrinsic component of cytoplasmic side of plasma membrane.
W06F12.3	*W06F12.3*	18.79 ± 5.212	0.0004	Protein kinase domain-containing protein; Belongs to the protein kinase superfamily.

C45G9.1	*ttbk-6*	18.68 ± 5.289	0.0006	Inactive tau-tubulin kinase ttbk-6.
D1044.3	*pqn-25*	18.59 ± 5.549	0.0011	Prion-like-(Q/N-rich) domain-bearing protein 25.
C01H6.9	*hasp-1*	18.43 ± 7.099	0.0106	Protein kinase domain-containing protein.
B0496.3	*unc-82*	18.25 ± 6.633	0.0068	Protein kinase domain-containing protein.
D1073.1	*trk-1*	18.06 ± 6.549	0.0067	Protein kinase domain-containing protein; Belongs to the protein kinase superfamily. Tyr protein kinase family.
Y42A5A.4	*cdkl-1*	17.18 ± 6.537	0.0097	Cyclin-dependent kinase-like 1; Modulates cilium assembly.
Y53C12A.1	*wee-1.3*	17.10 ± 6.542	0.0101	Membrane-associated tyrosine- and threonine-specific cdc2-inhibitory kinase wee-1.3; Acts as a negative regulator of entry into mitosis (G2 to M transition) by phosphorylation of the CDK1 kinase during oocyte maturation. Required for oocyte maturation, embryonic development, germline proliferation and initiation of meiosis during spermatogenesis. Required for chromosome structure during mitosis and negative regulation of nuclear envelope breakdown. Belongs to the protein kinase superfamily. Ser/Thr protein kinase family. WEE1 subfamily.
F32D8.1	*F32D8.1*	17.03 ± 6.570	0.0107	Protein kinase domain-containing protein; Belongs to the protein kinase superfamily.
T06C10.3	*T06C10.3*	16.96 ± 6.756	0.0134	Tyrosine-protein kinase.
C56C10.6	*C56C10.6*	16.84 ± 6.957	0.017	Protein kinase domain-containing protein.
H37N21.1	*hpo-11*	16.83 ± 6.653	0.0127	Protein kinase domain-containing protein.
C09G4.2	*pkg-2*	16.52 ± 6.920	0.0185	Protein Kinase, cGMP-dependent.
Y106G6A.1	*Y106G6A.1*	16.46 ± 6.644	0.0146	Protein kinase domain-containing protein.
W04G5.6	*kin-23*	16.01 ± 6.952	0.023	Protein kinase domain-containing protein; Belongs to the protein kinase superfamily. Tyr protein kinase family.
F29C4.1	*daf-1*	15.83 ± 4.956	0.0018	Predicted to enable activin binding activity; activin receptor activity, type I; and transforming growth factor beta receptor activity. Involved in several processes, including dauer entry; determination of adult lifespan; and response to bacterium. Predicted to be located in plasma membrane. Predicted to be part of activin receptor complex. Expressed in several structures, including distal tip cell; ganglia; inner labial sensillum; neurons; and somatic nervous system. Human ortholog(s) of this gene implicated in several diseases, including carcinoma (multiple); glaucoma (multiple); and hypertension (multiple). Is an ortholog of human ACVR1B (activin A receptor type 1B); ACVR1C (activin A receptor type 1C); and TGFBR1 (transforming growth factor beta receptor 1).
C35D10.4	*coq-8*	15.38 ± 5.517	0.0061	Atypical kinase coq-8, mitochondrial; Atypical kinase involved in the biosynthesis of coenzyme Q, also named ubiquinone, an essential lipid-soluble electron transporter for aerobic cellular respiration. Its substrate specificity is unclear: either acts as protein kinase that phosphorylates other proteins in the CoQ complex to stabilize their interactions or acts as a small molecule kinase that phosphorylates a prenyl lipid in the ubiquinone biosynthesis pathway.

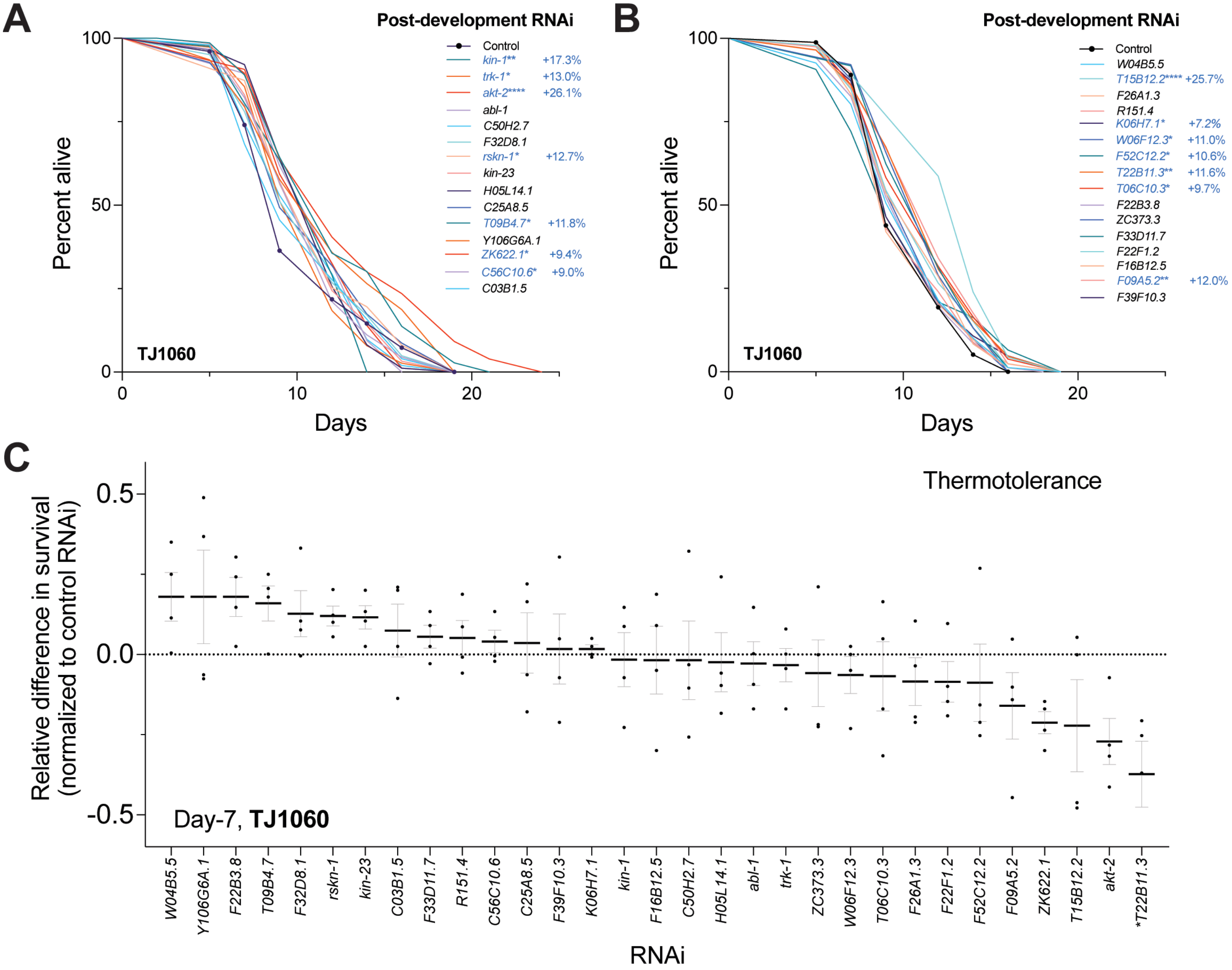
T17E9.1	*kin-18*	14.69 ± 4.834	0.0029	Serine/threonine-protein kinase SULU; Acts as a negative regulator of cortical contractions during early embryonic cell division, possibly by regulating rho-1-dependent actomyosin contractility. Plays a role in polarity establishment in early embryos by regulating the size of the anterior and posterior cortex in the first asymmetric cell division. Might play a role in cell cycle progression. In the germline, involved in the regulation of meiotic progression during oogenesis, possibly by modulating the timing of mpk-1 activation. Plays a role in meiotic recombination events. Involved in [...]
T23G7.5	*pir-1*	14.35 ± 6.528	0.0298	RNA/RNP complex-1-interacting phosphatase homolog; Possesses RNA 5'-triphosphatase and diphosphatase activities, but displays a poor protein-tyrosine phosphatase activity (By similarity). Binds RNA (By similarity). During small-RNA-mediated gene- silencing or RNA interference (RNAi), involved in the dcr-1-mediated processing of an amplified dsRNA intermediate.
B0523.1	*kin-31*	14.26 ± 4.998	0.0051	Expressed in body wall musculature.
C44C8.6	*mak-2*	14.25 ± 4.852	0.0039	MAP kinase-activated protein kinase mak-2; Serine/threonine-protein kinase which is involved in maintaining synapse and axon morphology as well as touch neuron axon regeneration after injury by regulating cebp-1 mRNA stability downstream of the dlk-1, mkk-4 and pmk-3 signaling cascade. May play a role in body wall muscle contraction. Plays a role in the formation of muscle connections, also called muscle arm extensions, between the body wall and the motor axons in the dorsal and ventral cord.
T17A3.8	*ver-2*	14.01 ± 5.336	0.0097	Protein ver-2; May be involved, downstream of pvf-1, in the positioning of ray 1, the most anterior ray sensillum in the male tail.
F09E5.1	*pkc-3*	13.98 ± 6.971	0.0472	Protein kinase C-like 3; Required for the normal progression of embryogenesis and viability of the organism. Plays an indispensable role in establishing embryonic polarity and in recruiting and maintaining par-6 to the periphery, through interaction with par-3. Required for epithelial cell polarity in the distal spermatheca. Phosphorylates serine residues of num-1. Required for the expression of antimicrobial peptide nlp-29 in response in response to fungal infection or physical injury.
F26A1.3	*F26A1.3*	13.47 ± 5.076	0.009	Protein kinase domain-containing protein.
F40G9.13		13.35 ± 6.663	0.0473	
H05L14.1	*H05L14.1*	12.40 ± 4.714	0.0096	Uncharacterized protein.
F16B12.5	*F16B12.5*	11.74 ± 4.868	0.0173	annotation not available
F08F1.1	*kin-9*	11.66 ± 5.505	0.0361	Protein kinase domain-containing protein.
PAR2.3	*aak-1*	11.56 ± 4.929	0.0205	Predicted to enable protein serine/threonine kinase activity. Involved in negative regulation of cell population proliferation and pharyngeal pumping. Predicted to be part of nucleotide-activated protein kinase complex.
F09A5.2	*F09A5.2*	10.82 ± 5.135	0.0371	Putative tyrosine-protein kinase F09A5.2; Belongs to the protein kinase superfamily. Tyr protein kinase family.

Knockdown supresses *daf-2(e1370)* dauer

EEED8.9	*pink-1*	-13.31 ± 6.621	0.0467	Serine/threonine-protein kinase pink-1, mitochondrial; Protects against mitochondrial dysfunction during cellular stress, potentially by phosphorylating mitochondrial proteins (By similarity). Plays a role in mitophagy.

ZK632.3	*riok-3*	-13.48 ± 6.513	0.0405	Serine/threonine-protein kinase RIO3.
F38E9.5	*twf-2*	-14.10 ± 6.516	0.0324	TWinFilin actin binding protein homolog.
F33D11.7	*F33D11.7*	-16.50 ± 6.535	0.0129	Protein kinase domain-containing protein.
M79.1	*abl-1*	-17.38 ± 6.510	0.0086	Tyrosine-protein kinase abl-1; Functions downstream of migratory protein mig-13 and is involved in Q neuroblast migration during larval development. Recruited by mig-13 to the leading edge of Q neuroblasts and their descendents to signal downstream, likely to the wve-1 pathway, and direct migration along the anteroposterior body axis. Promotes germline cell apoptosis in response to oxidative, osmotic and heat shock stresses. Belongs to the protein kinase superfamily. Tyr protein kinase family. ABL subfamily.
C09B8.7	*pak-1*	-17.96 ± 6.498	0.0066	Serine/threonine-protein kinase pak-1; Required for hypodermal cell fusion, together with cdc-42 and ced-10, leading to embryonic body elongation, which involves dramatic cytoskeletal reorganization. Plays a redundant role with max-2 in dorsal axonal guidance in ventral cord commissural motoneurons and in P neuroblast migration. Acts probably downstream of Rho GTPases mig-2 and ced-10 to regulate these 2 processes. Involved in orientating axonal growth of HSN neurons. During gonad morphogenesis and probably in association with pix-1 and git-1, involved in the migration of distal tip ce [...]
R13H8.1	*daf-16*	-26.46 ± 2.177	<0.0001	Enables several functions, including 14-3-3 protein binding activity; beta-catenin binding activity; and enzyme binding activity. Involved in several processes, including defense response to other organism; regulation of dauer larval development; and regulation of gene expression. Located in cytosol and nucleus. Expressed in several structures, including germ cell; gonad; hypodermis; neurons; and somatic cell. Used to study Parkinson's disease and diabetes mellitus. Human ortholog(s) of this gene implicated in several diseases, including Alzheimer's disease; alveolar rhabdomyosarcoma; reproductive organ cancer (multiple); and rheumatoid arthritis. Is an ortholog of human FOXO4 (forkhead box O4).

Figure 3


A
Post-development RNAi
Percent alive
100
50
0
Days
0
10
20
TJ1060
Control
kin-1** +17.3%
trk-1* +13.0%
akt-2**** +26.1%
abl-1
C50H2.7
F32D8.1
rskn-1* +12.7%
kin-23
H05L14.1
C25A8.5
T09B4.7* +11.8%
Y106G6A.1
ZK622.1* +9.4%
C56C10.6* +9.0%
C03B1.5
B
Post-development RNAi
Percent alive
100
50
0
Days
0
10
20
TJ1060
Control
W04B5.5
T15B12.2**** +25.7%
F26A1.3
R151.4
K06H7.1* +7.2%
W06F12.3* +11.0%
F52C12.2* +10.6%
T22B11.3** +11.6%
T06C10.3* +9.7%
F22B3.8
ZC373.3
F33D11.7
F22F1.2
F16B12.5
F09A5.2** +12.0%
F39F10.3
C
Thermotolerance
Relative difference in survival (normalized to control RNAi)
0.5
0.0
-0.5
Day-7, TJ1060
W04B5.5
Y106G6A.1
F22B3.8
T09B4.7
F32D8.1
rskn-1
kin-23
C03B1.5
F33D11.7
R151.4
C56C10.6
C25A8.5
F39F10.3
K06H7.1
kin-1
F16B12.5
C50H2.7
H05L14.1
abl-1
trk-1
ZC373.3
W06F12.3
T06C10.3
F26A1.3
F22F1.2
F52C12.2
F09A5.2
ZK622.1
T15B12.2
akt-2
*T22B11.3
RNAi

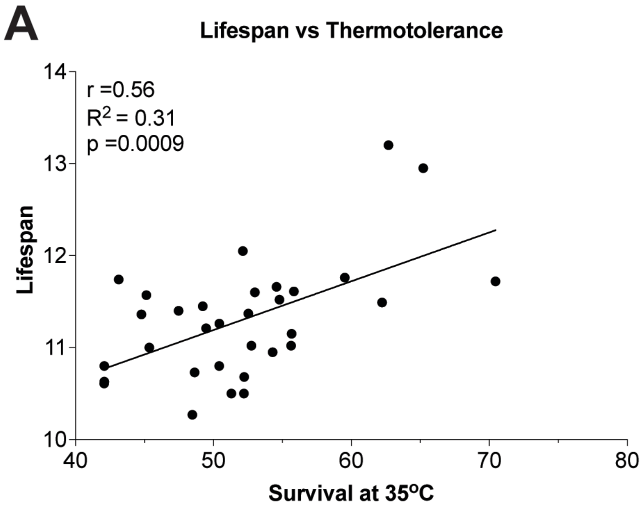
Knockdown signifciantly extends lifespan

Sequence	**Gene name**	**Human Ortholog**	**Description (ortholog)**
ZK909.2	*kin-1*	*PRKACB*	protein kinase cAMP-activated catalytic subunit beta
D1073.1	*trk-1*	*NTRK3*	neurotrophic receptor tyrosine kinase 3
F28H6.1	*akt-2*	*AKT/PKB*	Protein kinase B/AKT
T01H8.1	*rskn-1*	*RPS6KA3*	ribosomal protein S6 kinase A3
ZK622.1	*ZK622.1*	*FER/FES*	FER tyrosine kinase
C56C10.6	*C56C10.6*	*TTBK2*	tau tubulin kinase 2
T15B12.2	*T15B12.2*	*CSNK1A1*	casein kinase 1 alpha 1
W06F12.3	*W06F12.3*	*TTBK2*	tau tubulin kinase 2
F52C12.2	*F52C12.2*	*TSR3*	TSR3 ribosome maturation factor
T22B11.3		*LTK*	leukocyte receptor tyrosine kinase
T06C10.3	*T06C10.3*	*FER*	FER tyrosine kinase
F09A5.2	*F09A5.2*	*TIE1*	tyrosine kinase with immunoglobulin like and EGF like domains 1
K06H7.1	*K06H7.1*		
T09B4.7	*T09B4.7*		

Strain: TJ1060; Temperature: 25C; post-devlopment RNAi

See Supplementary. Table 2 for lifespan stats

Figure 4


A
Lifespan vs Thermotolerance
r =0.56
R2 = 0.31
p =0.0009
Lifespan
14
13
12
11
10
40
50
60
70
80
Survival at 35°C
B
Dauer vs Lifespan
r =0.45
R2 = 0.20
p =0.009
Lifespan
14
13
12
11
-20
0
20
40
Dauer

Supplementary Table 1

RNAi	Percent dauer formation					
C09B8.7	4.76190476	4	4.34782609	13.6363636	14.2857143	10
Y111B2A.b	4.54545455	8.69565217	13.0434783			
M79.1	4.76190476	8.69565217	5.55555556	3.22580645	10.5263158	21.7391304
F33D11.7	12.5	0	0	25	14.2857143	8
F22D6.5				11.1111111		
T14E8.1	5.88235294	0	7.69230769	15.7894737	12.5	28.5714286
F38E9.5	0	15	11.5384615	8.69565217	17.8571429	21.0526316
ZK632.3	4.34782609	14.2857143	4.54545455	16.2790698	21.7391304	16.6666667
EEED8.9	37.5	0	21.4285714	0	20	0
C36B7.1	19.2307692	8	4.34782609	14.2857143	23.5294118	12.5
C08H9.5	0	10	9.52380952	15	24	26.3157895
B0205.7	20	6.66666667	5.88235294	12.5	23.0769231	18.75
C24G7.5	15.7894737	16.6666667	0	24.3902439	17.6470588	16.1290323
K08F8.1	14.8148148	7.14285714	10.5263158	10.7142857	20	27.7777778
C16D9.2				25	10	11.1111111
C04A11.3	21.0526316	4.76190476	9.09090909	9.09090909	26.6666667	21.875
W03F8.2	0	12.5	11.7647059	20	26.6666667	22.2222222
T11F8.4	0	6.25	5.55555556	16.6666667	33.3333333	33.3333333
F17E5.1	25	27.2727273	14.2857143	5.88235294	7.14285714	17.3913043
C14A4.13	6.25	10.5263158	17.6470588	10.5263158	26.9230769	30.7692308
C39H7.1	6.25	10.5263158	5	23.0769231	24.2424242	35.483871
C05H8.1	0	11.7647059	9.52380952	28.125	26.6666667	30
C08H9.8	14.2857143	7.14285714	5	28	20	31.8181818
F59F5.3	0	28.5714286		25.8064516	13.3333333	20.8333333
K08H2.5	7.69230769	25	13.3333333	28.5714286	5.55555556	30.4347826
D2045.5	16.6666667	32.2580645		16.6666667	10	16.6666667
M03C11.1	5.55555556	10	31.25	32.1428571	18.75	13.5135135
F35G12.3	18.1818182	11.7647059	13.0434783	17.8571429	29.1666667	25
Y69E1A.3	9.52380952	13.3333333	9.375	17.6470588	22.8571429	43.75
ZC504.3	22.5	5.88235294	22.2222222	14.2857143	20	33.3333333
W09C5.5	10	16.6666667	29.4117647	17.6470588	33.3333333	15.3846154
C29H12.5	46.6666667	25	35.7142857	0	9.09090909	6.25
R03G5.2	28	10	19.2307692	27.2727273	16.6666667	21.7391304
W03G1.6	7.14285714	42.8571429	22.2222222	16	21.4285714	13.3333333
B0218.3	22.2222222	0	8.33333333	22.7272727	31.5789474	38.8888889
F59A6.4	31.5789474	12.5	14.8148148	18.5185185	8.57142857	40.625
C01G6.8	16.6666667	13.3333333	26.3157895	5.55555556	32	34.4827586
R06A10.4	32.3529412	35	23.0769231	18.1818182	8	12
F35H8.7	8.33333333	12	10	20.5882353	44.4444444	33.3333333
F36H12.8	0	12.5	5.55555556	75	17.3913043	19.047619

ZK354.2	14.2857143	12.5	13.0434783	21.7391304	40	30.7692308
ZC416.4	9.52380952	6.4516129	11.7647059	45.8333333	28.125	30.7692308
F45H7.4	26.6666667	18.5185185	25	22.7272727	18.1818182	22.7272727
DC2.7	5.26315789	4.34782609	16.6666667	60	20	27.7777778
Y81G3A.3	22.7272727	15	28.5714286	30.4347826	11.1111111	26.3157895
B0478.1	7.69230769	19.2307692	15	30.4347826	39.1304348	23.5294118
R05H5.4	19.2307692	21.4285714	9.09090909	45.4545455	36	6.25
ZC504.4	17.3913043	29.4117647	14.2857143	23.8095238	28.5714286	24
K06H7.8	17.6470588	14.8148148	3.38983051	43.3333333	29.0322581	29.4117647
E02H4.6	17.6470588	15.3846154	10	47.3684211	29.6296296	18.75
C05D2.1	40	11.7647059	31.8181818	10	22.7272727	22.7272727
C44H4.6	18.1818182	15.3846154	20	22.2222222	32.1428571	33.3333333
ZC581.1				17.6470588	36.3636364	16.6666667
R02C2.1	6.06060606	3.57142857	14.2857143	45.8333333	26.3157895	45.7142857
R13H9.6	33.3333333	5.55555556	7.69230769	33.3333333	15.3846154	47.3684211
F36H12.9	12.5	8.33333333	10.5263158	31.5789474	42.8571429	37.5
C34B2.3	33.3333333	30	44.4444444	0	23.0769231	12.5
K11H12.9	8.33333333	0	20	38.0952381	42.8571429	34.7826087
K07A9.2	4.34782609	0	5	34.7826087	50	50
C09B9.4	21.0526316	28.5714286	36.8421053	15	15	18.1818182
T27E9.3	0	8.69565217	11.1111111	22.5806452	47.0588235	55.5555556
F46G11.3	12	17.3913043	6.66666667	53.3333333	25	30.7692308
F13B9.4	12.9032258	20.6896552	26.0869565	25	24	36.8421053
T01H10.4	18.1818182	11.5384615	15.7894737	31.8181818	33.3333333	35
F26A1.4	55.5555556	28.5714286	26.6666667	5.88235294	6.66666667	23.3333333
F22H10.5	23.8095238	0	0	27.2727273	43.75	51.8518519
F46F6.2	20.8333333	41.9354839	23.3333333	13.3333333	15.3846154	33.3333333
F53G12.6	25	40	46.1538462	27.2727273	10	0
Y119D3_455.b	29.16667	57.14286	37.5	5	16.6666667	5
T05G5.3	26.6666667	10.5263158	15.7894737	39.2857143	35.2941176	23.3333333
M176.7	20.8333333	9.52380952	9.09090909	40	18.75	53.3333333
C24A1.3	13.0434783	0	9.09090909	36.3636364	32	61.1111111
F43C1.2	28.57143	22.22222	25	30	14.2857143	31.8181818
R13H9.5	40	18.75	30.7692308	17.8571429	33.3333333	12
C07A9.3	30	25	22.7272727	15.3846154	28.5714286	22.7272727
C29F9.7	11.7647059	10.5263158	5	40.7407407	52.173913	34.6153846
R03D7.5	9.52380952	4.34782609	13.3333333	31.25	42.8571429	54.5454545
R13F6.7	7.69230769	9.09090909	9.52380952	50	35	50
F31E3.2	14.2857143	19.2307692	20	50	35	40
R09D1.12	21.0526316	10.5263158	15.7894737	28.5714286	47.826087	40
F57B9.8	6.25	15	0	10	10	17.3913043

F25F2.1	33.3333333	31.0344828	26.6666667	15.7894737	26.3157895	23.5294118
C03C10.1	16.6666667	16.6666667	11.7647059	16.6666667	36.8421053	66.6666667
ZC449.3	13.0434783	25	13.7931034	18.75	36.3636364	59.2592593
F35C8.3	9.52380952	0	15.3846154	76.9230769	19.047619	45.4545455
T05A7.6	4	6.66666667	9.09090909	0	27.2727273	21.0526316
B0545.1a	29.4117647	41.6666667	23.0769231	33.3333333	17.1428571	22.7272727
C25F6.4	20	14.8148148	11.7647059	55.2631579	28.125	38.0952381
ZK1067.1	11.1111111	13.3333333	11.7647059	50	40.9090909	41.1764706
W02B12.12	14.2857143	26.3157895	16.6666667	4.16666667	61.1111111	47.0588235
B0464.5	6.25	24.3243243	22.2222222	33.3333333	30.4347826	55
K04C1.5	0	21.0526316	0	41.9354839	37.5	72.2222222
M176.6	13.6363636	35.2941176	18.75	33.3333333	47.826087	24.137931
F19H6.1	5	0	16	9.67741935	3.125	8.57142857
C28A5.6	50	30	33.3333333	25	12.5	22.7272727
C05D10.2	57.14286	26.66667	27.77778	27.7777778	15.3846154	19.2307692
F19C6.1	30.2325581	15.7894737	18.9189189	36.3636364	35.2941176	37.5
C06E8.3	30	15.7894737	19.047619	40	32	38.0952381
W10G6.2	0	20	10	30	65.5172414	50
F59F3.1	14.8148148	25	27.2727273	41.025641	41.6666667	26.0869565
C18H7.4	11.1111111	10.5263158	25.9259259	40	46.6666667	42.3076923
Y39G8C.2	33.3333333	13.3333333	50	25	37.5	18.75
F59E12.2	50	45.8333333	57.1428571	12.5	13.3333333	0
F58A3.2	33.3333333	7.14285714	18.4210526	21.875	48.1481481	50
C55C3.4	19.047619	18.75	14.2857143	36.3636364	46.1538462	44.4444444
T05E8.2	31.25	44.8275862	59.2592593	16.6666667	13.3333333	14.2857143
R107.4	27.7777778	33.3333333	42.8571429	35.483871	16.6666667	23.8095238
F46C3.1	5.88235294	8.69565217	24.2424242	53.8461538	44.4444444	42.8571429
H25P06.2a	60.8695652	55	50	0	0	14.2857143
C41C4.4	33.3333333	68.75	54.5454545	13.3333333	5.26315789	5.55555556
F54F7.5	20	13.6363636	14.8148148	50	48	35
C03C10.2	20	13.0434783	29.1666667	44.4444444	42.8571429	32.1428571
F49C5.4	22.22222	8.333333	29.16667	41.6666667	26.9230769	53.8461538
ZK596.2	32.3529412	32.2580645	43.75	22.2222222	31.25	20.8333333
F42A10.4	25	25	42.1052632	38.8888889	31.25	21.4285714
Y4C6A.k	20.8333333	10.5263158	46.6666667	50	43.4782609	13.7931034
ZK930.1	6.66666667	13.3333333	10.5263158	67.7419355	13.0434783	42.3076923
F28B12.3	41.9354839	35.2941176	30	25	36.8421053	18.1818182
R11E3.1						
D2024.1	15.625	18.75	10	55.8823529	53.8461538	35.2941176
F55A8.2	20	4.16666667		43.4782609	43.75	47.0588235
F15A2.6	33.3333333	23.8095238		41.3793103	34.7826087	25.9259259

M176.9	5	30	24	41.6666667	50	59.2592593
F35C8.2	7.69230769		7.40740741	55.5555556	38.7096774	51.6129032
C24G6.2	23.0769231	6.66666667	9.67741935	41.6666667	55	57.1428571
C35E7.10	20	0	25	55.5555556	34.7826087	61.1111111
T25E12.4	15.3846154	7.40740741	14.2857143	44	58.8235294	57.1428571
M04C9.5	62.962963	51.8518519	42.8571429	20	0	21.4285714
T04C10.1	10	20	31.5789474	63.1578947	25	50
W02B3.2	31.5789474	21.4285714	25		38.8888889	50
K08A8.1	23.5294118	25	53.3333333	26.3157895	33.3333333	39.2857143
E02H4.3	22.2222222	36	19.047619	50	27.7777778	48.1481481
ZK354.6	27.2727273	16.6666667	25	44.8275862	40.625	50
Y54E10B_152.	75	51.3513514	44.4444444	11.7647059	5.88235294	17.6470588
W03A5.1	11.1111111	5.26315789	12.5	65	65.2173913	47.0588235
F40A3.5	41.1764706	10.7142857	26.9230769	46.4285714	40.5405405	40.7407407
F42G10.2	40.9090909	35	11.5384615	56.25	28.5714286	34.2857143
F49E11.1				65.5172414	9.09090909	29.4117647
K08B4.5	25	26.3157895	8.33333333	31.5789474	51.7241379	65.2173913
F22H10.1	12.5	18.1818182		40.9090909	18.1818182	26.0869565
F59F3.5	10.5263158	23.8095238	29.6296296	52.9411765	38.0952381	57.6923077
T01C8.1	22.2222222	33.3333333	23.0769231	43.75	37.5	52.9411765
ZK938.5	7.14285714	18.75	26.6666667	15.3846154	30.4347826	50
C44C10.7	42.1052632	26.0869565	52.173913	27.2727273	27.5862069	39.2857143
K11E8.1	23.3333333	10.7142857	22.2222222	27.2727273	52.173913	44.4444444
Y52D3.1	23.8095238	33.3333333	22.2222222	52.173913	50	10
Y51B9A.9	8.33333333	6.4516129	18.1818182	33.3333333	22.2222222	15
T27C10.5	41.3793103	67.5675676	59.2592593	25	14.2857143	15
D2045.7	44.4444444	8.33333333	30.4347826	71.875	34.6153846	33.3333333
F09A5.2	15	12.5	12.5	13.3333333	15.3846154	35.7142857
C34F11.5	51.7241379	71.4285714	57.1428571	9.09090909	22.2222222	13.3333333
T07A9.3	23.8095238	22.7272727	14.2857143	30.4347826	50	85.7142857
Y39G8B.f	30.7692308	35.7142857	26.6666667	58.0645161	40	36.3636364
PAR2.3	4.76190476	27.7777778	17.6470588	52.173913	40	54.8387097
VZC374L.1	22.2222222	14.8148148	9.52380952	64.7058824	52.9411765	64.2857143
B0207.4	38.0952381	30	34.7826087	37.1428571	60.6060606	28
F08F1.1	10.5263158	8	4.34782609	43.75	30	27.5862069
F16B12.5	61.9047619	50	64.7058824	51.8518519	43.75	42.3076923
H05L14.1	40	62.5	47.3684211	33.3333333	39.1304348	56.25
K02E10.7	60.8695652	55	50	38.8888889	17.6470588	11.7647059
F18H3.5	10.5263158	31.5789474	37.5	54.8387097	52.173913	47.826087
Y38H8A.3	75	51.3513514	44.4444444	30	15.7894737	20
F35C8.1	27.7777778	38.8888889	6.66666667	60	59.0909091	44.4444444

F40G9.13	15.7894737	21.7391304	37.5	53.8461538	60	50
ZK370.5	18.75	21.0526316	20	59.2592593	46.1538462	73.6842105
F26A1.3	9.09090909	7.69230769	9.52380952	68.1818182	46.1538462	54.5454545
ZC581.9	63.8888889	76.9230769	63.1578947	25	0	11.1111111
F09E5.1	72.7272727	60	69.2307692	21.0526316	7.14285714	12.5
T17A3.8	10	5.55555556	0	33.3333333	35	52
C44C8.6	25	26.9230769	35	30.4347826	19.2307692	45
B0523.1	41.66667	44.44444	44.44444	31.25	29.4117647	25
T23G7.5	36.3636364	29.4117647	36.8421053	55	42.1052632	45.1612903
T17E9.1	60	52.9411765	42.1052632	32.1428571	38.0952381	52.173913
C35D10.4	11.7647059	12	5.55555556	30	40	80
F29C4.1	11.7647059	28.5714286	28.5714286	55.2631579	56.5217391	60.8695652
W04G5.6	33.3333333	6.66666667	12.5	66.6666667	77.7777778	57.8947368
Y106G6A.1	66.6666667	44.4444444	54.5454545	42.8571429	19.047619	30
C09G4.2	63.8888889	76.9230769	63.1578947	27.2727273	10	16.6666667
H37N21.1	75	50	40	30	37.5	27.2727273
C56C10.6	75	84.2105263	40	12.5	25	23.0769231
T06C10.3	28.5714286	73.6842105	53.8461538	27.7777778	16.6666667	60
F32D8.1	63.3333333	37.9310345	43.4782609	36.6666667	28.125	51.4285714
Y53C12A.1	52.9411765	50	52.6315789	39.1304348	27.2727273	39.3939394
Y42A5A.4	30	41.1764706	57.1428571	46.6666667	36.8421053	50
D1073.1	47.0588235	46.1538462	38.0952381	64	35.7142857	36.1111111
B0496.3	16.6666667	44.8275862	38.4615385	65	53.3333333	50
C01H6.9	64.2857143	66.6666667	92	10	23.0769231	13.3333333
D1044.3	16.6666667	0	0	47.826087	68.4210526	70
C45G9.1	16.6666667	3.84615385	7.69230769	70	73.3333333	60
W06F12.3	13.3333333	11.7647059	9.09090909	36.3636364	62.962963	54.5454545
R151.4	50	42.1052632	58.8235294	47.0588235	18.1818182	56.7567568
ZC373.3	55.5555556	33.3333333	46.1538462	36.3636364	39.1304348	64
C50H2.7	62.962963	51.8518519	42.8571429	37.5	44.4444444	36.3636364
W08D2.8	41.6666667	90	77.7777778	21.4285714	7.14285714	38.4615385
T09B4.7	28.5714286	73.6842105	53.8461538	37.5	41.6666667	42.8571429
F22B3.8	75	50	40	50	30	33.3333333
H39E23.1	33.3333333	30	44.4444444	43.75	81.8181818	45.4545455
F39F10.3	48.4848485	64.2857143	5.26315789	44.4444444	44.4444444	75
W06F12.1	39.3939394	48.5714286		47.6190476	47.6190476	52
C54G4.1	0	8.33333333	15.3846154	25	25	31.5789474
W04B5.5	17.6470588	15.7894737	17.3913043	26.6666667	41.1764706	52
F55G1.8	21.7391304	38.8888889	38.0952381	61.9047619	64.2857143	66.6666667
E01H11.1	38.8888889	21.4285714	60.7142857	56.5217391	63.3333333	58.3333333
ZK622.1	66.6666667	66.6666667	90.9090909	25	23.5294118	26.6666667

B0198.3	5.55555556	21.0526316	33.3333333	21.0526316	12.5	26.9230769
T20F10.1	57.1428571	100	95.6521739	11.7647059	16.6666667	22.2222222
C10C6.1	64.2857143	66.6666667	92	21.4285714	30	33.3333333
T01H8.1	31.5789474	52.173913	58.3333333	48.1481481	66.6666667	52.6315789
F22F1.2	62.962963	54.1666667		50	47.0588235	44.4444444
R166.5	21.4285714	42.8571429	5.26315789	50	66.6666667	76
F20B6.8	41.3793103	67.5675676	59.2592593	22.2222222	63.6363636	60
R10D12.10	66.6666667	44.4444444	54.5454545	47.6190476	18.1818182	83.3333333
B0285.1	9.52380952	11.7647059	15.3846154	73.3333333	86.9565217	88.8888889
T22B11.3	20	26.92308	38.09524	61.9047619	62.0689655	57.8947368
R06C7.8	41.6666667	90	77.7777778	46.1538462	31.25	36.3636364
F18F11.5	72.2222222	88.2352941	93.75	9.09090909	29.1666667	47.5
C25A8.5	33.3333333	68.75	54.5454545	29.4117647	25	42.1052632
ZK909.2	21.4285714	23.3333333		84.8484848	74.0740741	72.4137931
T15B12.2	10	40	9.09090909	100	95.2380952	68.1818182
C46C2.1				45.4545455	66.6666667	
K06H7.1	28.5714286	6.25	20.8333333	95.2380952	91.6666667	91.6666667
F52C12.2	14.2857143	5	10	72.7272727	76.1904762	78.3783784
C32D5.2	95.8333333	87.5	88.8888889	40	26.6666667	25
F46F2.2	56.25	55	55	70	76.9230769	55.1724138
C03B1.5	58.974359	51.8518519	52	90	60	56.5217391
F28H6.1	50	50	51.8518519	66.6666667	68.9655172	87.0967742
Y106G6E.6	90.9090909	93.75	85.7142857	26.6666667	42.8571429	36.3636364
ZK617.1	60.8695652	57.6923077	54.5454545	87.8787879	73.0769231	85.1851852
K09A9.1	100	100	95	100	78.2608696	85.7142857

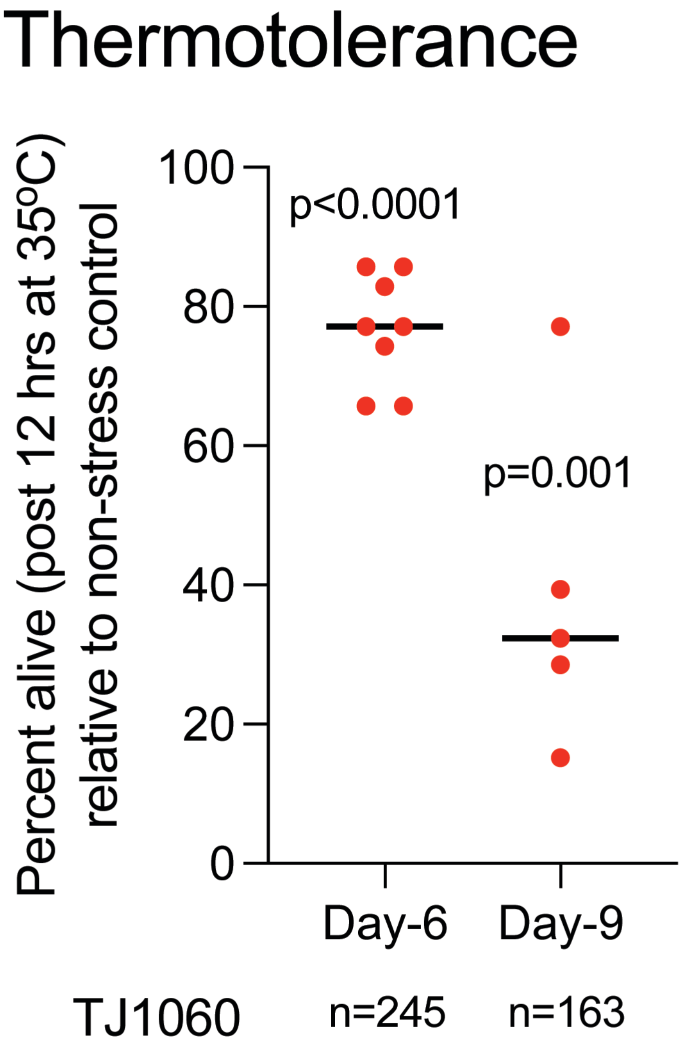
Supplementary Table 2

	Mean lifespan in days ± SEM	Median lifespan in days	N	% Mean lifespan change wrt Control	p-value wrt control RNAi
EV-Control RNAi	10.27 ± 0.42	9.00	74		
abl-1	11.02 ± 0.35	12.00	90	7.3	0.2598
akt-2	12.95 ± 0.52	12.00	80	26.1	0.0001
C25A8.5	11.21 ± 0.47	12.00	68	9.2	0.1792
C50H2.7	10.68 ± 0.36	9.00	101	4.0	0.6729
F32D8.1	11.36 ± 0.57	12.00	42	10.6	0.2401
H05L14.1	11.37 ± 0.29	12.00	87	10.7	0.1389
kin-1	12.05 ± 0.46	12.00	79	17.3	0.0066
kin-23	11 ± 0.42	12.00	64	7.1	0.3529
rskn-1	11.57 ± 0.37	12.00	87	12.7	0.0388
trk-1	11.6 ± 0.54	12.00	70	13.0	0.0387
EV-Control RNAi	10.5 ± 0.28	9.00	80		
T09B4.7	11.74 ± 0.39	12.00	43	11.8	0.0183
Y106G6A.1	10.8 ± 0.33	12.00	76	2.9	0.4593
ZK622.1	11.49 ± 0.32	12.00	86	9.4	0.0153
C56C10.6	11.45 ± 0.31	12.00	75	9.0	0.0348
W04B5.5	10.61 ± 0.35	9.00	80	1.0	0.5413
T15B12.2	13.2 ± 0.43	14.00	46	25.7	p<0.00001
F26A1.3	11.02 ± 0.32	12.00	84	5.0	0.1935
R151.4	10.73 ± 0.32	9.00	79	2.2	0.4103
K06H7.1	11.26 ± 0.36	12.00	80	7.2	0.0433
W06F12.3	11.66 ± 0.43	12.00	38	11.0	0.0379
F52C12.2	11.61 ± 0.30	12.00	84	10.6	0.0111
T22B11.3	11.72 ± 0.35	12.00	85	11.6	0.0048
T06C10.3	11.52 ± 0.37	12.00	79	9.7	0.0171
F22B3.8	10.63 ± 0.32	12.00	84	1.2	0.5846
C03B1.5	11.4 ± 0.46	12.00	45	8.6	0.0674
F22F1.2	11.15 ± 0.35	12.00	79	6.2	0.0848
F16B12.5	10.5 ± 0.32	9.00	86	0.0	0.7626
F09A5.2	11.76 ± 0.35	12.00	85	12.0	0.0026
F39F10.3	10.8 ± 0.34	9.00	85	2.9	0.3600
ZC373.3	10.95 ± 0.30	12.00	82	4.3	0.2698
F33D11.7	10.27 ± 0.56	9.00	41	-2.2	0.9727

Strain: TJ1060; Tmeprature: 25C; post-devlopment RNAi

The cumulative results of two independent trials, each scored by two investigators who were blinded to clone identit

Supplementary Figure 1



Supplementary Table 3

	Trial 1 alive	Trail 1 dead	Trial 2 alive	Trial 2 dead	Trial 3 alive	Trial 3 dead
c2-control	16	24	17	19	24	17
T09B4.7	16	22	11	16	15	24
H05L14.1	17	11	18	14	19	16
rskn-1	18	26	18	21	18	22
Y106G6A.1	24	20	12	25	16	13
kin-23	17	20	16	23	21	21
ZK622.1	24	16	24	12	20	14
C56C10.6	16	20	22	20	17	18
W04B5.5	10	16	14	28	24	23
T15B12.2	27	9	22	7	17	18
F26A1.3	17	20	19	12	23	14
R151.4	19	16	15	14	15	21
K06H7.1	19	20	15	14	21	21
W06F12.3	24	14	18	18	20	19
F52C12.2	27	15	23	14	19	13
T22B11.3	26	16	35	6	26	11
C25A8.5	18	24	26	17	16	24
T06C10.3	18	12	27	13	20	21
F22B3.8	14	22	14	14	14	18
C50H2.7	20	11	18	16	17	13
C03B1.5	15	22	19	19	16	23
trk-1	17	19	21	14	21	20
F22F1.2	22	14	20	14	22	17
abl-1	23	18	14	18	22	21
F16B12.5	21	24	21	18	15	21
F09A5.2	23	8	24	17	21	22
akt-2	25	13	29	11	22	18
kin-1	22	18	17	10	22	25
F39F10.3	20	21	22	18	15	27
ZC373.3	22	13	17	25	30	18
F32D8.1	17	20	12	23	17	16
F33D11.7	16	20	17	17	14	16

Trial 4 alive	Trial 4 dead	Total Trial 1	Total Trial 2	Total Trial 3
22	15	40	36	41
21	20	38	27	39
14	22	28	32	35
16	17	44	39	40
11	31	44	37	29
12	15	37	39	42
26	15	40	36	34
17	16	36	42	35
15	18	26	42	47
19	18	36	29	35
17	15	37	31	37
15	17	35	29	36
20	19	39	29	42
21	18	38	36	39
3	5	42	37	32
9	5	42	41	37
18	15	42	43	40
15	20	30	40	41
10	18	36	28	32
8	15	31	34	30
14	10	37	38	39
15	13	36	35	41
19	22	36	34	39
15	10	41	32	43
12	6	45	39	36
26	20	31	41	43
25	12	38	40	40
21	27	40	27	47
23	14	41	40	42
19	18	35	42	48
18	20	37	35	33
19	17	36	34	30

Total Trial 4	% alive Trial 1	% alive Trial 2	% alive Trial 3	% alive Trial 4
37	40	47.22222222	58.53658537	59.45945946
41	42.10526316	40.74074074	38.46153846	51.2195122
36	60.71428571	56.25	54.28571429	38.88888889
33	40.90909091	46.15384615	45	48.48484848
42	54.54545455	32.43243243	55.17241379	26.19047619
27	45.94594595	41.02564103	50	44.44444444
41	60	66.66666667	58.82352941	63.41463415
33	44.44444444	52.38095238	48.57142857	51.51515152
33	38.46153846	33.33333333	51.06382979	45.45454545
37	75	75.86206897	48.57142857	51.35135135
32	45.94594595	61.29032258	62.16216216	53.125
32	54.28571429	51.72413793	41.66666667	46.875
39	48.71794872	51.72413793	50	51.28205128
39	63.15789474	50	51.28205128	53.84615385
8	64.28571429	62.16216216	59.375	37.5
14	61.9047619	85.36585366	70.27027027	64.28571429
33	42.85714286	60.46511628	40	54.54545455
35	60	67.5	48.7804878	42.85714286
28	38.88888889	50	43.75	35.71428571
23	64.51612903	52.94117647	56.66666667	34.7826087
24	40.54054054	50	41.02564103	58.33333333
28	47.22222222	60	51.2195122	53.57142857
41	61.11111111	58.82352941	56.41025641	46.34146341
25	56.09756098	43.75	51.1627907	60
18	46.66666667	53.84615385	41.66666667	66.66666667
46	74.19354839	58.53658537	48.8372093	56.52173913
37	65.78947368	72.5	55	67.56756757
48	55	62.96296296	46.80851064	43.75
37	48.7804878	55	35.71428571	62.16216216
37	62.85714286	40.47619048	62.5	51.35135135
38	45.94594595	34.28571429	51.51515152	47.36842105
36	44.44444444	50	46.66666667	52.77777778

	Normalized Survival				relative Differe
51.3045668	0.77965769	0.92042922	1.14096247	1.15895062	0.22034231
	0.820692305	0.7940958	0.74967086	0.99834216	0.17930769
	1.183408994	1.09639363	1.05810686	0.75800053	-0.183409
	0.797377183	0.89960503	0.8771149	0.94503962	0.20262282
	1.063169577	0.63215488	1.07538992	0.51049015	-0.0631696
	0.895552752	0.79964891	0.97457211	0.86628632	0.10444725
	1.169486535	1.29942948	1.14655543	1.23604268	-0.1694865
	0.866286322	1.02098031	0.94672719	1.0041046	0.13371368
	0.749670856	0.64971474	0.99530769	0.88597465	0.25032914
	1.461858168	1.47866114	0.94672719	1.0009119	-0.4618582
	0.895552752	1.19463678	1.21163019	1.03548287	0.10444725
	1.058106865	1.00817805	0.81214343	0.91366136	-0.0581069
	0.949583084	1.00817805	0.97457211	0.99956114	0.05041692
	1.231038458	0.97457211	0.99956114	1.0495392	-0.2310385
	1.253021287	1.21163019	1.15730438	0.73092908	-0.2530213
	1.206613091	1.66390361	1.36966891	1.25302129	-0.2066131
	0.835347525	1.17855232	0.77965769	1.06316958	0.16465248
	1.169486535	1.31567235	0.95080206	0.83534752	-0.1694865
	0.758000532	0.97457211	0.8527506	0.69612294	0.24199947
	1.257512403	1.03189988	1.10451506	0.67796321	-0.2575124
	0.790193605	0.97457211	0.79964891	1.1370008	0.2098064
	0.920429217	1.16948653	0.99834216	1.04418441	0.07957078
	1.191143693	1.14655543	1.09951725	0.90326196	-0.1911437
	1.09342237	0.8527506	0.99723658	1.16948653	-0.0934224
	0.909600638	1.0495392	0.81214343	1.29942948	0.09039936
	1.446139263	1.14096247	0.95190764	1.10169021	-0.4461393
	1.282331727	1.41312956	1.07202932	1.31698934	-0.2823317
	1.072029324	1.22723896	0.91236538	0.8527506	-0.0720293
	0.950802061	1.07202932	0.69612294	1.21163019	0.04919794
	1.22517637	0.78893933	1.21821514	1.0009119	-0.2251764
	0.895552752	0.66827802	1.0041046	0.92327884	0.10444725
	0.866286322	0.97457211	0.90960064	1.02871501	0.13371368

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0.07957078	-0.1409625	-0.1589506
0.2059042	0.25032914	0.00165784
-0.0963936	-0.0581069	0.24199947
0.10039497	0.1228851	0.05496038
0.36784512	-0.0753899	0.48950985
0.20035109	0.02542789	0.13371368
-0.2994295	-0.1465554	-0.2360427
-0.0209803	0.05327281	-0.0041046
0.35028526	0.00469231	0.11402535
-0.4786611	0.05327281	-0.0009119
-0.1946368	-0.2116302	-0.0354829
-0.008178	0.18785657	0.08633864
-0.008178	0.02542789	0.00043886
0.02542789	0.00043886	-0.0495392
-0.2116302	-0.1573044	0.26907092
-0.6639036	-0.3696689	-0.2530213
-0.1785523	0.22034231	-0.0631696
-0.3156724	0.04919794	0.16465248
0.02542789	0.1472494	0.30387706
-0.0318999	-0.1045151	0.32203679
0.02542789	0.20035109	-0.1370008
-0.1694865	0.00165784	-0.0441844
-0.1465554	-0.0995173	0.09673804
0.1472494	0.00276342	-0.1694865
-0.0495392	0.18785657	-0.2994295
-0.1409625	0.04809236	-0.1016902
-0.4131296	-0.0720293	-0.3169893
-0.227239	0.08763462	0.1472494
-0.0720293	0.30387706	-0.2116302
0.21106067	-0.2182151	-0.0009119
0.33172198	-0.0041046	0.07672116
0.02542789	0.09039936	-0.028715