The longevity-promoting factor, TCER-1, widely represses stress resistance and innate immunity

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Stress resistance and longevity are positively correlated but emerging evidence indicates that they are physiologically distinct. Identifying factors with distinctive roles in these processes is challenging because pro-longevity genes often enhance stress resistance. We demonstrate that TCER-1, the *Caenorhabditis elegans* homolog of human transcription elongation and splicing factor, TCERG1, has opposite effects on lifespan and stress resistance. We previously showed that *tcer-1* promotes longevity in germline-less *C. elegans* and reproductive fitness in wild-type animals. Surprisingly, *tcer-1* mutants exhibit exceptional resistance against multiple stressors, including infection by human opportunistic pathogens, whereas, TCER-1 over-expression confers immuno-susceptibility. TCER-1 inhibits immunity only during fertile stages of life. Elevating its levels ameliorates the fertility loss caused by infection, suggesting that TCER-1 represses immunity to augment fecundity. TCER-1 acts through repression of PMK-1 as well as PMK-1-independent factors critical for innate immunity. Our data establish key roles for TCER-1 in coordinating immunity, longevity and fertility, and reveal mechanisms that distinguish length of life from functional aspects of aging.
In many organisms, a positive correlation has been noted between increased longevity and enhanced tolerance against environmental stressors such as high temperature, oxidative damage and pathogen attack (reviewed in refs. 1–3). Indeed, stress resistance has been used as a surrogate for lifespan extension in model organisms to identify several longevity genes. However, mutants that exhibit increased lifespan without enhanced stress resilience, and vice versa, have been reported intermittently in literature. In fact, in yeast, nematodes, flies and plants, only a fraction of mutants selected for increased stress resistance also exhibit enhanced longevity. This incomplete correlation implies that stress resistance alone is not sufficient to extend lifespan; other unknown processes may be induced coordinately with stress resistance in many long-lived mutants which may underlie their longevity. Importantly, these observations, and other emerging evidence, suggest that stress resilience is physiologically distinct from lifespan. This is an important distinction because stress resilience is also a major determinant of “healthspan”, the multiparametric measure of overall health in aging animals. With increasing emphasis on healthspan in the aging field, it is especially urgent to identify genetic and molecular pathways that uncouple stress resistance from lifespan. However, most known longevity-promoting genes also increase stress resistance (reviewed in ref. 1). Genes that promote longevity but widely inhibit stress resistance or other aspects of healthspan have not been identified.

There is widespread evidence from many species that increased reproduction is accompanied by reduced stress resistance, especially immune resistance, and reciprocally, pathogen infection impairs fertility (reviewed in ref. 16). But, while fertility and immunity appear to be mutually antagonistic, both diminish with age. Advanced maternal age is a major cause of reduced human reproductive fitness. Immunosenescence, the loss of immune resistance with age, underlies increased morbidity and mortality in older organisms. Hence, age is an important consideration in the immunity–fertility dynamic, but the molecular mechanisms governing this tripartite relationship are poorly understood.

The nematode Caenorhabditis elegans, similar to other organisms including humans, faces numerous stressors at the cellular (e.g., protein damage) and organismal (e.g., pathogens, high temperatures) levels, and responds via conserved, well-characterized systems such as the heat-shock response (HSR), oxidative stress response (OSR), hypoxia response (HR), unfolded protein response in the mitochondria (UPRmt) or endoplasmic reticulum (UPRer) and others (reviewed in refs. 19, 20). The response to pathogen threat in C. elegans is spearheaded by an innate immune system that includes conserved signaling pathways such as the mitogen-activated protein kinase (MAPK) cascade. The C. elegans p38 MAPK, PMK-1, activated by pathogenic stimuli as well as other stressors such as oxidative damage, governs the activity of multiple transcription factors to facilitate pathogen-specific responses. Significant overlap exists between innate immune responders and other canonical stress-response factors. For instance, ATFS-1, SKN-1, HSF-1 and HIF-1, key mediators of UPRer, OSR, HSR and HR, respectively, also upregulate innate immunity genes and confer pathogen resistance. Many of these proteins, and other such stress-response mediators, also enhance lifespan in C. elegans and other species. Additionally, their inactivation not only shortens lifespan but accelerates age-related decline in morphology, physiology and behavior i.e., healthspan parameters. So, while studying such factors has enriched our knowledge of stress-response mechanisms and longevity paradigms immensely, it has not advanced discovery of the molecular distinctions between the quantitative and qualitative measures of aging. In this study, we describe a role for TCER-1, C. elegans homolog of the human transcription elongation and splicing factor, TCERG1, in having discrete and opposite impacts on longevity and stress resilience.

We first identified TCER-1 as a factor essential for the lifespan extension caused by germline loss in C. elegans. In C. elegans, removal of the totipotent population of germline-stem cells increases lifespan, dependent on a network of transcription factors including TCER-1 and the conserved longevity determinant, DAF-16/FOXO3A (reviewed in ref. 26). TCER-1 overexpression increases the lifespan of normal, fertile animals underscoring its role as a pro-longevity gene. Recently, we showed that TCER-1 and DAF-16 act coordinately to extend the lifespan of germline-less animals by preserving lipid homeostasis. We also discovered that, in normal fertile C. elegans, TCER-1 is critical for optimal reproduction as well as prevention of age-related reproductive decline. tcer-1 mutants produce fewer, and less viable, eggs than their wild-type counterparts, and exhibit signs of premature reproductive senescence. Thus, under normal physiological conditions TCER-1 promotes reproductive fitness.

In this report, we demonstrate that TCER-1 inhibits resistance against multiple biotic and abiotic stressors, including immuneresistance against the opportunistic Gram-negative human pathogen Pseudomonas aeruginosa and the Gram-positive pathogen Staphylococcus aureus. tcer-1 mutants show increased survival upon infection and, reciprocally, TCER-1 overexpression increases susceptibility towards P. aeruginosa. TCER-1 acts cell non-autonomously in somatic tissues to mediate both its anti-immunity and prolongevity functions. We also find that TCER-1 inhibits immunity during the fertile stages of life and not in post-reproductive animals. Additionally, elevating TCER-1 levels partially arrests the decline in progeny production that follows infection, suggesting that TCER-1 may repress immunity to promote reproductive fitness. tcer-1 mutants exhibit increased expression of numerous known, as well as novel, antibacterial genes, at least four of which are essential for protection against infection. Our data suggest that TCER-1 inhibits immunoresistance by repressing PMK-1, as well as PMK-1-independent, innate immunity pathways. They reveal TCER-1 as a key factor governing the relationship between the intimately linked processes of immunity, lifespan and fertility.

Results

TCER-1 widely suppresses stress resistance. TCER-1 is critical for the extended lifespan of glp-1 mutants, the temperature sensitive, sterile model of germline-less longevity. tcer-1 single mutants exhibit a lifespan similar to normal, fertile animals. Since most longevity-promoting genes also augment stress resistance, we initially hypothesized that loss of tcer-1 would make animals more susceptible to environmental stressors. However, upon testing for resilience against the human opportunistic pathogen, P. aeruginosa PA14 strain (henceforth PA14), using the Slow Killing (SK) paradigm (wherein PA14 is grown on a low osmorality, minimal medium and causes C. elegans to die over the course of several days), we found that tcer-1 mutants showed a dramatic increase in survival as compared to wild-type adults (Fig. 1a, Supplementary Table 1A, B). Expectedly, glp-1 mutants survived longer than the fertile controls, but the tcer-1; glp-1 mutants survived even longer than glp-1 (Fig. 1a, Supplementary Table 1A, B). In contrast, and as expected, daf-16 inactivation significantly reduced survival in both wild-type and glp-1 strains (Supplementary Fig. 1a–c, Supplementary Table 1B). We checked if tcer-1 mutants exhibited aberrant feeding that may explain their resistance. However, no difference was observed in the pharyngeal pumping rates of the wild-type and mutant strains, either when fed Escherichia coli OP50, the regular lab diet.
Loss of TCER-1 enhances resistance against multiple biotic and abiotic stressors. Survival of L4 stage, wild-type *C. elegans* (WT, black), tcer-1 (blue), glp-1 (green) and tcer-1;glp-1 (orange) mutants exposed to different stressors. (a) Pathogen exposure. WT refers to number of animals analyzed/total number in experiment (see Methods for details). P values adjusted for multiplicity where applicable. Asterisks indicate statistical significance: <0.01 (**), <0.001 (***) and <0.0001 (****) and their color denotes the strain of comparison. Data from additional trials presented in Supplementary Tables 1A, B (panel a), 2A, B (b, c), 3A (e) and Supplementary Fig. 1d (f).

(henceforth OP50) or PA14 (Supplementary Fig. 2a). The two strains’ uptake of GFP-labeled OP50 was similar as well, although GFP-labeled PA14 uptake was slightly reduced in the mutants (Supplementary Fig. 2b). Notably, tcer-1 mutants infected with PA14 showed a dramatic reduction in colony-forming units (CFUs) as compared to wild type (Supplementary Fig. 2c), indicating that their increased survival could not be attributed to differences from wild type in the rate of bagging. tcer-1 mutants also showed increased survival upon exposure to another *P. aeruginosa* strain, PA01[43], in the SK paradigm as well as the Gram-positive pathogen *S. aureus*[44] (Fig. 1b, c, Supplementary Table 2). Thus, loss of tcer-1 function appeared to broadly inhibit *C. elegans* immunity.

We next asked if the dichotomy between the effect of tcer-1 inactivation on longevity and immunity extended to other environmental challenges and found similar results with heat, oxidative stress and DNA damage paradigms. Wild type, day 2 adults exposed to 35 °C for 6 h n = 50–100 animals per strain per each of six biological replicates analyzed using unpaired two-tailed t test. In (a–c) and (e) survival data analyzed using Kaplan–Meier test, shown as mean lifespans in hours (m ± standard error of the mean (SEM)). ‘n’ refers to number of animals analyzed/total number in experiment (see Methods for details). P values adjusted for multiplicity where applicable. Asterisks indicate statistical significance <0.01 (**), <0.001 (***) and <0.0001 (****) and their color denotes the strain of comparison. Data from additional trials presented in Supplementary Tables 1A, B (panel a), 2A, B (b, c), 3A (e) and Supplementary Fig. 1d (f).
oxidative stress induced by 7 mM tert-Butyl Hydroperoxide (tBOOH) by 30–50% (Fig. 1e, Supplementary Table 3A) and were significantly more resistant to DNA damage produced by exposure to γ-irradiation (Fig. 1f, Supplementary Fig. 1d). Upon experiencing ER stress mediated by Tunicamycin exposure, the mutants did not survive significantly longer (though a trend towards increased survival was evident; Supplementary Fig. 1e, Supplementary Table 3B). Thus, tcer-1 loss of function did not reduce resistance to stress, rather it dramatically and widely increased resilience against multiple biotic and abiotic stressors, indicating it to be a longevity gene that functions as a “anti-stress-resistance” factor.

**TCER-1 acts cell non-autonomously to inhibit immunity.** The incongruity of tcer-1’s effect on lifespan vs. stress resistance could possibly be explained if TCER-1 exerted longevity effects in some tissues and anti-stress resistance effects in others. To test this possibility, we expressed TCER-1 in individual tissues of tcer-1 mutants and examined the effect on their survival on PA14. Our previous studies showed that TCER-1 is ubiquitously expressed in the nuclei of all somatic tissues35. Expressing TCER-1 using its endogenous promoter significantly diminished the PA14 resistance of tcer-1 mutants (Fig. 2a, Supplementary Table 4A). When it was expressed selectively in the neurons of tcer-1 mutants (using the rgef-1 promoter), their enhanced survival on PA14 was completely abolished. TCER-1 expression in the intestine, muscles or hypodermis (using gly-19, myo-3 or col-12 promoters, respectively) also drastically shortened tcer-1 mutants’ survival on PA14 (Fig. 2b–e, Supplementary Table 4A). The enhanced PA14 resistance of tcer-1;glp-1 mutants was also suppressed by expressing TCER-1 under its endogenous promoter or in any one of these tissues (Fig. 2f–j, Supplementary Table 4B). Indeed, TCER-1 expression in the intestine or muscles rendered the animals hyper-susceptible to PA14 (Fig. 2g, i). Thus, TCER-1 expression in any somatic tissue was sufficient to cell non-autonomously repress the increased PA14 resistance of tcer-1 mutants.

**TCER-1 acts cell non-autonomously to promote longevity.** tcer-1;glp-1 mutants are significantly shorter-lived than glp-1 on normal OP50 lawns35,37. We next checked how TCER-1 expression in individual tissues of tcer-1;glp-1 mutants influenced their lifespan on a diet of OP50. Expectedly, endogenous promoter-driven TCER-1 rescued lifespan to glp-1 levels (Fig. 2k, Supplementary Data 1A). Additionally, expression in the intestine, neurons, muscle or hypodermis (using the same promoters as above) also completely rescued the lifespan of tcer-1;glp-1 mutants (Fig. 2l–o, Supplementary Data 1B–E). Thus, TCER-1 presence in any somatic tissue is sufficient to cell non-autonomously promote the longevity of germline-less adults under normal food conditions. These data also overrule the possibility that the increased PA14 susceptibility of these strains (described in the paragraph above) is a consequence of transgene toxicity or general sickness. Taken together, our results suggest that TCER-1 functions in a cell non-autonomous manner to influence both stress resistance and longevity, and it can act in any tissue to increase lifespan or reduce innate immunity.

**TCER-1 does not repress immunity in post-reproductive adults.** Like many species, *C. elegans*’ resistance against PA14 decreases with age45. To determine if TCER-1’s impact on immunity was age dependent, we compared the PA14 resistance of tcer-1 mutants as late L4 larvae (pre-adulthood), day 2 adults (peak reproduction), day 4 adults (reproductively active), day 6 adults (reproduction cessation) and day 9 adults (post-reproductive) to that of age-matched, wild-type controls (Fig. 3a). Both wild-type and tcer-1 mutants showed a decline in survival time with increasing age, but tcer-1 mutants survived significantly longer than their age-matched, wild-type counterparts when exposed to PA14 as L4 larvae, or day 2 or day 4 adults. However, by day 6, when most animals cease laying eggs, the mutants were not more resistant than wild type (no statistical difference in 5/7 trials). By post-reproductive Day 9, tcer-1 mutants were either as susceptible to PA14 as wild-type animals or even more sensitive (Fig. 3a, Supplementary Table 5). Thus, tcer-1 mutants exhibited increased survival on PA14 during the fertile stages of adulthood; its impact coincided with, and paralleled, the reproductive profile of the animal raising the possibility that TCER-1’s functions in fertility and immunity may be related.

**TCER-1 overexpression curbs fertility loss caused by PA14.** Upon pathogen attack, fertility decline and reproduction arrest are usually one of the first consequences experienced by the host in most species16,46. Based on our previous discovery, that TCER-1 promotes reproductive fitness and germline health in normal, fertile animals37, and since TCER-1 appeared to exert the strongest repression on immunity at the peak of the animal’s fertility but not during the post-reproductive phase of life, we speculated if the protein repressed stress resistance to divert cellular resources towards progeny production. To test this, we first documented how PA14 affects *C. elegans* reproduction. We assessed egg laying in wild-type animals grown on normal food for ~65-h post-hatching and then exposed to PA14 as L4 larvae. A decline in the number of eggs laid by infected animals became apparent within 8 h and by 12 h infected *C. elegans* laid ~65% fewer eggs as compared to normal adults (Fig. 3b). We next asked if, and how, the dynamics of this decline were altered if TCER-1 levels were elevated. The 12 h post-infection timepoint was selected for these experiments as this stage was early enough to exhibit a strong reduction in egg production but well before the animal was overwhelmed by infection (48 h). We compared the decline in egg laying upon PA14 exposure between wild-type adults and those overexpressing TCER-1 in the intestine, muscle, hypodermis, neurons or under control of tcer-1 endogenous promoter. While normal animals exhibited ~65% decline in the number of eggs laid 12 h post-infection, the decline in transgenic strains was only ~35–55% (Fig. 3c, d). The protective effect was strongest, and achieved statistical significance, in a strain overexpressing TCER-1 in intestinal cells and in one of two strains wherein TCER-1 was driven by its own promoter (Fig. 3c, d). Neuronal, hypodermal and muscle overexpression showed a consistent and perceptible rescue in fertility, but did not achieve statistical significance. Thus, upregulating TCER-1 partly counteracted the fertility decline that followed pathogen attack in *C. elegans*. These observations support the premise that TCER-1 may promote the allocation of resources towards reproduction by repressing cellular investment in stress resilience.

**TCER-1 overexpression increases susceptibility to infection.** If TCER-1 was indeed promoting reproduction by repressing immunity, overexpression of the protein would conversely be expected to increase susceptibility to the pathogen. Interestingly, when TCER-1 was overexpressed in individual somatic tissues of wild-type animals, no consistent alteration in PA14-sensitivity was observed (Supplementary Table 4c). But a transgenic strain overexpressing TCER-1 widely under control of its endogenous promoter37 showed extraordinarily high susceptibility to PA14, as compared to the wild type (Fig. 3e) despite having similar pharyngeal pumping rates as the wild-type and tcer-1 mutants
expression. WT (47.61), by the presence of pathogens and/or reproductive age. Indeed, we
mutants expressing TCER-1 (red) under control of tcer-1;glp-1 (29.32), glp-1 (79.2),
(17.11) and
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m
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f, h). Within 12 h of exposure of day 1 adults to
survival (hours) of L4 larvae transferred to PA14. a–e Expression in tcer-1 mutants. Wild type (WT, black, 60.68), tcer-1 (blue, 96.31) and
tcer-1;glp-1 mutants expressing TCER-1 (red) under control of a endogenous promoter (70.91) or promoters expressed in b intestine (gly-19, 55.3), c neurons (rgef-1, 62.32), d muscles (myo-3, 64.0) or e hypodermis (col-12, 71.67). f–j Expression in tcer-1;glp-1 mutants. f Native expression. Wild type (WT, 60.68), glp-1 (79.2), tcer-1;glp-1 (orange, 80.3) and tcer-1;glp-1 mutants expressing TCER-1 under the control of endogenous promoter (44.0). g–j Tissue-specific expression. WT (47.61), glp-1 (59.52), tcer-1;glp-1 (67.98) and tcer-1;glp-1 mutants expressing TCER-1 (red) under the control of promoters expressed in g intestine (22.45), h–i muscles (25.03) or j hypodermis (47.15). h Neurons: WT (75.3), glp-1 (102.34), tcer-1;glp-1 (126.59) and neuron-expressed TCER-1 (84.31). k–o TCER-1 expression in any somatic tissue suppresses PA14 resistance. Mean survival (days) on OP50. k–m WT (18.7), glp-1 (29.32), tcer-1;glp-1 (17.11) and tcer-1;glp-1 mutants expressing TCER-1 under k endogenous promoter (24.14) and l tissue-specific expression in intestine (25.66). m–o WT (18.44), glp-1 (27.35), tcer-1;glp-1 (21.46) and tcer-1;glp-1 mutants expressing TCER-1 in m neurons (25.98), n muscles (29.26) and o hypodermis (28.14). Survival data analyzed using Kaplan–Meier test. P values adjusted for multiplicity where applicable. Asterisks indicate statistical significance <0.05 (*), <0.001 (****) and <0.0001 (*****) and their color denotes the strain of comparison. Assays in some panels were conducted in the same biological replicate so their controls are shared. Details of number of animals and data from additional trials in Supplementary Tables 4A (panels a–e), 4B (f–j) and Supplementary Data 1 (k–o)

(Supplementary Fig. 2a). This susceptibility was abrogated upon tcer-1 RNAi (Fig. 3e).

Since altering TCER-1 levels impacted pathogen susceptibility and fertility, it is conceivable that the protein is in turn influenced by the presence of pathogens and/or reproductive age. Indeed, we found that TCER-1::GFP expression in both the somatic tissues and germ cells was significantly diminished with age, and upon PA14 exposure. TCER-1::GFP expression was highest on day 1 of adulthood. In intestinal nuclei, expression was dramatically reduced by day 4 (Fig. 4a–g); by day 6 no GFP was detectable in any intestinal cell. In the germ cells, expression was reduced in day 4 as well, but the decline was not as conspicuous as in the intestine (Fig. 4a–f, h). Within 12 h of exposure of day 1 adults to PA14, we observed a reduction in TCER-1::GFP levels in both the

Fig. 2 TCER-1 acts cell non-autonomously to suppress immunity and promote longevity. a–j TCER-1 expression in any somatic tissue suppresses PA14 resistance. Mean survival (hours) of L4 larvae transferred to PA14. a–e Expression in tcer-1 mutants. Wild type (WT, black, 60.68), tcer-1 (blue, 96.31) and tcer-1;glp-1 mutants expressing TCER-1 (red) under control of a endogenous promoter (70.91) or promoters expressed in b intestine (gly-19, 55.3), c neurons (rgef-1, 62.32), d muscles (myo-3, 64.0) or e hypodermis (col-12, 71.67). f–j Expression in tcer-1;glp-1 mutants. f Native expression. Wild type (WT, 60.68), glp-1 (79.2), tcer-1;glp-1 (orange, 80.3) and tcer-1;glp-1 mutants expressing TCER-1 under the control of endogenous promoter (44.0). g–j Tissue-specific expression. WT (47.61), glp-1 (59.52), tcer-1;glp-1 (67.98) and tcer-1;glp-1 mutants expressing TCER-1 (red) under the control of promoters expressed in g intestine (22.45), h–i muscles (25.03) or j hypodermis (47.15). h Neurons: WT (75.3), glp-1 (102.34), tcer-1;glp-1 (126.59) and neuron-expressed TCER-1 (84.31). k–o TCER-1 expression in any somatic tissue extends tcer-1;glp-1 mutant’s lifespan on OP50. Mean survival (days) on OP50. k–l WT (18.7), glp-1 (29.32), tcer-1;glp-1 (17.11) and tcer-1;glp-1 mutants expressing TCER-1 under k endogenous promoter (24.14) and l tissue-specific expression in intestine (25.66). m–o WT (18.44), glp-1 (27.35), tcer-1;glp-1 (21.46) and tcer-1;glp-1 mutants expressing TCER-1 in m neurons (25.98), n muscles (29.26) and o hypodermis (28.14). Survival data analyzed using Kaplan–Meier test. P values adjusted for multiplicity where applicable. Asterisks indicate statistical significance <0.05 (*), <0.001 (****) and <0.0001 (*****) and their color denotes the strain of comparison. Assays in some panels were conducted in the same biological replicate so their controls are shared. Details of number of animals and data from additional trials in Supplementary Tables 4A (panels a–e), 4B (f–j) and Supplementary Data 1 (k–o)
intestinal nuclei (Fig. 4i) and germ cells (Fig. 4j). Interestingly, tcer-1 mRNA levels did not change significantly upon PA14 exposure (Supplementary Fig. 7), implying the existence of post-transcriptional regulatory mechanisms.

TCER-1 represses PMK-1 and its targets to impede immunity. How does TCER-1 repress immunity? Previously, we identified genes repressed by TCER-1 ("DOWN" class) upon germline loss37. Upon closer examination of this dataset, we found that ~15% of the genes with >1.5 fold expression change (42/295) were associated with immunity and stress resistance functions and 24/42 were predicted to be upregulated upon PA14 exposure (Supplementary Fig. 3a). This led us to explore the potential overlap between TCER-1 DOWN targets and genes reported to be
elevated upon PA14 infection. Of the 317 and 288 genes found to be upregulated 4 and 8 h after PA14 infection, respectively, by Troemel et al.,48 23 were included in the TCER-1 DOWN37 group (8 h exposure—< 1.753e-07, Representation factor: 4.2). Indeed, the TCER-1 DOWN37 class included pmk-1 itself strengthening the possibility that it may inhibit the expression of immunity genes and do so through PMK-1 repression.

Quantitative PCR (Q-PCR) analysis showed a significant increase in pmk-1 mRNA levels in tcer-1 mutants, both on OP50 as well as upon PA14 infection (Fig. 5a). Strains expressing PMK-1::GFP or PMK-1::mCherry translational reporters52,53 both displayed increased fluorescence upon tcer-1 RNAi (Fig. 5b–d). To test the functional relevance of this interaction, the possibility that it may inhibit the expression of immunity genes and do so through PMK-1 repression.
Fig. 5 TCER-1 suppresses immunity by inhibiting PMK-1. **a** mRNA levels of pmk-1 measured by QPCR in day 1 wild-type (black) and tcer-1 mutant (blue) adults maintained on OP50 (solid bars) or exposed as L4s to PA14 for 8 h (hashed bars). **b-d** PMK-1::mCherry reporter strain grown on **b** empty control vector or **c** tcer-1 RNAi from egg until larval stage L4 then transferred to PA14 for 24 h. **d** PMK-1::mCherry quantification from (c) and similar imaging of PMK-1::GFP strain upon tcer-1 RNAi. Data combined from three biological replicates with 15–20 animals imaged per strain per replicate. **e** pmk-1 null mutation suppresses tcer-1 mutant’s PA14 resistance. Survival of wild-type animals (WT, black m = 63.49 ± 1.3, n = 75/100), tcer-1 (blue, m = 93.82 ± 4.4, n = 84/100, P vs. WT <0.0001), pmk-1 (m = 23.77 h ± 0.8, n = 92/100, P vs. WT <0.001) and tcer-1;pmk-1 mutants (m = 45.95 ± 1.8, n = 97/102, P vs. tcer-1 <0.001, P vs. pmk-1 <0.0001, P vs. WT <0.0001) on PA14 after development on OP50 till L4 stage. **f-i** Expression of PMK-1 target genes is upregulated in tcer-1 mutants. Q-PCR analysis of mRNA levels of known PMK-1-upregulated genes **f** dod-3, **g** F55G11.4, **h** irg-5 and **i** C14C6.5 measured after wild-type (WT, black) and tcer-1 mutants (blue) grown on OP50 till L4 stage were transferred to PA14 plates for 12 h (hashed bars) or continued on OP50 (solid bars). Data in (a) and (f–i) combined from three independent biological replicates, each including three technical replicates. Error bars represent standard error of the mean (SEM). Survival data in panel (e) calculated using the Kaplan–Meier test and shown as mean lifespan in hours (m) ± SEM (see Methods for details). In (a), (d) and (f–i) statistical significances were calculated using a one-tailed t test. P values were adjusted for multiplicity where applicable. Asterisks indicate the degree of statistical significance <0.05 (*), <0.01 (**), <0.001 (***) and <0.0001 (****) and their color denotes the strain/condition of comparison.
we examined the effect of pmk-1(km25) null mutation on the PA14 resilience of tcer-1 mutants. tcer-1;pmk-1 mutants exhibited significantly reduced survival on PA14 as compared to tcer-1 mutants alone but their resistance was not completely abolished compared to the pmk-1 single mutant (Fig. 5e).

We next asked if the repression exerted by TCER-1 on PMK-1 impacted the expression of the latter’s target genes. A comparison of our TCER-1 “DOWN” class with genes predicted by previous studies to be upregulated by PMK-1 following PA14 infection also revealed a significant overlap (Supplementary Fig. 3f–h). Indeed, in Q-PCR assays, 6/7 of the top genes shared between the two groups showed increased expression 12 h after PA14 infection (Fig. 5f–i, Supplementary Fig. 4) and of these, five were upregulated in tcer-1 mutants (three achieved statistical significance; dod-3, F55G11.4 and ilys-10). Overall, these data suggest that TCER-1 may repress pathogen resistance, in part, through inhibition of the PMK-1-dependent innate immunity pathways.

TCER-1 lowers expression of PMK-1-independent immunity genes. Our pmk-1;tcer-1 epistasis data suggested that the PA14 resistance of tcer-1 mutants could only be partially explained by PMK-1 repression. In addition, 17/42 TCER-1-repressed immunity genes were expected to be induced in a PMK-1-independent fashion (Supplementary Fig. 3a). Hence, we explored the regulation of the PMK-1-independent genes predicted to be repressed by TCER-1. In accordance with the RNA Seq data, 7/8 of the top genes from this class that we tested showed elevated expression in tcer-1 mutants and 6/8 were also upregulated in wild-type adults upon PA14 exposure (five achieved statistical significance; C08E3, C50F7.5, fbxa-59, C49C8.5 and spp-18) (Fig. 6, Supplementary Fig. 5). Thus, TCER-1 repressed the expression of both PMK-1-dependent as well as -independent genes induced by PA14 infection.

We next asked if either the PMK-1-dependent and/or -independent genes repressed by TCER-1 had any functional roles in immunity against PA14. Mutants available for four of the genes described above, dod-3, dod-24, irg-5 and ilys-3, were tested for their resistance to PA14 upon tcer-1 knockdown. Wild-type C. elegans subjected to tcer-1 RNAi throughout their development and then exposed to PA14 at the L4 stage exhibited a significant increase in survival compared to control animals (Fig. 7a–d, Supplementary Table 6A). However, dod-3, irg-5, dod-24, and ilys-3 mutants did not exhibit any lifespan extension under the same conditions (Fig. 7a–d, Supplementary Table 6A). tcer-1;ilys-3 double mutants were also significantly more susceptible to PA14 than tcer-1 mutants alone, confirming the observations made through RNAi knockdown of these genes (Fig. 7e, f, Supplementary Table 6B and 6C). Overall, these findings indicate that TCER-1 represses the expression of multiple genes critical for conferring resistance against PA14 infection and these include genes operating in the PMK-1 pathway as well as potentially novel immunity effectors.

tcer-1 mutants exhibit improved healthspan parameters. Immunoresistance is a prominent hallmark of improved healthspan. Based on the stress-resistance phenotypes of tcer-1 mutants, we asked if they showed improvement in other aspects of healthspan too. We focused on mobility, a key function that undergoes age-related decline both in C. elegans and humans. Using a standard “thrashing” assay, we found that young (day 2) tcer-1 mutants performed significantly better than their wild-type counterparts (Fig. 8a). Since tcer-1 mutants exhibited increased immunoresistance during the reproductive phase but at post-reproductive ages became more sensitive (Fig. 3a), we tested if this was true for thrashing efficiency too. Indeed, by day 5, there was no difference in thrashing capacity of tcer-1 mutants and wild

Fig. 6 TCER-1 inhibits immunity by repressing PMK-1-independent genes. Gene expression measured by Q-PCRs of wild-type animals (black) and tcer-1 mutants (blue) grown on OP50 till L4 stage and then exposed to PA14 (hashed bars) or retained on OP50 for 12 h. a–d Expression of PMK-1-independent immunity genes C08E3.3 (a), fbxa-59 (b), C50F7.5 (c) and ilys-3 (d). Data combined from 2 to 5 independent biological replicates, each including three technical replicates. Statistical significance of the differences in expression was calculated using a one-tailed t test. Error bars represent standard error of the mean. *P < 0.05, **P < 0.01, ***P < 0.001
were performed in the same biological replicate so their controls are shared. Details of number of animals and data from additional trials are presented in Supplementary Table 6 indicate statistical signi

β amyloid improvement (Fig. 8b decrease in some swimming activity. We tested the impact of though, post-reproductive (day 10) mutants showed marked

proteotoxic degenerative disease, using a mutation on another healthspan feature, the ability to combat

parameters of swimming locomotion, using the software CeleST (Fig. 8a). Interestingly, when we examined several individual

tracks age-related decline in mobility and muscle function55, that tracks age-related decline in mobility and muscle function55, young mutants did not show a consistent or signi

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type, whereas post-reproductive days 7 and 9 mutants showed significantly impaired thrashing as compared to wild type (Fig. 8a). Interestingly, when we examined several individual parameters of swimming locomotion, using the software CeleST that tracks age-related decline in mobility and muscle function55, young mutants did not show a consistent or significant improvement (Fig. 8b–d). As seen with thrashing capacity though, post-reproductive (day 10) mutants showed marked decrease in some swimming activity. We tested the impact of tcer-1 mutation on another healthspan feature, the ability to combat proteotoxic degenerative disease, using a C. elegans model of amyloid β(Aβ) proteotoxicity. The strain GMC101 expresses human Aβ1–42 constitutively in muscle cells and, when transferred from 20 to 25 °C, animals exhibit full body paralysis56. We found that tcer-1 mutants exhibited a striking delay in developing paralysis. In one trial, while 50% of animals of the control strain were fully paralyzed by 33 h, and 100% by 50 h, the Aβ;tcer-1 strain did not show onset of paralysis till 30 h, and 50% and 100% of the population were only paralyzed by 95 and 125 h, respectively (Fig. 8e, Supplementary Fig. 9). Thus, tcer-1 mutation dramatically improved the ability of C. elegans to combat proteotoxicity and aggregation-related pathology. Together, the mobility and paralysis data suggest that TcER-1 may have a widespread impact on stress response and a nuanced in

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Fig. 8 Young tcer-1 mutants exhibit improvement in some healthspan parameters. a Threshing efficiency compared between wild-type (WT, black) and tcer-1 mutants (blue) at different ages. Data shown are from six independent biological replicates with 20 animals tested per strain, per replicate. Statistical significance was calculated using an unpaired two-tailed t test. b–d Measurement of change in swimming parameters with age in wild-type C. elegans (WT, black) and tcer-1 mutants (blue) quantified using CeleST55. In this longitudinal assay, the differences seen between the strains on day 1 of adulthood were not replicated but a clear reduction in day 10 tcer-1 mutants’ wave initiation rate (b, number of body waves initiated per minute) and activity index (c, measure of overall swimming activity) was observed. Brush stroke (d, depth of movement) was also reduced in the mutants but not in a statistically significant manner. Data combined from three independent biological replicates with number of animals tested for each genotype indicated on the bars. Statistical significance was calculated using two-way Anova, error bars represent standard error of the mean. e Loss of tcer-1 delays paralysis in Aβ proteotoxicity model. Onset of paralysis measured in the amyloid β proteotoxicity model strain GM101 (Aβ, black) and Aβ strain carrying a tcer-1 mutation (Aβ:tcer-1, blue). Kaplan–Meier analysis for mean paralysis time: Aβ m = 33.1 h, n = 67; Aβ:tcer-1 m = 95 h, n = 25. Asterisks indicate statistical significance <0.05 (*), <0.01 (**), and <0.0001 (****), ns not significant. Data from additional trials in Supplementary Fig. 9

Discussion

The innate immune system is an ancient and conserved system of defense against pathogens, and MAPK signaling, through PMK-1, has a well-established role in driving transcriptional changes that mediate immunity21. Our data suggest that TCER-1 may counter such changes directly or indirectly. PMK-1 was predicted to be a TCER-1-repressed gene and confirmed by both molecular and transgenic approaches. Several known PMK-1 targets were also upregulated in tcer-1 mutants. Yet, the pmk-1 null mutation did not completely abrogate tcer-1 mutants’ resistance suggesting that TCER-1-mediated immunity suppression is only partially through PMK-1-repression. What other processes may be impacted by TCER-1 to impair immunity? Several cellular surveillance pathways are activated by pathogen exposure, either partially or completely independent of PMK-122–26. In light of this, and TCER-1’s role in inhibiting multiple stress modalities, a logical prospect is that cellular stress response factors may be inhibited by TCER-1. Indeed, several genes with roles in UPRmt (e.g., mrps-5)57 and HR (e.g., cysl-1)58 were included in the TCER-1 DOWN37 group. tcer-1 mutants showed higher expression of the central UPRmt regulator, atfs-122,23, hsp-6, the UPRmt-specific chaperone29 (Supplementary Fig. 6a, b) and hif-128, the key HR mediator (Supplementary Fig. 6c). Accordingly, we also noted a significant overlap between the TCER-1 DOWN37 genes and ATFS-1-regulated genes (Supplementary Fig. 6d–f)22,23.

Sequence-based computational approaches have led to the identification of numerous immune effectors in C. elegans, including conserved and invertebrate-specific lysozymes (LYS and ILYS proteins, respectively) that may digest bacterial cell walls60,61. TCER-1-repressed genes comprised some of these factors as well as novel proteins with predicted antibacterial functions. For instance, C50F7.5, a TCER-1-repressed gene upregulated >50-fold upon infection (Fig. 6c), encodes a protein that shares ~60% sequence similarity with a cell-surface glycoprotein in Clostridium thermocellum. Cell-surface glycoproteins function in pathogen recognition, the most well known being the family of Toll-like receptors—receptors that sense pathogen associated molecular patterns expressed by infective agents62. Notably, no pathogen-specific receptors have been identified yet in nematodes. Similarly, the TCER-1-repressed gene, fbxa-59, which encodes an F-box protein, is highly upregulated upon PA14 exposure (Fig. 6b). F-Box proteins are E3 ligase components that mediate proteasomal protein degradation and influence longevity63. Natural allelic variations of the HECT-domain E3 ligase, HECW-1, have been implicated in PA14 avoidance64, and the CUL-6 E3 ligase complex is involved in the response to Nematodica parisii65. But, E3 ligases activated by Pseudomonas infection remain unknown. Characterization of the TCER-1-repressed transcriptome can reveal novel insights into the molecular repertoire of nematode immunity.
Cell non-autonomous mechanisms that govern longevity and stress resistance have been demonstrated recently in many contexts. In *C. elegans*, protein-folding imbalance in muscles induces transcellular chaperone signaling that evokes stress responses in intestine and neurons69, XBP-1 and retrograde Wnt signaling in neurons to coordinate the organisnal UPRmt and UPRER responses (reviewed in refs. 67,68), whereas intestinal DAF-16 expression is sufficient to confer longevity in germline-less mutants69. Similarly, dFOXO activity in *Drosophila* fat body regulates brain insulin signaling as well as lifespan70, whereas Activin disruption in muscles impacts systemic insulin metabolism71. In mice, Xbp1s activity in Pmc neurons is sufficient to improve hepatic glucose metabolism and protect against diet-induced obesity72. An interesting finding of this study is that TCER-1 can function in any of the four somatic tissues we tested to impact longevity and stress response. Expressing TCER-1 in any somatic tissue of tcer-1 mutants suppressed their PA14 resistance. While it is possible that this is simply a consequence of toxicity caused by TCER-1 overexpression, the fact that these tissues (a) do not cause lifespan shortening in normal animals fed either OP50 (Supplementary Data 1) or PA14 (Supplementary Table 4C), (b) do not shorten glp-1 mutants longevity on OP50 (Supplementary Data 1) and (c) extend the lifespan of tcer-1;glp-1 mutants on OP50, supports TCER-1’s non-autonomous mode of action in its anti-immunity and longevity functions. It is, indeed, intriguing that TCER-1 overexpression has profoundly different consequences for the animal depending on consumption of benign food or noxious pathogen. It implies that TCER-1’s presence in any somatic tissue can convert it into a coordinating center for orchestrating an animal-wide outcome, and that it may contextually direct the release of vastly different signals from the same tissue. Such molecular versatility can be highly beneficial to an animal in the wild facing rapidly fluctuating conditions to coordinate different aspects of its physiology.

TCER-1 is a longevity-promoting factor because it is essential for the lifespan extension conferred by germline loss, and because its overexpression in normal, fertile adults increases their lifespan55. Hence, the widespread, enhanced stress resistance of tcer-1 mutants was surprising considering the strong correlation between longevity and stress tolerance. It suggested that mechanisms that confer stress resistance do not directly confer longevity, although we cannot rule out this possibility for an as-yet untested stress modality. Though infrequently, mutations that increase thermal or oxidative stress resistance but do not increase lifespan (e.g., pep-2)10 as well as ones that enhance lifespan without improving stress endurance (e.g., cep-1)11 have been described. Germline-less daf-16 mutants are shorter lived than wild type but exhibit greater thermostolerance69, and daf-2 mutants subjected to RNAi inactivation of the prolongevity gene, smk-1, continue to exhibit increased thermostolerance73. But, it is noteworthy that so many instances of uncoupling reported so far have been exceptions and prolongevity genes largely act as pro-stress-resistance factors. The thermostolerance uncoupling notwithstanding, daf-16 and smk-1 confer resistance against numerous stressors in long-lived and wild-type animals4. Importantly, the knockdown of neither gene improves stress resistance, whereas tcer-1 inactivation does, widely and consistently. tcer-1 not only uncouples longevity from multiple stress modalties, it broadly antagonizes stress endurance. Additionally, while our previous study demonstrated that DAF-16 and TCER-1 collaborated to establish lipid homeostasis and promote longevity in germline-less adults37, these data reveal that, for stress resistance, TCER-1 and DAF-16 have an antagonistic relationship. They highlight the complexity of the links between lifespan and stress biology and suggest that the relationship is context dependent and plastic.

In most species, infections reduce fertility, whereas increased reproduction is accompanied by immunosuppression16. Alterations to a woman’s immune system are critical during pregnancy to tolerate fetal tissue, but are associated with increased susceptibility to infectious agents45. In insects, infections reduce fecundity while mating diminishes infection resistance16. In *C. elegans* too, sterile mutants have been reported to exhibit increased pathogen resistance40. This study, however, indicated that embryonic development, rather than absence of gametes per se, repressed immunity. It is also salient that while fertility and immunity appear to be mutually antagonistic, both diminish with age. In *C. elegans*, PMK-1 activity declines with age and thought to underlie immunosenescence53. Since TCER-1 acts, in part, to repress PMK-1, it is possible that the age-related loss of tcer-1 mutants’ PA14 resistance is linked to this reduction. Interestingly, the immune resistance of some sterile mutants is also reduced at post-reproductive ages45, emphasizing that the association of immunity and fertility is multifaceted and governed by factors such as age and resource allocation. The fact that TCER-1 is essential for reproductive health and exerts a repressive influence on immunity only during the reproductive phase, and the observation that raising its levels allows the animal to escape some of the fertility loss inflicted by infection, suggest that TCER-1’s primary molecular function may be to promote fertility. Indeed, we found that both somatic and germline levels of TCER-1 are highest in young animals and decline with age. Interestingly, human oocytes also express high levels of TGER1 mRNA and its levels decline in older oocytes54, so it may have a conserved role in promoting reproductive fitness. Identification of both PMK-1-dependent and -independent TCER-1 immunity functions also implies that multiple pathways may link immunity and fertility. TCER-1 can be a useful handle to decipher the mechanistic differences between these vital processes.

**Methods**

*C. elegans* strains and culture. All strains were grown and maintained on standard nematode growth medium (NGM) at 20 °C using *E. coli* strain OP50 as the food source. For experiments involving RNAi, NGM plates supplemented with 1 ml per liter of 1 M IPTG (isopropyl β-D-1-thiogalactopyranoside) and 1 ml per liter of 100 μm ampicillin. The strains used in this study include N2 (wild type), CG1652, mu86, daf-16(mu60) I, CF1903 (gfp-lwc1452) III, CF2154 (tcer-1(tk1452) II, gfp-lwc1441) III, CF1880 [daf-16(mu60b) I, gfp-lwc1441] III, CF2858 [tcer-1(tm1452); Pterc-1:TCER-1::GFP, Pdr-1::RFP] AGP214 [daf-16 (mu60b) I; tcer-1(tm1452) II] AGP215 [daf-16 (mu60b) I; tcer-1(tm1452) II; gfp-lwc1441] III, AGP1 [glmls (Pterc-1::tcer-1::GFP::Pdr-1::RFP) obtained by integration into *C. elegans* genome in CF2022 strain (CAGC65)], IT213 (tcer-1::GFP::3' utr)37, ZDI193 (gfp-ex101 [Ppwb-si-1:pmk-3::GFP::pmk-3::mCherry])33, PR1112 (mu47 Ex270 [pmk-1::GFP::rol-6(su1006)])32, AGP97 [pmk-1::msk-2])75 (obtained by outcrossing to Ghazi lab N2), AGP213 (tcer-1(tk1452) II; pmk-1 (km25) IV), RB2356 (dod-3(ok3202) V), RB2478 (eg::ok3418) II, RB1994 (dod-28 (ok2629) IV), VC2498 (ilys-3 (ok3222) V), AGP256 (tcer-1(tk1452) II; ilys-3 (ok3222) V). AGP258 (dod-3 (ok3202) V) outcrossed to Ghazi lab N2), AGP257 [tcer-1(tk1452) II; dod-3 (ok3202) V], GM101 (dvl100 (unc-54p::Afp-1:42:unc-54 3'-UTR::mtl-2p::GFP)) and AGP275 [tcer-1(tm1452) II; dvl100 (unc-54p::Afp-1:42:unc-54 3'-UTR::mtl-2p::GFP)]. Transgenic strains expressing TCER-1 under control of tissue-specific promoters in different genetic backgrounds generated for this study are listed in Supplementary Table 7.

Transgenic strain generation. To generate the Pterc-1::tcer-1::GFP construct, 5.6 kb region of tcer-1 gene (4.0 kb comprising the coding region covering all tcer-1 transcripts and 1.5 kb sequence upstream of the first tcer-1 exon) was amplified with primers modified to introduce SplII and Xmal restriction sites (forward 5′-ggctagcGAGaGgcAaAgGttagacctgac3′; reverse 5′-taagcCGCGGCTTCTGAAggtgacctcgcgacgggcg3′). The amplified product was cloned into the GFP expression vector pPD95.77 (Addgene plasmid 1495). The full length tcer-1 fragment was inserted upstream of, and in frame with, GFP at the SplII site (pAG10). To generate tissue-specific TCER-1 expressing constructs the 4 kb tcer-1 coding region was inserted into plasmids created previously in the lab for another gene, nhr-49 (Ratnappan and Ghazi, unpublished). The tcer-1 coding region was amplified with primers modified to introduce SalI and Acc65I restriction sites (forward 5′-ggtggtgCGCGGGTTTCTGAAggtgacctcgcgacgggcg3′; reverse 5′-taagcCGCGGCTTCTGAAggtgacctcgcgacgggcg3′). The nhr-49 coding region was removed from the tissue-specific promoter plasmids using
restriction enzymes, Sall and Asc6SlI, and with PCR amplified and digested tcer-1 coding region by ligation into the respective plasmids in frame with the GFP. The plasmids thus obtained were pAG11 (a 2.4 kb muscle promoter)77, Pnpyo-3::TCER-1::GFP), pAG12 (1.9 kb intestinal promoter77, Pgly-19:: TCER-1::GFP), pAG13 (400 bp hydropemal promoter78, Pcel-12:: TCER-1::GFP), and pAG14 (4.2 kb neuronal promoter77, Pregf-1::TCER-1::GFP). Transgenic strains were generated by injecting the plasmids at a concentration of 25 ng per µl or 100 ng per µl of Pyr2-18 and syringe by injection marker, respectively. Three to six independent stable transgenic lines were generated for each of the genetic backgrounds in which the transgene was injected. Transgenic strains were maintained by picking fluorescent animals in each generation.

**Lifespan assays.** All lifespan experiments were conducted at 20 °C on *E. coli* OP50 plates unless otherwise noted. Between 20 and 30 L4 hermaphrodites were transferred to each of 5 – 6 plates per experiment and observed at 24 – 48 h intervals to document live, dead or censored (animals that exploded, bagged or could not be located) animals. Animals were scored as dead when they failed to respond to gentle prodding with a platinum wire pick. Fertile strains were transferred every other day to fresh plates until progeny production ceased. For lifespan assays of strains with the temperature sensitive gfp-1 mutation, eggs were picked and maintained at 20 °C for 2 – 4 h, transferred to 25 °C to induce sterility and then returned to 20 °C. One day later, at the age of adulthood, 30 – 40 L4 hermaphrodites were transferred to each of ~5 plates, growing at 20 °C and then shaking and then ~10 µl of this was spread onto BHI-agar plates. Plates were streaked plate were inoculated and grown in BHI broth overnight at 37 °C with shaking. For survival every 24 h.

**Pathogenic stress assays.** Pathogenic bacterial strains used in this study include *Pseudomonas aeruginosa* (strains PA14 and PA01) and *Staphylococcus aureus* (NCTC8325). These strains were streaked from frozen stocks onto Luria Bertani (LB) agar (PA14 and PA01) or Brain Heart Infusion (BHI) agar (NCTC8325) plated, growing at 20 °C and then transferring them onto PA14 SK plates at 37 °C. For PA01 seeded SK plates and assaying for survival at 25 °C. Kaplan – Meier analysis were used for the quantification of eggs laid per animal per strain during its lifetime. Unpaired t test was used to calculate statistical differences in egg laying between strain/conditions.

**Microscopy and fluorescence imaging.** For imaging age-related change in Tcer-1::GFP levels, the IT213 strain was grown at 20 °C on OP50 till day 2 or 4 of adulthood before dissection. For documenting PA14-induced changes, L4 larvae grown on OP50 at 20 °C were transferred to PA14 or OP50 plates and kept at 25 °C for 12 – 14 h before dissections. Dissections were performed in 3 μl 1x sperm salts (50 mM PIPES, pH 7.0, 25 mM KCl, 1 mM MgSO4, 45 mM NaCl, 2 mM CaCl2) with –0.2 μl 10 mM Levamisole, one or two animals at a time. Both gonads and intestines were imaged within 3 min of dissection. Images were acquired on a Nikon A1r confocal microscope with 0.5 μm Z-stacks with identical imaging parameters for all samples. Image analysis was performed using Velocity3D imaging software (Perkin Elmer). The number of nuclei was counted using Velocity3D imaging software (Perkin Elmer). The number of nuclei was counted using Velocity3D imaging software (Perkin Elmer). 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Averaged intensities per genotype or condition were plotted with their respective gentamicin (1 mg per ml) for 30 min. Finally, the nematodes were lysed and the were then transferred to a new NGM plate containing ampicillin (1 mg per ml) and for 15 min. To further ensure elimination of any residual To eliminate mM levamisole in order to paralyze nematodes and inhibit pharyngeal pumping.

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The probability of overlapping genes overlap between two groups of genes was calculated on Nemates.org (http://www.nemates.org/MA/progs/overlap_stats.html). The probability of overlapping genes

The study are available within the paper and its supplementary information files. All data are available from the corresponding author upon request. The raw data for Figs. 3c–e, 4g–j, 5, 6 and 8b–d are included in the Source Data file.

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21. Kim, D. H. & Ewbank, J. J. Signaling in the innate immune lumen, animals were transferred from OP50 plates to ones containing GFP-labeled PA14 bacteria for 24 h and maintained at 25 °C. GFP intensity in the pharynx was visualized as described above and quantified using the Fiji (ImageJ) software. Briefly, the corrected total cell fluorescence (CTCF) values for all strains, conditions were measured. The average CTCF value obtained for WT animals fed GFP-OP50 was then used as the reference to which all the other conditions were compared.

CeleST swimming assay. Both wild-type and the tcer-1 strains were maintained at 15 °C on OP50 bacteria seeded on NGM plates. l4 animals were picked for age synchronization and transferred to 20 °C for the assay. Swimming was recorded under dark field by a Lumenera camera (Lt225M) installed on a trinocular dissecting microscope, and the StreamPix 7 software was used for camera control and video recording (18 frames per second for a total of 30 s in each video). In each measurement, four crawling animals were randomly picked and transferred into 30 pl M9 solution contained in a 1 cm circle imprinted on a microscope slide. Swimming parameters, including wave initiation rate, brush stroke and activity index, were measured using CeleST software.

Thrashing assay. To measure thrashing rate with age, L4-stage larvae were picked and maintained at 20 °C. On day 2, adults were transferred to an unseeded NGM plate (to remove excess OP50 from the body of the animal), then transferred one at a time into 1 ml of M9 media in an unseeded 3 cm plate. The animals were allowed to acclimate for 5 min in the liquid, then the number of body bends counted for a 30-s interval. A body bend constituted the movement of the head and/or tail beyond the midline of the body. Thrashing rate was measured similarly on animals aged to 5, 7, and 9 days of adulthood at 20 °C.

Paralysis assay. L4-staged animals of strains maintained at 20 °C were picked and shifted to 25 °C. Plates were scored after 24 h and then at 24 h intervals till the entire population was paralyzed. An animal was scored as paralyzed if it did not move upon prodding with a pick.

Statistical analyses. All data in this article are expressed as mean (m) ± standard error of mean (SEM) unless otherwise noted. Graphs were plotted and statistical analyses performed using Prism, OASIS2 or Microsoft Excel. Probability levels of 0.05 or below were considered statistically significant. Statistical significance of overlap between two groups of genes was calculated on Namates.org (http://www.nemates.org/MA/progs/overlap_stats.html). The probability of overlapping genes was calculated using the hypergeometric probability formula and the representation factor (RF) was calculated as the number of overlapping genes divided by the expected number of overlapping genes drawn from two independent groups.

Data availability

The authors declare that all data generated or analyzed supporting the findings of this study are available within the paper and its supplementary information files. All data are available from the corresponding author upon request. The raw data for Figs. 3c–e, 4g–j, 5, 6 and 8b–d are included in the Source Data file.
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A.G. and F.R.G.A. conceived the project; A.G., F.R.G.A., M.D., and J.L.Y. designed different experiments; F.R.G.A., N.N., R.R., J.L., A.G., J.L.Y., C.M., L.S., T.B.M. and G.W. performed the experiments; A.G. and F.R.G.A. wrote the manuscript with input from the other authors.

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