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A hierarchy of causes of death in senescent *C. elegans*

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Abstract

Interventions that extend lifespan in animal models could, in principle, decelerate the aging process as a whole. Alternatively, they could act by suppressing one or more individual late-life pathologies that contribute to mortality. Here we show how, in the nematode *Caenorhabditis elegans*, late-life pathologies can compete in a hierarchical fashion to cause death, such that removal of one cause of death can unmask another. Under standard culture conditions, a major cause of death in elderly *C. elegans* is infection by their bacterial food source. We report that only when such infection is prevented is lifespan extended by suppression of a second senescent pathology, teratoma-like uterine tumors. Thus, as in mammals, lifespan in wild-type *C. elegans* can be limited by naturally-occurring neoplasia. By contrast, blocking bacterial infection attenuated the life-shortening effects of vitellogenesis, and did not unmask a life-shortening effect of distal gonad degeneration. Thus, depending on the masking or unmasking of competing causes of mortality in the hierarchy of causes of death, nematode lifespan limitation in different contexts can reflect action of distinct life-limiting senescent pathologies. This underscores how increases in lifespan do not necessarily reflect a reduction in overall aging rate.

Keywords Aging, *C. elegans*, Competing causes, Infection, Mortality, Pathology, Teratoma, Tumor

Introduction

Although there is little consensus within the field of biogerontology about the causes of senescence (aging)[1,2], it is at least clear that its principal, ultimate cause is the process of evolution[3,4]. Evolutionary physiology seeks to understand the evolved proximate mechanisms by which genes cause senescence[5,6]. Research guided by an emerging explanatory framework, programmatic theory, has yielded useful insights into the causes of senescence in the short-lived nematode *C. elegans*[7-15].

According to one concept within programmatic theory, lifespan is limited by hyperfunctional (i.e. excessive) levels of wild-type mechanistic target of rapamycin (mTOR) signaling[16]. The originator of the hyperfunction theory, Misha Blagosklonny, suggested that although stochastic molecular damage accumulates throughout life, this is not a major, primary cause of late-life mortality. This is because mTOR hyperfunction causes death before damage accumulation can substantially promote life-limiting senescent pathology (with the exception of cancer)[17]. Thus, the hyperfunction theory includes a view of the causes of lifespan limitation by senescence in which different causes of mortality compete with one another, and where one cause of mortality can mask another - a competing causes of mortality model.

The evolutionary biologist George C. Williams once asked the senior author of the present study: "What do senescent *C. elegans* die from?" While aging people die mainly from cardiovascular disease, cancer and chronic obstructive pulmonary disease, the causes of death in aging *C. elegans* are less well understood. However, it is clear that death for elderly *C. elegans* under standard culture conditions[18] is a consequence of a combination of extrinsic and intrinsic factors.

A major extrinsic cause is infection with the *Escherichia coli* bacteria that is supplied as a food source, to which all individuals succumb in later life[19-21]. This occurs due to a combination of at least two intrinsic changes: mechanical senescence of the pharyngeal cuticle in early adulthood, and later processes of immune senescence[22]. Another possible internal cause is vitellogenesis (yolk synthesis), whose inhibition by RNA-mediated interference (RNAi) reduces several senescent changes, including intestinal atrophy and formation of pseudocoelomic lipoprotein pools (accumulations of lipid and yolk in the body cavity), and also extends lifespan[8,23,24]. However inhibition of several other major senescent pathologies does not increase lifespan under standard culture conditions: degeneration and fragmentation of the distal gonad[7], and formation of uterine tumors[25].

In this study, we explore a competing causes hypothesis of *C. elegans* lifespan determination (Figure 1a, left), in which causes of mortality are, to some degree, nested, in a manner akin to a Russian matryoshka doll, or the nested skins of an onion. According to this hypothesis, extrinsic causes contribute particularly to the outer skin, underneath which lie partially or fully masked intrinsic causes. Arguably, first among the latter will come programmatic causes of mortality (e.g. due to hyperfunction). Then, ultimately, according to Blagosklonny's scheme, after programmatic causes will come accumulation of stochastic molecular damage. According to this model, the identity of the senescent pathologies that limit lifespan is context dependent[8]. Here a counter-hypothesis is that senescence is, as it were, all one thing, happening all at once, and limiting life (Figure 1a, right).

Among the most striking pathologies in senescent *C. elegans* hermaphrodites are the uterine tumors (Figure 1b, bottom), that frequently grow so large as to fill the entire width of the body cavity in the mid-body region. Uterine tumors (also referred to as oocyte clusters) develop in all hermaphrodites after depletion of self-sperm stocks[26]. Completion of meiosis II in terminal oocytes requires fertilization, and in its absence, diploid oocytes are ovulated into the uterus.

There they undergo numerous rounds of DNA endoreduplication, leading to formation of disordered chromatin masses, and to cellular hypertrophy[27].

Older uterine tumors show signs of recapitulation of later embryonic gene expression[10], suggesting that their formation represents a futile attempt by diploid oocytes to execute a program of embryogenesis (or embryogenetic quasi-program). This and the similarity in etiology to that of ovarian teratomas (ovarian cysts, also arising from failure of meiosis II) in female mammals suggests that uterine tumors are a form of teratoma[10,28].

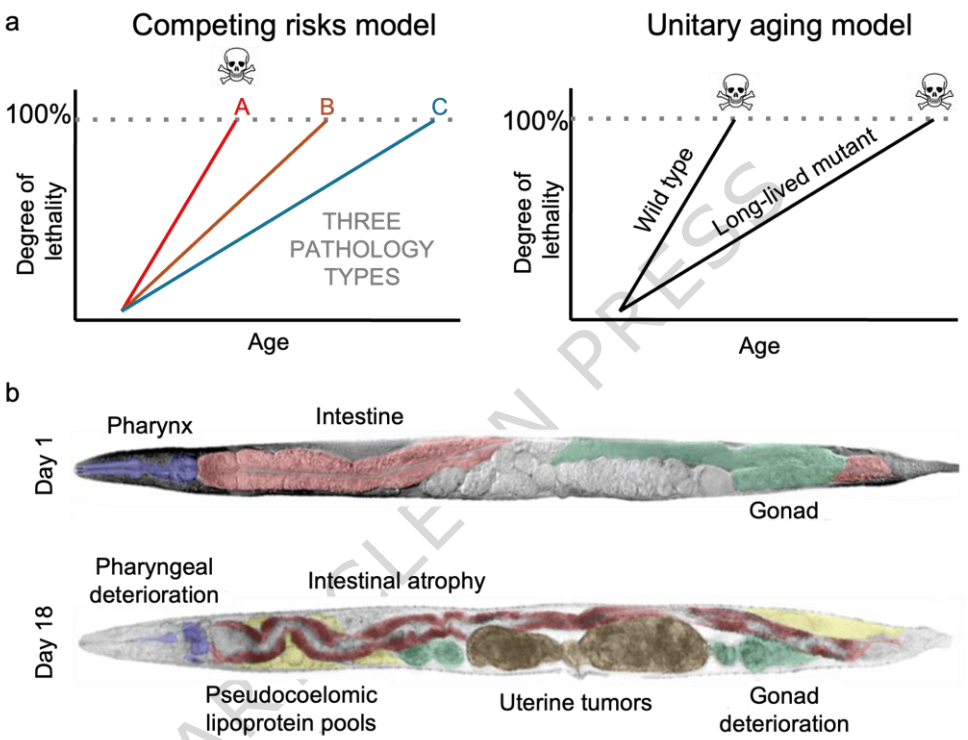

Figure 1. (a) Left, the competing causes of mortality model (standard culture conditions). Senescent pathologies develop, gradually become more severe, until they become lethal, causing death. Here pathology A is life limiting. If A is prevented, then pathology B becomes life limiting. Right, A counter-hypothesis, where a unified overall process of senescence limits life. Only according to the right hand scheme can lifespan be viewed as a metric of the overall aging process. (b) Senescent pathologies in *C. elegans* hermaphrodites, including uterine tumors (brown). Top, young adult hermaphrodite, with no uterine tumors (day 1 of adulthood). Bottom: Elderly hermaphrodite showing typical paired uterine tumors, one in each branch of the uterus. Several other pathologies are also visible.

Uterine tumor development can be prevented using 5-fluoro-2'-deoxyuridine (FUDR, or floxuridine), a drug used for the treatment of colorectal cancer. Surprisingly, under otherwise standard culture conditions, this does not increase lifespan, i.e. these large tumors have no effect on late-life mortality[25]. However, results of a recent study employing machine learning to assess the role of individual senescent pathologies in lifespan limitation suggest that under conditions that extend lifespan, uterine tumors may contribute to late-life mortality[29]. Here we explore the competing causes of mortality model by examining effects of preventing senescent pathology development on late-life mortality under different conditions. Our results provide an example of a

programmatic pathology, uterine tumor development, that contributes to late-life mortality in a context-dependent fashion.

Results

FUDR extends lifespan when bacterial infection is prevented

Human populations with higher death rates throughout life from infectious pathogens will experience concomitantly lower death rates from cancer (which predominantly occurs in later life). We wondered whether, by the same token, uterine tumors contribute to late-life mortality in the absence of bacterial infection. To this end, we first compared the effects on lifespan of suppressing tumor growth, using 50 μM FUDR, administered from the late-larval L4 stage or D2 (day 2) of adulthood, in the presence and absence of an antibiotic (4 mM carbenicillin, Carb). The following account describes summed data from three trials, unless otherwise stated; for full statistics, see Supplementary Tables; for raw data for all lifespan trials in this study, see Dataset S1).

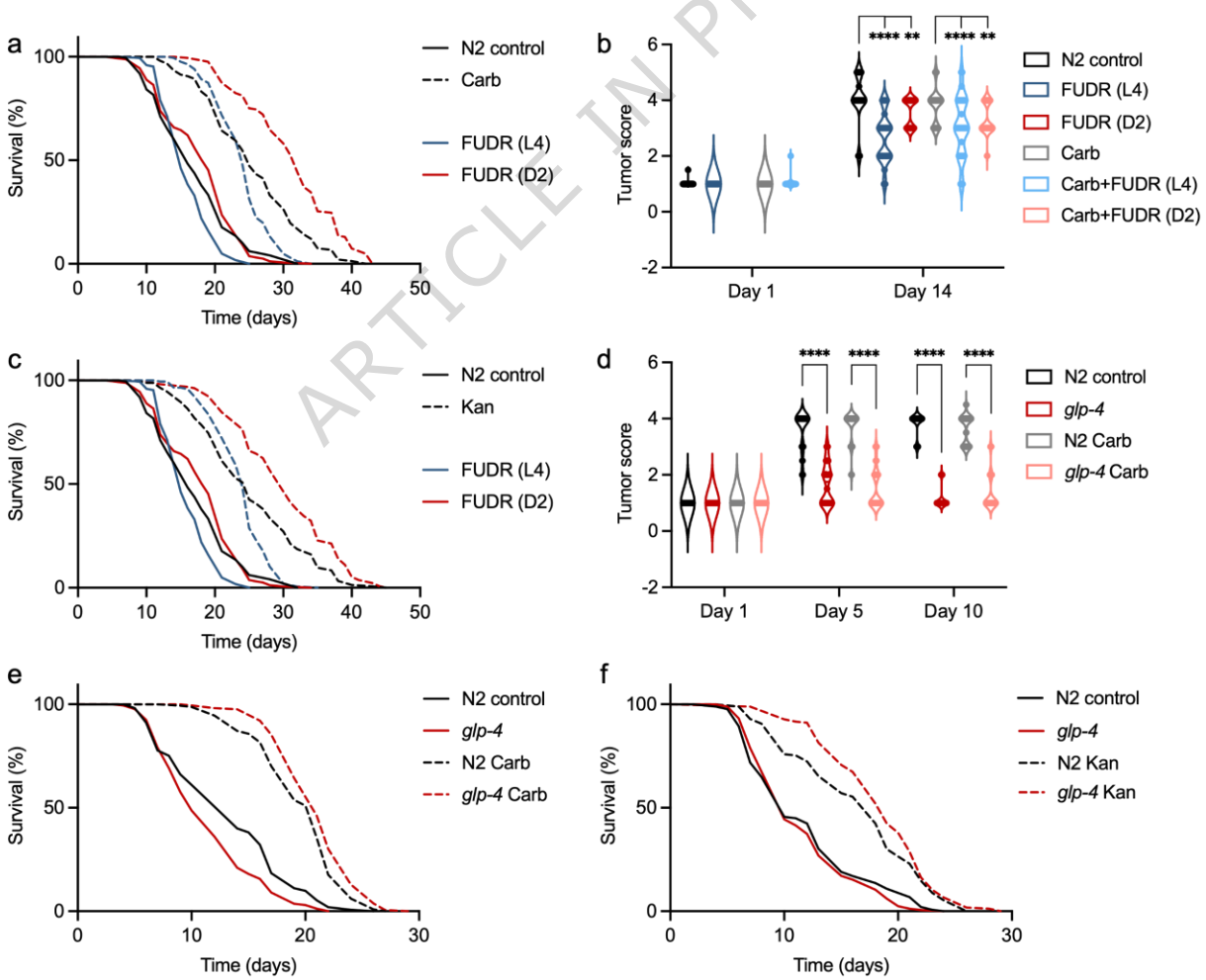

Figure 2. 50 μM FUDR increases lifespan when bacterial infection is prevented. (a) FUDR from day 2 of adulthood (D2) increases lifespan in the presence of Carb. (b) FUDR suppresses uterine tumor development. Dunnett's multiple

comparisons test, **p<0.01, ****p<0.0001. n = 20-36 per time point. (c) FUDR increases lifespan in the presence of Kan. (a-c) Animals maintained at 20˚C. (d) *glp-4(bn2)* suppresses uterine tumor development, with or without Carb. Sidax's multiple comparisons test, ****p<0.0001. n = 10-39 per time point. (e, f) *glp-4(bn2)* extends lifespan when bacterial infection is prevented using Carb (e), or Kan (f). (d-f) Animals raised at 15˚C to L4 stage and then shifted to 25˚C. For individual trials and statistical comparisons in lifespan and pathology analyses, see Tables S1 (a, c), S2 (b), S3 (d) and S4 (e, f).

In the absence of Carb, FUDR did not increase longevity: it slightly shortened mean lifespan when administered from L4 but not from D2 (respectively -6.1%, +6.0%, p = 0.0024, p = 0.27, log rank test; Figure 2a; Table S1). Antibiotic treatment extended lifespan (+52.2%, p < 0.0001, Figure 2a), as previously seen[20]. In the presence of Carb, FUDR from L4 again slightly shortened lifespan (-5.8%, p < 0.0001), but FUDR from D2 significantly increased it (+22.0%, p < 0.0001, Figure 2a). The life-shortening effects of FUDR from L4 may reflect a deleterious effect of drug on later development at this relatively high drug concentration. In all subsequent trials, FUDR was administered from D2 onwards unless otherwise stated.

The life-extending effect of FUDR could be attributable to tumor suppression, amelioration of other senescent pathologies, or some other effect. To explore the second possibility, effects of FUDR on several major senescent pathologies were assessed: uterine tumor development, pharyngeal deterioration, distal gonad degeneration, and intestinal atrophy. In the presence of Carb, FUDR from D2 significantly reduced tumor development, but not the other pathologies (Figure 2b, Figure S1a, b, Table S2).

To verify that the putative unmasking effect of Carb is attributable its antibiotic properties, we performed similar tests using an alternative means to block bacterial infection: a different class of antibiotic (~100 µM kanamycin, Kan) which, like Carb, increases *C. elegans* lifespan[20]. Here again, in the presence of Kan, FUDR extended lifespan (+17.8%, p < 0.0001; Figure 2c, Table S1). These findings are consistent with prevention of mortality from bacterial infection unmasking effects of uterine tumors on late-life mortality. However, it remains possible that the life-extending effect of FUDR is caused by something other than tumor suppression.

glp-4(bn2) extends lifespan when bacterial infection is prevented

One way to investigate this is to test whether suppression of tumor development by other means also increases lifespan when bacterial infection is prevented. *glp-4(bn2)* (germline proliferation defective) is a temperature-sensitive mutation that blocks germline proliferation at 25˚C but not 15˚C. In the similar *glp-1(e2141ts)* mutant, when animals are raised from egg at 25˚C, the resulting strong inhibition of germline signaling markedly extends lifespan; however, if raised at 15˚C and shifted to 25˚C at the L4 stage, some germline proliferation occurs and lifespan is only weakly extended[30]. *glp-4(bn2)* mutant adults raised from egg at 25˚C are also long-lived, but only when *E. coli* proliferation is prevented[31], suggesting increased susceptibility to *E. coli* infection in this mutant. This is an idiosyncrasy, given that *glp-1* and *glp-4* mutants show resistance to the life-shortening effects of several other bacterial species, both Gram positive and Gram negative[31-34].

Shifting to 25˚C at L4 blocks germline development from L4 onwards, and is therefore expected to prevent tumor growth, and this was confirmed, and tumor suppression was found to be unaffected by Carb (Figure 2d, Figure S2a, Table S3). Notably, preventing bacterial

proliferation caused *glp-4(bn2)* to extend mean lifespan (Table S4): on Carb by +7.6% (p = 0.0012, Figure 2e), and on Kan by +11.8% (p = 0.022, Figure 2f).

One possible interpretation here is that *glp-4(bn2)* (L4 shift to 25˚C) leads to a slight inhibition of signals emanating from the germline, whose life-extending effects are masked by the life-shortening effects of *E. coli*[31]. Now, the life-extending effects of inhibiting germline development are dependent on the *daf-16* FOXO class forkhead transcription factor and the *daf-12* dafachronic acid receptor[35,36]. We therefore tested the capacity of *glp-4(bn2)* (L4 shift to 25˚C) to extend lifespan in *daf-16(mgDf50)* and *daf-12(m20)* mutant backgrounds.

Both *daf-16(mgDf50)* and *daf-12(m20)* alone moderately reduced lifespan at 25˚C (-31.5% and -23.6%, respectively, $p < 0.0001$, $p < 0.0001$; Table S5, S6), consistent with prior observations[37,38]. Moreover, in the presence of Carb, *glp-4(bn2)* again extended mean lifespan, by +8.4% (*daf-16* trials, $p < 0.0001$) or +9.1% (*daf-12* trials, $p < 0.0001$). Notably, in a *daf-16(mgDf50)* background, *glp-4(bn2)* increased mean lifespan by +21.5% ($p < 0.0001$), while in a *daf-12(m20)* background, it increased mean lifespan by +10.7% ($p < 0.0001$) (Figure 3a, b; Table S5, S6). This implies that life extension by *glp-4(bn2)* (L4 shift, Carb present) is not attributable to reduced germline signaling, consistent with a life-shortening effect of uterine tumor development. However, we note that in a previous comparison of N2 vs *glp-4(bn2)* (egg shift to 25˚C), subjected to *daf-16* RNAi, and maintained on non-proliferating *E. coli*, *glp-4(bn2)* shortened rather than extended lifespan[31]. We postulate that complete failure of germline development combined with loss of *daf-16* shortens lifespan by mechanisms unrelated to germline signaling and tumor development.

A further possibility is that *glp-4(bn2)* extends lifespan by suppressing other senescent pathologies. However, *glp-4(bn2)* (Carb present) in some cases worsened them, on both D5 and D10 for gonadal degeneration and intestinal atrophy. (Figure S2b, Table S3). That an intervention other than FUDR that blocks tumor development also extends lifespan when bacterial proliferation is prevented is further evidence for the unmasking hypothesis.

Further tests of the life-limiting tumor hypothesis

However, it still remains possible that life-extending effects of FUDR and *glp-4(bn2)* are due to changes other than tumor suppression. To test this further we used a triangulation strategy: the use of multiple, orthogonal tests to address a given question[39]. First, we reasoned that if FUDR extends lifespan by suppressing tumor development then, in the presence of Carb, it should not extend lifespan in *glp-4(bn2)* populations, since they are tumor-less. To test this, effects of 50 μM FUDR on N2 and *glp-4(bn2)* populations were compared (L4 shift to 25˚C, Carb present). FUDR increased lifespan in N2, again, but not in *glp-4(bn2)* (+9.50%, p = 0.0032, -2.58%, p = 0.12, respectively; summed data; Figure 3c, Table S7).

We also checked that FUDR efficiently suppresses tumor development in N2 at 25˚C. To our surprise, 50 μM and even 100 μM FUDR only marginally reduced tumor size (Figure S3a). This could partially reflect the fact that uterine tumors are smaller at 25˚C (Figure S3b). Noting that FUDR only increased N2 lifespan (Carb present) in 2/3 trials (Table S7), we conducted 3 further trials to verify this. A significant lifespan increase was seen in only 1/3 cases (Table S8);

combined data from all 6 trials indicates a significant 6.62% increase in mean lifespan after FUDR treatment ($p = 0.0065$; Table S8). We postulate that the smaller life-extending effect of FUDR at 25˚C could reflect its weaker effect on tumor growth, and perhaps their being smaller in the absence of treatment (Figure S3b).

As a further test, we compared the effect of 50 μM FUDR on lifespan in hermaphrodites and males. Males, lacking either a uterus or the oocytes from which uterine tumors develop, do not develop uterine tumors[29]. Thus if life extension by FUDR is attributable to tumor suppression, then this treatment should not extend male lifespan. To prevent life-shortening male-male interactions[19] animals were cultured individually in liquid culture[40]. In the presence of Carb addition of FUDR significantly increased lifespan in hermaphrodites (+22.0%, $p < 0.0001$) but not in males (+5.1%, $p = 0.588$) (Figure 3d, Table S9). Unexpectedly FUDR also increased lifespan in the absence of Carb, in both hermaphrodites (+27.5%, $p < 0.0001$) and males (+12.3%, $p < 0.0027$) (Figure 3d); the former could imply that in liquid culture *E. coli* infection for some reason no longer masks the life-shortening effect of tumors.

Next we tested the effect on lifespan of initiating exposure to FUDR at later ages, comparing initiation at D2, D12 and D18 of adulthood (Carb present). Our expectation was that treatment starting at the two later ages, after tumors have already formed, would not extend lifespan. This proved to be the case: FUDR from D2 increased lifespan, but from D12 and D18 did not (+13.0%, +3.08%, -1.0%, respectively, $p < 0.0001$, $p = 0.069$, $p = 0.84$, respectively, $N = 4$; Figure 3e, Table S10).

Finally we tested effects of FUDR on lifespan in other nematode species, comparing two sibling species pairs where one species is androdioecious (hermaphrodites [H] and males) and the other gonochoristic (females [F] and males). Aging *C. tropicalis* and *Pristionchus pacificus* (hermaphrodites) develop uterine tumors while females of their respective sibling species, *C. wallacei* and *P. exspectatus*, do not[13]. If FUDR extends lifespan by preventing tumor growth, then it should extend lifespan in species with hermaphrodites but not females.

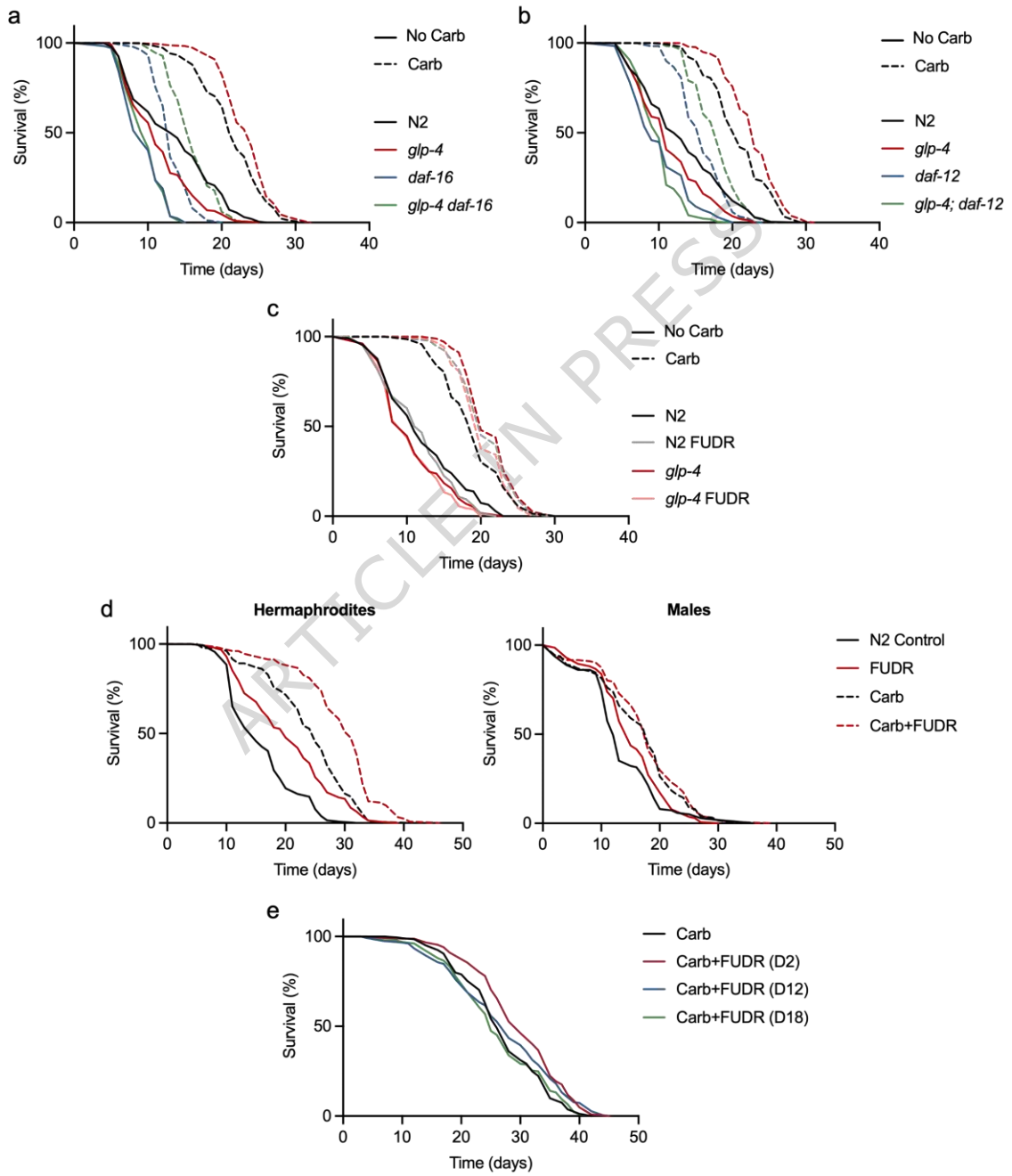

Figure 3. Evidence that uterine tumors shorten hermaphrodite lifespan when bacterial proliferation is suppressed. (a, b) Life extension by *glp-4(bn2)* on Carb is not suppressed by (a) *daf-16(mgDf50)* or (b) *daf-12(m20)*. These results suggest that the mechanisms of life-shortening by *daf-16(mgDf50)* and *daf-12(m20)* do not mask those of uterine

tumors. (c) FUDR does not increase lifespan in tumor-less *glp-4(bn2)* hermaphrodites. (a-c) Animals raised at 15˚C to L4 stage and then shifted to 25˚C. (d) On Carb, 50 μM FUDR increases lifespan of N2 hermaphrodites but not males. Animals maintained singly in monoxenic liquid culture in microtitre wells, 20˚C. (e) FUDR treatment fails to extend lifespan when initiated after tumor development (20˚C). For individual trials and statistical comparisons, see Tables S5 (a), S6 (b), S7 (c), S9 (d), and S10 (e).

On Carb, FUDR increased lifespan in *C. tropicalis* hermaphrodites but also in *C. wallacei* females (+20.7%, +13.3%, respectively, $p = 0.0022$, $p = 0.046$; Figure S4a, Table S11), and the effect in the former was not significantly greater than in the latter ($p = 0.181$, Cox Proportional Hazard [CPH] analysis). On Carb, FUDR increased lifespan in *P. pacificus* hermaphrodites but decreased it in *P. exspectatus* females (+7.1%, -15.6%, respectively, $p = 0.0001$, $p < 0.0001$; Figure S4b, Table S12). Thus, findings from only the *Pristionchus* sibling species pair support the view that FUDR extends lifespan by inhibiting tumor development. We speculate that the life-extending effect of FUDR in *C. wallacei* females involves some mechanism peculiar to this species. Finally, a formal possibility is that life-extension by FUDR is attributable to suppression of death due to internal hatching of larvae (matricide). However, matricide frequency in the absence of FUDR was very low and not increased by Carb, ruling out this possibility.

Taken together, with the exception of the *Caenorhabditis* sibling species pair tests, results of these additional tests support the view that the life-extending effects of FUDR and *glp-4(bn2)* in the presence of Carb are attributable to tumor suppression.

Uterine tumors partially mask the life-shortening effect of vitellogenesis

Next we explored the position of other causes and types of pathology in the hierarchy of death. The *C. elegans* intestine is the site of synthesis of yolk, which is transported across the body cavity to provision developing oocytes[41,42]. Vitellogenins (yolk proteins) are the products of the genes *vit-1 - vit-6*, and RNAi knockdown of *vit* expression blocks vitellogenin accumulation, reduces intestinal atrophy and yolk pool accumulation, and extends lifespan[8,23,24]. To test the possibility that the effect on late-life mortality of blocking yolk synthesis is partially masked by either bacterial infection or uterine tumor development, we subjected N2 populations to *vit-5,-6* RNAi, in the presence of either the antibiotic kanamycin (Kan), or FUDR, or both. Kan was used because the plasmid in the *E. coli* RNAi feeding strains confers Carb resistance. To exclude the possibility that Kan interferes with RNAi knockdown (given its impact on the *E. coli*), effects of *gfp* RNAi on a transgenic *Pftn-1::GFP* strain[43] was compared in the absence and presence of Kan, but no effect of Kan on RNAi knockdown of GFP was detected (Figure S5a).

We first examined effects on pathology. Tumor size was reduced by FUDR under all conditions (± Kan, ± RNAi) on D7 but, unexpectedly, not on D14 (Figure 4a; for all statistical comparisons, see Table S13), in contrast to prior tests (Figure 2b). This may reflect the use here of the *E. coli* RNAi feeding strain HT115, rather than OP50 as previously. This was confirmed using a direct test (Figure 4b): suppression of tumor growth by FUDR was greater on *E. coli* OP50 than HT115, possibly reflecting differences in FUDR biotransformation[44].

vit-5,-6 RNAi by itself reduced intestinal atrophy and yolk pool accumulation (Figure 4c, Table S13), as previously seen[8,24]. Kan alone did not significantly reduce pathologies, and on Kan

or FUDR rescuing effects of *vit-5,-6* RNAi were still detectable (Figure 4c, Table S13). However, at some ages FUDR alone reduced yolk pool size (Figure 4c, Table S13), and also pharyngeal and distal gonad pathology (Figure S5b, Table S13).

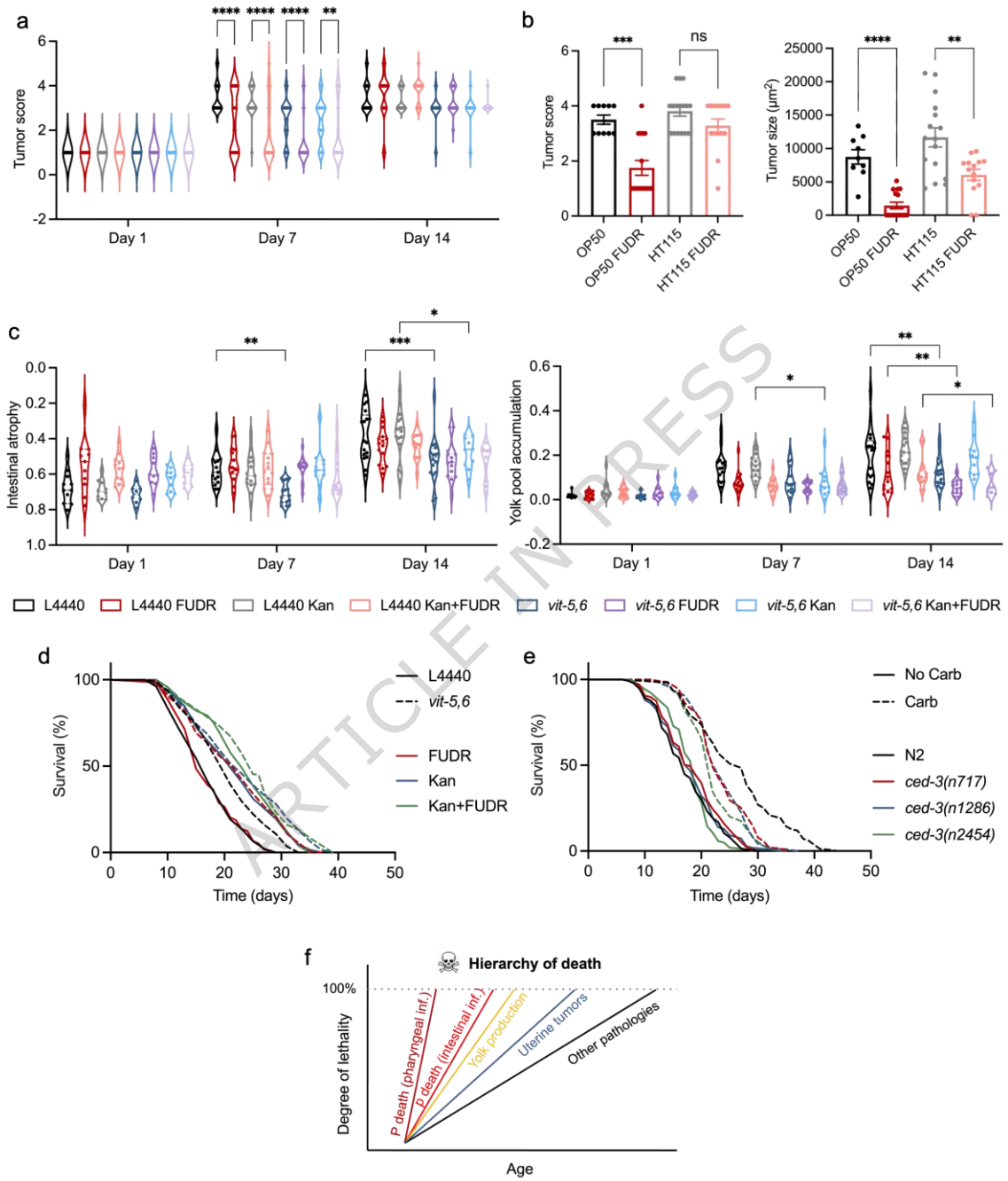

Figure 4. Condition-dependent effects of *vit-5,-6* RNAi and *ced-3* (20˚C). (a) Suppression of tumor growth by FUDR on D7 but not D14 under all conditions. Sidax's multiple comparisons test, ***p*<0.001, *****p*<0.0001. n=16-37 per time point. (b) Weaker tumor suppression by 50 µM FUDR on *E. coli* HT115 (RNAi strain) than OP50 (D8 of adulthood). Error bars, standard error. Mann-Whitney test, ***p*<0.01, ****p*<0.001, *****p*<0.0001. n=9-16 per time point. (c) *vit-5,-6* RNAi reduced intestinal atrophy (left) and yolk pool accumulation (right). Sidax's multiple comparisons test, **p*<0.05, ***p*<0.01, ****p*<0.001. n=7-18 per time point. (d) Effects of *vit-5,-6* RNAi on lifespan ± FUDR ± Kan. (a-d) L4440, RNAi negative control (RNAi plasmid without insert). Trials performed with FUDR from

L4 onwards. Effects on lifespan of FUDR from L4 showed some variability, such that in earlier trials, life extension was seen with Carb present. We were unable to identify the cause of this variability. (e) Effects of *ced-3* on lifespan ± Carb. For individual trials and statistical comparisons in lifespan and pathology analyses, see Tables S13 (a, c), S14 (d) and S15 (e). (f) Hierarchy of death competing causes (onion) model. The evidence presented clearly supports the view that death from infection masks life-limiting effects of uterine tumors. The weaker life-extending effects of blocking yolk synthesis when Kan is present suggests an approximate position for vitellogenesis higher up the hierarchy than uterine tumors. P death, where corpses have a swollen, bacterially-infected pharynx; p death, where corpses have an atrophied pharynx[22]. In both cases death is due to *E. coli* infection, and increased infection susceptibility caused by senescence.

Next, effects on lifespan were examined, first considering those of *vit-5,-6* RNAi. In the absence of Kan or FUDR, *vit-5,-6* RNAi extended mean lifespan (+20.4%, $p < 0.0001$, Figure 4b; Table S14), as previously seen [24]. Notably, life extension by *vit-5,-6* RNAi was smaller on Kan (+5.34%, $p = 0.019$; Figure 4b; Table S14), though comparing effects of *vit-5,-6* RNAi with and without Kan, the difference in this diminution did not quite reach statistical significance ($p = 0.0659$, CPH analysis). Thus, in contrast to uterine tumor development, prevention of bacterial infection does not unmask a greater life-shortening effect of vitellogenesis. The apparent reduction in the life-extending effect in the presence of Kan could imply that *vit-5,-6* RNAi increases infection resistance, e.g. by suppressing intestinal atrophy.

The impact of FUDR on life extension by *vit-5,-6* RNAi was also assessed, first in the absence of Kan. Notably, the presence of FUDR increased the life-extending effect of *vit-5,-6* RNAi to +28.2% ($p < 0.0001$ log rank test; CPH, $p = 0.0133$; Figure 4b; Table S14). Thus, blocking vitellogenesis causes uterine tumors to become life-limiting, even in the presence of proliferative bacteria. This again suggests that *vit-5,-6* RNAi increases infection resistance. Consistent with this, in the presence of Kan and FUDR, the life-extending effect of *vit-5,-6* RNAi is again reduced, to +5.38% ($p = 0.014$ log rank test; CPH, $p = 0.0241$; Figure 4b; Table S14).

Taken together, these results suggest that the life-extending effect of *vit-5,-6* RNAi is attributable to increased resistance to late-life *E. coli* infection. In the presence of such protective changes, uterine tumors contribute to late-life mortality, such that their suppression enhances the life-extending effect of *vit-5,-6* RNAi.

Delayed distal gonad degeneration does not extend lifespan in the absence of infection

During aging of *C. elegans* hermaphrodites, the distal gonad undergoes marked atrophy and then fragments (Figure 1b)[7,20]. Notably, this pathology was reduced by FUDR, significantly so in the absence of Carb (Figure S1b; D14 of adulthood). One contributor to such atrophy is futile, post-reproductive continuation of physiological apoptosis in the distal gonad, which in young adults contributes to oogenesis[45]. Supporting this, prevention of physiological apoptosis using *ced-3* mutations, including *ced-3(n717)*, which block both somatic and germline apoptosis, significantly delays gonad degeneration[7]. Prior reports of effects of inhibition of *ced-3* on lifespan are conflicting, for reasons unknown, describing either no effects using *ced-3(n1286)*[20], *ced-3(n717)* or *ced-3* RNAi[7], or increased lifespan using *ced-3(n717)*[46] or *ced-3* RNAi[47].

To test the possibility that effects of distal gonad degeneration on late-life mortality are masked by death due to infection, lifespan of N2 and *ced-3* populations were compared in the

absence or presence of Carb. Three *ced-3* mutants were tested, *ced-3(n717)*, *ced-3(n1286)* and *ced-3(n2454)*, but no extension of lifespan relative to N2 controls was seen under either condition (Figure 4e; Table S15). This suggests that distal gonad disintegration does not become life-limiting in the absence of infection. In fact, preventing bacterial infection unmasked a life-shortening effect of *ced-3* (-10.8% to -16.1%, $p < 0.0001$; Figure 4e; Table S15).

glp-4(bn2) extends *daf-2(m577)* lifespan by increasing infection resistance

daf-2 insulin/IGF-1 signaling (IIS) mutants are long-lived[48] and exhibit increased resistance to bacterial infection[21,49-51]. *daf-2* also strongly suppresses several senescent pathologies, but only weakly reduces uterine tumor size [8], though it does substantially reduce tumor DNA content[27,52]. We noted a previous observation that *glp-4(bn2)* (L4 temperature upshift) extends lifespan in *daf-2* mutant backgrounds (no Carb), including the class 1 (less pleiotropic) *daf-2(m577)* mutant[53,54]. This could imply that, as with *vit-5,-6* RNAi treatment (see above), *daf-2* mutant infection resistance causes uterine tumors to become life limiting.

To investigate this we re-examined the effect of *glp-4(bn2)* on lifespan in a *daf-2(m577)* background (no Carb). This confirmed that *glp-4(bn2)* extends *daf-2(m577)* mean lifespan (+61.0%, $p < 0.0001$; Figure S6a; Table S16), as previously reported[54]. If *glp-4(bn2)* enhances *daf-2* longevity by preventing tumor formation, then this enhancing effect should still be present in the presence of Carb. However, this proved not to be the case: *glp-4(bn2)* only marginally increased lifespan in a *daf-2(m577)* background (+0.45%, $p = 0.0015$, Figure S6b, Table S16).

glp-4(bn2) enhancement of infection resistance could involve prevention of either of the two forms of infection-related death in *C. elegans*. These are the P ("big P") deaths with a swollen, infected pharynx, and the later p ("small p") deaths with an atrophied pharynx[22]. Necropsy analysis comparing *daf-2(m577)* and *glp-4(bn2); daf-2(m577)* populations showed in the latter a modest reduction in P death frequency, and strong increases in both P and p lifespan (Figure S6c-e, Table S17; $N = 2$). This implies that in a *daf-2(m577)* background *glp-4(bn2)* enhances resistance to both pharyngeal and intestinal infection.

These results suggest the presence of a weak reduction in germline signaling in *glp-4(bn2)* (L4 shift) animals, that in a *daf-2*(+) background is not sufficient to increase lifespan, but which synergises with the reduction in IIS in *daf-2* mutants, leading to increased infection resistance and, consequently, increased lifespan. They also suggest that uterine tumors do not contribute to late-life mortality in *daf-2* mutants (at least in class 1 mutants).

Discussion

This study deals with the question of how to interpret the effects of treatments that extend lifespan in model organism studies. One interpretation is that an increase in lifespan represents a slowing of the overall aging process, unless it is clearly the result of some form of environmental disruption, as in the case of life-shortening *E. coli* infection in *C. elegans*. The existence of interventions that extend lifespan in animal models has been taken to suggest that human aging as whole may be slowed down, providing an efficient means of preventing diverse late-life illnesses[55-57]. Our findings here underscore that life extension in *C. elegans* can result

from suppression of individual pathologies. This, of course, does not rule out the existence of life-extending interventions that slow aging as a whole, and studies of temporal scaling for one support this possibility[58]. We show that a senescent pathology of intrinsic and quasi-programmed origin, uterine tumors, can contribute to late-life mortality. Notably, this contribution is condition dependent: under standard laboratory conditions (with proliferating *E. coli*) it is masked, presumably by lethal late-life infection.

This represents a distant mirror of human mortality: in centuries past, the major causes of death were infectious pathogens (e.g. tuberculosis, smallpox, bubonic plague), made more lethal by malnutrition, cold and poor hygiene. Thanks to improvements in conditions in the developed world, the main causes of death are now cardiovascular disease, cancer and lung disease (particularly chronic obstructive pulmonary disease). Hypothetically, a cure for all cancer would cause a greater increase in lifespan in the 21st than in the 14th century; similarly, preventing uterine tumor development extends *C. elegans* lifespan in the absence but not presence of bacterial infection.

Mapping out the hierarchy of death

These findings draw attention to the utility of viewing *C. elegans* lifespan as the product of competing causes of mortality. Such competing causes are a possible determinative factor in the outcome of tests of interactions between interventions that extend *C. elegans* lifespan, that can be difficult to interpret[59]. For example, *rsks-1(ok1255),* affecting S6 kinase in the mTOR pathway, modestly increases mean lifespan (+20%), but in a *daf-2(e1370)* background it causes a massive ~360% increase relative to *daf-2* alone[60,61]. This could imply, in a competing causes interpretation, that IIS more than mTOR signaling limits wild-type lifespan, but reducing IIS unmasks a greater life-limiting role for mTOR.

Thus, interpretation of *C. elegans* lifespan data would be aided by a description of the competing causes of late-life mortality, both extrinsic and intrinsic. Part of such a description is an account of how such competing causes mask one another, and their position in an onion-like, nested hierarchy of causes. Here we present the beginnings of such an account (Figure 4f).

The outer-most layer of the onion, in the presence of proliferating *E. coli*, was defined in a previous study in which necropsy (post-mortem examination) revealed two forms of corpse: one with a swollen, infected pharynx (P death) and the other with an atrophied pharynx (p death)[22]. Notably, P deaths occur earlier than p deaths. Here death from pharyngeal infection is the outer skin of the onion for P but not p individuals. This is evident since mutation of *eat-2*, which lowers pharyngeal pumping rate, reduces P death frequency[22]. The resulting increase in frequency of p deaths, which occur later, causes an increase in overall lifespan. The lifespan of the p subpopulation, like that of the P subpopulation, is evidently limited by *E. coli* infection, since preventing the latter increases p lifespan[22]. Given the findings in the present study, we can confidently state that uterine tumor development becomes life-limiting when bacterial infection is prevented. Key evidence for this is the life-extending effects of two interventions that prevent tumor formation, FUDR and *glp-4(bn2)*, but only when bacterial proliferation is prevented (Figure 2), and only in comparison to control strains in which tumors are present (Figure 2, 3).

Our analysis allowed additional pathologies to be placed, tentatively, in the hierarchy of death (Figure 4f). Blocking vitellogenesis by *vit* gene RNAi modestly increases lifespan[23,24], i.e. the presence of proliferating *E. coli* does not mask an effect on lifespan; however, it remained possible that partial masking occurs. In fact, antibiotic treatment reduced rather than increased the life-extending effect of inhibiting vitellogenesis (Figure 4d). This implies that life-extension here is largely due to increased infection resistance, placing vitellogenesis above tumor development in the hierarchy of death (Figure 4f).

Distal gonad atrophy and fragmentation is an anatomically striking pathology, yet its inhibition by mutation of *ced-3* did not extend lifespan, even in the presence of Carb (Figure 4e). This could imply either that distal gonad atrophy never contributes to mortality, or that its effects on mortality are masked by competing causes. A further consideration is that *ced-3(-)* delays but does not prevent distal gonad atrophy, to which declining germline stem cell proliferation likely also contributes[7,26]; thus, full suppression of distal gonad atrophy might reveal a contribution to late-life mortality.

Uterine tumors as a distant mirror of cancer

C. elegans uterine tumor is a form of age-related neoplasia that in certain respects resembles its mammalian counterparts. First, it has etiological and phenotypic similarities to mammalian teratomas, particularly ovarian cysts[10]. Second, like mammalian neoplasia, it appears largely as part of organismal senescence, and can contribute to late-life mortality (this study). Third, *C. elegans* uterine tumor can be treated with FUDR, a drug also used for the treatment of colorectal cancer in humans[62]. These similarities support the presence of underlying, general principles of senescent pathophysiology, that are operative across the animal kingdom during aging, from nematodes to humans[8,63]. Hence we may learn about such general principles from studying organisms even as primitive as *C. elegans*.

It is worth noting too that a screen for compounds that prevent uterine tumor growth could have identified FUDR, a useful anticancer drug. Thus, the capacity to prevent *C. elegans* uterine tumor growth could in principle be used to identify novel anti-cancer agents. A further deduction is that if a given treatment extends lifespan in *C. elegans* in the absence but not the presence of proliferating *E. coli*, this could reflect action through suppression of uterine tumor growth.

Also of interest is the mechanism by which uterine tumors contribute to late-life mortality, which could be informative with respect to how cancer causes illness and death. Uterine tumors often press against and flatten the intestine, providing one potential mechanism. A second possibility is that uterine tumors secrete molecular species (e.g. proteins) that have deleterious effects on other tissues; the *C. elegans* uterus does contain secreted proteins whose levels increase with age and shorten life[64]. A third is that uterine tumors absorb vital nutrients in a sponge-like fashion, leading to health-impairing deficiencies in other tissues; consistent with this, uterine tumors accumulate vitellogenin[10]. Impact on mortality of any such mechanisms are likely to entail an interaction with age-related frailty due to other aspects of organismal aging.

More broadly, this study illustrates how interventions that extend lifespan in model organisms may not be assumed to affect the aging process as a whole. Rather, the interventions characterized here may be understood as antigeroid in nature, either unimodal (involving suppression of a single senescent pathology)[65] or segmental (involving suppression of several pathologies)[66]. It also underscores the utility of programmatic theory in guiding research to useful conclusions, here its emphasis on competing causes of late-life mortality[17], and our demonstration that uterine tumors, a pathology of programmatic origin[10], can contribute to late-life mortality.

Methods

Culture methods and strains

C. elegans maintenance was performed using standard protocols[18]. Except where noted, all strains were grown at 20˚C on agar plates containing nematode growth media (NGM, containing Bacto Peptone [Gibco, USA]), seeded with *E. coli* OP50 as a food source. In certain experiments (as specified), bacterial lawns were treated an antibiotic (4 mM carbenicillin, unless otherwise stated) to inhibit bacterial infection of *C. elegans*.

An N2 hermaphrodite stock recently obtained from the Caenorhabditis Genetics Center was used as wild type (N2H)[67]. Other *C. elegans* strains used included: DR20 *daf-12(m20) X*, DR1567 *daf-2(m577) III*, GA134 *glp-4(bn2) I; daf-2(m577) III*, GA633 *wuIs177*[*Pftn-1::GFP, lin-15(+)*] *daf-2(m577) III*, GA1952 *daf-16(mgDf50) I*, GA6001 *glp-4(bn2) I*; *daf-12(m20) X*, GA6002 *glp-4(bn2) daf-16(mgDf50) I*, MT1522 *ced-3(n717) IV*, MT3002 *ced-3(n1286) IV*, MT8354 *ced-3(n2454) IV*, SS104 *glp-4(bn2) I*. Other nematode species strains (wild type): JU1373 *C. tropicalis*, JU1873 *C. wallacei*, PS312 *Pristionchus pacificus*, RS5522 *Pristionchus exspectatus*.

Survival analysis

Nematodes were maintained at a density of 25-30 per plate, and transferred daily during the egg laying period, followed by every 6-7 days thereafter. The L4 stage was defined as day 0. Mortality was scored every 1-2 days, with worms scored as alive if they showed any movement, spontaneously or in response to gentle touch with a worm pick. Corpses were checked for possible death from internal hatching (matricide), but this was rarely seen. Raw data for all lifespan trials in this study is presented in Dataset S1.

Due to high frequency of plate leaving by solitary males, it is difficult to maintain them on agar plates. To circumvent this, lifespan measurements in trials including males were performed in liquid medium. Animals were cultured singly in 96-well microtitre plates, in 50 μL of a suspension of *E. coli* in S medium (concentration $1 \times 10^8 - 1 \times 10^9$ cells mL^{-1}), as described[40].

Mortality deconvolution analysis

This analysis is based on the presence of two forms of death in aging *C. elegans* cultured on proliferating *E. coli*: earlier death with an infected, swollen pharynx (P death) and later death with an atrophied pharynx (p death)[22]. Mortality deconvolution involves analysis of P and p lifespans separately. Alterations in lifespan can result from altered percentages of P (and p) deaths, and/or altered P and/or p lifespan. Deconvolved mortality statistics include each of these values. Corpses were scored by necropsy as P or p using the highest magnification of a dissecting microscope, as described[22].

Treatment with FUDR

NGM plates were seeded with *E. coli* OP50, and left overnight at room temperature for lawns to grow. FUDR was then added using a 5 mM stock to give a final concentration of 50 μM. FUDR treatment was initiated on D2 of adulthood unless otherwise stated.

RNA-mediated interference (RNAi)

E. coli HT115 RNAi-producing and control bacteria (plasmid L4440), were cultivated as previously described[68], seeded onto NGM plates containing carbenicillin and IPTG. IPTG was added to cultured bacteria and induced at 37˚C for 1 hr before seeding plates. RNAi treatment was started at the L4 stage, after larval development on HT115 (no carbenicillin). RNAi feeding strains used for *vit-5* and *vit-6* RNAi were as described[24].

For RNAi trials in the absence of *E. coli* infection, 103.2 μM kanamycin (Kan) was added (thus plates contained both Carb and Kan). To test effects of Kan on RNAi efficacy, GA633 animals bearing a *Pftn-1::GFP* transgene were used, which show high levels of green fluorescence in the intestine[43]. *gfp* RNAi was initiated at the L4 stage, and epifluorescence images captured on day 2, 4 and 7 of adulthood. Fluorescence levels were estimated as mean pixel density using Fiji software.

Analysis of senescent pathology

This was performed as previously described[8,29]. At each age assayed, 10-15 animals were mounted onto 2% agar pads and anesthetized with 0.2% levamisole. Images were acquired using differential interference contrast (DIC, or Nomarski) optics, with a Zeiss ApoTome.2 microscope. A Hamamatsu C13440 ORCA-Flash4.0 V3 digital camera and Zen software were used for image acquisition. For pharynx, distal gonad and tumor pathologies, all images first randomized and blinded, and then examined independently by two trained scorers, assigned severity scores of 1-5, and mean values calculated. Scoring criteria were: 1 = youthful, healthy appearance; 2 = early signs of mild deterioration; 3 = clearly discernible, mild pathology; 4 = well-developed pathology; and 5 = very severe pathology (e.g. gonad completely disintegrated), or reaching a maximal level (e.g. large uterine tumor filling the entire body cavity in the mid-body region). Intestinal atrophy was quantified by measuring the intestinal width in a region posterior to the uterine tumors, subtracting the width of the intestinal lumen and dividing by the body width. Yolk accumulation was measured by dividing the yolk pool area by the area of the body visible in the field of view as captured at 630x magnification.

Statistical analysis

Statistical tests were performed on raw data using GraphPad Prism 9.0 (GraphPad Software, USA) and JMP Pro 15 (JMP Statistical Discovery LLC, USA) unless otherwise stated, with the specific tests and post hoc corrections performed as described in the figure legends. No statistical methods were used to predetermine sample size. The experiments were not randomized. The investigators were not blinded to allocation during experiments and outcome assessment.

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Author contributions

Conceptualization, D.G.; methodology, C.C.K., D.G., H.W.; investigation, A.S.A., M.E., Y.F., C.C.K., C.N.H., J.Q., H.W., A.Z.; writing – original draft, D.G.; writing – review and editing, D.G., H.W.; funding acquisition, D.G.; supervision, D.G.

Declaration of interests

C.C.K. is CEO of LinkGevity. The other authors do not have a competing interest.

Data availability

All relevant data are available from the authors on request.

Supplementary information

Supplementary information can be found online.

References

1 de Magalhães, J. P. An overview of contemporary theories of ageing. *Nat Cell Biol* **27**, 1074-1082 (2025).
2 Medvedev, Z. A. An attempt at a rational classification of theories of ageing. *Biol Rev* **65**, 375-398 (1990).
3 Williams, G. C. Pleiotropy, natural selection and the evolution of senescence. *Evolution* **11**, 398-411 (1957).
4 Rose, M. R. *Evolutionary Biology of Aging*. (Oxford University Press, 1991).

5 Arnold, K. R. & Rose, M. R. *Conceptual Breakthroughs in The Evolutionary Biology of Aging* (Academic Press, 2023).

6 Gems, D. & Kern, C. C. Biological constraint, evolutionary spandrels and antagonistic pleiotropy. *Ageing Res Rev* **101**, 102527 (2024).

7 de la Guardia, Y. *et al.* Run-on of germline apoptosis promotes gonad senescence in *C. elegans*. *Oncotarget* **7**, 39082-39096 (2016).

8 Ezcurra, M. *et al.* C. elegans eats its own intestine to make yolk leading to multiple senescent pathologies. *Curr Biol* **28**, 2544-2556 (2018).

9 Gems, D. & de la Guardia, Y. Alternative perspectives on aging in C. elegans: reactive oxygen species or hyperfunction? *Antioxid Redox Signal* **19**, 321-329 (2013).

10 Wang, H. *et al.* A parthenogenetic quasi-program causes teratoma-like tumors during aging in wild-type C. elegans. *NPJ Aging Mech. Disease* **4**, 6 (2018).

11 Galimov, E. R. & Gems, D. Shorter life and reduced fecundity can increase colony fitness in virtual C. elegans. *Aging Cell* **19**, e13141 (2020).

12 Gems, D., Kern, C. C., Nour, J. & Ezcurra, M. Reproductive suicide: similar mechanisms of aging in *C. elegans* and Pacific salmon. *Front Cell Dev Biol* **9**, 688788 (2021).

13 Kern, C. C. *et al. C. elegans* ageing is accelerated by a self-destructive reproductive programme. *Nat Commun* **14**, 4381, doi:10.1038/s41467-023-40088-1 (2023).

14 Kern, C. C. *et al. C. elegans* feed yolk to their young in a form of primitive lactation. *Nat Commun* **12**, 5801 (2021).

15 Slade, L., Etheridge, T. & Szewczyk, N. L. Consolidating multiple evolutionary theories of ageing suggests a need for new approaches to study genetic contributions to ageing decline. *Ageing Res Rev* **100**, 102456 (2024).

16 Blagosklonny, M. V. Aging and immortality: quasi-programmed senescence and its pharmacologic inhibition. *Cell Cycle* **5**, 2087-2102 (2006).

17 Blagosklonny, M. V. Aging: ROS or TOR. *Cell Cycle* **7**, 3344-3354 (2008).

18 Brenner, S. The genetics of *Caenorhabditis elegans*. *Genetics* **77**, 71-94 (1974).

19 Gems, D. & Riddle, D. L. Genetic, behavioral and environmental determinants of male longevity in *Caenorhabditis elegans*. *Genetics* **154**, 1597-1610 (2000).

20 Garigan, D. *et al.* Genetic analysis of tissue aging in *Caenorhabditis elegans*: a role for heat-shock factor and bacterial proliferation. *Genetics* **161**, 1101-1112 (2002).

21 Podshivalova, K., Kerr, R. & Kenyon, C. How a mutation that slows aging can also disproportionately extend end-of-life decrepitude. *Cell Reports* **19**, 441–450 (2017).

22 Zhao, Y. *et al.* Two forms of death in aging Caenorhabditis elegans. *Nat Commun* **8**, 15458 (2017).

23 Murphy, C. T. *et al.* Genes that act downstream of DAF-16 to influence the lifespan of *C. elegans*. *Nature* **424**, 277-284 (2003).

24 Sornda, T. *et al.* Production of YP170 vitellogenins promotes intestinal senescence in C. elegans. *J Gerontol A* **74**, 1180-1188 (2019).

25 Riesen, M. *et al.* MDL-1, a growth- and tumor-suppressor, slows aging and prevents germline hyperplasia and hypertrophy in C. elegans. *Aging* **6**, 98-117 (2014).

26 Luo, S., Kleemann, G. A., Ashraf, J. M., Shaw, W. M. & Murphy, C. T. TGF-beta and insulin signaling regulate reproductive aging via oocyte and germline quality maintenance. *Cell* **143**, 299-312 (2010).

27 Golden, T. R. *et al.* Dramatic age-related changes in nuclear and genome copy number in the nematode Caenorhabditis elegans. *Aging Cell* **6**, 179-188 (2007).

28 Wang, H., Zhang, Z. & Gems, D. Monsters in the uterus: teratoma-like tumors in senescent C. elegans result from a parthenogenetic quasi-program *Aging* **10**, 1188-1189 (2018).

29 Kern, C. C. *et al.* Machine learning predicts lifespan and suggests underlying causes of death in aging *C. elegans*. *Comms Biol* **8,** 1630 (2025).

30 Arantes-Oliveira, N., Apfeld, J., Dillin, A. & Kenyon, C. Regulation of life-span by germ-line stem cells in *Caenorhabditis elegans*. *Science* **295**, 502-505 (2002).

31 TeKippe, M. & Aballay, A. C. elegans germline-deficient mutants respond to pathogen infection using shared and distinct mechanisms. *PLoS One* **5**, e11777 (2010).

32 Alper, S. *et al.* The Caenorhabditis elegans germ line regulates distinct signaling pathways to control lifespan and innate immunity. *J Biol Chem* **285**, 1822-1828 (2010).

33 Miyata, S., Begun, J., Troemel, E. & Ausubel, F. DAF-16-dependent suppression of immunity during reproduction in Caenorhabditis elegans. *Genetics* **178**, 903-918 (2008).

34 Evans, E. A., Kawli, T. & Tan, M. W. Pseudomonas aeruginosa suppresses host immunity by activating the DAF-2 insulin-like signaling pathway in Caenorhabditis elegans. *PLoS Pathog* **4**, e1000175 (2008).

35 Hsin, H. & Kenyon, C. Signals from the reproductive system regulate the lifespan of *C. elegans*. *Nature* **399**, 362-366 (1999).

36 Motola, D. L. *et al.* Identification of Ligands for DAF-12 that Govern Dauer Formation and Reproduction in C. elegans. *Cell* **124**, 1209-1223 (2006).

37 Lin, K., Hsin, H., Libina, N. & Kenyon, C. Regulation of the *Caenorhabditis elegans* longevity protein DAF-16 by insulin/IGF-1 and germline signaling. *Nat Genet* **28**, 139-145 (2001).

38 Larsen, P. L., Albert, P. S. & Riddle, D. L. Genes that regulate both development and longevity in *Caenorhabditis elegans*. *Genetics* **139**, 1567-1583 (1995).

39 Munafò, M. R. & Davey Smith, G. Robust research needs many lines of evidence. *Nature* **553**, 399-401 (2018).

40 McCulloch, D. & Gems, D. Evolution of male longevity advantage in nematodes. *Aging Cell* **2**, 165-173 (2003).

41 Kimble, J., Sharrock, WJ. Tissue-specific synthesis of yolk proteins in *Caenorhabditis elegans*. *Dev Biol* **96**, 189-196 (1983).

42 Perez, M. & Lehner, B. Vitellogenins - yolk gene function and regulation in *Caenorhabditis elegans*. *Front Physiol* **10**, 1067 (2019).

43 Ackerman, D. & Gems, D. Insulin/IGF-1 and hypoxia signaling act in concert to regulate iron homeostasis in C. elegans. *PLoS Genet* **8**, e1002498 (2012).

44 Scott, T. *et al.* Host-microbe co-metabolism dictates cancer drug efficacy in C. elegans. *Cell* **169**, 442-456 (2017).

45 Gumienny, T. L., Lambie, E., Hartwieg, E., Horvitz, H. R. & Hengartner, M. O. Genetic control of programmed cell death in the Caenorhabditis elegans hermaphrodite germline. *Development* **126**, 1011-1022 (1999).

46 Judy, M. E. *et al.* A shift to organismal stress resistance in programmed cell death mutants. *PLoS Genet* **9**, e1003714 (2013).

47 Curran, S. & Ruvkun, G. Lifespan regulation by evolutionarily conserved genes essential for viability. *PLoS Genet.* **3**, e56 (2007).

48 Kenyon, C., Chang, J., Gensch, E., Rudener, A. & Tabtiang, R. A *C. elegans* mutant that lives twice as long as wild type. *Nature* **366**, 461-464 (1993).

49 Zhao, Y. *et al.* Mutation of daf-2 extends lifespan via tissue-specific effectors that suppress distinct life-limiting pathologies. *Aging Cell* **20**, e13324 (2021).

50 Garsin, D. A. *et al.* Long-lived C. elegans daf-2 mutants are resistant to bacterial pathogens. *Science* **300**, 1921 (2003).

51 Troemel, E. R. *et al.* p38 MAPK regulates expression of immune response genes and contributes to longevity in C. elegans. *PLoS Genet* **2**, e183 (2006).

52 McGee, M. D., Day, N., Graham, J. & Melov, S. cep-1/p53-dependent dysplastic pathology of the aging C. elegans gonad. *Aging* **4**, 256-269 (2012).

53 Gems, D. *et al.* Two pleiotropic classes of daf-2 mutation affect larval arrest, adult behavior, reproduction and longevity in Caenorhabditis elegans. *Genetics* **150**, 129-155 (1998).

54 McElwee, J. J., Schuster, E., Blanc, E., Thomas, J. H. & Gems, D. Shared transcriptional signature in *C. elegans* dauer larvae and long-lived *daf-2* mutants implicates detoxification system in longevity assurance. *J Biol Chem* **279**, 44533-44543 (2004).

55 Kennedy, B. *et al.* Geroscience: linking aging to chronic disease. *Cell* **159**, 709-713 (2014).

56 Gems, D. Tragedy and delight: The ethics of decelerated ageing. *Phil Trans R Soc* **366**, 108-112 (2011).

57 Butler, R. N. *et al.* New model of health promotion and disease prevention for the 21st century. *BMJ* **337**, a399 (2008).

58 Stroustrup, N. *et al.* The temporal scaling of *Caenorhabditis elegans* ageing. *Nature* **530**, 103-107 (2016).

59 Gems, D., Pletcher, S. & Partridge, L. Interpreting interactions between treatments that slow ageing. *Aging Cell* **1**, 1-9 (2002).

60 Chen, D. *et al.* Germline signaling mediates the synergistically prolonged longevity produced by double mutations in *daf-2* and *rsks-1* in *C. elegans*. *Cell Rep* **5**, 1600–1610 (2013).

61 Selman, C. *et al.* Ribosomal protein S6 kinase 1 signalling regulates mammalian lifespan. *Science* **326**, 140-144 (2009).

62 Allen-Mersh, T. G., Earlam, S., Fordy, C., Abrams, K. & Houghton, J. Quality of life and survival with continuous hepatic-artery floxuridine infusion for colorectal liver metastases. *Lancet* **344**, 1255-1260 (1994).

63 Gems, D. & de Magalhães, J. P. The hoverfly and the wasp: A critique of the hallmarks of aging as a paradigm. *Ageing Res Rev* **70**, 101407 (2021).

64 Zimmerman, S., Hinkson, I., Elias, J. & Kim, S. Reproductive aging drives protein accumulation in the uterus and limits lifespan in C. elegans. *PLoS Genet* **11**, e1005725 (2015).

65 Hisama, F. M., Oshima, J. & Martin, G. M. How research on human progeroid and antigeroid syndromes can contribute to the longevity dividend initiative. *Cold Spring Harb Perspect Med.* **6**, a025882 (2016).

66 Zhang, A. *et al.* Characterization of effects of mTOR inhibitors on aging in *C. elegans*. *J Gerontol A* **79**, glae196 (2024).

67 Zhao, Y., Wang, H., Poole, R. J. & Gems, D. A *fln-2* mutation affects lethal pathology and lifespan in *C. elegans*. *Nat Commun* **10**, 5087 (2019).

68 Kamath, R. *et al.* Systematic functional analysis of the *Caenorhabditis elegans* genome using RNAi. *Nature* **421**, 231-237 (2003).

Figure legends

Figure 1. (a) Left, the competing causes of mortality model (standard culture conditions). Senescent pathologies develop, gradually become more severe, until they become lethal, causing death. Here pathology A is life limiting. If A is prevented, then pathology B becomes life limiting. Right, A counter-hypothesis, where a unified overall process of senescence limits life. Only according to the right hand scheme can lifespan be viewed as a metric of the overall aging process. (b) Senescent pathologies in *C. elegans* hermaphrodites, including uterine tumors (brown). Top, young adult hermaphrodite, with no uterine tumors (day 1 of adulthood). Bottom: Elderly hermaphrodite showing typical paired uterine tumors, one in each branch of the uterus. Several other pathologies are also visible.

Figure 2. 50 μM FUDR increases lifespan when bacterial infection is prevented. (a) FUDR from day 2 of adulthood (D2) increases lifespan in the presence of Carb. (b) FUDR suppresses uterine tumor development. Dunnett's multiple comparisons test, $^{**}p<0.01$, $^{****}p<0.0001$. n = 20-36 per time point. (c) FUDR increases lifespan in the presence of Kan. (a-c) Animals maintained at 20˚C. (d) *glp-4(bn2)* suppresses uterine tumor development, with or without Carb. Sidax's multiple comparisons test, $^{****}p<0.0001$. n = 10-39 per time point. (e, f) *glp-4(bn2)* extends lifespan when bacterial infection is prevented using Carb (e), or Kan (f). (d-f) Animals raised at 15˚C to L4 stage and then shifted to 25˚C. For individual trials and statistical comparisons in lifespan and pathology analyses, see Tables S1 (a, c), S2 (b), S3 (d) and S4 (e, f).

Figure 3. Evidence that uterine tumors shorten hermaphrodite lifespan when bacterial proliferation is suppressed. (a, b) Life extension by *glp-4(bn2)* on Carb is not suppressed by (a) *daf-16(mgDf50)* or (b) *daf-12(m20)*. These results suggest that the mechanisms of life-shortening by *daf-16(mgDf50)* and *daf-12(m20)* do not mask those of uterine tumors. (c) FUDR does not increase lifespan in tumor-less *glp-4(bn2)* hermaphrodites. (a-c) Animals raised at 15˚C to L4 stage and then shifted to 25˚C. (d) On Carb, 50 μM FUDR increases lifespan of N2 hermaphrodites but not males. Animals maintained singly in monoxenic liquid culture in microtitre wells, 20˚C. (e) FUDR treatment fails to extend lifespan when initiated after tumor development (20˚C). For individual trials and statistical comparisons, see Tables S5 (a), S6 (b), S7 (c), S9 (d), and S10 (e).

Figure 4. Condition-dependent effects of *vit-5,-6* RNAi and *ced-3* (20˚C). (a) Suppression of tumor growth by FUDR on D7 but not D14 under all conditions. Sidax's multiple comparisons test, $^{**}p<0.001$, $^{****}p<0.0001$. n=16-37 per time point. (b) Weaker tumor suppression by 50 μM FUDR on *E. coli* HT115 (RNAi strain) than OP50 (D8 of adulthood). Error bars, standard error. Mann-Whitney test, $^{**}p<0.01$, $^{***}p<0.001$, $^{****}p<0.0001$. n=9-16 per time point. (c) *vit-5,-6* RNAi reduced intestinal atrophy (left) and yolk pool accumulation (right). Sidax's multiple comparisons test, $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$. n=7-18 per time point. (d) Effects of *vit-5,-6* RNAi on lifespan ± FUDR ± Kan. (a-d) L4440, RNAi negative control (RNAi plasmid without insert). Trials performed with FUDR from L4 onwards. Effects on lifespan of FUDR from L4 showed some variability, such that in earlier trials, life extension was seen with Carb present. We were unable to identify the cause of this variability. (e) Effects of *ced-3* on lifespan ± Carb. For individual trials and statistical comparisons in lifespan and pathology analyses, see Tables S13 (a, c), S14 (d) and S15 (e). (f) Hierarchy of death competing causes (onion) model. The evidence

presented clearly supports the view that death from infection masks life-limiting effects of uterine tumors. The weaker life-extending effects of blocking yolk synthesis when Kan is present suggests an approximate position for vitellogenesis higher up the hierarchy than uterine tumors. P death, where corpses have a swollen, bacterially-infected pharynx; p death, where corpses have an atrophied pharynx[22]. In both cases death is due to *E. coli* infection, and increased infection susceptibility caused by senescence.