Supplementary Information

Machine learning predicts lifespan and underlying causes of death in aging *C. elegans*

Contents Summary

Supplementary results

Effects of axenic culture on senescent pathology

We examined how interventions that alter lifespan affect the rate of development of senescent pathology. Dietary restriction (DR) increases lifespan and delays senescent pathology in a number of organisms[1]. Several putative *C. elegans* DR regimens have been described[2]. One such is culture on nutrient-rich, semi-defined axenic medium (i.e. lacking a microbial food source), which causes retarded development and large increases in lifespan and therefore resembles a DR effect[3,4]. We employed a solid medium protocol where larvae are raised on UV-killed *E. coli* OP50 to support normal growth and then transferred onto axenic medium plates (no *E. coli*) at adulthood[5]. This resulted in almost complete suppression of pharyngeal deterioration, PLPs and intestinal atrophy, and partial suppression of uterine tumors (Supplementary Figure 2a-d). Gonad atrophy was not measured as axenic culture inhibited gonadal development.

Selective effects on pathology of different *daf-2* alleles

Reduction of insulin/IGF-1 signaling (IIS), as in *daf-2* insulin/IGF-1 receptor mutants, greatly increases *C. elegans* lifespan, and this effect is dependent upon the DAF-16 FOXO transcription factor[6]. Mutation of *daf-2* suppresses development of diverse pathologies, including gonadal and pharyngeal deterioration[7], late-life bacterial infection in the pharynx [8,9], intestinal atrophy and yolk accumulation[10,11]. Inhibiting bacterial infection or yolk synthesis is sufficient to extend lifespan[12-14], suggesting that reduced IIS increases lifespan by blocking development of pathology, at least partly.

We previously compared effects on intestinal atrophy and yolk pool accumulation of two *daf-2* mutations, a less pleiotropic (class 1) allele *daf-2(e1368)*, and a more pleiotropic (class 2) allele *daf-2(e1370)*[15]. This showed that *e1368* modestly delayed both pathologies, but *e1370* strongly suppressed them[11]. Here we extend this analysis to the remaining three pathologies. Notably, the five pathologies were not regulated by IIS as a group, in tandem with one another. For example, in *e1370* mutants, development of pharyngeal and gonadal pathology was delayed but eventually reached maximal wild-type levels, whereas for intestinal atrophy and yolk pools they did not. Moreover, little reduction in uterine tumor growth was detected in either mutant (Supplementary Figure 3a-e). The lack of effect was also somewhat unexpected, given the previous observation that *daf-2* also inhibits formation of chromatin masses within uterine tumors[16]; it suggests that *daf-2* reduces chromatin mass formation without reducing tumor size.

All suppression of pathology by *daf-2(e1370)* was fully dependent upon *daf-16* FOXO, as is the case for *daf-2* longevity[17], consistent with the possibility that IIS shortens lifespan by causing senescent pathology (Supplementary Figure 3f-j).

These findings imply that although wild-type IIS promotes all five senescent pathologies, it does not do so equally. Rather, IIS strongly promotes intestinal atrophy and yolk accumulation, modestly promotes gonadal atrophy and pharyngeal deterioration, and has little effect on uterine tumor formation. Also, different alleles differ in terms of severity of suppression of pathology as well as mortality: *e1370* has stronger effects than *e1368* on both pathology (including pharyngeal infection, gut atrophy and PLPs) and lifespan[8,9,11].

Next we investigated the role of the *daf-16* FOXO transcription factor gene in development of senescent pathology. Mutation of *daf-16* slightly shortens lifespan[18] and fully suppresses *daf-2* longevity[17]. The null mutation *daf-16(mgDf50)* reduced pharyngeal deterioration slightly and did not significantly affect gonad atrophy or tumor formation, but did aggravate PLPs and intestinal atrophy

(Supplementary Figure 4a-e). This is in agreement with an earlier study showing little effect of *daf-16* on the pharynx and germline[7]; and our observation that *daf-16(0)* accelerates PLPs and intestinal aging[11].

We also examined the effects of the *hsf-1* heat shock factor, another transcription factor that mediates the effect of *daf-2* on lifespan[19]. It was previously shown that *hsf-1* RNAi substantially shortens lifespan and accelerates pharyngeal pathology[7]. Although we could not confirm the effects of *hsf-1* RNAi on pharyngeal pathology, we did observe accelerated gonad atrophy and intestinal atrophy (Supplementary Figure 4f-j). These results could imply that loss of *daf-16* or *hsf-1* shortens lifespan by promoting pathology development, and support the earlier deduction that *hsf-1* RNAi causes a progeroid condition[7].

Next we examined whether effects of *daf-2(e1370)* on pathology are *daf-16* dependent, and found that they are, fully (Figure 4f-j). This raises the question of where, within *daf-2(e1370)* mutants, *daf-16(+)* acts to prevent pathology development. With respect to lifespan, effects of *daf-16* are exerted most strongly in the intestine, as shown using transgenic strains where *daf-16* is expressed from tissue-specific promoters in a *daf-16; daf-2* background[20]. We used the same strains to ask: is this also true of the action of *daf-16* against senescent pathologies?

Expressing *daf-16* with its own promoter restored the capacity of *daf-2(e1370)* to suppress pathology in all four cases, confirming the anti-pathogenic role of *daf-16* (Supplementary Figure 5a-f). In most cases, *daf-16* expression in muscle or in the nervous system had little effect on any pathologies (Supplementary Figure 5a-f). By contrast, intestinal rescue of *daf-16* partially restored *daf-2* suppression of intestinal atrophy and yolk steatosis, suggesting an organ autonomous effect of *daf-16*. Intestine-limited rescue of *daf-16* did not suppress gonadal or pharyngeal pathology, suggesting that *daf-16* acts in a tissue- or organ-autonomous manner to suppress pathology.

That *daf-16* rescue in the intestine suppressed both intestinal atrophy and PLP formation (Supplementary Figure 5d,e) is consistent with tissue autonomous suppression of conversion of intestinal biomass into yolk by *daf-16*[11]. By contrast, intestinal atrophy is also modestly suppressed by neuronal rescue of *daf-16* (Supplementary Figure 5d,e), implying tissue non-autonomous action of *daf-16*. Notably, neuronal (as well as intestinal) knockdown of DAF-2 auxin-induced DAF-2 degradation increases lifespan[21-23] while neuronal rescue of *daf-2* shortens it[24]; possibly intestinal *daf-16* effects on biomass conversion play a role in this.

Effects on senescent pathology of other interventions that extend lifespan

Next we investigated effects on senescent pathology of life-extending genetic interventions that inhibit protein synthesis, the mTOR pathway and mitochondrial function. Knockdown of genes involved in protein translational machinery can increase lifespan, including the initiation factor 4F (eIF4F), *ife-2*, the translation initiators eIF2β (*iftb-1*) and eIF4G (*ifg-1*), and the small ribosomal subunit *rps-15*[25]. We found that *ife-2* and *iftb-1* RNAi had little effect on pharyngeal pathology, gonad atrophy or uterine tumors. By contrast, both interventions suppressed intestinal atrophy, particularly *ife-2* RNAi (Supplementary Figure 6a-e). Possibly this reflects reduction of vitellogenin synthesis, which is coupled to intestinal atrophy [11,14]. *iftb-1* RNAi also markedly increased PLP levels (Supplementary Figure 6d) which was somewhat unexpected; however, inhibition of *iftb-1* can reduce brood size by 50%[26], which is predicted to cause yolk retention due to reduced efflux via egg laying[14].

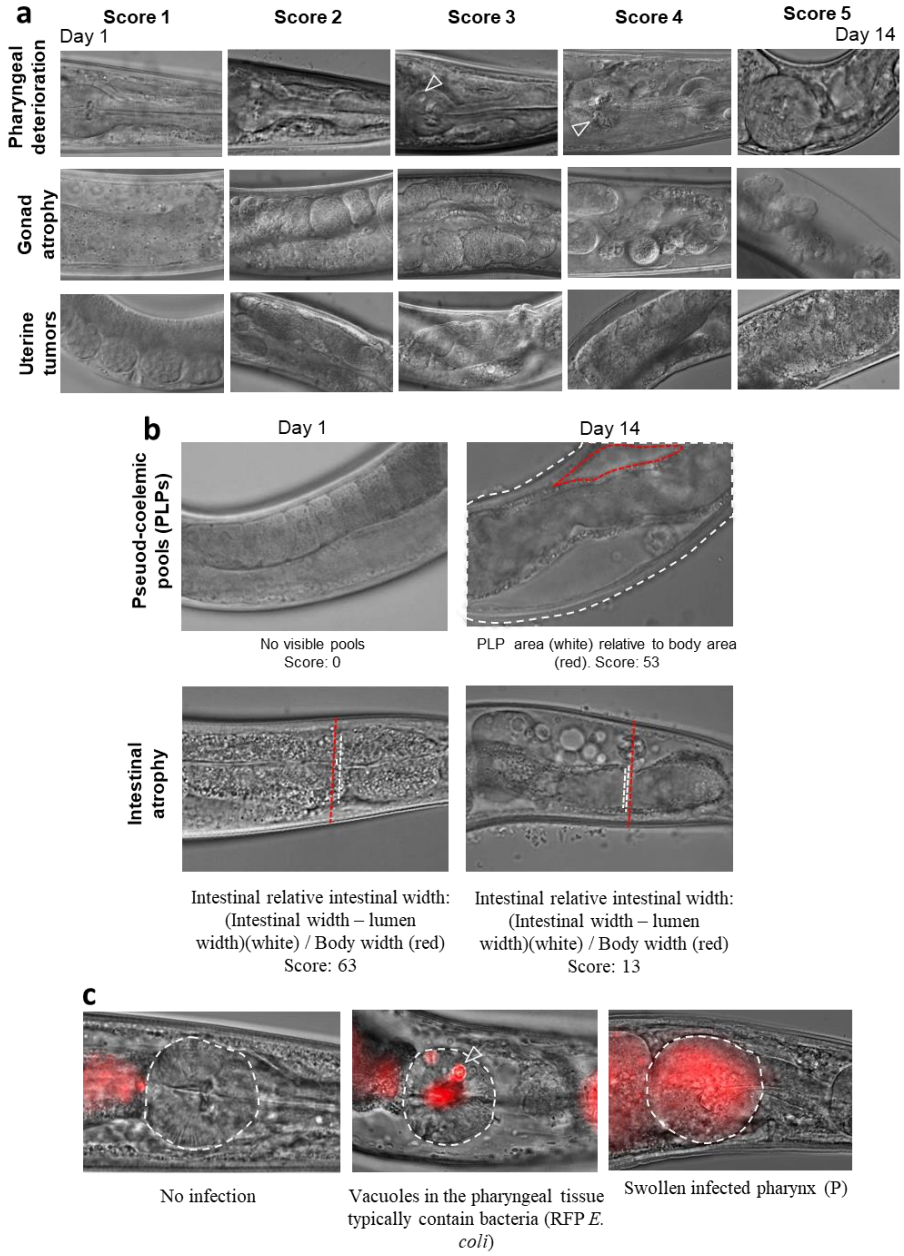
Protein synthesis is promoted by the target of rapamycin (TOR) pathway, acting via ribosomal protein S6 kinase (S6K), encoded by *rsks-1* in *C. elegans*. Lifespan is increased by loss of function of several genes in the TOR pathway, including *rsks-1(ok1255)*[27], which also reduces protein synthesis rate[28]. *rsks-1(ok1255)* slightly alleviated pathology development, with significant effects

on pharyngeal decline and tumor formation. Intestinal atrophy appeared delayed but the effect was not statistically significant (Supplementary Figure 7). The modest effects seen are consistent with the small magnitude of effects of *rsks-1* on lifespan, e.g. a 6% increase in mean lifespan in one study (20˚C, *E. coli* HT115)[28].

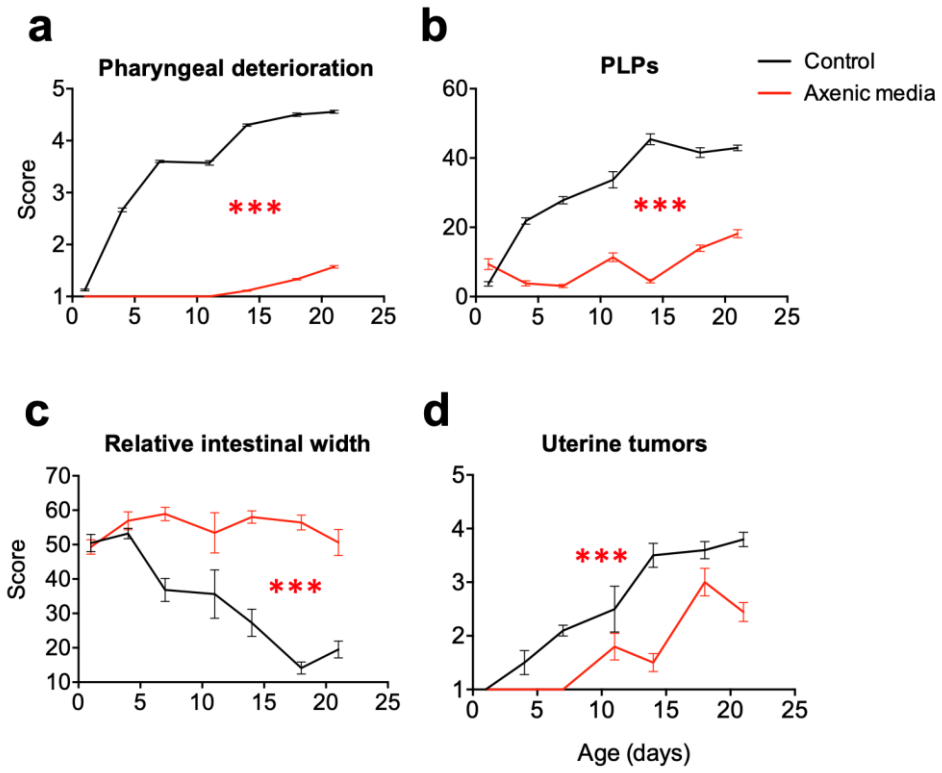
Finally, we tested mutations that perturb mitochondrial function and also delay development and increase lifespan, the Mit phenotype[29], specifically point mutations in *isp-1*, encoding a catalytic subunit of mitochondrial complex III[30] and *nuo-6*, a subunit of mitochondrial complex I[31]. *isp-1(qm150)* and *nuo-6(qm200)* both inhibited pathology development of most but not all of the five pathologies (Supplementary Figure 6f-j), indicating a major role of mitochondrial function in senescent pathogenesis.

One possibility is that mitochondrial metabolism supports processes that contribute to pathogenesis, such as feeding and reproduction. E.g., pharyngeal pumping contributes to pharyngeal pathology[32], production of oocytes past the reproductive period results in uterine tumors[33], and yolk synthesis results in PLP accumulation and intestinal atrophy[11]. Decreasing mitochondrial function reduces both pharyngeal pumping and reproduction[31,34,35].

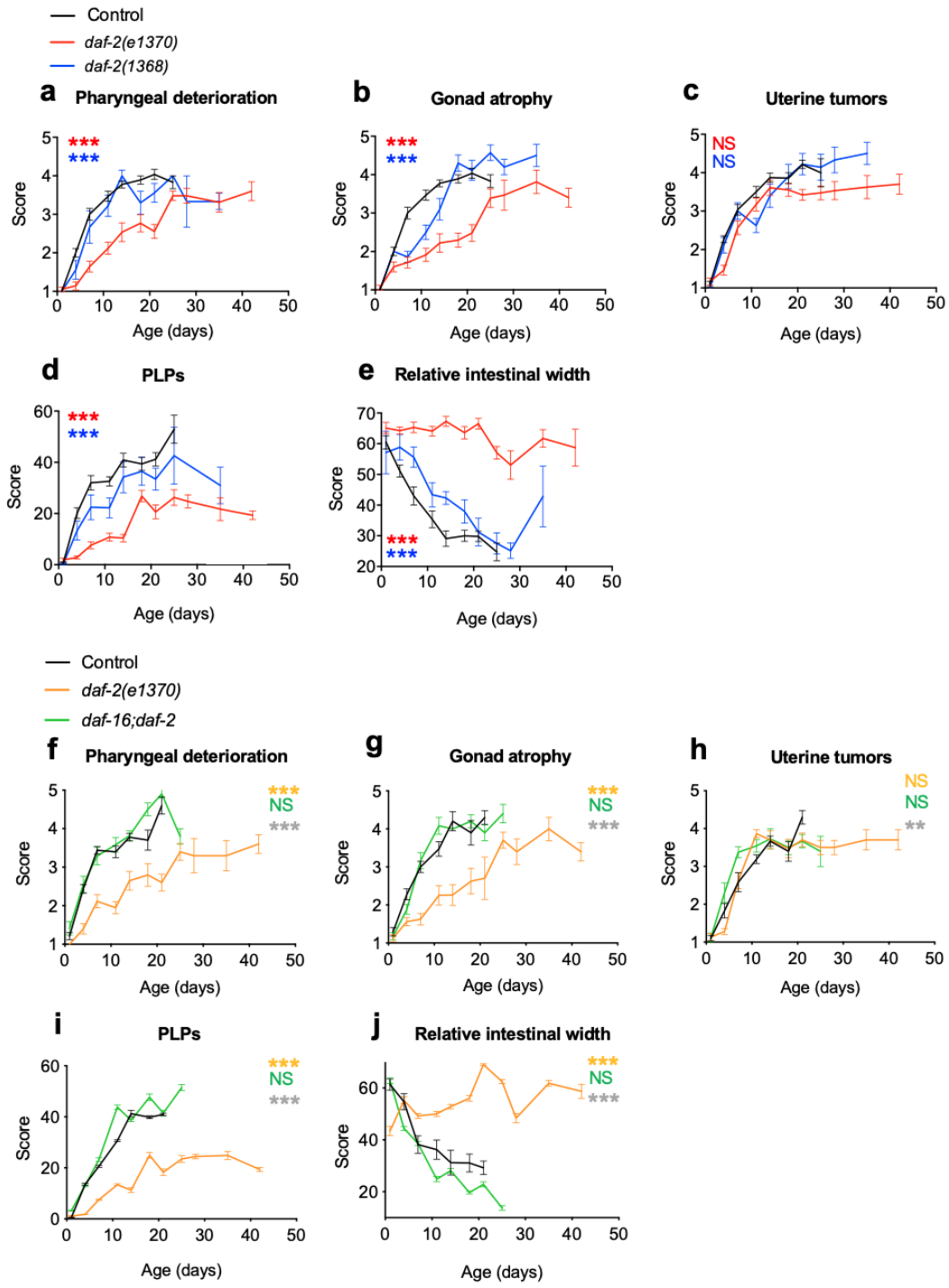
Supplementary figures



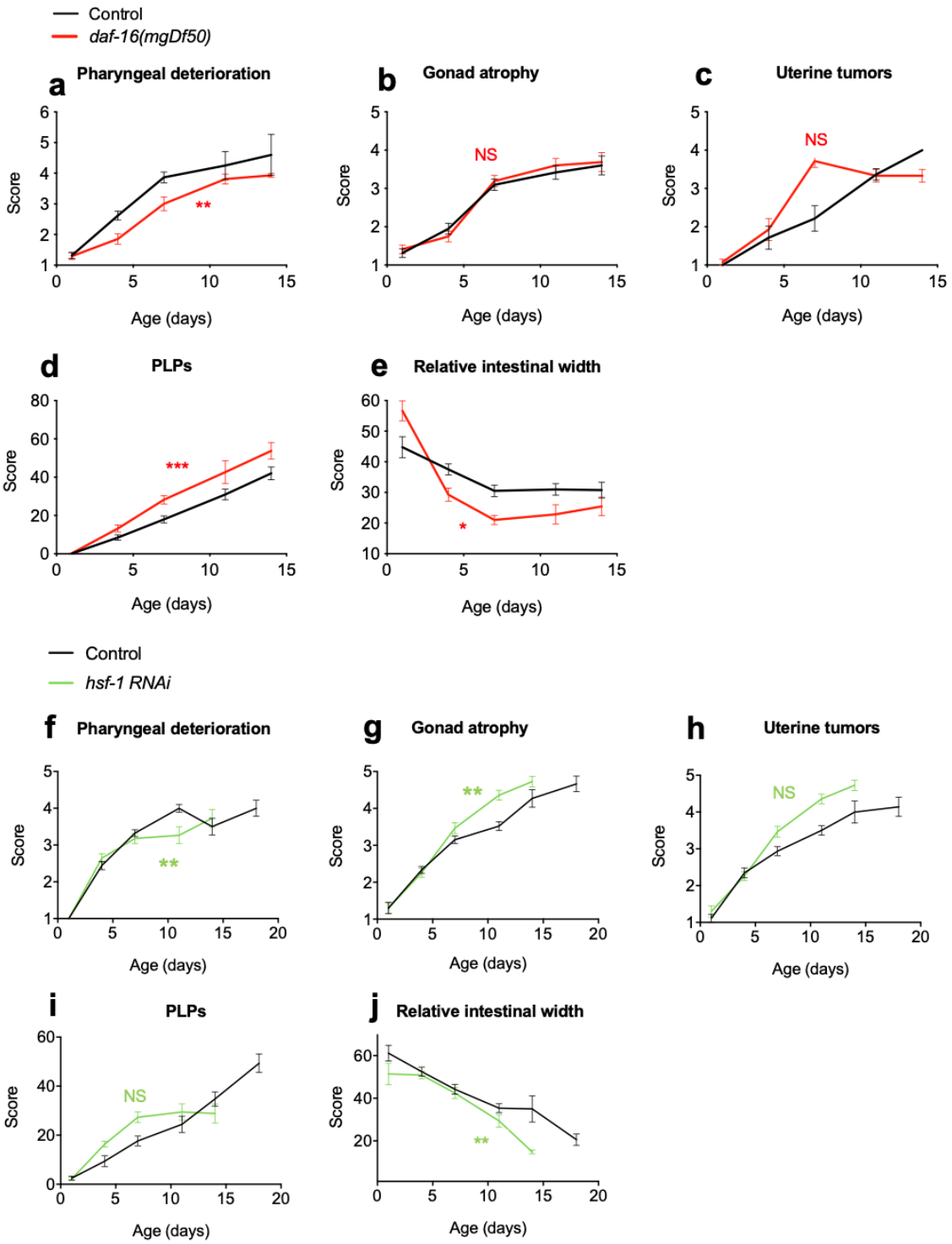
Supplementary Figure 1. Quantification of age-related pathologies in aging *C. elegans.* (a) Images showing age-related pathologies in *C. elegans* hermaphrodites: Pharyngeal deterioration, gonad atrophy, uterine tumors, PLPs, and intestinal atrophy. Images were randomized, and given scores of 1-5. Here 1 = a youthful, healthy appearance; 2 = showing subtle signs of deterioration; 3 = clearly discernible, low level pathology; 4 = well developed pathology; and 5 = tissue so deteriorated as to be barely recognizable (e.g. gonad completely disintegrated), or reaching a maximal level (e.g. large tumor filling the entire body diameter). (b) Pseudocoelomic lipoprotein pool (PLP) formation (yolk accumulation) and intestinal atrophy. PLPs were measured by dividing the total area of yolk pools with the area of the body visible in the field of view. Intestinal atrophy was measured by calculating the width of intestinal tissue relative to body width. (c) Bacterial infection in the pharynx shown via RFP::*E. coli.* Arrow: vacuole site of infection. Terminal bulb of the pharynx is circled.



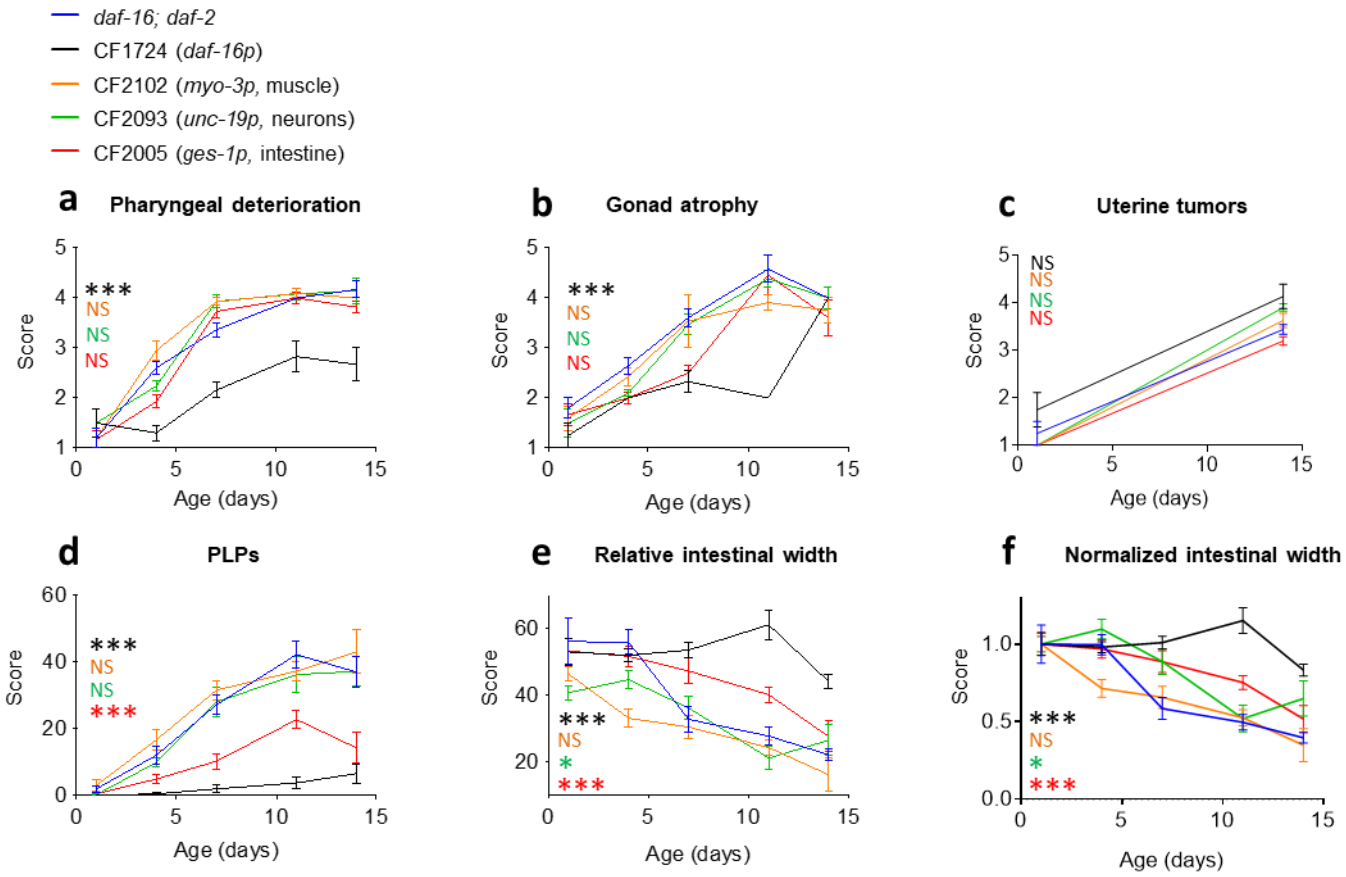
Supplementary Figure 2: Effects on senescent pathologies of axenic culture (putative DR regimen). (a-d) Culture on solid axenic medium suppresses all age-related pathologies. (a) Pharyngeal deterioration, (b) PLPs, (c) gut atrophy, (d) uterine tumors. (a-d) 2 trials (n=10). Stars show differences in pathology progression from day 1 to 14; ANCOVA (Tukey correction).



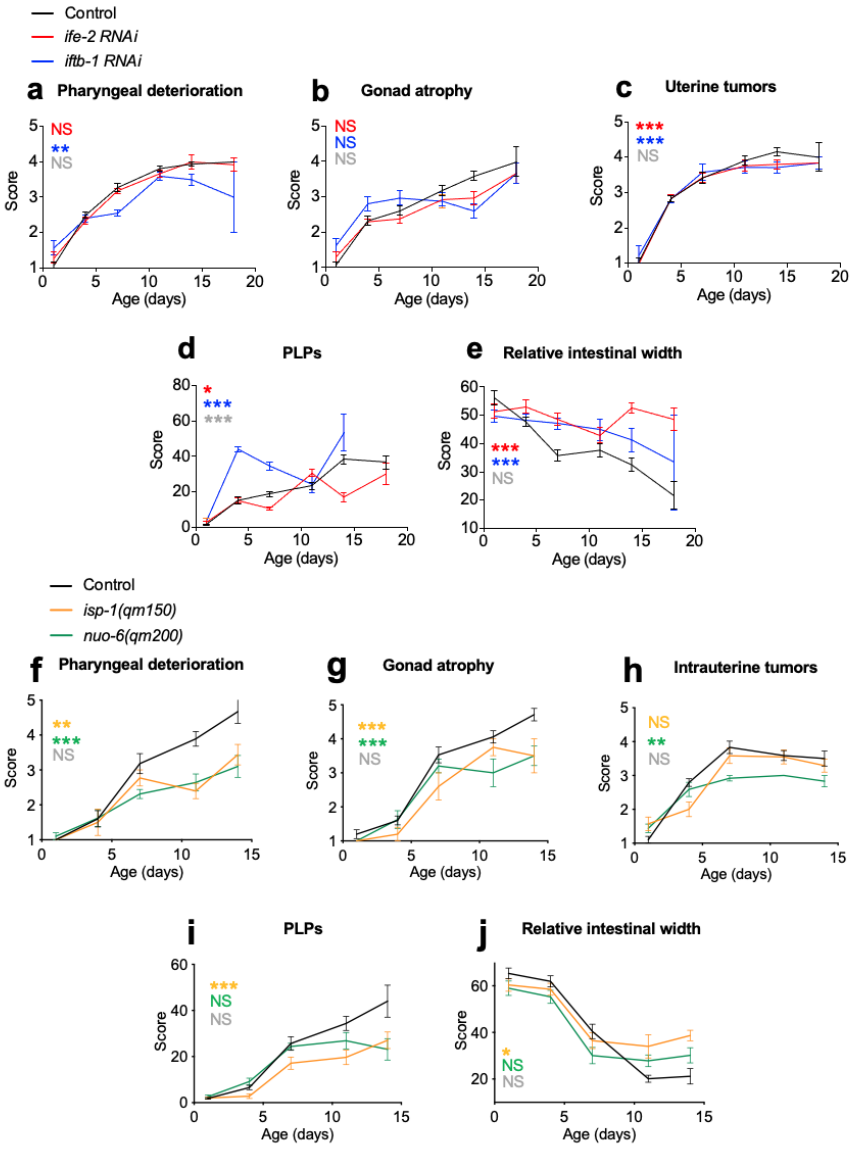
Supplementary Figure 3. Mutation of *daf-2* differentially suppresses senescent pathology. (a-e) *daf-2(e1370)* suppresses pathology development more strongly than *daf-2(e1368)*. (a) Pharyngeal deterioration, (b) gonad disintegration, (c) uterine tumors, (d) PLPs (yolk pools), (e) gut atrophy. (f-i) Pathology suppression by *daf-2(e1370)* is dependent on DAF-16. (f) Pharyngeal deterioration, (g) gonad disintegration, (h) uterine tumors, (i) PLPs, (j) gut atrophy. 2 trials (n=10). Two-way ANOVA (Tukey correction). Stars denote differences in pathology progression via ANCOVA (Tukey correction). **** $p < 0.00001$; *** $p < 0.0001$; ** $p < 0.001$; * $p < 0.01$.



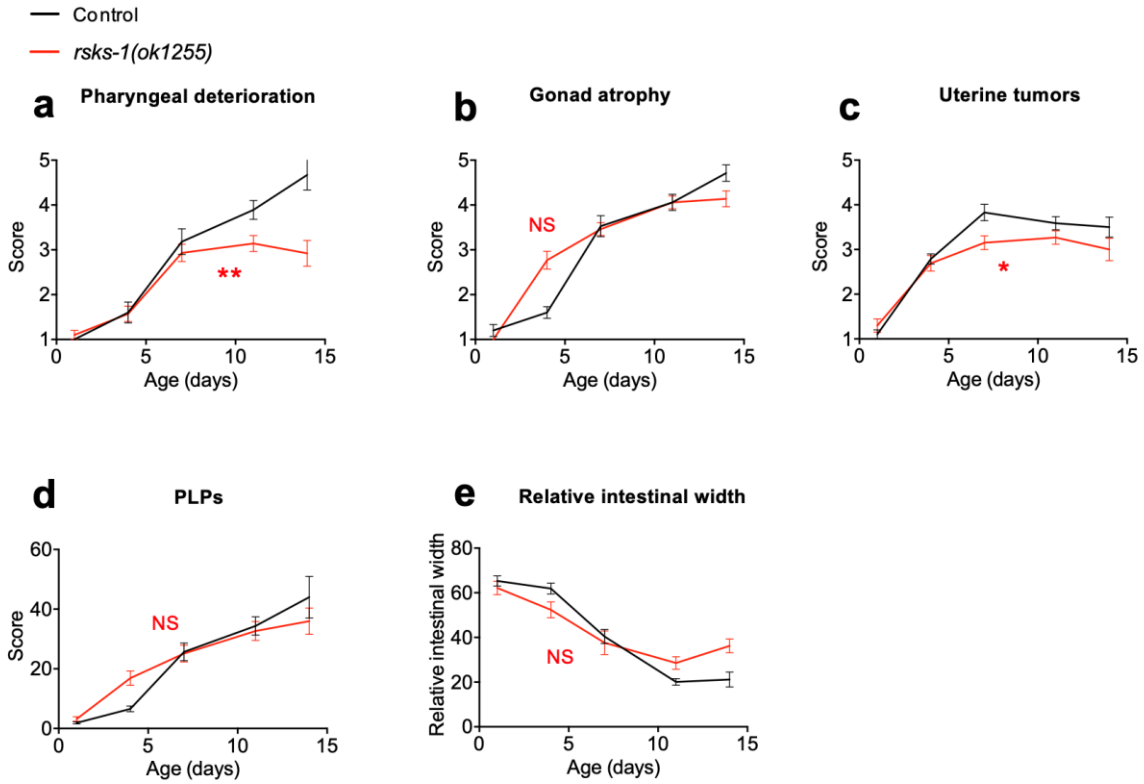
Supplementary Figure 4. Effects on pathology development of tissue-specific *daf-16* rescue and *hsf-1* inhibition. (a-e) *daf-16(mgDf50)* accelerates some but not all pathologies. (a) Pharyngeal deterioration (b) Gonad atrophy. (c) Uterine tumors. (d) PLPs. (e) Relative intestinal width. (f-j) *hsf-1* RNAi accelerates some but not all pathologies. (f) Pharyngeal deterioration. (g) Uterine tumors. (h) PLPs. (i) Relative intestinal width. 2 trials (n=10). Two-way ANOVA (Bonferroni correction), and stars show differences in pathology progression via ANCOVA (Tukey correction). * p<0.01; ** p<0.001; *** p<0.0001; **** p<0.00001.



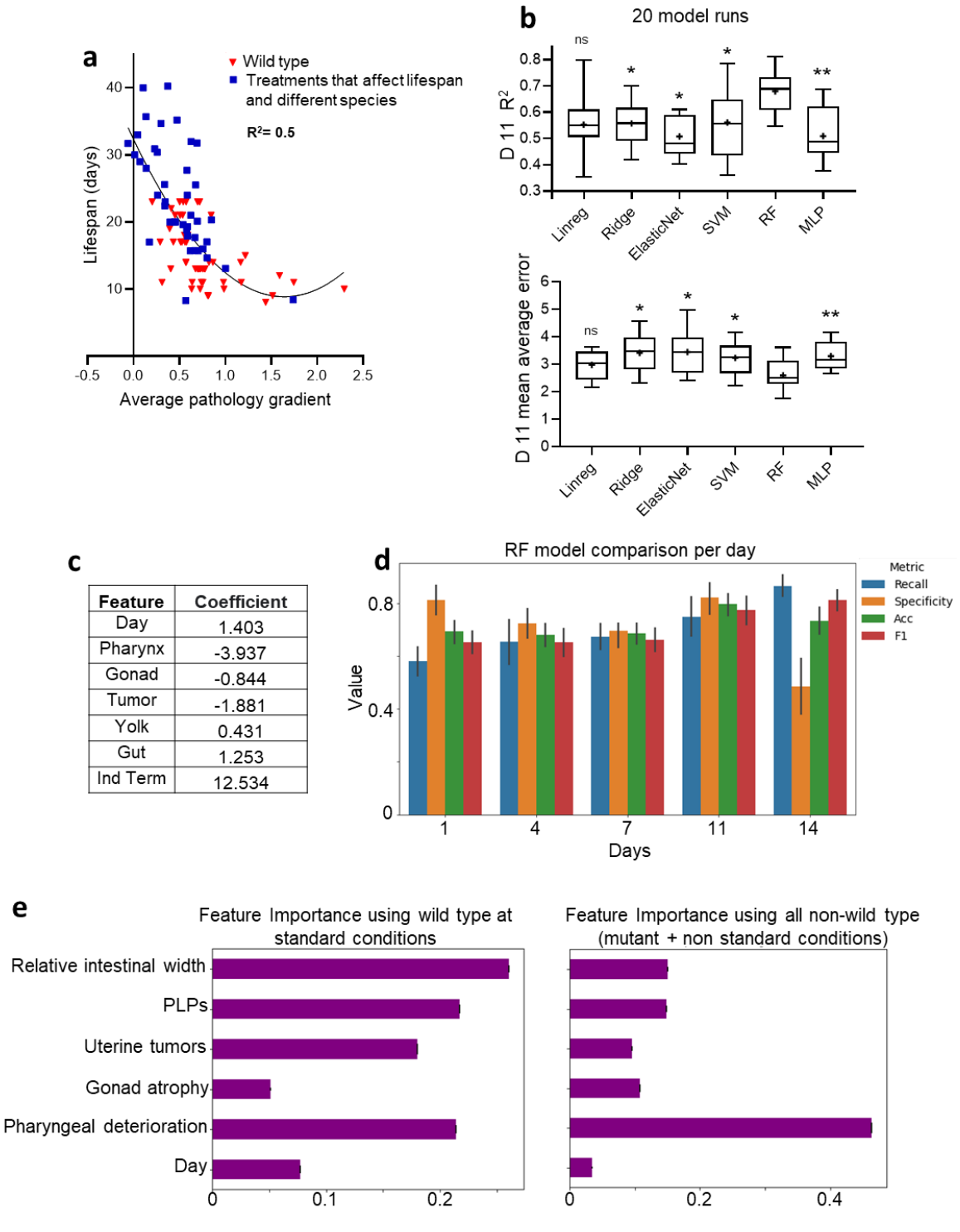
Supplementary Figure 5. Effects of tissue-specific rescue of *daf-16* on senescent pathology in *daf-16; daf-2(e1370)* mutants. *myo-3p::* muscle*; ges-1p::* intestine; and *unc-119p::* neurons. (a) Pharyngeal deterioration, (b) gonad atrophy, (c) uterine tumors, (d) PLPs, (e) gut atrophy. (f) Relative intestinal width normalised to intestinal score on day 1 per condition. Two-way ANOVA (Tukey correction), and stars show differences in pathology progression via ANCOVA (Tukey correction). * p<0.01; ** p<0.001; *** p<0.0001; **** p<0.00001.



Supplementary Figure 6. Evidence that protein synthesis and mitochondrial function promote pathology development. (a-e) *ife-2* and *iftb-1* RNAi inhibits gut atrophy but not most other pathologies. (a) Pharyngeal deterioration, (b) gonad disintegration, (c) uterine tumors, (d) PLPs (yolk pools), (e) gut atrophy. (f-j) *isp-1(qm150)* and *nuo-6(qm200)* inhibit multiple age-related pathologies. (f) Pharyngeal deterioration, (g) uterine tumors, (h) PLPs (yolk pools), (i) gut atrophy. 2 trials (n=10). Two-way ANOVA (Tukey correction). Stars show differences in pathology progression via ANCOVA (Tukey correction). The color of the stars represents treatment color vs control. Grey stars: between treatment comparison. * $p < 0.01$; ** $p < 0.001$; *** $p < 0.0001$; **** $p < 0.00001$.



Supplementary Figure 7. Ribosomal protein S6 kinase has small effects on pathology development. (a-e) *rsks-1(ok1255)* suppresses pharyngeal decline and tumor formation but not other pathologies. (a) Pharyngeal deterioration. (b) Gonad atrophy. (c) Uterine tumors. (d) PLPs. (e) Relative intestinal width. Two-way ANOVA (Tukey correction), and stars show differences in pathology progression via ANCOVA (Tukey correction). * $p<0.01$; ** $p<0.001$; *** $p<0.0001$; **** $p<0.00001$.



Supplementary Figure 8. Senescent pathology can be used to predict lifespan. (a) Broad correlation between pathology and lifespan. Pathology progression through time is converted into a gradient and then transformed into Z-scores (which describe a value's relationship to the mean of a group of values) for standardisation. This allows comparison between different pathologies by normalising levels of a given pathology to the average level of that pathology in the group (represented by a Z-score of 0, with a Z-score of +2

representing the maximum pathology severity in the group and -2 the healthiest animals). Only intestine scores were normalised to the respective mean intestine score on day 1 prior to being converted to a gradient, so the normalised intestinal pathology scores represent the change in percentage of intestinal volume and this accounts for differences in terms of the ratio of intestinal width to whole body width between different treatments and species. The average of all 5 pathology Z-scores is shown (x-axis) plotted against real observed lifespan (y-axis). (b) R^2 and mean average error (days) with different ML models (linear regression, ElasticNet regression, Support Vector Machine [SVM], random forest [RF], multilayer perceptron [MLP]) and pathology progression accounted until day 11 of adulthood. pval comparing RF model to other models. *** $p<0.0001$; **** $p<0.00001$. (c) Coefficients generated by the linear regression model created by accounting for pathology progression to day 11(c.f. Figure 6a). (d) Specificity and sensitivity analysis of our model derived from predicting long lived (> 18 days survival) vs short lived (<18 days survival animals). Recall =Sensitivity; F1 = F1 score; Acc = Accuracy. (e)) Feature importance: RF model Mean Decrease in Impurity for individual pathologies and day of scoring. Right: mutant animals and animals at non-standard culture conditions; Left: wild type at standard culture conditions.

Score	Pharyngeal deterioration	Gonad atrophy	Uterine tumours
1	Healthy pharynx. Borders are smooth, radial muscle lines are visible and intact.	Healthy gonad. Gonad arms are intact. Edges are smooth. Germline cells are surrounded by cytoplasm. Gonad arms fill up the body cavity and are mostly touching each other.	Healthy uterus, no tumours.
2	Borders of the pharynx are uneven. Radial muscle lines are starting to fade. Small vacuoles/cavities appear.	Thinned gonad. Starting to show deterioration. Diameter of gonad arm is decreased and are touching less than in a healthy gonad. Edges of distal arm are starting to shrivel.	Abnormal uterus; possible beginning of tumour
3	Aged pharynx. One of the following ageing features: bacterial plug present, terminal bulb starting to swell or cavities in bulb.	Aged gonad. Gonad even thinner. Further decrease of diameter and further shrivelling.	Small tumours; Area/(diameter)^2 < 0.9
4	Very aged. Two or more of the ageing features above.	Very aged. Fragmented gonad. Disruption/displacement/ twisting of gonad	Large tumours but do not fill entire cavity; A/d^2 > 0.9
5	Posterior pharyngeal bulb completely swollen or disintegrated.	Gonad not recognisable.	Massive tumours; fills the entire diameter of body cavity

Supplementary Table 1. Pathology scoring system. c.f. Supplementary Figure 1.

Treatment, species, genotype	**Pathology data source**	**Lifespan data source**
Culture at 15˚C	This study	[36]
Culture at 25˚C	This study	[36]
Culture on solid axenic medium	This study	[5]
Mated hermaphrodites	This study	[37]
Male	This study	[12]
C. elegans	[38]	[38]
C. inopinata	[38]	[38]
C. briggsae	[38]	[38]
C. nigoni	[38]	[38]
C. remanei	[38]	[38]
C. tropicalis	[38]	[38]
C. wallacei	[38]	[38]
C. remanei	This study	This study
Pristionchus exspectatus	[38]	[38]
Pristionchus pacificus	[38]	[38]
Laser ablation z1-4 *C. elegans*	[38]	[38]
Laser ablation z23 *C. briggsae*	[38]	[38]
Laser ablation z23 *C. elegans*	[38]	[38]
Laser ablation z23 *C. tropicalis*	[38]	[38]
Laser ablation z23 *P. pacificus*	[38]	[38]
glp-1(bn4)	[38]	[32]
glp-4(bn2)	[38]	[39]
daf-2(e1370)	This study	[40]
daf-2(e1368)	This study	[11]
daf-16; daf-2	This study	[40]
daf-16(mgDf50)	This study	[40]
Pdaf-16::daf-16; daf-16	This study	[20]
Pges-1::daf-16; daf-16	This study	[20]
Pmyo-3::daf-16; daf-16	This study	[20]
Punc-119::daf-16; daf-16	This study	[20]
mab-3	[11]	[11]
mab-3 males	[11]	[11]
him-5	This study	[11]
him-5 males	This study	[11]
fem-3	[38]	[38]
fog-2	[38]	[38]
rps-15 RNAi	This study	[25]
iftb-1 RNAi	This study	[25]
rsks-1(ok1255)	This study	[28]
isp-1	This study	[31]
nuo-6	This study	[31]
hsf-1 RNAi	This study	[41]
ife-2 RNAi	This study	[27]
iftb-1 RNAi	This study	[25]
vit-5 RNAi	[14]	[14]
vit-6 RNAi	[14]	[14]
vit-5, vit-6 RNAi	[14]	[14]

Supplementary Table 2. All lifespan data sources and pathology data sources collated and used in ML analysis. C.f. Figure 3.

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