Research Paper

Healthspan pathway maps in *C. elegans* and humans highlight transcription, proliferation/biosynthesis and lipids

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ABSTRACT

The molecular basis of aging and of aging-associated diseases is being unraveled at an increasing pace. An extended healthspan, and not merely an extension of lifespan, has become the aim of medical practice. Here, we define health based on the absence of diseases and dysfunctions. Based on an extensive review of the literature, in particular for humans and *C. elegans*, we compile a list of features of health and of the genes associated with them. These genes may or may not be associated with survival/lifespan. In turn, survival/lifespan genes that are not known to be directly associated with health are not considered. Clusters of these genes based on molecular interaction data give rise to maps of healthspan pathways for humans and for *C. elegans*. Overlaying healthspan-related gene expression data onto the healthspan pathway maps, we observe the downregulation of (pro-inflammatory) Notch signaling in humans and of proliferation in *C. elegans*.

We identify transcription, proliferation/biosynthesis and lipids as a common theme on the annotation level, and proliferation-related kinases on the gene/protein level. Our literature-based data corpus, including visualization, should be seen as a pilot investigation of the molecular underpinnings of health in two different species. Web address: http://pathways.h2020awe.eu.

INTRODUCTION

For a long time, an active, targeted intervention to maintain health into old age was *terra incognita*. It had no priority, and few, if any, reliable data were available to implement it in everyday life. Today, however, systematically established diagnostic hints become available for the individual, based on family history and biomarker data, including genetic variants (polymorphisms). To assess and prevent premature health deterioration successfully, it would therefore be useful (1) to dissect "health" into a set of its most important features, (2) to specify biomarkers and corresponding supportive interventions for the various features of health and for health itself, and (3) to detail its molecular basis and to map out molecular "healthspan pathways". Arguably, the increase in life expectancy in the last 100 years has not been accompanied by an increase in disease-free life expectancy [1, 2]. Cardiovascular disease, type-2 diabetes and neurodegenerative disorders are highly prevalent in the elderly, and these diseases frequently coexist in the same aged individual, often with mutual reinforcement [3, 4]. Extending healthspan may thus enable economic, societal and individual gains on a large scale [5, 6].

Intervention studies to prolong healthspan based on compound exposure in humans are limited to relatively few compounds. Resveratrol for instance, being one of the best-studied polyphenols in humans and animals, has been tested in several clinical studies [7]. These include studies focused on biomarkers, like the level of blood glucose [8] and cholesterol [9], or glutathione S-transferase expression [10]. Moreover, data about long-term effects on overall health in human are missing in general, and given the average human life expectancy, they are difficult to obtain. Therefore, model organisms are of great relevance to uncover the molecular basis of healthspan and to identify supporting compounds. The nematode *Caenorhabditis elegans (C. elegans)* is a widely used model organism for studying ageing which guided the discovery of fundamental ageing-related findings, e.g., on calorie restriction and Insulin/IGF-1 like signaling [11]. Last not least, around 40% of the genes found in *C. elegans* have human orthologs and, vice versa, about 50% of the human protein-coding genome has recognizable worm orthologs [12]. Studies revealing the role of metabolism on health conducted in *C. elegans* have been subsequently strengthened in murine models [13, 14], rendering this nematode a valuable model for human ageing processes. Furthermore, the effects on lifespan, when manipulating orthologous lifespan-associated genes in different model organisms, are mostly concordant, despite high evolutionary distances between them [15]. Most recently, *C. elegans* has come to enjoy increasing popularity as a model for health [16, 17], and an ever increasing number of compounds [18–21] and diets [22, 23] are tested in *C. elegans* for their anti-ageing and health effects.

Here, we assemble and explore "healthspan pathway maps", that is, annotated sets of interacting genes implicated in health. To create these, we follow a stepwise procedure: first, we dissect health into its various features, based on disease and dysfunction. Second, we compile lists of genes associated with health based on the literature, for humans and *C. elegans*. Third, we organize these genes into maps of healthspan pathways, based on gene/protein interaction and annotation data. Fourth, we create an overlay of health-related gene expression data onto the resulting healthspan pathway maps, highlighting corroborating knowledge that was not used as input. Finally, we investigate the overlap of the healthspan pathways in humans and *C. elegans.*

Health is a term in biology and medicine that is hard to define. We propose that the best definition of health must be based on an aggregation of the literature, see also Fuellen et al. [5] and Luyten et al. [17]. Then, healthspan is simply the time spent in good health. Supplementary Tables 1–3 list features of human health as discussed in the literature, referring to lack of dysfunction, lack of multiple diseases, and lifespan/longevity mediated by lack of disease. In principle, at least for human, dysfunction can be operationalized with the help of a codified classification of function (such as the ICF, the International Classification of Functioning, Disability and Health, https//:www.who.int/classifications/icf/en/). This classification provides criteria to establish that an individual is affected by a dysfunction. As described and discussed in Fuellen et al. [5], we can filter the "body function" part of the ICF by looking for follow-up in the literature on health and healthspan. The result is a pragmatic community consensus definition of dysfunction, centering around the lack of physiological, physical,

cognitive and reproductive function; a lack of physiological, physical and cognitive functions is often called frailty. To a large degree, this consensus definition can be used for non-human species as well. Further, *disease* can also be operationalized by a codified classification (such as ICD-11, International Statistical Classification of Diseases and Related Health Problems, https//:www.who.int/classifications/icf/en/). Again, the classification provides criteria to establish that an individual is affected by a disease. In this paper, affection by a single disease is not considered, as in old age, single-disease morbidity rarely exists, and in terms of interventions, we are interested in preventing more than one disease. As described and discussed in Fuellen et al. [5], not all parts of the ICD feature diseases related to health and healthspan. However, we note that all diseases referred to in Supplementary Tables 1–3 qualify as age-associated diseases.

The main sources of knowledge about health, that is, about features, biomarkers and interventions regarding health-related phenotypes, are

(a) observational genetic investigations, usually in the form of genome-wide association studies, looking for associations between health and polymorphisms of specific genes [24],

(b) observational studies of non-genetic biomarkers, which are dynamic in time and are usually related to known canonical pathways, and their longitudinal or cross-sectional correlation with health [25],

(c) interventional studies, most often in model organisms, where interventions affecting health may be genetic or based on food or (pharmaceutical) compounds, and the intervention effects are measured on the molecular level, implicating particular genes or pathways [26].

Like genetic studies, compound intervention studies can, in principle, elucidate the causative basis of health. Studies of type (b) may only be revealing correlative evidence and can sometimes not be linked to particular genes; therefore, we will not consider these further. A biomarker of health is any (composite) feature that allows to predict future health better than chronological age [5]; it may be genetic (polymorphisms; such a biomarker is essentially static over lifetime), molecular but not genetic (epigenetic or transcript or protein or metabolic markers, etc.), cellular (blood counts, etc.) or organismic (such as grip strength). Based on studies of types (a) and (c), in this work we will only deal with genes and sets of genes (that is, genes organized into networks or pathways) as candidate biomarkers of health.

For humans, we thus consider that knowledge of the causal basis of health may be best derived from genetic association studies [27]. Based on an extensive review of the literature, we identify a core set of 12 genes that are genetically associated with a lack of frailty [28, 29] and the Healthy Aging Index [30], and another set of 40 genes genetically associated with (a lack of) multiple diseases, or with longevity mediated by a lack of disease (see Supplementary Tables 1–3). In contrast to humans, genetic intervention studies on healthspan are available for *C. elegans*, as well as compound intervention data. A lack of dysfunction exemplified by stress resistance, locomotion, pharyngeal pumping and reproduction are taken as the key health features in *C. elegans* [31]. On this basis, a core set of 11 genes is directly implicated in improvements of locomotion by genetics, and another set of 20 genes is indirectly implicated in improvements of the key health features by studies that investigate effects of compounds (see Supplementary Tables 4, 5). While there is a strong overlap between genes affecting healthspan and genes affecting lifespan, the genes we selected may or may not be associated with survival/lifespan. In turn, we do not consider survival/lifespan genes that are not (known to be) directly associated with health. In other words, what we selected is a list of genes related to health(span), without explicitly considering lifespan.

We then place the genes implicated in health into context by adding gene/protein interaction and gene annotation knowledge. Specifically, we turn the lists of genes into gene/protein interaction networks, to which 20 closely interacting genes are added, employing GeneMania [32]. Gene ontology annotation data are then used to annotate clusters of strongly connected genes within the network, employing AutoAnnotate [33]. Then, we elaborate how the resulting healthspan pathways can be interpreted in plausible ways, specifically in the light of independent health-related gene expression data describing effects of caloric restriction and of rapamycin, and in the light of gene expression data describing aging and disease. Given the incomplete and sometimes inaccurate knowledge we use as input, our healthspan pathway map, and its interpretation can only be a first sketch to drive the development of models of this polygenetic phenotype. For example, not much weight should be given to the small pathways (clusters of 2-3 genes) in the pathway maps, as the clustering is entirely based on high-throughput data such as protein interaction data.

We also predict microRNAs that may be potential regulators of healthspan [34, 35]. Finally, we find that if we construct an overlap between the healthspan pathways in *C. elegans* and humans, genes involved in transcription, proliferation/biosynthesis and lipids are

highlighted, but this overlap is not straightforward to interpret in the light of the independent health-related gene expression data that we used to test plausibility of the single-species healthspan pathway maps. Further, "lipids" come up by way of the Gene Ontology annotation data for both species.

All healthspan pathways discussed in this manuscript, as well as the overlaps we found between species, are available for interactive exploration at http://pathways.h2020awe.eu.

RESULTS AND DISCUSSION

Based on the considerations in the introduction, we first justify the gene lists we used to construct the healthspan pathway maps for humans and *C. elegans*. Second, we describe the healthspan pathway maps in detail, specifically in light of gene expression data that we overlaid onto the pathway maps. We then consider the human - *C. elegans* overlap, followed by some general discussion of our approach, including its strengths and limitations.

Genes associated with health

Health genes in humans may be discovered based on genetic association, and with some probability it can be assumed that these correlations indicate a causal relevance. This is not a certain inference, because of the intrinsic ambiguities in assigning genetic polymorphisms (in the form of SNPs, single-nucleotide polymorphisms) to genes, e.g. in intergenic regions or in intronic regions with overlapping non-coding RNA on the complementary strand [36]. In turn, for *C. elegans*, only few studies report effects of genetic interventions on health, although these are increasingly becoming available. However, as of early 2018, Sutphin et al. [16] is the only large-scale genetic study that we could identify, even though it is basically a small-scale study of healthspan based on a large-scale study of lifespan. Many more studies in *C. elegans* refer to canonical aging-related pathways, and in contrast to studies in humans, these studies often directly report the molecular effects of compound intervention. The *C. elegans* genes listed in the Supplementary Tables 4, 5 are thus based on the effects of genetic intervention and on the effects (on the gene level) of compound intervention, and we can assume a high probability of causality in both cases. For *C. elegans*, Supplementary Tables 4, 5 list features of health based on the literature, referring to lack of dysfunction in the form of stress resistance (in response to thermal and oxidative stress), (stimulated) locomotion, pharyngeal pumping, and reproduction. These features dominate the literature, and they cover the aspects of physiological function, physical and cognitive function, and reproductive function, as in human [5]. Of note, genetic analyses of health in *C. elegans* have focused up to now mostly on (stimulated) locomotion. Stimulated locomotion integrates some aspects of strength (physical function) and cognition (cognitive function).

Additional genes associated with *C. elegans* health

For *C. elegans,* we generated an additional list (see Supplementary Table 8) of health-associated genes (which differ from genes listed in Supplementary Tables 4, 5 to a large extent) that cannot be generated for humans (see Methods), using WormBase to systematically identify health-related compound interventions with associated gene expression data, and compiling the list of genes with strongest differential expression that are well-annotated by Gene Ontology terms.

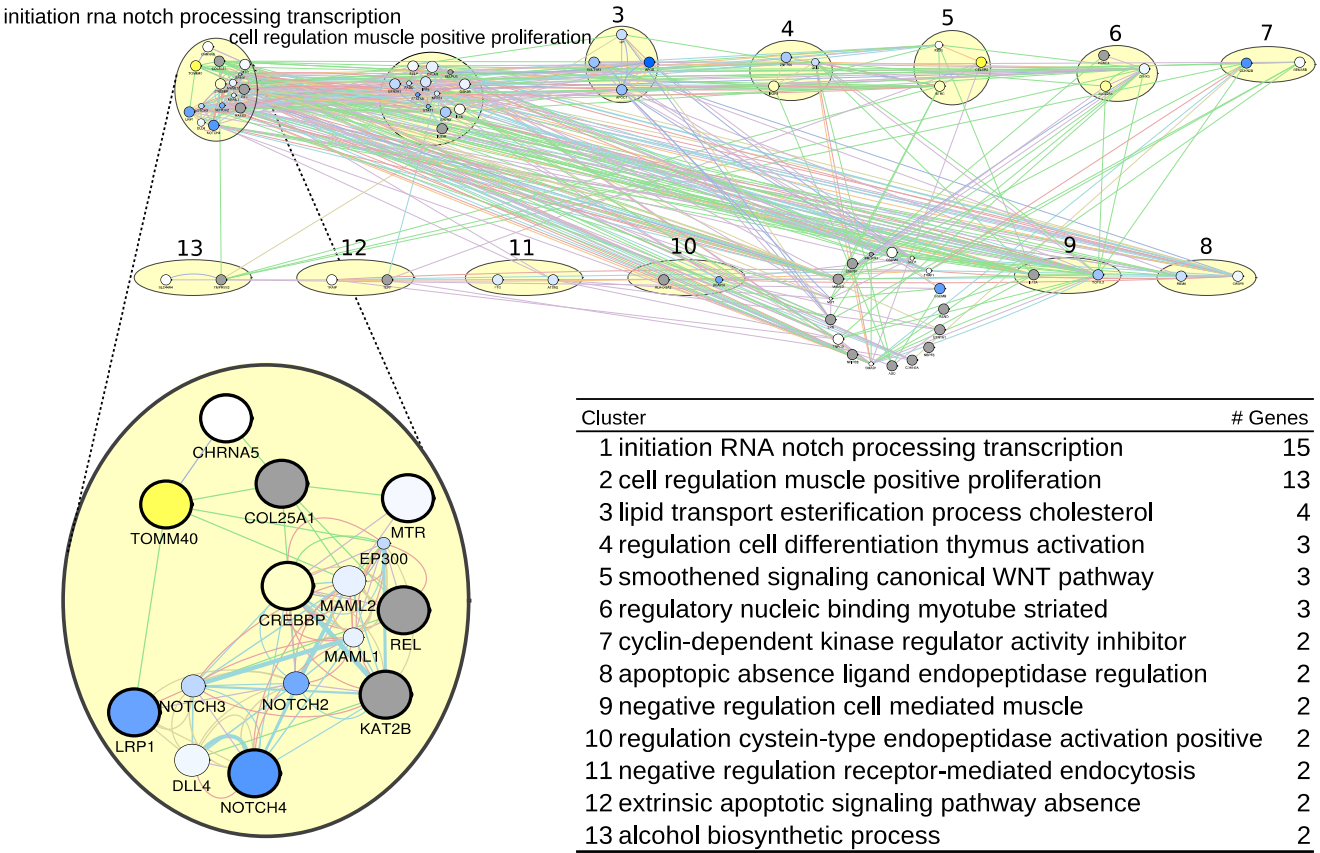
From gene lists to maps of healthspan pathways

We used Cytoscape with selected plugins to obtain and annotate a connected network of the human healthspan associated genes from Supplementary Tables 1–3 and the *C. elegans* genes from Supplementary Tables 4, 5. Specifically, we used GeneMANIA to establish a gene/protein interaction network and to add connecting genes, and subsequently we clustered all genes based on their connectivity, and added GeneOntology-based annotations using AutoAnnotate. The resulting healthspan pathway maps are presented in the following. Moreover, health-related gene expression data are overlaid onto all healthspan pathway maps and will be discussed as well; these data are describing the effects of caloric restriction (CR) in humans [37] and of rapamycin in *C. elegans* [38], as examples of health-promoting interventions, or they describe the effects of aging and disease in specific tissues.

For humans, we derived a gene list (Supplementary Table 6) summarizing all genes associated with healthspan. (see Supplementary Tables 1–3 to trace back these genes to their origin). This list yielded the network of Figure 1, where the two largest pathways/clusters (15 and 13 genes) are specifically labeled by NOTCH and transcription initiation, and by proliferation, and the smaller pathways/clusters (4, 3, 3 and 3 genes) are labeled by cholesterol and lipid processes, by thymus activation, by myotube (striate muscle) regulation, and by Wnt signaling. In Figure 1 bottom, the list of pathways/clusters is given, and the details of the largest pathway are zoomed in.

In the largest pathway/cluster, in light of the CR-triggered gene expression changes, the most prominent findings are an induced downregulation of NOTCH4

(and to a lesser extent of NOTCH 2 and 3), as well as of LRP1, and an upregulation of TOMM40 and CREBBP (also known as CBP). The family of NOTCH proteins has various functions, including a pro-inflammatory one [39, 40]. NOTCH4 is upregulated in kidney failure [41], and promotes vascularization/angiogenesis, which includes its upregulation in malignancy [40, 42]. A downregulation of NOTCH4 by CR can thus be taken as beneficial effect. This is less obvious for LRP1, the low-density lipoprotein receptor-related protein 1, which is responsible for membrane integrity and membrane cholesterol homeostasis, thus being involved in proper myelination [43] and vascular integrity [44]. A downregulation of LRP1 during CR could therefore be seen as deleterious. However, LRP1 expression mainly depends on cholesterol levels [45] – and these are lower during fasting. Hence, lower LRP1 expression actually reflects a lower LDL level, which per se has been found to be protective. The upregulations observed for TOMM40 and CREBBP during CR can also be seen as protective. TOMM40 is part of a mitochondrial membrane protein translocase, supporting mitochondrial function [46], and low expression and/or particular risk alleles of this protein are associated with Huntington's and Alzheimer's Disease [47, 48]. Of note, TOMM40 upregulation during CR goes together with APOE4 downregulation. Although both genes are closely located on chromosome 19, prompting the speculation that this linkage could imply concordant expression changes, this is obviously not the case here. CREBBP is a transcriptional co-activator with histone-acetyltransferase activity [49], acting primarily on histones 3 and 4, and thus it acts in concert with a range of transcription factors. Its downregulation is

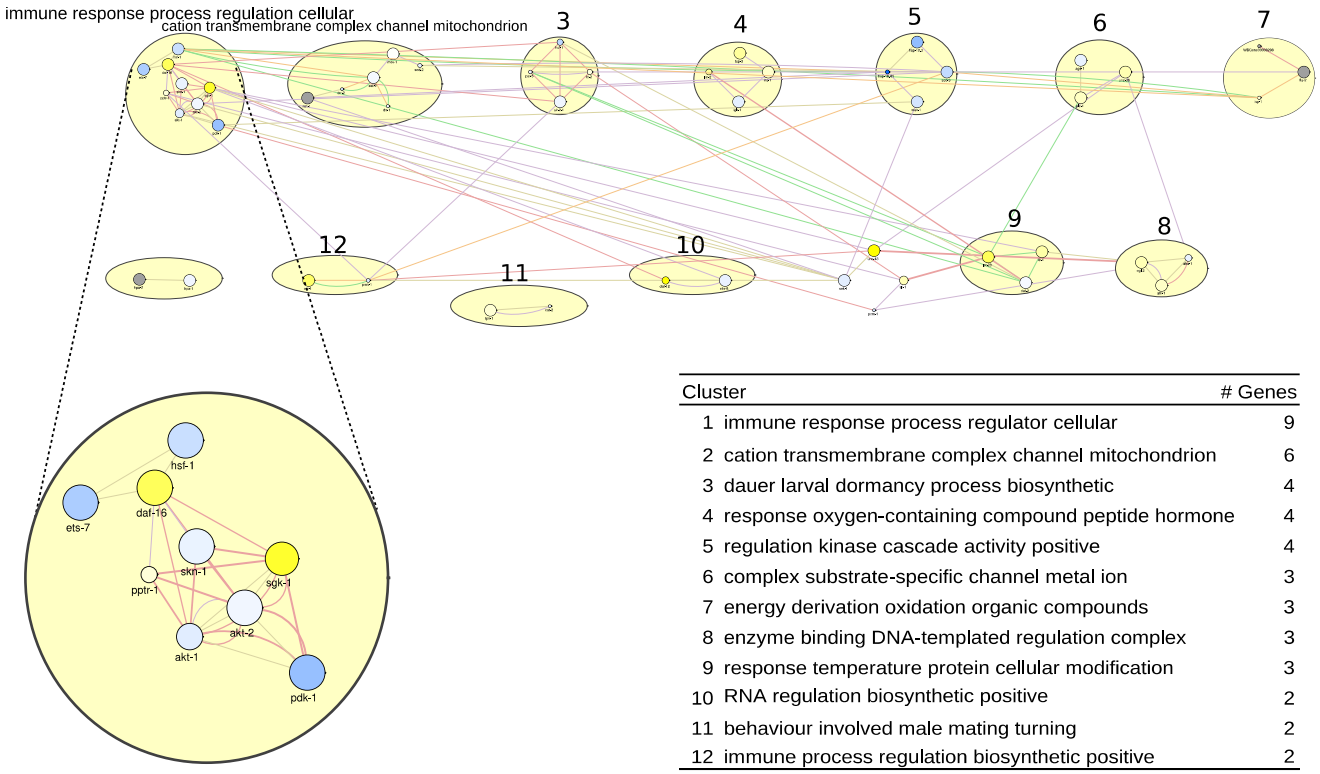


Cluster	# Genes
1 initiation RNA notch processing transcription	15
2 cell regulation muscle positive proliferation	13
3 lipid transport esterification process cholesterol	4
4 regulation cell differentiation thymus activation	3
5 smoothened signaling canonical WNT pathway	3
6 regulatory nucleic binding myotube striated	3
7 cyclin-dependent kinase regulator activity inhibitor	2
8 apoptopic absence ligand endopeptidase regulation	2
9 negative regulation cell mediated muscle	2
10 regulation cystein-type endopeptidase activation positive	2
11 negative regulation receptor-mediated endocytosis	2
12 extrinsic apoptotic signaling pathway absence	2
13 alcohol biosynthetic process	2

Figure 1. A healthspan pathway map for humans, based on Supplementary Tables 1–3, including the list of pathways/clusters with their labels as assigned by AutoAnnotate and their size (number of genes). The largest pathway is zoomed in to reveal details. The size of a gene node is proportional to its GeneMANIA score, which indicates the relevance of the gene with respect to the original list of genes to which another 20 genes are added by GeneMANIA, based on the network data. Genes upregulated by CR are shown in yellow, downregulated genes are shown in blue, and grey denotes genes for which no expression values are available in the caloric restriction dataset [37]. The color of an edge refers to the source of the edge in the underlying network, that is co-expression (pink), common pathway (green), physical interactions (red), shared protein domains (brown), co-localization (blue), predicted (orange), and genetic interaction (green). The thickness of an edge is proportional to its GeneMANIA "normalized max weight", based on the network data. Genes from the GeneMANIA input list feature a thick circle, while genes added by GeneMANIA do not.

deleterious, resulting in, e.g., MHCII expression loss on lymphocytes [50], rendering the lymphocytes dysfunctional for antigen presentation, and in inflammatory signaling [51]. An upregulation of CREBBP by CR is thus likely beneficial. We further investigated the miRNAs that are statistically enriched in the largest healthspan pathway using the TFmir webserver [52], revealing regulation of NOTCH genes implicated in the epithelial-mesenchymal transition, cancer, heart failure and obesity, see Supplementary Results. The genes in the next-largest pathway/clusters, related to cell proliferation and lipids, are also described there in detail, as well as further evidence provided by mapping aging- and disease-related gene expression data onto them, as published or collected by Aramillo Irizar et al. [53].

For *C. elegans*, the gene list representing all healthspan associated genes is shown in Supplementary Table 7 (see Supplementary Tables 4–5 to trace back these genes to their origin). This list yielded the network of Figure 2, where the largest clusters (9 and 6 genes, respectively) are labeled by immune response process and by terms related to the mitochondrion. Three clusters (of 4 genes each) specifically feature dauer/dormancy, hormone response, and regulation. In Figure 2 bottom, the list of pathways/clusters, and the details of the largest pathway are zoomed in. Regarding the first pathway, rapamycin reduces ets-7 transcription, which was shown to be necessary for the healthspan-promoting effects of salicylamine [54]. Furthermore, rapamycin upregulates the transcription factor daf-16 (a homolog to Foxo) and downregulates the daf-16 inhibitors akt-1 and akt-2, putatively leading to an improved stress- and immune-response and prolonged lifespan via the Insulin/IGF-1 pathway [55]. Along the same lines, the akt-1 and akt-2 activator pdk-1 is also downregulated by rapamycin, further promoting daf-16 activity [56]. In contrast, the daf-16 inhibitor sgk-1 (a homolog to Nrf) is upregulated; however, its inhibitory role is subject of discussion [57]. Finally, the transcription factors hsf-1 and skn-1, both important in stress response processes [58, 59], are slightly downregulated in rapamycin-treated *C. elegans*. Thus, the stress defense system of *C. elegans* seems to play a central role in healthspan prolongation. Indeed, stress resistance is frequently discussed as a key to a long and healthy life. Vitagenes, which are genes involved in preserving cellular homeostasis during stress conditions, were shown to be crucial for the beneficial effects of dietary phytochemicals [60]. Furthermore, mild stress, which stimulates repair pathways and the stress defense



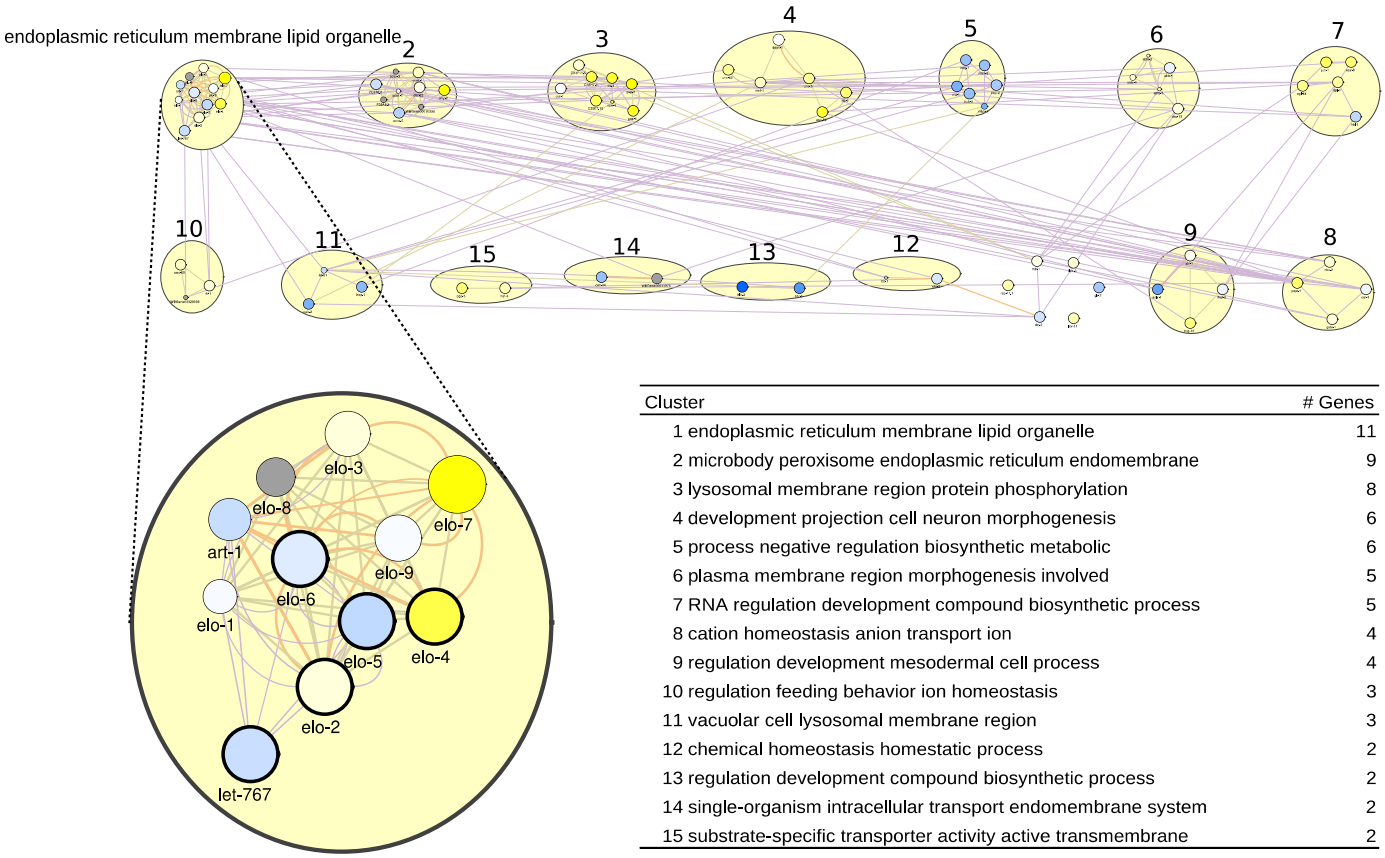
Cluster		# Genes
1	immune response process regulator cellular	9
2	cation transmembrane complex channel mitochondrion	6
3	dauer larval dormancy process biosynthetic	4
4	response oxygen-containing compound peptide hormone	4
5	regulation kinase cascade activity positive	4
6	complex substrate-specific channel metal ion	3
7	energy derivation oxidation organic compounds	3
8	enzyme binding DNA-templated regulation complex	3
9	response temperature protein cellular modification	3
10	RNA regulation biosynthetic positive	2
11	behaviour involved male mating turning	2
12	immune process regulation biosynthetic positive	2

Figure 2. A healthspan pathway map for *C. elegans*, based on Supplementary Tables 4, 5. See also Figure 1. Gene expression data reflect the effect of rapamycin [38].

of an organism including vitagenes, is able to promote healthy ageing in numerous ways [61]. This phenomenon, called hormesis, was held responsible for beneficial effects observed by many compound interventions [62–64]. More specific concepts, like mitohormesis which explains how reactive oxygen species can increase life- and healthspan [65] or the xenohormesis hypothesis which links evolutionary processes to the health-promoting abilities of plant-derived food [66] allow deeper insights into the entanglement of stress and health. In the Supplementary Results, the next-largest pathway/clusters, related to the mitochondrion, to dauer/dormancy, to regulation, and to hormone response are described in detail.

For *C. elegans*, we also derived a gene list from WormBase, taking the genes that are most differentially regulated by healthspan-extending interventions and, at the same time, are annotated with a sufficient number of GO terms (see Methods; Supplementary Table 8). We obtained the network of Figure 3. Curiously, the top healthspan pathways of 11, 9 and 8 genes are related to the endoplasmic reticulum (ER), lipid and membrane, to the peroxisome, macrobody and ER, and to the lysosome. The endoplasmic reticulum, the peroxisome and the lysosome are part of the endomembrane system, together with the mitochondria, contributing to healthspan and longevity in mammals and beyond [67]. Peroxisomal and lysosomal functions connect this pathway to dietary effects on lifespan [68, 69], and to liver disease [70]. The second tier of healthspan pathways (6 or 5 genes) are related to morphogenesis, biosynthesis and transcription.

For the WormBase data, the list of pathways/clusters, and the details of the largest pathway, are given in Figure 3, bottom. The ER/lipid-related pathway includes genes involved in fatty acid elongation/production (*elo-1* to *elo-9; let-767; art-1*). Overlaying the rapamycin gene expression data, the well-characterized *elo-1* and *let-767* genes show some downregulation. However, the importance of elongase genes for health maintenance in general was repeatedly documented. Vásquez and colleagues [71] demonstrated the impairment of touch response in *elo-1* mutants. They argue that elo-1 has a crucial role in the synthesis of C20 polyunsaturated fatty acids which are required for mechanosensation. Moreover, *elo-1* mutants showed increased resistance



Cluster	# Genes
1 endoplasmic reticulum membrane lipid organelle	11
2 microbody peroxisome endoplasmic reticulum endomembrane	9
3 lysosomal membrane region protein phosphorylation	8
4 development projection cell neuron morphogenesis	6
5 process negative regulation biosynthetic metabolic	6
6 plasma membrane region morphogenesis involved	5
7 RNA regulation development compound biosynthetic process	5
8 cation homeostasis anion transport ion	4
9 regulation development mesodermal cell process	4
10 regulation feeding behavior ion homeostasis	3
11 vacuolar cell lysosomal membrane region	3
12 chemical homeostasis homestatic process	2
13 regulation development compound biosynthetic process	2
14 single-organism intracellular transport endomembrane system	2
15 substrate-specific transporter activity active transmembrane	2

Figure 3. A healthspan pathway map for *C. elegans*, based on genes affected the most by healthspan-extending interventions, using WormBase gene expression data. See also Figures 1, 2.

to *Pseudomonas aeruginosa* infections due to the accumulation of gamma-linolenic acid and stearidonic acid [72] and knockdown of *elo-1* or *elo-2* extend survival during oxidative stress [73]. Finally, *art-1* is a steroid reductase that is downregulated by rapamycin in our case, but also in long-lived *eat-2* mutants [74]. In the Supplementary Results, the next-largest pathway/clusters, related to the ER, the peroxisome, the lysosome, morphogenesis, biosynthesis and transcription, are described in detail.

Overlap between human and *C. elegans* health genes and healthspan pathways

Based on reciprocal best orthologs, we found no direct overlap between the human health genes based on genetic associations and the *C. elegans* healthspan genes based in part on genetic interventions, but mostly on expert analysis of intervention effects (Figure 2), or on gene expression changes related to healthspan-extending interventions (Figure 3). We found some hints at an overlap on the level of the healthspan pathway annotations, considering that "proliferation" is listed for human, and "biosynthesis", "immune response", and "mitochondrion" for *C. elegans*, while "transcription" as well as "lipid" are found for both. Due to the post-mitotic nature of the adult *C. elegans*, proliferation processes have only minor impact on healthspan in *C. elegans*. In contrast, given that deregulated cell proliferation is the basis for cancer [75] and that cancer is one of the four main reasons for morbidity and mortality in humans according to the WHO (https://www.who.int/gho/ncd/mortality_morbidity/en/; status as of August 2019), it is not surprising that proliferation is a fundamental part of the human healthspan map. Furthermore, since *C. elegans* is usually fed on bacteria, which cause pathogenic stress in older nematodes [76, 77], the immune system is of particular importance for the health of nematodes. Finally, differences of the healthspan pathway maps regarding annotations such as "mitochondrion" could also be due to differences in how the underlying data were generated, in addition to species-specific differences.

Regarding lipids, for humans, specific reference is made to APOE/APOC (implicated in cholesterol metabolism); for *C. elegans*, specific reference is made to the elo set of genes (implicated in fatty acid elongation). The dysregulation of cholesterol and its different manifestations such as high- and low-density lipoprotein cholesterol (HDL-C and LDL-C) are one of the main causes for atherosclerotic cardiovascular diseases (CVD), a top ageing-related deadly disease [78, 79]. In contrast to mammals, *C. elegans* does not exhibit a heart or blood vessels and it cannot synthesize cholesterol by itself. Furthermore, a transgenic cholesterol-heterotrophic line lives 31% longer [80]. Another interesting difference is that cholesterol's main task in nematodes is probably not its role as a crucial membrane component, but rather its role as a signaling molecule [81, 82]. Further discrepancies regarding the function and regulation of lipids in humans and *C. elegans* are summarized in Mullaney and Ashrafi [83]. Nevertheless, and quite surprisingly, numerous key components, functions and regulatory pathways regarding lipid metabolism are indeed comparable in *C. elegans*: Similarities in the regulation of membrane fluidity [84], of fat depletion after consumption of oats [85], legumes [86], and fibrates [87] as well as after exercise [88], and in the genetic background of obesity [89–91] and fat storage [92] are only a few examples. The adult worm is post-mitotic [93] but also many human diseases and cell senescence processes are associated with tissues that no longer divide, e.g., in the brain [94, 95].

In search for other modes of overlap, we additionally constructed and compared two interaction networks, based on mapping genes to their respective orthologs in the other species. Each of the two interaction networks is based on the union set of the health genes of human (based in turn on genetics, Supplementary Tables 1–3, Figure 1) and of *C. elegans* (based in turn on the gene expression analysis of healthspan-extending interventions using WormBase, Figure 3). Specifically, as outlined in Figure 4, we added the *C. elegans* orthologs of the human health genes to the list of *C. elegans* health genes and *vice versa,* yielding two separate input gene lists for GeneMANIA to enable the construction of the two interaction networks, one per species. We used strict ortholog mapping rules (only reciprocal best hits were accepted). By design, the two gene lists feature a high degree of overlap (with differences due to missing orthologs), and their subsequent comparison, consisting of the partial network alignments that are based on ortholog mapping on the one hand and the species-specific network data on the other hand can only reveal hypotheses for common healthspan pathways, as long as explicit experimental evidence for a relation to health is only found for one species. Moreover, interaction points between a healthspan pathway with evidence in one species and a healthspan pathway with evidence in the other species may be revealed, if a partial alignment of the interaction networks consists of interacting genes for which the relationship to health was demonstrated only in one species for each pair of orthologs.

Of the two interaction networks to be aligned, the first network is based on *C. elegans* health genes, the *C. elegans* orthologs of human health genes, and *C. elegans* gene interaction information provided by GeneMANIA. The second network is based on human

health genes, the human orthologs of *C. elegans* health genes, and human gene interaction information provided by GeneMANIA. Despite using similar lists of genes (with differences due to missing orthologs and due to the genes added by GeneMANIA), we can expect that the two GeneMANIA networks are quite different because the interaction data sources employed by GeneMANIA are strongly species-specific. Moreover, we observe that in both cases, the 20 closely interacting genes added by GeneMANIA for one species included no orthologs of the other species. Nevertheless, to identify joint healthspan pathways and interaction points between healthspan pathways, we used GASOLINE [96] to align the two networks wherever feasible, obtaining two partial (subnetwork) alignments as output, as shown in Figure 5.

In the first alignment (Figure 5, left), we see an alternating pattern of demonstrated health-relatedness, since pak-2, sad-1 and pig-1 are considered health-related by gene expression analysis using WormBase, while CDKN2B and GSK3B are known to be human health genes (Supplementary Tables 1–3; GSK3B was implicated by a GWAS of the Healthy Aging Index, while CDKN2B was in fact one of the few genes implicated by two independent health studies). The *C. elegans* genes belong to three small clusters in the healthspan pathway map of Figure 3 (pak-2: lysosomal, sad-1: neural, pig-1: biosynthesis), while the human genes belong to one large (GSK3B: proliferation) and one small (CDKN3B: cyclin-dependent kinase) cluster in the human healthspan pathway map of Figure 1. Interactions in *C. elegans* are all based on shared domains (kinase signaling, except for the predicted interaction of gsk-3 and C25G6.3, which is based on the Interologous Interaction Database), while interactions in human are based on shared domains, genetic interaction (i.e., large-scale radiation hybrid) and pathway data. Essentially, the healthspan pathway overlap suggested by our analysis involves proliferation-related serine/tyrosine kinase signaling (pak-2/sad-1/pig-1 and PAK4/BRSK2/MELK), Wnt signaling (GSK3) and cyclin-dependent kinase signaling (CDKN2B). Both alignments are described further in detail in the Supplementary Results, and a functional analysis of the genes is given in Supplementary Tables 11, 12.

Given lists of genes, there is a plethora of possibilities to organize the genes into groups of related ones. Motivated by the idea of a "healthspan pathway", we hypothesized that the genes should be known to interact based on functional gene/protein interaction data (provided by GeneMANIA). Here, as in most other studies, pathways are not assumed to be linear [97]. The

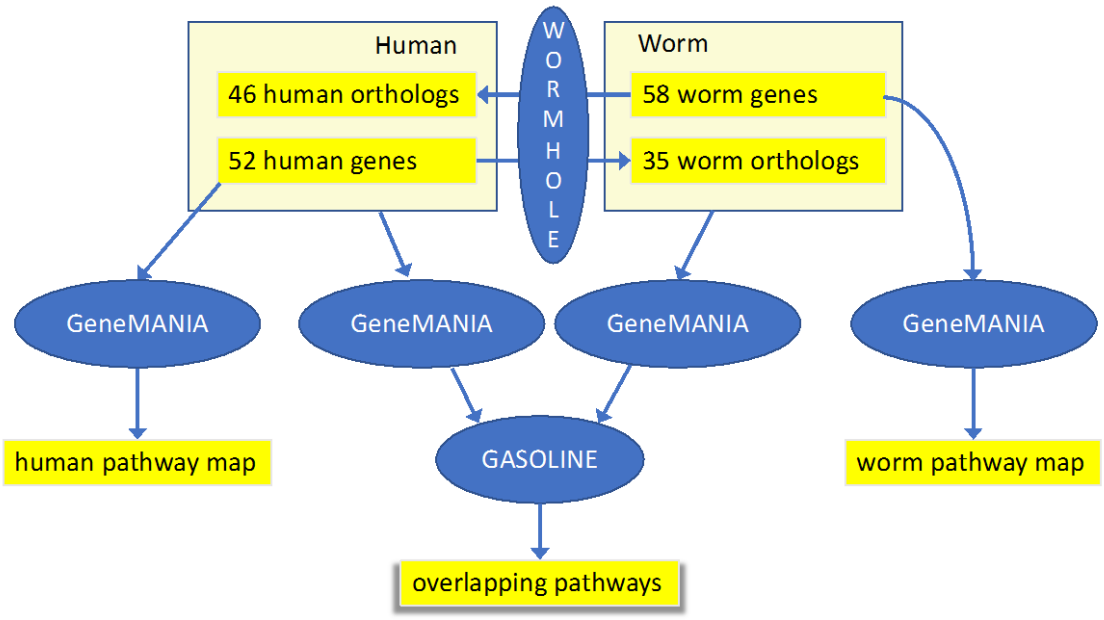


Figure 4. Workflow of the main analysis steps. First, 52 human health genes (Supplementary Tables 1–3) were processed with GeneMANIA and AutoAnnotate to determine the human healthspan pathway map (left, see also Figure 1). Analogously, 58 worm health genes (based on gene expression analysis using Wormbase) were studied, yielding the *C. elegans* healthspan pathway map (right, see also Figure 3). Then, to determine overlap across species, the gene lists were extended by the orthologs (calculated by WORMHOLE, see Supplemental Methods) from the respective other species. We then employed GeneMANIA as before, to generate two interaction networks (one per list). and overlaps between these two networks of health genes were determined by GASOLINE (middle, see also Figure 5).

(higher-level) interaction among the clusters/healthspan pathways (i.e., the pathway map) is given by the individual gene/protein interactions that are shown *between* the clusters in Figures 1–3. However, we did not investigate these further.

The small amount of healthspan gene/pathway overlap that we found may be seen from a pessimistic or an optimistic perspective, depending in part on expectations. From the pessimistic perspective, the molecular processes may be completely different, and the *C. elegans* orthologs of the human health genes are involved in different processes as compared to the human health genes, and *vice versa*. From the optimistic perspective, it may just be that the number and scope of the investigations that yielded the health genes we studied is still insufficient, annotations are still incomplete, and considering only reciprocal best orthologs may be too restrictive. (We tried a less restrictive mapping of orthologs by relaxing the condition that orthologs must be reciprocal, but the overlap was still negligible; results not shown). Nevertheless, future genetic studies are expected to yield more health genes in both species, and their characterizations are expected to improve. Moreover, when we analyze in detail the effects of intervention studies in *C. elegans*, we do find clear hints to some mechanisms that underlie healthspan also in human [98]. For example, changes in the Ins/IGF-1 pathway genes daf-2 and daf-16 are found to be associated with many of the features described in Supplementary Table 5, suggesting a fundamental role for immune defense mechanisms (and proliferation) in health maintenance, as described by Ermolaeva et al. [99].

Since *C. elegans* only exhibits an innate immune system and is missing the adaptive immune response, one could argue that the biological relevance of "immune response" in the *C. elegans* healthspan pathway map is negligible. However, the strict separation of the immune response into an innate and an adaptive system was questioned by Kvell et al. [100] and more recently by Penkov et al. [101], not least because of the discovery of the trained innate immune response [102]. Furthermore, the suitability of *C. elegans* as a model for the mammalian immune system and for pathogen response was summarized in several reviews [99, 103, 104]. Indeed, based on the expression of antifungal or antibacterial polypeptides in response to pathogenic stress, this nematode is used to find new antimicrobial drugs [105, 106]. Finally, it was demonstrated that immunosenescence, which is one of the most important healthspan parameters, affects the innate immune system in both organisms, nematodes [107–109] and humans [110].

Of course, the precise definition of phenotype is crucial. If the samples are not really about (lack of) health, in human or in *C. elegans*, then any subsequent molecular or bioinformatics analyses will compare apples and oranges and may thus fail. Therefore, it is important to use a good phenotyping of health in human as well as in *C. elegans,* and on this basis, to collect data as genome-wide as possible. For most of the age-related diseases that we use to define health in humans, there is no *C. elegans* counterpart. E.g., as *C. elegans* has no heart, it cannot have any heart diseases. In addition, the aging process that may underlie most of these age-related diseases is poorly characterized and hard to quantify in

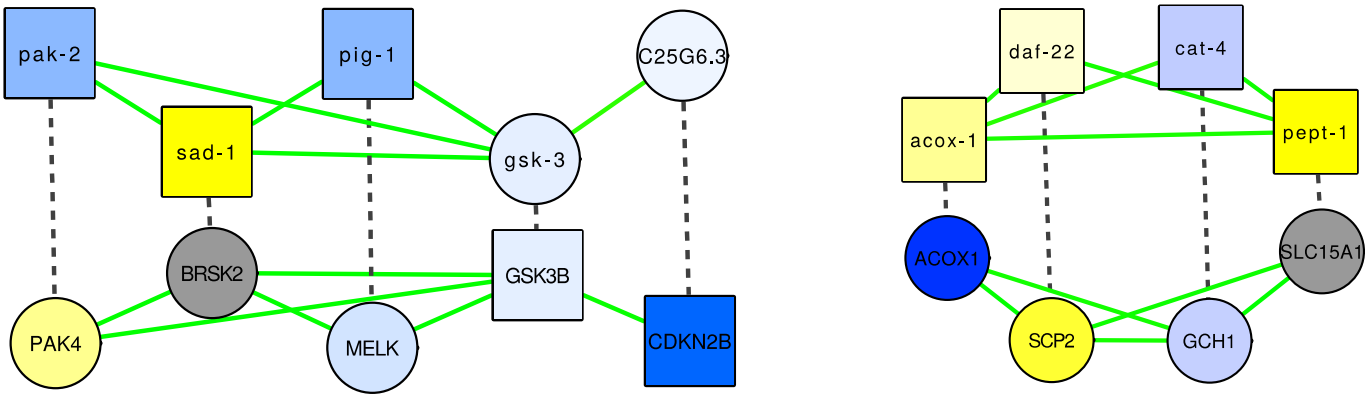


Figure 5. The two alignments demonstrating overlap of (putative) healthspan pathways in human and *C. elegans,* based on a GASOLINE alignment of the network of genes implicated in health-related gene expression changes in WormBase (top), and in human health based on genetic studies (bottom), and of corresponding orthologs. Dashed edges indicate orthologs, green edges indicate interactions based on GeneMANIA known for the respective species; the node shape is square if the gene originates from the original lists of health genes and it is circular if the gene is an ortholog, and node colors are based on gene expression changes triggered by rapamycin (in case of *C. elegans*) or by caloric restriction (in case of human), as in Figures 1–3.

humans. Nonetheless, locomotion degrades with age in both species, due to changes at the muscle as well as neural level. Two related features of physical function, that is, grip strength [111] and the ability to sit and rise from the floor [112] are good predictors of all-cause mortality in humans. Likewise, both in humans and in *C. elegans*, the ability to withstand various forms of stress decreases with age [113, 114]. Thus, at the level of organs or functional systems, both *C. elegans* and humans show age-related declines in performance, that may well be due to underlying processes that are similar at the cellular and molecular level. Moreover, the investigation of healthspan in *C. elegans* already identified additional ageing-related genes, e.g. for EGF signaling, which is known for its connection to ageing in mammals [115, 116]. Interestingly, in *C. elegans*, the EGF-regulator HPA-2 was identified by analyzing locomotion but not lifespan [117] highlighting the usefulness for phenotyping-assays distinct from lifespan. This is underlined by the observation that locomotion is impaired during ageing in mammals and *C. elegans* in a similar way [118].

Overall, we suggest that within the limitations of currently available data, the health genes we assembled, the healthspan pathways we constructed based on these, and the overlap we then found between species, are a first glimpse of the species-specific and cross-species molecular basis of health.

MATERIALS AND METHODS

Gene sets associated with health, literature-based

In this work, we conducted a semi-systematic review, including publications until 2018, using health, healthspan and healthy aging, for human and *C. elegans,* as search terms in Google Scholar, initially filtering for recent reviews and considering only the top hits. For humans, genetic studies of Supplementary Tables 1–3 are often not found using health-related keywords, so we included terms related to dysfunction (such as frailty) and disease (such as multi-morbidity) as well. For the genetics of human frailty, we identified two publications [28, 29]. Overall, a list of 52 genes (Supplementary Table 1, 12 genes; Supplementary Tables 2, 3, 40 genes) was taken as the starting point in humans. For the genetics of *C. elegans* health, we followed a similar approach (Supplementary Table 4). For compound interventions in *C. elegans*, we identified a specific set of recent reviews (see Supplementary Table 5). Overall, a list of 31 genes (Supplementary Table 4, 11 genes; Supplementary Table 5, 20 genes) was taken as the starting point in *C. elegans*. From the original publications and reviews, we extracted the gene names, using *iHOP* [119] to assign HUGO nomenclature names if necessary. In the Supplement, we describe in detail how a second set of health-associated genes in *C. elegans* was identified using WormBase.

Construction of maps of clusters/pathways

For all gene sets analyzed, we used the Cytoscape 3.5.1 application GeneMANIA [32], version 3.4.1, downloaded October 2017, with default settings, to create a functional interaction network that is complemented with the GeneMANIA default of 20 connecting genes. For clustering, and for annotating the clusters based on the "annotation name" column of GO annotations collected by GeneMANIA, we used AutoAnnotate [33] v1.2, downloaded October 2017, in Quick start mode to enable to "layout network to prevent cluster overlap", so that a map of disjoint clusters (i.e., healthspan pathways) was generated. This was supplemented by a second advanced annotation step to increase the "max. number of words per cluster label" to the largest possible value of 5. Cluster annotations were generated using WordCloud [120] v3.1.1, downloaded January 2018.

In the Supplement, we further describe in detail how we overlaid expression data onto the pathway maps, constructed the overlap of healthspan pathways in *C. elegans* and humans, and programmed the web presentation.

Data accessibility

The accompanying web presentation uses CytoscapeJS to present the pathways, which also offers all pathway maps for download that were exported from Cytoscape. All files contributing to the analysis and to the website are freely available from https://bitbucket.org/ibima/healthspannetworkscytoscapejs. The above described generation of pathway maps was performed manually by interacting with the respective tools. Genes can be selected via their cluster or by the GeneOntology terms they are annotated with. Any such selection of genes is referenced to the MEM [121] and g:Profiler [122] web services.

AUTHOR CONTRIBUTIONS

Study design: GF, WL, SM, NS. Collection of data: GF, NS, SM. Analysis of data: GF, NS, RK, SSe, HME, CJ, SS, IB, MH. Website: SM, PAd, JV. Manuscript writing: GF, NS, SM, AAC, RK, SSe, HME, LS, BW, FC, AB, PAn, HJG, DR, MB, LJ. All authors reviewed and approved the final manuscript.

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CONFLICTS OF INTEREST

Alan A. Cohen is founder and CSO at Oken Health. All other authors declare that they have no conflicts of interest.

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SUPPLEMENTARY MATERIALS

Supplementary Results

Human, largest healthspan pathway, further details

We further investigated the miRNAs that are statistically enriched in the largest healthspan pathway using the TFmir webserver [1]. Notably, hsa-mir-34 stands out as a regulator of the *Notch* genes, and it is implicated in cancer, intracranial aneurysm and heart failure (Supplementary Figure 1, Supplementary Table 9). Additionally, hsa-mir-30 regulates many genes of this healthspan cluster/pathway, including NOTCH2, and it is implicated in the epithelial-mesenchymal transition (EMT), cancer, heart failure and obesity. In fact, the EMT is known to be involved in kidney disease and cancer, mediated by Notch signalling [2–4]. It is also associated with human longevity [5]. Furthermore, miRNA 34 has been associated with schizophrenia [6], and miRNA 34a has been associated with Alzheimer´s Disease [7], and its upregulation was found 30 minutes after fear conditioning in the amygdala, transiently downregulating Notch signaling. Finally, according to Wikipedia's community annotation facilitated by miRBase and Rfam, hsa-mir-34 and hsa-mir-30 are both linked to cancer, see also [8, 9].

Human, second-largest healthspan pathway

The genes in the second-largest pathway/cluster, related to cell proliferation (with links to inflammation and apoptosis) feature downregulation as expected, affecting NFKB1, STAT1, STAT5a and GSK3B, with likely beneficial effects. Specifically, JAK/STAT pathway inhibition is considered to alleviate the cellular senescence-associated Zhang et al. [11] secretory phenotype and frailty in old age [10]. Furthermore, [11] demonstrated that the hypothalamus is important for the development of whole-body ageing in mice, and that the underlying basis involves hypothalamic immunity mediated by IKBKB (IkB kinase-b, IKK-b) and NFKB. Zhang et al. developed several interventional models and could show that ageing retardation and lifespan extension were achieved in mice by preventing ageing-related hypothalamic or brain IKBKB and NFKB activation. Further mechanistic studies revealed that IKBKB and NFKB inhibit gonadotropin-releasing hormone (GnRH) to mediate ageing-related hypothalamic GnRH decline, and GnRH treatment amends ageing-impaired neurogenesis and decelerates ageing. For the second-largest pathway, a miRNA enrichment analysis by TFmir highlights hsa-mir-146a, which interacts with NFKB1 and STAT1 in particular, and is implicated in many immunity-related diseases (Supplementary Figure 2, Supplementary Table 10). According to Wikipedia's community annotation, miR-146 is primarily involved in the regulation of inflammation and other processes related to the innate immune system, see also [12].

Human, third-largest healthspan pathway

The third-largest healthspan pathway/cluster features the strong downregulation of APOE, and to a lesser extent also of APOC1. The APO family proteins are all lipid transporters, and severe decreases are detrimental, as they lead to hypercholesterinemia [13]. On the other hand, APOE4 has been widely implicated in the formation of amyloid plaques in Alzheimer's Disease [14], and experimental downregulation showed a protective effect in an Alzheimer Disease mouse model [15]. A supplementary interpretation of the CR-related downregulation found for this healthspan pathway is that fasting reduces lipid load, and hence induces a downregulation of the corresponding transporter proteins.

Human, further gene expression data mapping

The three largest healthspan clusters/pathways were further investigated by mapping aging- and disease-related gene expression data onto them, as published or collected by [16], see Supplementary Methods. In the largest (Notch-related) healthspan pathway (see Supplementary Figure 3), gene expression changes in aging blood clearly show the expected upregulation of Notch genes and LRP1, and the same holds for skin except for Notch3. In the second-largest (proliferation-related) healthspan pathway (see Supplementary Figure 4), most genes are upregulated as expected; again, the signal is stronger in blood than in skin. Finally, the downregulation of lipid-associated genes by CR we observed in the third pathway (see Supplementary Figure 5) is matched by an upregulation of all 4 genes in blood as well as in skin, with the single exception of APOE in skin, the downregulation of which may impair wound healing, since it does so in mice [17]. Furthermore, we mapped disease-related gene expression changes onto the healthspan pathway map, including one cancer entity (pancreatic cancer), coronary disease and Alzheimer disease (AD), see Supplementary Figure 6. We found the genes in the Notch-related healthspan pathway upregulated most consistently in case of Alzheimer disease, whereas genes in the proliferation-related and the lipid-related healthspan pathways were upregulated most consistently in coronary disease. All three healthspan pathways discussed here consist mostly of genes that are, for higher values of gene expression, affecting health in negative ways; they are mostly downregulated by CR and upregulated by aging and disease.

C. elegans, next-largest healthspan pathways based on genetic/intervention data

In case of the mitochondrial and the hormone response cluster, the rapamycin-induced gene expression changes are not discussed since these are weak; in the *dauer*/dormancy cluster, the daf-16 inhibitor hcf-1 [18] is the most strongly downregulated gene. In the regulation cluster, the strongest changes consist of the downregulation of heat shock response genes hsp-16.41 and hsp-12.3.

C. elegans, next-largest healthspan pathways based on *WormBase* gene expression effect of intervention data

The ER/peroxisome-related pathway features upregulation of phy-2, daf-22 and acox-1. Specifically, phy-2 is essential for survival and embryonic development [19], while daf-22 catalyzes the final step in the peroxisomal β-oxidation pathway and is essential for *dauer* pheromone production [20], whereas acox-1 is essential for the prevention of fat accumulation [21]. The correlation of fat content and health maintenance was described several times: Fat accumulation in *C. elegans* was found to be decreased in phytochemically treated healthy nematodes [22] [23], during life-prolonging CR [24], or during increased autophagy [25]. Moreover, *ectopic* fat deposition is found in ageing worms and is discussed as a cause of ageing itself [26]. On the other hand, increased fat content was described in long-lived daf-2 mutants [27] and in germline ablated nematodes [28]. Moreover, acox-5 (aka drd-51), which is implicated in starvation-sensing and is downregulated by dietary restriction [29], is downregulated by rapamycin as well. The lysosome-related pathway is dominated by upregulated genes involved in fertility/development (gsp-3, gsp-4, frk-1 and spe-1). Finally, the six genes in the cluster related to morphogenesis are all upregulated (unc-52, involved in neuron differentiation [30] and muscle development [31], is upregulated the strongest), whereas the six genes in the cluster related to biosynthesis and transcription are all downregulated by rapamycin.

Overlap of healthspan pathways, first network alignment (Figure 5, left)

Notably, the serine/tyrosine kinases involved in the alignment are all known to be involved in proliferative processes, albeit in complex ways. The five kinases highlighted by our analysis include pro- and anti-proliferative genes, that is, tumor drivers as well as tumor suppressors. Control of proliferation is arguably the most important aspect of staying healthy, enabling stem cells to perform their function, while avoiding cancer. Accordingly, the independent expression data based on caloric restriction/rapamycin intervention data reflect that proliferation/biosynthesis is generally, but not completely, going down by pro-longevity interventions. Naturally, the phosphorylation status of these kinases would be more informative than their expression at the transcript level. Also, not much is known about the role of PAK4, BRSK2 and MELK in human health or aging. PAK4 is considered to protect cells from apoptosis [32], and a positive role in supporting stem cells is possible [33]. In context of cancer, however, upregulation of PAK4 has been associated with high-grade human breast cancer [34] and with malignancy in a variety of cancer cell lines [35], [36]. PAK4 can positively mediate cell survival and proliferation as well as enhance cell migration and invasion [35], [37], [38]. The inhibition of PAK4 reduced cell proliferation, migration and invasion of gastric cancer cells [35]. Further, depletion of PAK4 is considered to increase cell adhesion dynamics in breast cancer cells; due to its RhoU stabilizing function, it promotes the focal adhesion disassembly via phosphorylation of paxillin [34]. Furthermore, PAK4 modulates Wnt signaling by ß-Catenin regulation, increasing cell proliferation. Concordantly, the Wnt signaling pathway itself promotes intrinsic processes such as cell migration, hematopoiesis and cell polarity, and organogenesis during embryonic development [39], [40] . Concerning BRSK2, which is usually expressed in brain, testis and pancreatic tissue, an enhanced activity in response to DNA damage was reported [41], [42], [43]. In brain, BRSK2 is significant for proper regulation and formation of neuronal polarity in the developing nervous system [43], [44]. Finally, MELK as a stem cell marker is expressed in several types of progenitor cells and hematopoietic stem cells, and it plays key roles in cell cycle, embryonic development and in other crucial cellular processes [45] supporting stem cell function. In turn, upregulation of MELK has been associated with tumor progenitor cells of different origin and direct knock down of MELK leads to significant apoptosis induction [46]. In general, MELK is preferentially upregulated in cancer [47]. Overall, the overlap described highlights a cluster of genes held together mostly by shared protein domains in both species, with alternating evidence for their relation to health.

Overlap of healthspan pathways, second network alignment (Figure 5, right)

In the second alignment, all genes are considered health-related based on the *C. elegans* gene expression data in *WormBase*. The genes acox-1 and daf-22 are involved in the ER, peroxisome and microbody health cluster (see above), whereas the genes cat-4 and pept-1 were found to be related to ion transport and homeostasis (in a smaller cluster of the original healthspan pathway map, Figure 3). All four genes are differentially

regulated in a long-lived sir-2.1 overexpression strain [48] and in nematodes suffering from down-regulation of nhr-49, a key regulator of fat metabolism [49]. Moreover, differential translational regulation of cat-4, pept-1, acox-1, and daf-22 was observed in wild types during osmotic stress [50], underlining their role in health- and lifespan regulation. Interactions in *C. elegans* are all based on co-expression, while in human they are based on co-expression, co-localization and physical interaction (except for the interaction of SLC5A1 and GCH1, which is genetic). The independent gene expression data describing the effect of rapamycin in *C. elegans* are plausible for the ER/peroxisome genes acox-1 and daf-22 (see above). For cat-4, no data related to its role in health or survival is available, though the gene is involved in dopamine biosynthetic processes and is a target of the transcription regulator skn-1, which is known to be indispensable for proper stress response [51]. Finally, healthspan-promoting treatments like tannic acid [52], colistin exposure [53], or life-prolonging fasting [54] were shown to induce pept-1 transcription. The expression data in case of human, reflecting caloric restriction effects, are matching expectations (SCP2), are not available (SLC15A1), or are of unknown significance (ACOX1, GCH1; see also [55]). Overall, the overlap described here highlights a cluster of genes held together mostly by co-expression in both species, with a demonstrated relation to health in *C. elegans* only.

Supplementary Discussion

Biological interpretation of the lack of evolutionary conservation

In some sense, the lack of overlap between healthspan pathways in *C. elegans* and humans should not be surprising, and relates to our definition of health as the absence of undesirable conditions (that is, disease and dysfunction). Biologically speaking, each such undesirable condition may have its own etiology, or may partially share an etiology with others, such that depending on environmental factors the prevalence may vary greatly. For example, heart disease appears to be largely absent in Tsimane hunter-gatherers [56], but is a major cause of mortality in modern societies. Any heart disease pathway would thus have a major impact on healthspan in modern societies, but not in the Tsimane. Similar challenges apply to the comparison of healthspan pathways across modern populations as well [57]. It is thus to be expected that healthspan pathways will differ not just across distantly related species, but also among populations of a given species, depending on the environmental factors that push some pathways to more or less important roles in determining healthspan.

Of course it is still possible that there are shared healthspan pathways that operate across populations and species. Indeed, the conservation of genetic pathways related to aging (mTOR, sirtuins, insulin signaling, etc.) [58], [59] strongly implies the existence of shared healthspan pathways, since it is expected that these known aging pathways are also healthspan pathways. The more interesting question is thus whether there might be conserved healthspan pathways that are not also lifespan pathways: pathways that affect health much more than survival. The preliminary answer from this study is that there are few, if any, though we must consider that variation in causes of healthspan deterioration across populations and species might hide some more subtle effects.

From an evolutionary perspective, the question is how selection might act to create and maintain pathways that regulate healthspan. In the case of lifespan, it has been suggested that conserved pathways regulate a mechanism to allow individuals to put reproduction on hold during lean times, increasing lifespan at a cost to reproduction (a "trade-off"), and leading to diverse downstream mechanisms of aging with a shared control switch [60]. One possibility is that healthspan might undergo a similar trade-off, with individuals sacrificing reproduction in order to maintain health, or vice versa, though there is not yet evidence one way or another. If such a trade-off were facultative (i.e., regulated within the lifespan of an individual), we should see variation in gene expression across individuals even in the absence of allelic variation. If it were an obligate trade-off, we might see allelic variation in healthspan pathways. Allelic variation in healthspan could thus either imply (a) that there is some unknown benefit, through the trade-off, to having a shorter healthspan; or (b) that the population is not at evolutionary equilibrium, i.e. is in an environment for which healthspan regulation has not been optimized [61].

Supplementary Methods

Gene sets associated with health, based on WormBase differentially expressed genes

The *basic search* for *expression clusters* in WormBase (http://www.wormbase.org/species/c_elegans/expression_cluster#1-0-5) was used (status: 13th December 2017), to find transcriptomic data for healthspan-promoting compounds in *C. elegans*. For this purpose, the search term "treated OR treatment OR exposure" was used, which resulted in a total of 323 expression clusters comprising about 100 different chemical, physical, and biological treatments of various kinds (differing by exposure time or dosage, and including RNAi treatment). In order to focus on small molecules,

only studies with RNAi-untreated wild type animals were selected. The treatment had to lead to at least one enhanced health-related endpoint such as stress resistance or locomotion. The data sets covered the following substances (with results each described in an accompanying WormBase paper): Allantoin (WBPaper 00048989), astaxanthin (WBPaper00049979), cocoa-peptide 13L (WBPaper00042404), colistin (WBPaper 00045673), 2-deoxy-D-glucose (WBPaper00044434 and WBPaper00031060), garlic extract (WBPaper00046741), hydrogen sulfide (WBPaper00040285), lithium (WBPaper00046415), quercetin (WBPaper00040963), rapamycin (WBPaper00048989), resveratrol (WBPaper00026929), rifampicin (WBPaper00046496), and tannic acid (WBPaper00040963). All differentially expressed genes (DEGs) in the selected gene expression studies were compiled and duplicates were deleted, resulting in 11312 genes that are mentioned in at least one data set. For all genes annotated to at least one GO term (based on the ontology browser in WormBase), the exact number of associated GO terms was determined, by entering all 7646 genes in the search field of the "MGI Gene Ontology Term Finder" (http://www.informatics.jax.org/gotools/MGI_Term_Finder.html). The number of GO terms per gene was counted, and the count of each gene in all DEG lists (regulated, up-regulated only or down-regulated only, respectively) was determined. Finally, all genes were chosen which appear in at least four DEG lists in total or in at least three lists of up-regulated DEGs or in at least three lists of down-regulated DEGs, and which are annotated to at least 14 GO terms. These filter criteria were used to yield a manageable number of annotated genes; the resulting list of 58 genes was then used further. For acox-1.1 and acox-1.5, their alternative nomenclature names acox-1 and acox-5 were used.

Overlaying of expression data onto pathway maps

We searched the GEO (Gene Expression Omnibus) database in December 2017 for datasets/series where effects on healthspan or healthy aging in human or *C. elegans* were actually observed following an intervention. We found two gene expression series describing the effects of caloric restriction, or its mimetic rapamycin, that featured at least 3 replicates, as follows. For *C. elegans,* from GSE64336, "Expression data of worms under different caloric restriction mimetic treatments", we selected 1) wild type versus 2) rapamycin treatment, since the accompanying paper [62] claimed the largest number of differentially expressed genes for this compound (in comparison to the other compound tested, allantoin). For humans, from GSE38012, we selected all 25 samples, 1) Western diet versus 2) caloric restriction diet (for both series, we checked the box plots but we found no outlier distribution of expression values for any sample). We then used the GEO2R tool [63] to compute fold-changes using default parameters, downloaded the resulting tables, imported these into Excel (using "Text" column format for the gene names), removed genes with logFC equal to NaN and sorted, smallest to largest, by absolute fold change so that for genes with more than one probe, the probe with the largest fold-change is taken when the table is imported using Cytoscape. Selecting the "Gene.symbol" column as key column of the table and selecting the "gene name" column created by GeneMANIA as the "Key column for network", we established matching gene names (in case-insensitive mode) in the GEO2R and GeneMANIA tables as the common reference, to then import the tables into Cytoscape. Finally, we adjusted the "Style" of the resulting networks so that the logFC values from GEO2R are mapped continuously to a yellow-blue color scale with the appropriate max/min settings, adding a handle to map a logFC of 0 to white.

Further, we took [16] as reference publication for aging- and disease-related datasets. We took the human aging data published alongside the article, contrasting blood and skin in 24-29 and 45-50 versus 60-65 and 75-80 year-old humans. We took publicly available disease-related datasets listed in Supplementary Table 5 of [16]: for cancer we selected pancreatic cancer (GSE28735) as the only entity with paired data available at GEO; as cardiovascular disease we selected coronary artery disease as the only entity with paired data (taking plaque biopsy data rather than blood), and for neurodegenerative disease, we took Alzheimer Disease data based on brain biopsies. In the latter two cases we chose the tissues directly affected by the respective disease.

Overlap of healthspan pathways

Figure 4 (middle) summarizes the overall approach. The human health-related gene list based on Supplementary Tables 1-3 (Supplementary Table 6) and the health-related gene-expression-based gene list from wormbase (Supplementary Table 8) were investigated jointly. More specifically, both lists were submitted to Wormhole (wormhole.jax.org) on Jan 29, 2018, with "Limit results to ortholog pairs" set to "Do not filter (keep all results)" and with the "Reciprocal best hits (RBHs) only" option, to map from human to *C. elegans* and from *C. elegans* to human, respectively. The two resulting tables were downloaded, and the ortholog genes were used to obtain two new gene lists: one list consisting of the human health gene list from Supplementary Tables 1-3 supplemented with the human orthologs of the *C. elegans* genes implicated by gene expression in WormBase, and a second list

consisting of the health-related gene-expression-based *C. elegans* gene list supplemented with the *C. elegans* orthologs of the human health genes. Both new gene lists were submitted to GeneMANIA with default parameters[1], and the two GeneMANIA reports were exported[2]. From the two GeneMANIA reports, the two interaction networks and the two new lists of genes/nodes in the network were extracted. As input for the GASOLINE network aligner [64], each network was then written to a text file, and a single table of ortholog mappings was created by (a) submitting each of the two new lists of genes/nodes to Wormhole (with parameters as above), converting the "WORMHOLE Score" from 0 (worst) to 1 (best) into an E-value-like score as expected by GASOLINE (using the ad-hoc formula E-Value-substitute=1/WORMHOLE_Score*1E-20, which results in values roughly the same as given in the BLAST-based tables offered by the GASOLINE website as sample input data), and (b) concatenating both Wormhole ortholog tables into a single text file. Submitting the two network files[3] and the single table of ortholog mappings to GASOLINE with default parameters resulted in no alignment of subnetworks, but changing the GASOLINE "density threshold" from 0.8 to 0.5 resulted in the two alignments presented. Finally, the gene expression data describing effects of caloric restriction and of rapamycin were both imported, mapping the expression data to the alignments, and setting node colors, all as described above. Whenever data were processed by Excel, column formats of gene names were set to "Text".

All genes displayed in Supplementary Tables 1-5 and those derived from WormBase gene expression data are summarized in Supplementary Tables 6–8, including GenAge information [65] (https://genomics.senescence.info/genes/; GenAge Build 19; release date: June 24, 2017), if available.

The **web presentation** accompanying this paper employed Cytoscape version 3.6.1 to export the networks and its views as a CytoscapeJS object, employing a library used in version 3.29 (http://js.cytoscape.org) together with an Apache 2 web server [66]. The dynamic highlighting of genes and GO terms in the pathway was implemented in JavaScript. The transcriptional profile of user-selected genes can be inspected in GEO expression data aggregated by the multi-experiment matrix (MEM, https://biit.cs.ut.ee/mem/) [67]. Queried with single genes, the MEM service shows all the transcriptomics experiments of a selected platform and, underneath, all the genes with which the query gene is correlating in its expression [68]. The resulting list is ranked and differences between experiments with respect to the observed correlation are indicated graphically. When queried with a set of genes, specifically with all genes of a healthspan pathway, only correlations of transcripts assigned to these genes are shown. This tells us, in which experiments the genes included in our healthspan pathways interact, and for which conditions there is no concerted action of the healthspan-associated genes. One can thus obtain a characterization of a healthspan pathway in the light of a large set of gene expression experiments.

[1] which ignores some duplications introduced into the lists by a wormhole bug; non-nomenclature gene names for IL-6 and IL-12 were processed correctly.

[2] due to a GeneMANIA feature, reports for the same input gene lists may vary slightly in the last decimal places of some of the scores.

[3] first the C. *elegans* and then the human network; uploading networks the other way around results in slightly different output due to a GASOLINE feature.

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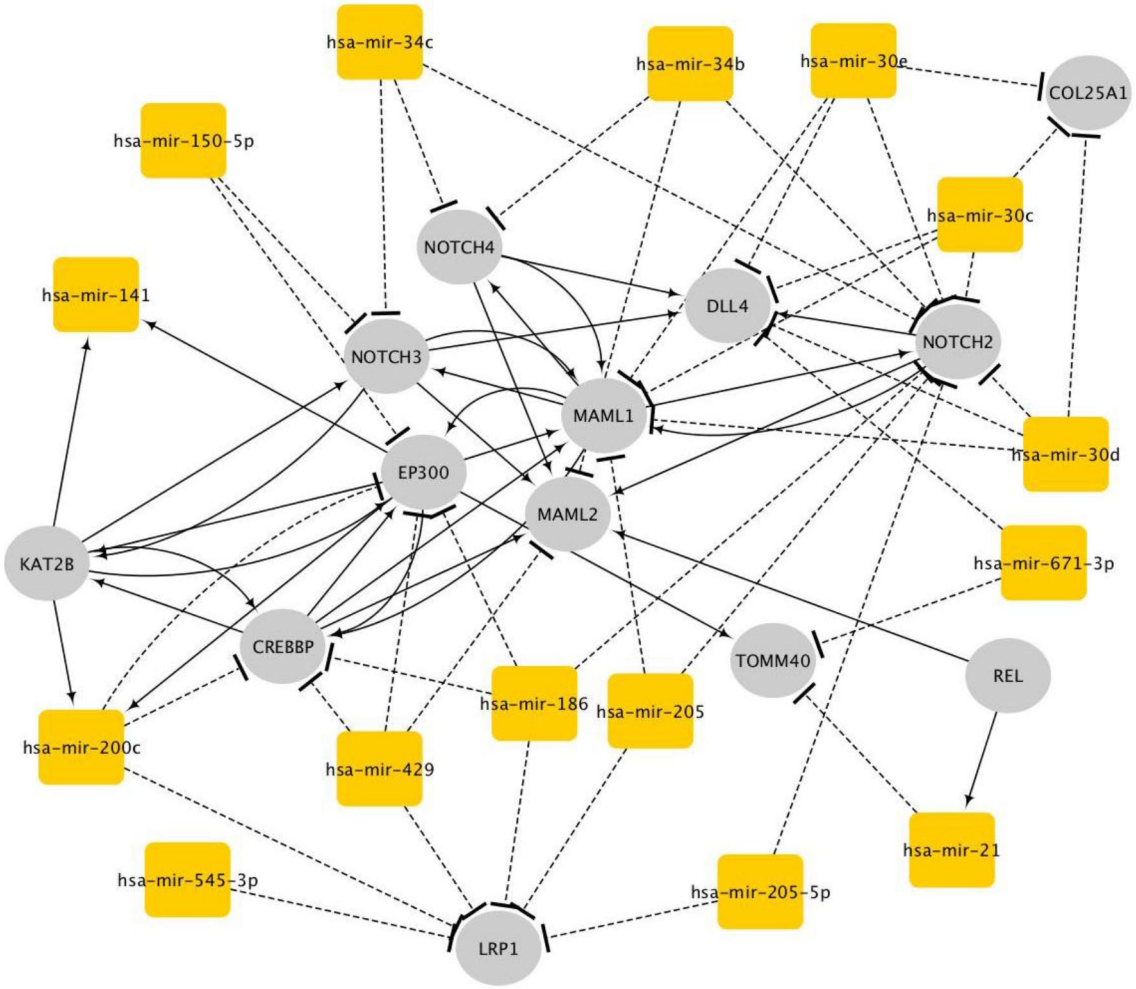
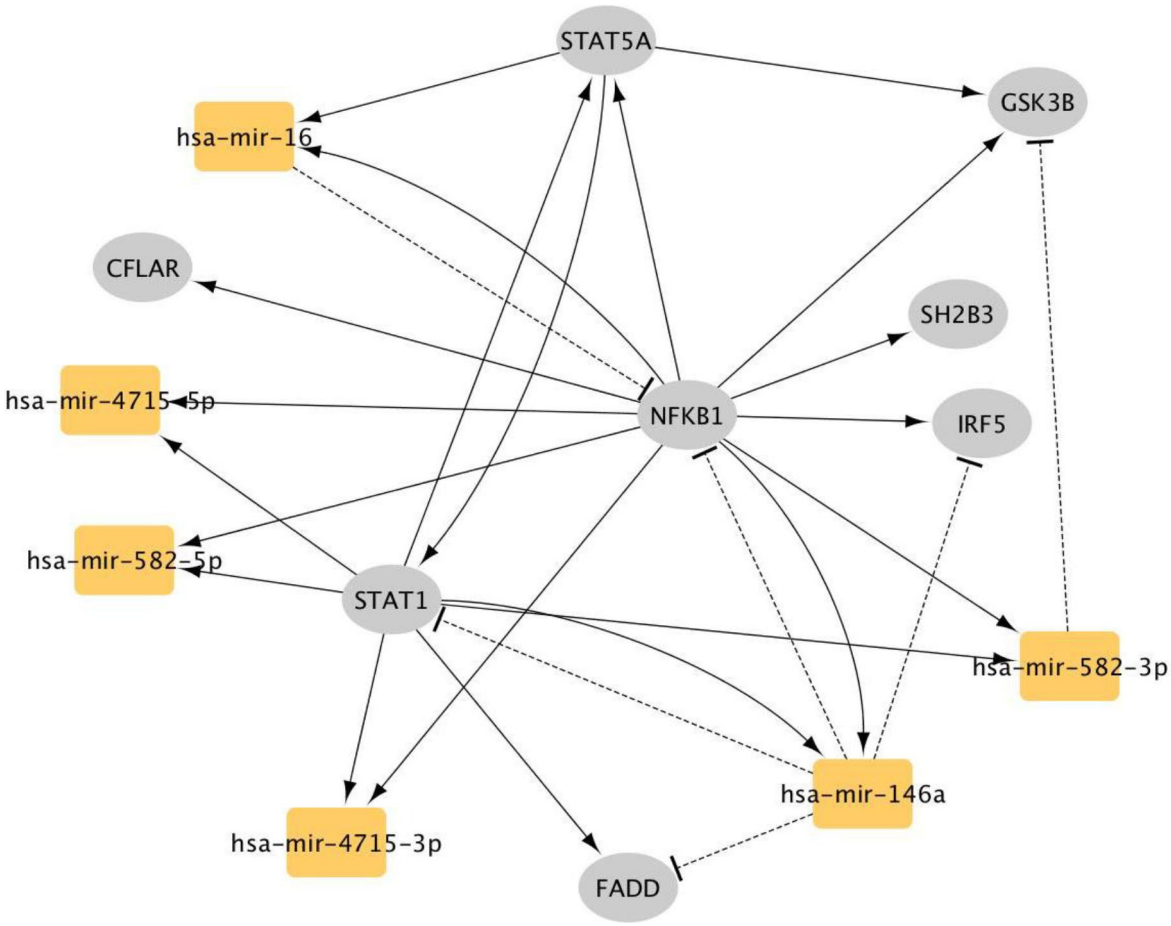
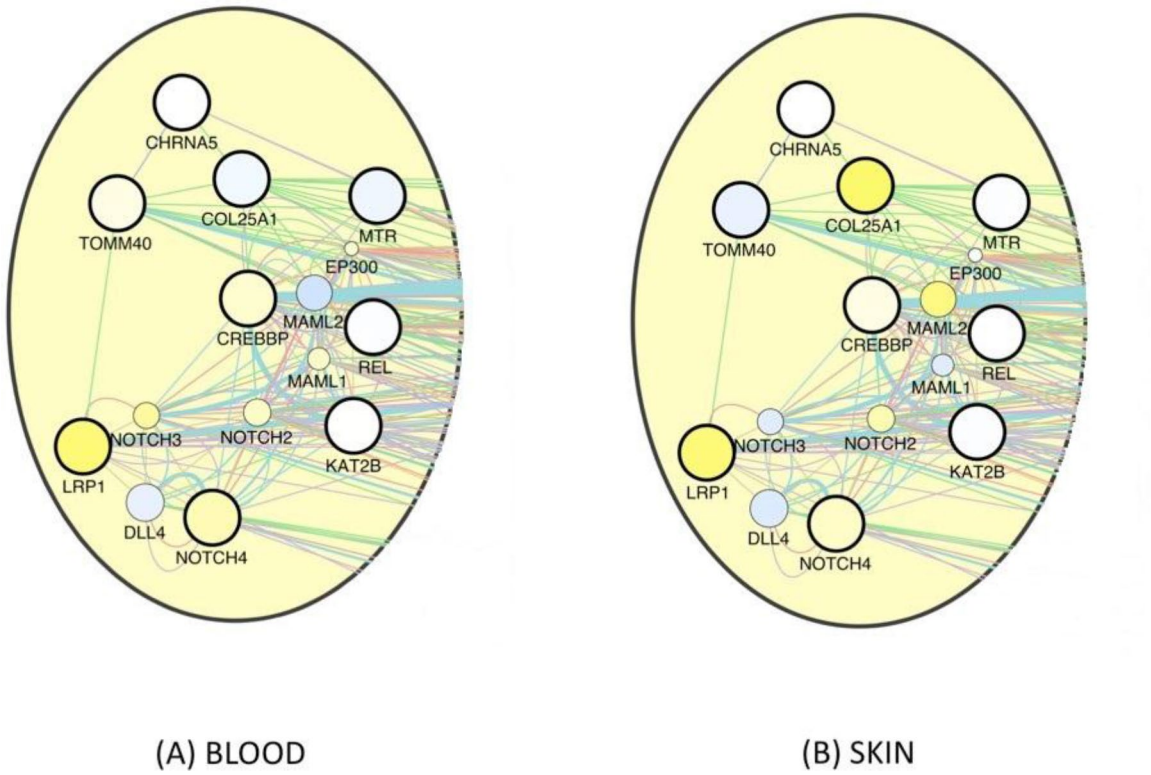
Supplementary Figures



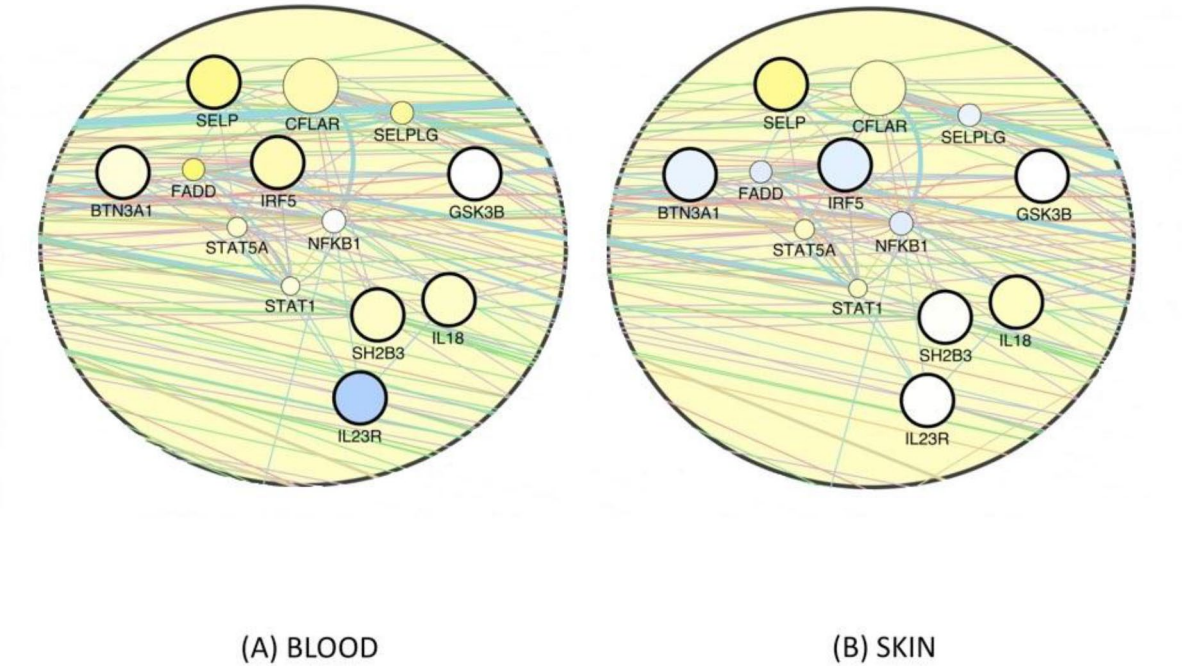
Supplementary Figure 1. The regulatory interactions between the largest human healthspan pathway and the corresponding enriched miRNAs.



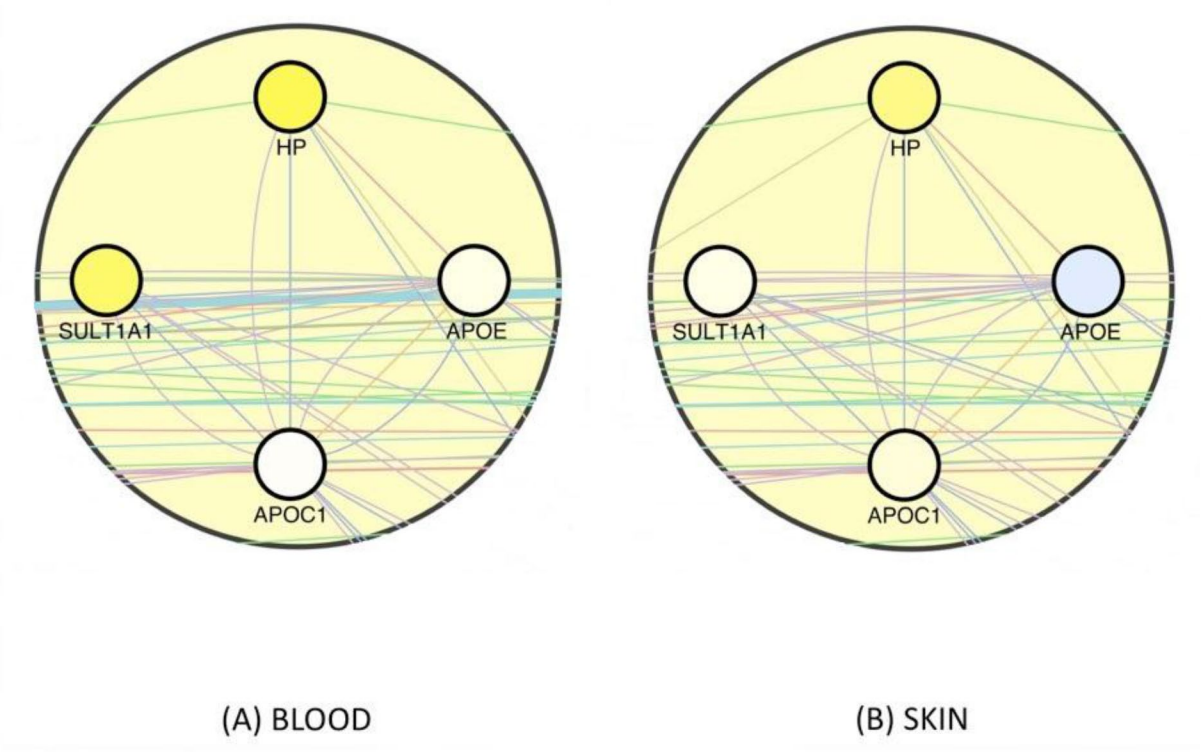
Supplementary Figure 2. The regulatory interactions between the second-largest hu-man healthspan pathway and the corresponding enriched miRNAs.



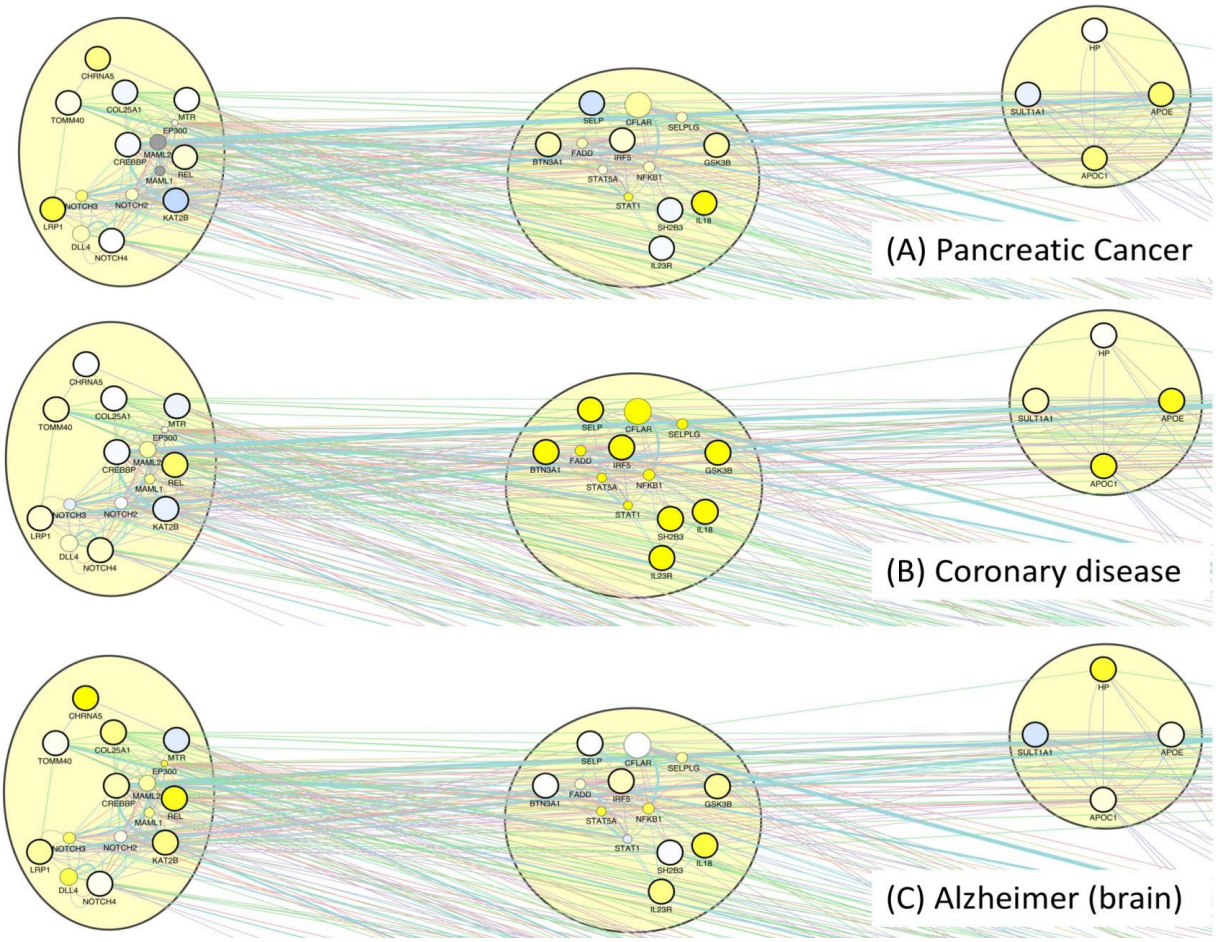
Supplementary Figure 3. Comparison of expression patterns in two aging tissues, largest human healthspan pathway.



Supplementary Figure 4. Comparison of expression patterns in two aging tissues, second-largest human healthspan pathway.



Supplementary Figure 5. Comparison of expression patterns in two aging tissues, third healthspan pathway.



Supplementary Figure 6. Comparison of expression patterns in three disease-affected tissues, top 3 healthspan pathways.

Supplementary Tables

Supplementary Table 1. Features of human health, lack of dysfunction(s), and associated genes.

Feature	Associated gene(s)	Reference	Remarks
Healthy Aging Index, defined by systolic blood pressure, pulmonary vital capacity, creatinine, fasting glucose, and Modified Mini-Mental Status Examination score	ZNF704	[69]	LLFS (Long life family study) cohort, suggestive association nominally replicated in CHS and FHS
Healthy Aging Index	GSK3B	[70]	candidate gene sequencing in LLFS cohort
grip strength, gait speed, physical activity, FEV from exceptional survivors	NBPF6	[71]	LLFS cohort, replicated in Health ABC cohort; phenotype defined based on first principal component of 28 physiologic measurements
lack of frailty	IL-18 IL-12A LRP1 SELP	[72]	association analysis of 620 polymorphisms with a frailty index (deficit count) in the English Longitudinal Study of Ageing; SNPs did not show genome-wide significance
lack of frailty	MTR CASP8 CREBBP KAT2B BTRC	[73]	association analysis of 1354 polymorphisms with a frailty index (deficit count) in Women's Health and Aging Studies I and II; SNPs were not significant genome-wide

Supplementary Table 2. Features of human health, (lack of) multiple diseases, and associated genes.

Feature	Associated gene(s) / pathways	Reference	Remarks
no chronic diseases (by list of ICD codes) and no chronic medications	* BTN1A1, BTN3A1 (MHC locus) * SLC22A4 (→carnitine) * KCNE4 * COL25A1 (→amyloid deposition?)	[74] (Wellderly)	whole-genome sequencing; molecular correlate SNPs were not significant genome-wide; sample (e.g., education) bias is likely [75]; no replication was done; COL25A1 was found based on rare variants
candidate SNP identification by disease (atrial fibrillation, cancer, coronary heart disease, diabetes, heart failure, stroke, death) in one study; *candidate confirmation* by the same, and also by other disease (neuronal, vascular, psychiatric and inflammatory), in 5 additional studies	* MAML3 * SEMA5B * TCF7L2 * ZFHX3 * TMPRSS2	[76]	genes based on summary statistics of disease
never diagnosed with cancer, cardiovascular disease, dementia, diabetes or major pulmonary disease	* APOE * HP (haptoglobin) → lipid and cholesterol maintenance	[77]	targeted genotyping
macular degeneration, multiple sclerosis. menopause onset, rheumatoid arthritis, systemic lupus erythematosus	SLC44A4	[78]	expression QTLs interacting with age implicated in multiple diseases
psoriasis, diabetes	HLA-DQA2		
Alzheimer dementia, cardiovascular disease	* Cholesterol transport endocytosis * Immune response	[79], cf. [80]	overlap of enrichment in GWAS pathway analyses
clustering of 372 disease polymorphisms in the genome	* NOTCH4 * CDKN2B (p15INK4b), CDKN2A (p16INK4a/p14ARF), *CDKN2BAS (ANRIL)* * IL23R * REL * TERT * IRF5, TNPO3 * IKZF3, GSDMA, GSDMB	[81]	meta-analysis of SNPs in age-associated diseases

Supplementary Table 3. Features of human health, lifespan/longevity mediated by lack of disease, and associated genes.

Feature	Associated gene(s) / pathways	Reference	Remarks
exceptional human longevity, weighted by chronic kidney disease, bone mineral density, LDL, triglycerides, total cholesterol	* APOE, TOMM40 (→Alzheimer's disease), * CDKN2B, *ANRIL* (→cellular senescence), * ABO (→ O blood group), * SH2B3, ATXN2	[82]	diseases/dysfunctions/traits used for weighting selected for their significant genetic overlap with longevity; "many of the SNPs found by iGWAS showed an association for not one, but many diseases which seem to have distinct etiologies"
parental lifespan mediated by participant's risk factors known to be disease-related, particularly education level, LDL, HDL, BMI, smoking, coronary artery disease, diabetes, schizophrenia, height, triglycerides, glucose	* RBM6 * SULT1A1 * CHRNA5 * BSND * CELSR2 * TRAIP * C5ORF67 * *FTH1P5 (pseudogene)* * LPA * *CDKN2BAS (ANRIL)* * NPIPB8 * FTO * APOC1	[83]	based on UK biobank data; using offspring genotypes as proxy for parental genotypes; replication in five independent longevity studies; the paper does not specify which genes are involved in lipid metabolism claimed to be enriched; SNPs in RBM6, SULT1A1, CHRNA5 are "nearby" brain expression QTLs

Supplementary Table 4. Features of *C. elegans* health, based on genetic studies of health, and associated genes.

Feature	Associated gene(s) / pathways	Reference	Remarks
(stimulated) locomotion	hpa-1, hpa-2, let-23, plc-1, itr-1 (→ EGF pathway)	[84]	
	ahr-1	[85]	
	* C15H9.7 (kynu-1) (also Alzheimer pathology delay) * iglr-1 * tsp-3 (→ hypoxic response, by pathway interaction) * rcn-1 (rcan-1) (→ mTOR, by pathway interaction) * unc-36 (→ mTOR, by pathway interaction)	[86]	association with stimulated locomotion *("motivated* movement") and thrashing, based on candidate genes of *C. elegans* orthologs of human genes differentially expressed with age

Supplementary Table 5. Features of *C. elegans* health, based on compound intervention studies affecting health, and associated genes.

Feature	Associated gene(s) / pathways	Reference
stress resistance	**eat-2, skn-1**	cf. [87]
thermal stress resistance	**ahr-1**	[85]
	osr-1, unc-43, sek-1	cf. [87]
	akt-2, mev-1, nhr-8	
	sir-2.1	
	age-1, skn- 1, mek-1	
	daf-2, daf-16, hsf-1, sod-3, hsp-12.3	
oxidative stress resistance	**osr-1, unc-43, sek-1**	cf. [87]
	sir-2.1	
	age-1, skn- 1, mek-1	
	daf-2, daf-16, hsf-1, sod-3, hsp-12.3	
	sek-1, daf-16, eat-2, mev-1	
	daf-2, akt-2, mev-1, nhr-8	cf. [88]
locomotion	* ins-1 (Ins/IGF-1)	cf. [89]
	* pdk-1 (→ pyruvate metabolism)	
	* hsf-1, skn-1 (nrf2)	
	daf-2, daf-16, **hsf-1,** sod-3, hsp-12.3	cf. [87]
	tph-1	
pharyngeal pumping	**ahr-1**	[85]
	akt-2, mev-1, nhr-8	cf. [87]
	sir-2.1	
	tph-1	
	daf-2, daf-16, hsf-1	
	ets-7	[90]
	age-1, **skn-1,** mek-1	cf. [89]
reproduction	eat-2	cf. [87]
	tph-1	
	*** Ins/IGF-1**	cf. [89]
	*** sirtuins**	

Supplementary Table 6. Human genes associated with health, listing all genes from Tables 1–3.

Human gene	GenAge information (Tacutu et al., 2018)
ZNF704	-
GSK3B	Target of genes previously linked to ageing
NBPF6	-
IL-18	-
IL-12A	-
LRP1	-
SELP	-
MTR	-
CASP8	-
CREBBP	Regulation or control of genes previously linked to ageing
KAT2B	-
BTRC	-
BTN1A1	-
BTN3A1	-
SLC22A4	-
KCNE4	-
COL25A1	-
MAML3	-
SEMA5B	-
TCF7L2	-
ZFHX3	-
TMPRSS2	-
APOE	Linked to human longevity and/or multiple age-related phenotypes
HP	-
SLC44A4	-
HLA-DQA2	-
NOTCH4	-
CDKN2B	Directly linked to ageing in a cellular model system
CDKN2A	Directly linked to ageing in a cellular model system
IL23R	-
REL	-
TERT	Directly linked to ageing in a cellular model system
IRF5	-
TNPO3	-
IKZF3	-
GSDMA	-
GSDMB	-
TOMM40	-
ABO	-
SH2B3	-
ATXN2	-
RBM6	-
SULT1A1	-
CHRNA5	-
BSND	-
CELSR2	-

TRAIP	-
C5ORF67	-
LPA	-
NPIPB8	-
FTO	-
APOC1	-

Supplementary Table 7. *C. elegans* genes associated with health, listing all genes from Tables 4, 5.

C. elegans gene	GenAge information (Tacutu et al., 2018)
hpa-1	Median lifespan is 30% higher in mutants
hpa-2	Median lifespan is 15% higher in mutants
let-23	19% decrease of median lifespan and 8% decrease of maximum lifespan in reduction-of-function mutants; 29% increase of median lifespan and 9% increase of maximum lifespan in gain-of-function mutants
plc-1	-
itr-1	Increased ITR-1 activity extends median and maximum lifespan (53% increase of median lifespan, 29% increase of maximum lifespan); reduced ITR-1 activity shortens culture survival (i.e., -11% increase of median lifespan, -31% increase of maximum lifespan)
ahr-1	-
C15H9.7	-
iglr-1	-
tsp-3	-
rcn-1	-
unc-36	-
ins-1	Increased dosage increases lifespan by 25%
pdk-1	Loss-of-function alleles extend lifespan by 60%
hsf-1	Transgenic overexpression-mutants live longer (median lifespan ~50% higher) and are more thermotolerant; RNAi resulted in a 74% decrease in median lifespan in daf-2 mutant background and a 45% decrease in lifespan in daf-2/daf-16 double mutant background
skn-1	RNA interference or mutations prevented the life-extension effects of dietary restriction; mean lifespan is 5-20% higher after overexpression
daf-2	Mutations double adult lifespan; post developmental RNAi resulted in a 79% increase in mean lifespan
daf-16	Average lifespan is 45% lower by using RNAi
sod-3	-
hsp-12.3	-
tph-1	-
akt-2	-
mev-1	Mutants had a decreased lifespan, are hypersensitive to raised oxygen concentrations, and their lifespan decreases dramatically as oxygen concentrations increase
nhr-8	Median lifespan is up to 35% lower in mutants
sir-2.1	Overexpression extends lifespan uo to 50%; sir-2.1(ok434) mutants show a slight decrease in lifespan as well as sensitivity to various stresses
ets-7	-
age-1	Maximum and average lifespan are up to 10-fold greater in mutants
mek-1	-
eat-2	Mutations result in partial starvation by disrupting the function of the pharynx and an approximately 50% extension of lifespan
osr-1	-
unc-43	-
sek-1	-

Supplementary Table 8. *C. elegans* genes associated with health, listing genes based on WormBase gene expression data.

C. elegans gene	GenAge information (Tacutu et al., 2018)
hop-1	-
frk-1	-
itr-1	Increased ITR-1 activity extends median and maximum lifespan (53% increase of median lifespan, 29% increase of maximum lifespan); reduced ITR-1 activity shortens culture survival (i.e., -11% increase of median lifespan, -31% increase of maximum lifespan)
jun-1	-
sad-1	-
unc-43	-
nhx-2	RNAi led to a loss of fat stores in the intestine and a 40% increase in lifespan
hlh-2	-
rab-11.1	-
mlk-1	-
pept-1	Deletion results in retarded development, reduced body size, and extended reproductive lifespan; it also further extends (60%) the life-extension caused by daf-2 mutations
pig-1	-
rig-6	-
let-767	-
egl-44	-
abts-1	-
elo-2	RNAi resulted in lifespan extension (mean lifespan 9% higher)
daf-22	-
pak-2	-
stn-1	-
mig-10	-
unc-68	-
unc-52	RNAi in adulthood extended mean lifespan by 11%
C35E7.10	-
nhr-8	Median lifespan is up to 35% lower in mutants
gsp-4	-
elo-6	-
gsp-3	-
C18H7.4	-
ilys-3	-
C34F11.5	-
elo-5	RNAi decreased median lifespan by 45% in wild type animals and 29% in daf-2 mutants
elo-4	-
bub-3	6% mean lifespan extension by using RNAi
unc-5	-
pgp-5	-
rhgf-2	-
hbl-1	-
ima-2	-
gsto-1	-
acly-1	-
mtm-6	-

mrg-1	-
rbr-2	rbr-2(tm1231) strain displays reduced longevity; rbr-2(ok2544) strain exhibits longer mean and maximum lifespan, both at 20°C (~14%) and 25°C (~15%); overexpression can extend lifespan of adult wild-type animals at 20°C; mean lifespan is 37% higher by using RNAi
acox-1	-
apl-1	Mean lifespan is 20% higher in overexpression conditions
lev-11	-
cat-4	-
acox-5	-
glr-2	-
ceh-44	-
unc-13	Mutation results in a 150% life-extension in males and 32% in hermaphrodites
haf-4	-
ddr-1	-
eps-8	-
phy-2	-
ZC376.2	-
sor-3	-

Supplementary Table 9. List of diseases and functional terms associated with the miRNAs enriched as regulators of the largest human healthspan pathway.

Associated Terms	miRNAs	Adjusted P-val
Epithelial-mesenchymal transition	hsa-mir-200c, hsa-mir-141, hsa-mir-429, hsa-mir-205, hsa-mir-30c, hsa-mir-30d, hsa-mir-30e, hsa-mir-21	0.0007
Nephrosclerosis	hsa-mir-141, hsa-mir-429, hsa-mir-205	0.0003
Kidney Neoplasms	hsa-mir-21, hsa-mir-200c, hsa-mir-141	0.0005
Lupus Erythematosus, Systemic	hsa-mir-200c, hsa-mir-205, hsa-mir-429, hsa-mir-141, hsa-mir-21	0.0005
Carcinoma, Non-Small-Cell Lung	hsa-mir-205, hsa-mir-21, hsa-mir-30d, hsa-mir-34b, hsa-mir-34c, hsa-mir-200c, hsa-mir-429, hsa-mir-30e, hsa-mir-186	0.0005
Esophagus	hsa-mir-205, hsa-mir-21	0.0006
Intracranial Aneurysm	hsa-mir-34b, hsa-mir-34c	0.0006
Helplessness, Learned	hsa-mir-141, hsa-mir-200c, hsa-mir-429	0.0007
Barrett Esophagus	hsa-mir-21, hsa-mir-200c, hsa-mir-141, hsa-mir-429	0.0011
Neoplasms	hsa-mir-21, hsa-mir-30d, hsa-mir-200c, hsa-mir-141, hsa-mir-429, hsa-mir-30e, hsa-mir-34b, hsa-mir-34c, hsa-mir-205	0.0018
Small Cell Lung Carcinoma	hsa-mir-34b, hsa-mir-34c	0.0019
Melanoma	hsa-mir-30d, hsa-mir-429, hsa-mir-200c, hsa-mir-205, hsa-mir-186, hsa-mir-30e, hsa-mir-21, hsa-mir-34b, hsa-mir-34c, hsa-mir-141	0.0021
Endometrial Neoplasms	hsa-mir-186, hsa-mir-21, hsa-mir-200c, hsa-mir-141, hsa-mir-429, hsa-mir-205	0.0028
Sarcoma	hsa-mir-34b, hsa-mir-34c	0.0037
Mouth Neoplasms	hsa-mir-200c, hsa-mir-141, hsa-mir-21, hsa-mir-205	0.0042
Cholangiocarcinoma	hsa-mir-141, hsa-mir-21, hsa-mir-200c	0.0053
Aortic Aneurysm, Thoracic	hsa-mir-30d, hsa-mir-30e, hsa-mir-21	0.0053
Ovarian Neoplasms	hsa-mir-21, hsa-mir-141, hsa-mir-429, hsa-mir-30d, hsa-mir-200c, hsa-mir-34b, hsa-mir-34c, hsa-mir-30e	0.0078
Aortic Valve Stenosis	hsa-mir-141, hsa-mir-21	0.0089

Adenocarcinoma	hsa-mir-205, hsa-mir-429, hsa-mir-200c, hsa-mir-21, hsa-mir-34b	0.0093
Carcinoma, Renal Cell	hsa-mir-141, hsa-mir-200c, hsa-mir-429, hsa-mir-34b, hsa-mir-34c, hsa-mir-205, hsa-mir-21, hsa-mir-30d	0.0095
Mesothelioma	hsa-mir-21, hsa-mir-30e, hsa-mir-34b, hsa-mir-34c	0.0096
Thyroid Neoplasms	hsa-mir-141, hsa-mir-21, hsa-mir-34b, hsa-mir-30d, hsa-mir-200c	0.0101
Urinary Bladder Neoplasms	hsa-mir-205, hsa-mir-21, hsa-mir-34b, hsa-mir-34c, hsa-mir-429, hsa-mir-200c, hsa-mir-141	0.0103
Heart Failure	hsa-mir-186, hsa-mir-200c, hsa-mir-205, hsa-mir-21, hsa-mir-30e, hsa-mir-34b, hsa-mir-429, hsa-mir-34c	0.0115
Carcinoma, Ehrlich Tumor	hsa-mir-429, hsa-mir-141	0.0123
HBV Infection	hsa-mir-34b, hsa-mir-34c	0.0123
Esophageal Neoplasms	hsa-mir-21, hsa-mir-200c, hsa-mir-141, hsa-mir-205, hsa-mir-34b, hsa-mir-34c	0.0125
Lymphoma, Large B-Cell, Diffuse	hsa-mir-21, hsa-mir-200c	0.0161
Huntington Disease	hsa-mir-34b, hsa-mir-200c	0.0204
Obesity	hsa-mir-21, hsa-mir-30e	0.0204
Carcinoma, Squamous Cell	hsa-mir-21, hsa-mir-205, hsa-mir-30d, hsa-mir-200c, hsa-mir-34b, hsa-mir-34c	0.0217
Leukemia, Promyelocytic, Acute	hsa-mir-34b, hsa-mir-34c	0.0251
Lung Neoplasms	hsa-mir-205, hsa-mir-21, hsa-mir-30d, hsa-mir-30e, hsa-mir-34b, hsa-mir-34c, hsa-mir-186, hsa-mir-200c	0.0267
ACTH-Secreting Pituitary Adenoma	hsa-mir-141, hsa-mir-21	0.0302
Diabetic Nephropathies	hsa-mir-21, hsa-mir-200c	0.0302
Prostatic Neoplasms	hsa-mir-21, hsa-mir-205, hsa-mir-34c, hsa-mir-200c, hsa-mir-141, hsa-mir-34b, hsa-mir-30d	0.0369

Supplementary Table 10. List of diseases associated with the miRNAs enriched as regulators of the second-largest human healthspan pathway.

Associated Terms	miRNAs	Adjusted P-val
Eczema	hsa-mir-146a	0.010
Chlamydia Infections	hsa-mir-146a	0.010
Creutzfeldt-Jakob Syndrome	hsa-mir-146a	0.010
Gerstmann-Straussler-Scheinker Disease	hsa-mir-146a	0.010
Arthritis, Psoriatic	hsa-mir-146a	0.021
Sjogren's Syndrome	hsa-mir-146a	0.021
Moyamoya Disease	hsa-mir-146a	0.021
Myocardial Reperfusion Injury	hsa-mir-146a	0.021
Behcet Syndrome	hsa-mir-146a	0.021
Psychotic Disorders	hsa-mir-146a	0.021
Prion Diseases	hsa-mir-146a	0.031
Influenza, Human	hsa-mir-146a	0.041

Supplementary Table 11. Overlap of healthspan pathway genes: first network alignment.

Human gene	Putative function	Kind of inter-action	GenAge information	Orthologues *C. elegans* gene	Putative function	Kind of inter-action	GenAge information
PAK4	protects from apoptosis [32] overexpression/hyperstimulation is associated with cancer [34]; [35]; [36] Wnt signaling [91] Cytoskeletal reorganization [92], [93]	serine/threonine kinase signaling	shared domains, genetic interaction, and pathway data	none available	*pak-2*	serine/threonine kinase signaling	differentially regulated during ageing l., [94]	shared domains (except for the predicted interaction of gsk-3 and C25G6.3, which is based on the Interologous Interaction Database)	none available
BRSK2	response to DNA damage [95]; [41] neuronal differentiation [43]; [44] regulator of glucose-stimulated insulin secretion [43]				*sad-1*		regulation of neuronal polarization and synapse organization [96]; [97] tau-protein kinase activity *in vitro* [98]		
MELK	regulation of cell cycle [45] involved in apoptosis [47]				*pig-1*		regulation of programmed cell death [99] neuronal development [100]; [101]		
GSK3B	Wnt signaling [102] associated with Alzheimer's and Parkinson's disease [103]; [104]			target of genes previously linked to ageing	*gsk-3*		Wnt signaling [105] Tau phosphorylation [106] apoptotic cell clearance [107]		
CDKN2B	cyclin-dependent kinase inhibitor [108] tumor suppressor via inhibition of cell cycle progression [109] involved in cardiovascular and metabolic diseases [110]			linked to ageing in a cellular model system	*C25G6.3*		increased expression during pathogenic stress [111]		

Underlined genes indicate that gene originates from the original lists of health genes. Not underlined genes are orthologs. Colors are based on gene expression changes triggered by rapamycin (in case of *C. elegans*) or by caloric restriction (in case of human), see Figures 1–3.

Supplementary Table 12. Overlap of healthspan pathway genes: second network alignment.

Human gene	Putative function	Kind of inter-action	GenAge information	Orthologues *C. elegans* gene	Putative function	Kind of inter-action	GenAge information
ACOX1	fatty acid beta-oxidation pathway [112] knockdown results in ROS overproduction [113]	co-expression, co-localization and physical interaction (except for the interaction of SLC5A1 and GCH1, which is genetic)	none available	*acox-1*	biosynthesis of the fatty acid component of dauer pheromones [114]	co-expression	none available
SCP2	intracellular lipid transfer [115] involved in Zellweger syndrome [116]			*daf-22*	biosynthesis of the fatty acid component of dauer pheromones [114] regulation of fat metabolism [117]		
GCH1	affects cardiovascular risk [118] associated with dopamine-responsive dystonia [119]			*cat-4*	dopamine biosynthetic processes [120] (target of the stress-related transcription regulator *skn-1* (Oliveira et al., 2009).		
SLC15A1	overexpressed in human cancer cell lines [121] nutrient transport processes [122]			*pept-1*	involved in insulin and TOR signalling [123] induced transcription in healthspan-promoting treatments (Ihara et al., 2017; Cai et al., 2014; Pietsch et al., 2012)		Deletion results in extended lifespan and reproductive lifespan

differentially regulated in a long-lived *sir-2.1* overexpression strain (Viswanathan and Guarente, 2011) and during osmotic stress (Burton et al., 2017)

Underlined genes indicate that gene originates from the original lists of health genes. Not underlined genes are orthologs. Colors are based on gene expression changes triggered by rapamycin (in case of *C. elegans*) or by caloric restriction (in case of human), see Figures 1–3.