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-related diseases is being unraveled at an increasing pace. More recently, a long *healthspan* is seen as an important goal. However, a precise definition of health and healthspan is not straightforward, and the causal molecular basis of health "per se" is largely unknown. Here, we define health based on 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. Clusters of these genes based on molecular interaction and annotation data give rise to maps of healthspan pathways for humans, featuring the themes *transcription initiation, proliferation* and *cholesterol/lipid processing*, and for *C. elegans*, featuring the themes i*mmune response* and the *mitochondrion*. Overlaying healthspan-related gene expression data (describing effects of metabolic intervention associated with improvements in health) onto the aforementioned healthspan pathway maps, we observe the downregulation of Notch signalling in humans and of proliferation/cell-cycle in *C. elegans*; the former reflects the proinflammatory role of the Notch pathway. Investigating the overlap of healthspan pathways of humans and *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, is available as a reference for future investigations, at http://www.h2020awe.eu/index.php/pathways/.

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 detail its molecular basis and to map out molecular "healthspan pathways", and (3) to specify biomarkers and corresponding supportive interventions for the various features of health and for health itself. Arguably, the increase in life expectancy in the last 100 years has not been accompanied by an increase in disease-free life expectancy (Crimmins 2015), (Robine, Jagger et al. 2013). Cardiovascular disease, type-2 diabetes and neurodegenerative disorders are highly prevalent in the elderly, and these disorders frequently coexist in the same aged individual, often with mutual reinforcement (Fillenbaum, Pieper et al. 2000). Extending healthspan may thus enable economic, societal and individual gains on a large scale (Fuellen, Schofield et al. 2016).

Intervention studies to prolong healthspan based on compound exposure in humans are not available or are of limited use. Resveratrol for instance, being one of the best-studied polyphenols in humans and animals, was tested in several clinical studies (Smoliga, Baur et al. 2011). However, almost all of them were focused on bioavailability or side effects and only a few studies focused on the treatment of single diseases or on biomarkers, like the level of blood glucose (Brasnyó, Molnár et al. 2011) and cholesterol (Chen, Zhao et al. 2015), or glutathione S-transferase expression (Ghanim, Sia et al. 2011). In fact, 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)* lacks the diseases mentioned in the previous paragraph, but it is a widely used ageing model which guided the discovery of fundamental ageing-related findings, e.g., on calorie restriction and Insulin/IGF-1 like signaling (Gruber, Chen et al. 2015), (Lees, Walters et al. 2016). Last not least, more than 80% of the *C. elegans* proteome has human homologues (Lai, Chou et al. 2000) and studies revealing the role of metabolism on healthspan conducted in *C. elegans* have been subsequently validated and strengthened in murine models (Berry and Cirulli 2013), rendering this nematode a valuable model for human ageing processes. Most recently, *C. elegans* has come to enjoy increasing popularity as a model for health (Sutphin, Backer et al. 2017), (Luyten, Antal et al. 2016), and an ever increasing number of compounds are tested in *C. elegans* for their anti-ageing and health effects (Chen, Barclay et al. 2015), (Luo, Wu et al. 2009), (Collins, Evason et al. 2006).

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, with an emphasis 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, corroborating knowledge that was not used as input. Finally, we investigate the overlap of the *healthspan pathways* in humans and *C. elegans.*

For humans, we consider that knowledge of the causal basis of health may be best derived from genetic studies. We identify a core set of 12 genes that are genetically associated with a lack of frailty (Mekli, Marshall et al. 2015), (Ho, Matteini et al. 2011) and the Healthy Aging

Index (Sanders, Minster et al. 2014), and another set of 40 genes genetically associated with (a lack of) multiple diseases, or with longevity mediated by a lack of disease (see the tables for references). For *C. elegans*, we consider the few genetic healthspan studies available, as well as compound intervention data that are not available for humans. A lack of dysfunction exemplified by stress resistance, locomotion, pharyngeal pumping and reproduction are taken as the key health features in *C. elegans* (Fischer, Hoffman et al. 2016) (Rollins, Howard et al. 2017). 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.

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 (Zuberi, Franz et al. 2013). Gene ontology annotation data are then used to define and annotate clusters of similarity within the network, employing AutoAnnotate (Kucera, Isserlin et al. 2016). Then, we demonstrate that 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. We also predict microRNAs that may be potential regulators of healthspan. Finally, we find that some overlap exists of healthspan pathways in humans and *C. elegans,* based on the health-related data presented in this paper. However, overlap is limited to genes involved in transcription and proliferation/biosynthesis, and it is not straightforward to interpret in light of the independent health-related gene expression data we used to test plausibility of the single-species healthspan pathway maps.

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

Results and Discussion

Studies of health. The main sources of knowledge about health, that is, about features, biomarkers and interventions regarding health-related phenotypes, are (a) observational studies of genetics, usually in the form of genome-wide association studies, looking for associations between health and polymorphisms of specific genes, (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, (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. Like genetic studies, intervention studies can, in principle, find out about 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. Candidate biomarkers of health can be many kinds of features with the potential to predict future health better than chronological age (Fuellen et al, in preparation); they may be genetic (polymorphisms; such biomarkers are 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.

Defining Health. *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, in preparation), (Luyten, Antal et al. 2016). In Tables 1-3, we 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, 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, in preparation), 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 function, physical and cognitive function, and reproductive function. 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-10, International Statistical Classification of Diseases and Related Health Problems, www.who.int/classifications/icd/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, in preparation), not all parts of the ICD feature diseases related to health and healthspan. However, we note that all diseases referred to in Tables#1-3 qualify as age-associated diseases.

For *C. elegans,* in Tables 4-5 we 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 (Fuellen et al, in preparation). Of note, genetic studies 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). The lack or failure of reproducing results as well as biases and pitfalls in measuring locomotion in *C. elegans* are discussed in (Melov 2016).

Genes associated with health. Our list of *health genes,* that is, genes with associations to health in humans and *C. elegans* in Tables 1-5 is based on genetic studies in both species, or on natural compound intervention in *C. elegans*. Very few studies in humans report the results (in molecular terms) of compound supplementation affecting health; canonical aging-related pathways like mTOR or Insulin/IGF-1 are often implicated, though (Kapahi, Chen et al. 2010), (Barbieri, Bonafe et al. 2003). Therefore, the human health genes that are listed in the tables are based on genetic association, and we can assume some probability of them being causal, leaving aside the intrinsic ambiguities to assign 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 (Schwarz et al., 2008). In turn, for *C. elegans*, few studies report results of genetic interventions, though these are increasingly becoming available; (Sutphin, Backer et al. 2017) is the only large-scale genetic study that we could identify as of early 2018, even though it is essentially 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 tables are thus based on genetic effects and gene-level effects of compound intervention, and we can assume a high probability of causality in both cases.

Additional genes associated with C. elegans health. For *C. elegans,* we generated an additional list of health-associated genes that cannot be generated for humans (see Methods), as follows. We used *WormBase* to systematically identify health-related compound interventions with associated gene expression data, and compiled the list of genes with strongest differential expression that are well-annotated by Gene Ontology terms.

Table 1. Features of human health, lack of dysfunction(s). *Italics* is applied to genes that are not known to encode a protein.

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	(Minster, Sanders et al. 2015)	LLFS (Long life family study) cohort, suggestive association nominally replicated in CHS and FHS
Healthy Aging Index	GSK3B	(Druley, Wang et al. 2016)	candidate gene sequencing in LLFS cohort
grip strength, gait speed, physical activity, FEV from exceptional survivors	NBPF6	(Singh, Minster et al. 2017)	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	(Mekli, Marshall et al. 2015)	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	(Ho, Matteini et al. 2011)	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

Table 2. Features of human health, (lack of) multiple diseases. Overlaps of genes (with Tables 1 or 3) are marked in boldface. Genes implicated for the same locus are listed in a single row; genes listed a second time in the same study but for a different locus are listed only once. Gene annotations noted in the original papers that refer to function are marked by "→". QTL, quanitative trait locus; GWAS, genome-wide association study. See also Table 1.

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?)	(Erikson, Bodian et al. 2016) (Wellderly)	whole-genome sequencing; molecular correlate SNPs were not significant genome-wide; sample (e.g., education) bias is likely (Christensen and McGue 2016); 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	(He, Kernogitski et al. 2016)	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	(Tindale, Leach et al. 2017)	targeted genotyping
macular degeneration, multiple sclerosis. menopause onset, rheumatoid arthritis, systemic lupus erythematosus	SLC44A4	(Yao, Joehanes et al. 2014)	expression QTLs interacting with age implicated in multiple diseases
psoriasis, diabetes	HLA-DQA2		
Alzheimer dementia, cardiovascular disease	* Cholesterol transport endocytosis * Immune response	(Kauwe and Goate 2016), cf. (Pilling, Harries et al. 2012)	overlap of enrichment in GWAS pathway analyses
clustering of 372 disease polymorphisms in the genome	* NOTCH4 * **CDKN2B** (p15INK4b), CDKN2A (p16INK4a/p14ARF), *CDKN2BAS (**ANRIL**)* * IL23R	(Jeck, Siebold et al. 2012)	meta-analysis of SNPs in age-associated diseases.

	* REL * TERT * IRF5, TNPO3 * IKZF3, GSDMA, GSDMB		

Table 3. Features of human health, lifespan/longevity mediated by lack of disease. See also Table 2.

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	(Fortney, Dobriban et al. 2015)	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	(McDaid, Joshi et al. 2017)	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

Table 4. Features of *C. elegans* health, based on genetic studies of health. See also Table 2. In contrast to Tables 1-3, the *first* occurrence of a duplicate is not marked in boldface, neither in this table nor in Table 5; only the second occurrence is marked.

Feature	Associated gene(s) / pathways	Reference	Remarks
(stimulated) locomotion	hpa-1, hpa-2, let-23, plc-1, itr-1 (→ EGF pathway)	(Iwasa, Yu et al. 2010)	
	ahr-1	(Eckers, Jakob et al. 2016)	
	* 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)	(Sutphin, Backer et al. 2017)	association with stimulated locomotion *("motivated* movement") and thrashing, based on candidate genes of *C. elegans* orthologs of human genes differentially expressed with age

Table 5. Features of *C. elegans* health, based on compound intervention studies affecting health. See also Table2. In the column listing the reference, „cf." refers to reviews.

Feature		Associated gene(s) / pathways	Reference	Remarks
stress resistance		**eat-2, skn-1**	cf. (Leonov, Arlia-Ciommo et al. 2015)	
	thermal stress resistance	**ahr-1**	(Eckers, Jakob et al. 2016)	
		osr-1, unc-43, sek-1	cf. (Leonov, Arlia-Ciommo et al. 2015)	thermo-tolerance was suggested to mark ***lack*** of health in short-lived mutants (Rollins, Howard et al. 2017)
		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** **sir-2.1** **age-1, skn- 1, mek-1**	cf. (Leonov, Arlia-Ciommo et al. 2015)	
		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. (Ding, Zheng et al. 2017)	
locomotion	* ins-1 (Ins/IGF-1) * pdk-1 (→ pyruvate metabolism) * hsf-1, skn-1 (nrf2)	cf. (Jafari 2015)	
	daf-2, daf-16, **hsf-1,** sod-3, hsp-12.3	cf. (Leonov, Arlia-Ciommo et al. 2015)	
	tph-1		
pharyngeal pumping	**ahr-1**	(Eckers, Jakob et al. 2016)	
	akt-2, mev-1, nhr-8	cf. (Leonov, Arlia-Ciommo et al. 2015)	
	sir-2.1		
	tph-1		
	daf-2, daf-16, hsf-1		
	ets-7	(Nguyen, Caito et al. 2016)	
	age-1, **skn-1,** mek-1	cf. (Jafari 2015)	
reproduction	eat-2	cf. (Leonov, Arlia-Ciommo et al. 2015)	
	tph-1		
	*** Ins/IGF-1** *** sirtuins**	cf. (Jafari 2015)	

From gene lists to maps of healthspan pathways. We used *Cytoscape* and some of its application plugins as the most straightforward tool for obtaining and annotating a connected network of the genes from Tables 1-3 and from Tables 4 and 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 GO annotations, using *AutoAnnotate*. The resulting healthspan pathway maps are presented in the following. 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 (Mercken, Crosby et al. 2013) and of rapamycin in *C. elegans* (Calvert, Tacutu et al. 2016), as examples of health-promoting interventions, or they describe the effects of aging and disease in specific tissues (Aramillo Irizar, Schäuble et al. 2018). CR is able to dramatically increase nematode lifespan by up to 150 % and provides survival benefits during oxidative and heat stress exposure (Houthoofd, Braeckman et al. 2002). Lifespan-independent consequences of CR are diverse and contrasting and include increased swimming speed but decreased crawling speed (Lüersen, Faust et al. 2014) improved short- and long-term memory in late adulthood but impaired long-term memory in young adulthood (Kauffman, Ashraf et al. 2010), (Kauffmann, Ashraf et al. 2010), and reduction of body size, fecundity, as well as fat accumulation (Bar, Charar et al. 2016). Treatment with 10-100 µM rapamycin prolongs *C. elegans* lifespan by about 20 %, and by more than 110 % during oxidative stress (Robida-Stubbs, Glover-Cutter et al. 2012). Furthermore, rapamycin treatment results in an increased pharyngeal pumping rate and autophagy rate. However, as in the case of CR, rapamycin does not enhance *C. elegans* locomotion on solid media (Robida-Stubbs, Glover-Cutter et al. 2012), (Calvert, Tacutu et al. 2016). Based on these and similar phenotypic as well as transcriptional observations, rapamycin is considered to be a suitable CR mimetic (Calvert, Tacutu et al. 2016).

For humans, the gene list derived from Tables 1-3 (Suppl. Table 1) yielded the network of Fig._A1, where the two largest pathways/clusters (15 and 13 genes) are specifically labeled by *Notch* & transcription initiation, and by proliferation, and the smaller pathways/clusters (4, 3, 3 and 3 genes) are labeled by cholesterol & lipid processes, by thymus activation, by myotube (striate muscle) regulation, and by Wnt signal-

ling. In Fig._A2, the list of pathways/clusters, and the details of the largest pathway are zoomed in.

In the largest pathway/cluster, the most prominent findings are CR-induced downregulation of NOTCH4 (and to a lesser extent of NOTCH 2&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 (via NFKB) (Balistreri, Madonna et al. 2016), (Zhang, Kuang et al. 2017). NOTCH4 is upregulated in kidney failure (Liu, Liang et al. 2017), and promotes vascularization/angiogenesis, which includes its upregulation in malignancy (Zhang, Kuang et al. 2017), (Kofler, Shawber et al. 2011). 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 (Lin, Mironova et al. 2017) and vascular integrity (Strickland, Au et al. 2014). A downregulation of LRP1 during CR could therefore be seen as deleterious. However, LRP1 expression depends on cholesterol levels (Llorente-Cortes, Otero-Nivas et al. 2002) – 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 (Zeitlow, Charlambous et al. 2017), and low expression and/or particular risk alleles of this protein are associated with Huntington's and Alzheimer's Disease (Shirendeb, Reddy et al. 2011), (Chong, Goh et al. 2013). 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 widely-active histone-acetyltransferase (Bedford and Brindle 2012), acting primarily on histones 3 and 4, and thus it acts in concert with a range of transcription factors. Its downregulation is deleterious, resulting in, e.g., MHCII expression loss on lymphocytes (Hashwah, Schmid et al. 2017), rendering the lymphocytes dysfunctional for antigen presentation, and in inflammatory signalling (Dixon, Nicholson et al. 2017). 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 (Hamed, Spaniol et al. 2015). Notably, hsa-mir-34 stands out as a regulator of the *Notch* genes, and it is implicated in cancer, intracranial aneurysm and heart failure (Suppl. Fig._D1, Suppl. Table_D1). 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 (Wang, Li et al. 2010), (Liu 2010), (Sharma , Sirin et al. 2011). It is also associated with human longevity (ElSharawy, Keller et al. 2012). Finally, according to Wikipedia's community annotation facilitated by miRBase and Rfam, hsa-mir-34 and hsa-mir-30 are both linked to cancer.

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 secretory phenotype and frailty in old age (Xu, Tchkonia et al. 2015). For this 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 (Suppl. Fig._D2, Suppl. Table_D2). 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.

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 (McNeill, Channon et al. 2010). On the other hand, APOE4 has been widely implicated in the formation of amyloid plaques in Alzheimer's Disease (Kivipelto, Helkala et al. 2002), and experimental downregulation showed a protective effect in an Alzheimer Disease mouse model (Huynh, Liao et al. 2017). 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.

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 (Aramillo Irizar, Schäuble et al. 2018), see Methods. In the largest (Notch-related) healthspan pathway (see Suppl. Fig._E1), 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 Suppl. Fig._E2), 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 Suppl. Fig._E3) 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 (Gordts, Muthuramu et al. 2014). 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 Suppl. Fig._E4. 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.

Fig._A1. A healthspan pathway map for humans, based on Tables 1-3. For the pathway/cluster labels, see also Fig._A2; in Fig._A2 (right), the top-left pathway is zoomed in. 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 (Mercken, Crosby et al. 2013). 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.

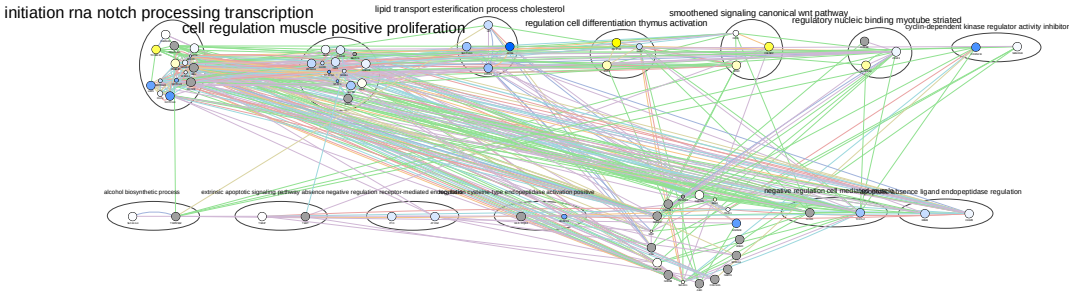
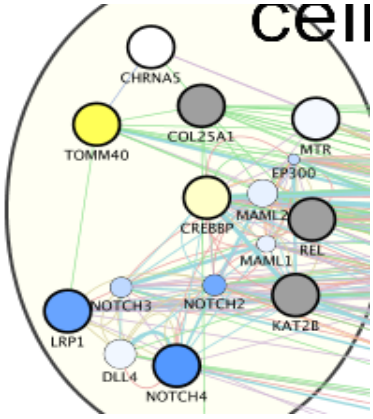


Fig._A2. Healthspan pathways/clusters for humans and their size (number of genes) (left) and details of the largest pathway (right). See also Fig._A1.

Cluster	...
initiation rna notch processing transcription	15
cell regulation muscle positive proliferation	13
lipid transport esterification process cholesterol	4
regulation cell differentiation thymus activation	3
regulatory nucleic binding myotube striated	3
smoothened signaling canonical wnt pathway	3
alcohol biosynthetic process	2
apoptotic absence ligand endopeptidase regulation	2
cyclin-dependent kinase regulator activity inhibitor	2
extrinsic apoptotic signaling pathway absence	2
negative regulation cell mediated muscle	2
negative regulation receptor-mediated endocytosis	2
regulation cysteine-type endopeptidase activation positive	2



For *C. elegans*, the gene list derived from Tables 4-5 (Suppl. Table 2) yielded the network of Fig._B1, where the largest clusters (9 and 6 genes, respectively) are labeled by immune response process and by terms related to the mitochondrion, and three clusters (of 4 genes each) specifically feature dauer/dormancy, regulation and hormone response. In Fig._B2, the list of pathways/clusters, and the details of the largest pathway are zoomed in. Regarding the first pathway, rapamycin induces ets-7 transcription, which was shown to be also necessary for the healthspan-promoting effects of salicylamine (Nguyen, Caito et al. 2016). 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 (Henderson and Johnson 2001). Along the same lines, the akt-1 and akt-2 activator pdk-1 is also downregulated by rapamycin, further promoting daf-16 activity (Paradis, Ailion et al. 1999). In contrast, the daf-16 inhibitor sgk-1 (a homolog to Nrf) is upregulated; however, its inhibitory role is subject of discussion (Mizunuma, Neumann-Haefelin et al. 2014). Finally, the transcription factors hsf-1 and skn-1, both important in stress re-

sponse processes (Morton and Lamitina 2013); (Wang, Robida-Stubbs et al. 2010), are slightly downregulated in rapamycin-treated *C. elegans*.

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 (Li, Ebata et al. 2008) 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.

Fig._B1. A healthspan pathway map for *C. elegans*, based on Tables 4 and 5. See also Fig._A1. Gene expression data reflect the effect of rapamycin (Calvert, Tacutu et al. 2016).

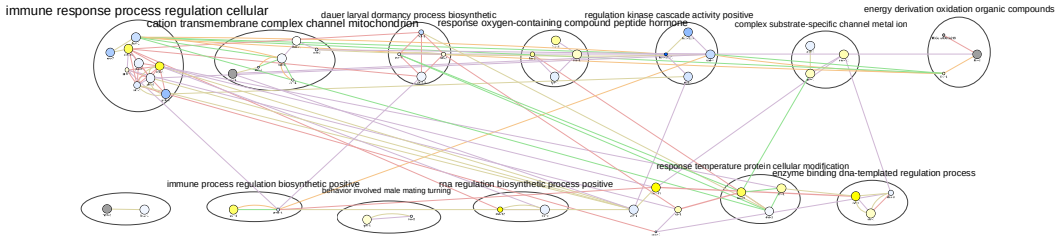
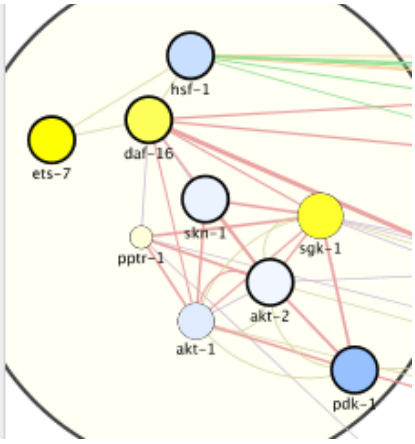


Fig._B2. Healthspan pathways/clusters and their size (number of genes) for *C. elegans* (left) and details of the largest pathway (right). See also Figs._A2 and _B1.

Cluster	...	...
immune response process regulation cellular		9
cation transmembrane complex channel mitochondrion		6
dauer larval dormancy process biosynthetic		4
regulation kinase cascade activity positive		4
response oxygen-containing compound peptide hormone		4
complex substrate-specific channel metal ion		3
energy derivation oxidation organic compounds		3
enzyme binding dna-templated regulation process		3
response temperature protein cellular modification		3
		2
behavior involved male mating turning		2
immune process regulation biosynthetic positive		2
rna regulation biosynthetic process positive		2



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; Suppl. Table 3). We obtained the network of Fig._C1. Curiously, the top healthspan pathways of 11, 9 and 8 genes are related to the *endoplasmic reticulum (ER)*, lipid & membrane, to the *peroxisome*, macrobody & 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 (Nisoli and Valerio 2014). Peroxisomal function connects this pathway to dietary effects on lifespan (Weir, Yao et al. 2017), and to liver disease (Cai, Sun et al. 2018). The second tier or 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 Fig._C2. 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 negligible downregulation. However, the importance of elongase genes for health maintenance was repeatedly documented. Vásquez and colleagues (Vásquez, Krieg et al. 2014) 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 to *Pseudomonas aeruginosa* infections due to the accumulation of gamma-linolenic acid and stearidonic acid (Nandakumar and Tan 2008) and knockdown of elo-1 or elo-2 extends survival during oxidative stress (Reis, Xu et al. 2011). In contrast, down-regulation of elo-2, elo-4, and elo-5 via RNAi resulted in less resistance against *E. coli* strain LF82 (Gonçalves 2014). The involvement of elongase genes in stress resistance and immune response is corroborated by their interaction with the insulin signaling cascade (Horikawa and Sakamoto 2010) which also explains the suppression of anoxia-resistance in daf-2 mutants through elo-1 knockdown (Garcia, Ladage et al. 2015). Moreover, art-1 is a steroid reductase that is downregulated by rapamycin in our case, but also in long-lived eat-2 mutants (Yuan, Kadiyala et al. 2012).

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 (Wang, Saar et al. 2018), while daf-22 catalyzes the final step in the peroxisomal β-oxidation pathway and is essential for *dauer* pheromone production as well as for the prevention of fat accumulation (Butcher, Ragains et al. 2009); the latter also applies to acox-1 (Zhang, Bakheet et al. 2011). The inverse 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 (Zarse, K., A. Bossecker et al. 2011; Shukla, Yadav et al. 2012; Zheng, Liao et al. 2014), during life-prolonging CR (Bar, Charar et al. 2016) or during increased autophagy (Schiavi, Torgovnick et al. 2013). Moreover, *ectopic* fat deposition is found in ageing worms and is discussed as a cause of ageing itself (Ackerman and Gems 2012). Thus, the appearance of genes involved in fat metabolism is not surprising here. On the other hand, acox-5 (aka drd-51), which is implicated in starvation-sensing and is downregulated by dietary restriction (Ludewig, Klapper et al. 2014), 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, is upregulated the strongest), whereas the six genes in the cluster related to biosynthesis and transcription are all downregulated by rapamycin.

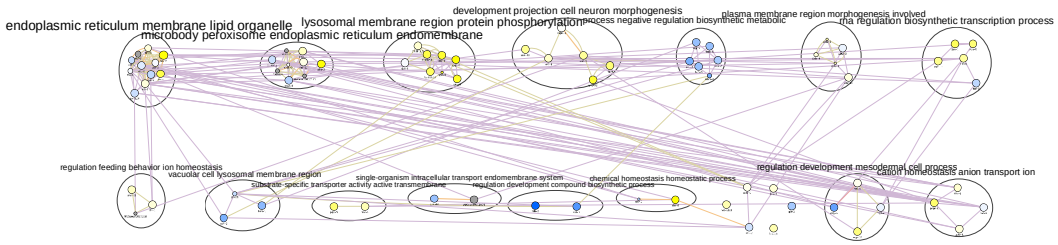
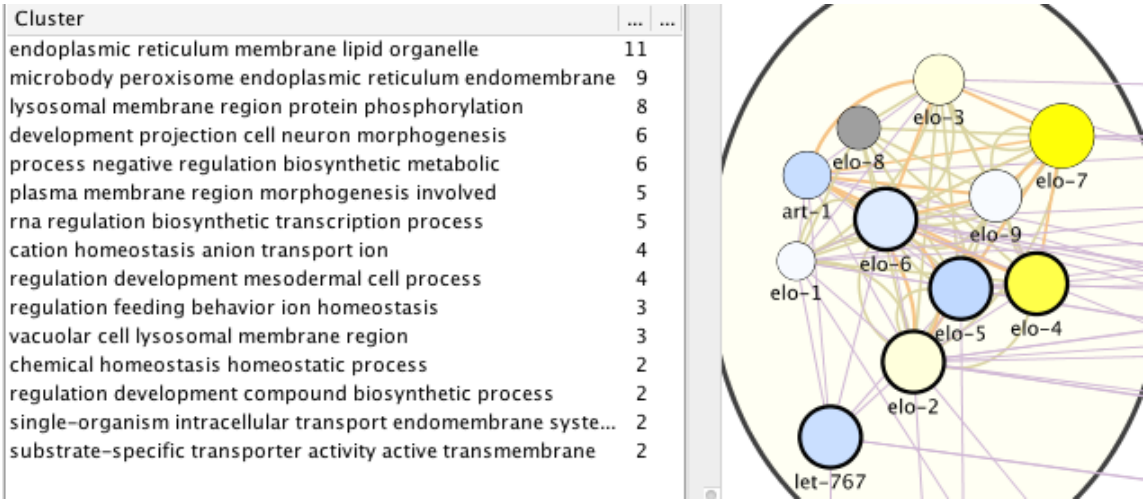


Fig._C1. A healthspan pathway map for *C. elegans*, based on genes affected the most by healthspan-extending interventions, using WormBase gene expression data. See also Figs._A1 and _B1.



Cluster	...	...
endoplasmic reticulum membrane lipid organelle	11	
microbody peroxisome endoplasmic reticulum endomembrane	9	
lysosomal membrane region protein phosphorylation	8	
development projection cell neuron morphogenesis	6	
process negative regulation biosynthetic metabolic	6	
plasma membrane region morphogenesis involved	5	
rna regulation biosynthetic transcription process	5	
cation homeostasis anion transport ion	4	
regulation development mesodermal cell process	4	
regulation feeding behavior ion homeostasis	3	
vacuolar cell lysosomal membrane region	3	
chemical homeostasis homeostatic process	2	
regulation development compound biosynthetic process	2	
single-organism intracellular transport endomembrane syste...	2	
substrate-specific transporter activity active transmembrane	2	

Fig._C2. Healthspan pathways/clusters and their size (number of genes) for *C. elegans* (left) and details of the largest pathway (right). See also Figs._A2 and _C1.

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 genetics and the *C. elegans* healthspan genes based in part on genetics, but mostly on expert analysis of intervention effects (Fig._B1), or on gene expression changes related to healthspan-extending interventions (Fig._C1). We found some hints at an overlap on the level of the healthspan pathway annotations, considering that “proliferation” is listed for human, and “biosynthesis” for *C. elegans*, and “transcription” as well as “lipid” for both.

In search for other modes of overlap, we therefore 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, Tables 1-3, Fig._A1) and of *C. elegans* (based in turn on the gene expression analysis of healthspan-extending interventions using WormBase, Fig._C1). Specifically, as outlined in Fig._D1, 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. 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 in one species and a healthspan pathway 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.

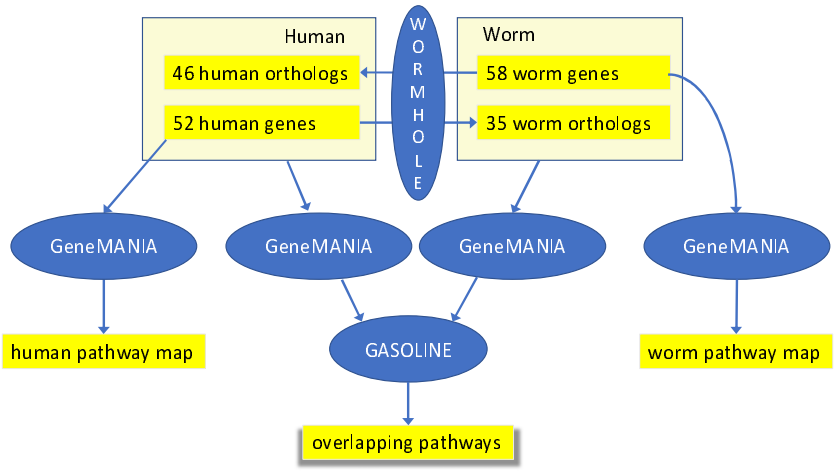


Fig._D1. In the preceding sections of the paper, 52 human health genes (Tables 1-3) were processed with GeneMANIA and AutoAnnotate to determine the human healthspan pathway map (left, see also Figs._A1,_A2). Analogously, 58 worm health genes (based on gene expression analysis using Wormbase) were studied, yielding the *C. elegans* healthspan pathway map (right, see also Figs._B1,_B2). Here, to determine overlap across species, the gene lists were extended by the orthologs (calculated by *WORMHOLE,* see 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 Figs_E1,E2).

Of note, 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 (Micale, Continella et al. 2014) to align the two networks wherever feasible, obtaining two partial (subnetwork) alignments as output, as shown in Figs._E1 and _E2.

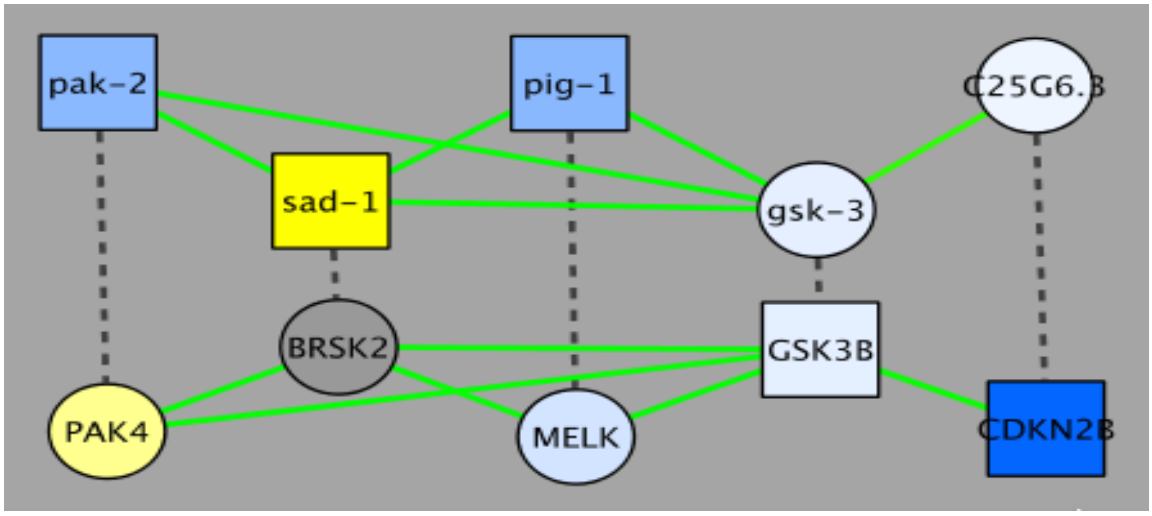


Fig._E1. First alignment demonstrating overlap of (putative) healthspan pathways in human and *C. elegans,* based on a GASOLINE alignment of the network of genes implicated in health-related gene expression changes in WormBase (top), and in human health based on genetic studies (bottom), and of corresponding 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 Figs._A1-_C2.

In the first alignment (Fig._E1), 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 (Tables1-3; 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 Figs._C1/_C2 (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 Figs._A1,_A2. Interactions in *C. elegans* are all based on shared domains (kinase signaling, except for the predicted interaction of gsk-3 & 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 serine/tyrosine kinase signalling (pak-2/sad-1/pig-1 & PAK4/BRSK2/MELK), Wnt signalling (GSK3) and cyclin-dependent kinase signalling (CDKN2B).

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, while avoiding cancer. Accordingly, the independent expression data based on caloric restriction/rapamycin intervention data reflect that proliferation/biosynthesis is generally, but not complete-

ly, 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 (Gnesutta, Qu et al. 2001), and a positive role in supporting stem cells is possible (Tyagi, Marimuthu et al. 2016). In context of cancer, however, upregulation of PAK4 has been associated with high-grade human breast cancer (Dart, Box et al. 2015) and with malignancy in a variety of cancer cell lines (Zhang, Wang et al. 2012), (Callow, Clairvoyant et al. 2002). PAK4 can positively mediate cell survival and proliferation as well as enhance cell migration and invasion (Zhang, Wang et al. 2012), (Siu, Chan et al. 2010), (Ahmed, Shea et al. 2008). The inhibition of PAK4 reduced cell proliferation, migration and invasion of gastric cancer cells (Zhang, Wang et al. 2012). 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 (Dart, Box et al. 2015). 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 (Chaker, Minden et al. 2018), (Komiya and Habas 2008) . Concerning BRSK2, which is usually expressed in brain, testis and pancreatic tissue, an enhanced activity in response to DNA damage was reported (Seoighe and Scally 2017), (Wang, Wan et al. 2012), (Nie, Liu et al. 2013). In brain, BRSK2 is significant for proper regulation and formation of neuronal polarity in the developing nervous system (Nie, Liu et al. 2013), (Kishi, Pan et al. 2005). 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 (Jiang and Zhang 2013) 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 (Giuliano, Lin et al. 2018). In general, MELK is preferentially upregulated in cancer (Ganguly, Mohyeldin et al. 2015). 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.

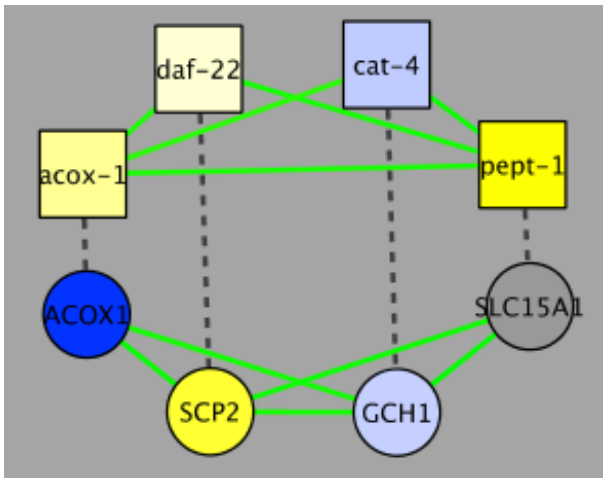


Fig._E2. Second alignment demonstrating overlap of (putative) healthspan pathways in human and *C. elegans.* See Fig._E1 for further explanations.

In the second alignment (Fig._E2), 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 & homeostasis (in a smaller cluster of the original healthspan pathway map, Fig._C1/_C2). All four genes are differentially regulated in a long-lived sir-2.1 overexpression strain (Viswanathan and Guarente 2011) and in

nematodes suffering from down-regulation of nhr-49, a key regulator of fat metabolism (Van Gilst, Hadjivassiliou et al. 2005). Moreover, differential translational regulation of cat-4, pept-1, acox-1, and daf-22 was observed in wildtypes during osmotic stress (Burton, Furuta et al. 2017), 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 various neuronal processes and is a target of the transcription regulator skn-1, which is known to be indispensable for proper stress response (Oliveira, Porter Abate et al. 2009). Finally, healthspan-promoting treatments like tannic acid (Pietsch, Saul et al. 2012), colistin exposure (Cai, Cao et al. 2014), or life-prolonging fasting (Ihara, Uno et al. 2017) 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 (Swindell 2009)). 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.

Biological interpretation of the lack of evolutionary conservation. In some sense, the lack of overlap between 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 (Gurven, Kaplan et al. 2009), 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 (Cohen, Legault et al. 2017). 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.) (Kapahi, Chen et al. 2010), (Barbieri, Bonafe et al. 2003) nearly guarantees 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 but not 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 (Partridge and Gems 2002). 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 (Pease, Lande et al.).

Healthspan pathway mapping, current possibilities and future opportunities. We took a rigorous yet pragmatic approach to investigate the molecular basis of health. For humans, there are only few alternatives to genetic association studies, to determine the genes responsible for the phenotype of lacking disease and dysfunction. Post-mortem analyses aside, studies in human are bound to the insights gathered from the etiology and treatment of diseases in patients. Genetic manipulation is possible in human cell lines, but this requires to break down the disease or dysfunction to a cellular phenotype. On the other hand, molecular insights can be gained with few ethical constraints in non-vertebrates such as *C. elegans.* A range of age-associated phenotypes can be observed in *C. elegans* that are similar to human, yet the nematode is still a much simpler organism with far fewer cells and a smaller genome. As noted, however, sequence similarity supports the mapping of ortholog genes across species, suggesting functional similarity or equivalence, and many core pathways are preserved.

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) and, at the same time, be involved in related processes as suggested by Gene Ontology annotation data. The latter was assured by the AutoAnnotate clustering. Here, as in most other studies, pathways are not assumed to be linear. The (higher-level) interaction among the clusters/healthspan pathways (i.e., the pathway map) is given by the number of individual gene/protein interactions that are preserved by AutoAnnotate and shown *between* the clusters in Figs._A1,_B1,_C1. However, we did not investigate these further.

Looking for models of human health, there is a choice of cell lines, organoids (“human-in-a-dish”), *in silico* simulations and model organisms. A major disadvantage of cell lines is their artefactual isolated cultivation. This disadvantage is addressed by organoid-based approaches, which, however, are still far removed from the human situation they are supposed to model, in terms of size, complexity, and the duration of the investigation. *In silico* simulations are limited in complexity, and their faithfulness may be questioned on many accounts. This faithfulness is also a big issue with model organisms, where ease of handling and a reasonable duration of the investigation usually result in difficult tradeoffs with faithfulness. A discussion of the various approaches just mentioned, and of the various choices of model organisms is beyond

the scope of this paper. But it should be mentioned that while health can be defined and investigated in *C. elegans*, this model is also fraught with an array of issues, some shared with organoid-based approaches, but also including some particularities of the species (post-mitotic tissues, artificial cultivation, hermaphrodite, genetic uniformity of strains, lack of organs present in humans like heart, brain, etc.) and of our modes of investigation (limitations of the data currently available, limitations of healthspan assays in terms of their design and the controlled environment in which these are conducted). Nevertheless, for studies in a living system to be completed in a short time-frame, *C. elegans* is arguably the best choice.

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; data 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. 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 Table 5, suggesting a fundamental role for immune defense mechanisms (and proliferation) in health maintenance, as described by (Ermolaeva and Schumacher 2014). Furthermore, in humans, an imbalance between inflammatory and anti-inflammatory networks has been hypothesized to affect healthspan (Franceschi, Capri et al. 2007).

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. It is evident that there is no *C. elegans* counterpart to most of the (age-related) diseases that we use to define health in humans, and that the aging process that may underlie most of these age-related diseases is poorly characterized and hard to quantify in 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 (Leong, Teo et al. 2015) and the ability to sit and rise from the floor (Brito, Ricardo et al. 2014) 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 (Dues, Andrews et al. 2016), (Bajorat, Oberacker et al. 2014). *C. elegans* does not suffer from dementia, but (like most humans) it shows a cognitive decline with age (Kauffman, Ashraf et al. 2010). 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. We claim that within the limitations of currently available data,

the health genes we assembled, the healthspan pathways we constructed based on these, and the overlap then we found between species, are a first glimpse of the species-specific and cross-species molecular basis of health.

Methods

Gene sets associated with health, literature-based. In this work, we conducted a semi-systematic review, 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, the various genetic studies of 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 (Mekli, Marshall et al. 2015), (Ho, Matteini et al. 2011). Overall, a list of 52 genes (Table 1, 12 genes; Tables 2 and 3, 40 genes) was taken as the starting point in humans. For the genetics of *C. elegans* health, we followed a similar approach (Table 4). For compound interventions in *C. elegans*, we identified a specific set of recent reviews (see Table 5). Overall, a list of 31 genes (Table 4, 11 genes; 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* (Hoffmann and Valencia 2004) to assign HUGO nomenclature names if necessary.

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 (WBPaper00048989), astaxanthin (WBPaper00049979), cocoa-peptide 13L (WBPaper00042404), colistin (WBPaper00045673), 2-deoxy-D-glucose (WBPaper00044434 & 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.

Construction of maps of clusters/pathways. For all gene sets analyzed, we used the Cytoscape 3.5.1 application *GeneMANIA* (Zuberi, Franz et al. 2013), 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 the "annotation name" column of GO annotations collected by GeneMANIA, we used *AutoAnnotate* (Kucera, Isserlin et al. 2016) v1.2, downloaded October 2017, for clustering, in Quick start modus to enable to "layout network to prevent cluster overlap", so that a map of disjoint clusters (healthspan pathways) was generated, 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 (Oesper, Merico et al. 2011) v3.1.1, downloaded January 2018.

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 (Calvert, Tacutu et al. 2016) 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 to compute fold-changes using default values, 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 (Aramillo Irizar, Schäuble et al. 2018) 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 (Aramillo Irizar, Schäuble et al. 2018): 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 neuro-

degenerative 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. Fig._D1 (middle) summarizes the overall approach. The human health-related gene list based on Tables 1-3 (Suppl. Table 1) and the health-related gene-expression-based gene list from wormbase (Suppl. Table 3) 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 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 (Micale, Continella et al. 2014), 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".

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 (Franz, Lopes et al. 2016). 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/) (Adler, Kolde et al. 2009). 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

[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 round results in slightly different output due to a GASOLINE feature

expression (Kolesnikov, Hastings et al. 2015). 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 tell 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.

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Competing interests

The authors declare that they have no competing interests.

Authors' 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, FC, AB, PAn, MB, LJ. All authors reviewed and approved the final manuscript.

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

ZNF704
GSK3B
NBPF6
IL-18
IL-12A
LRP1
SELP
MTR
CASP8
CREBBP
KAT2B
BTRC
BTN1A1
BTN3A1
SLC22A4
KCNE4
COL25A1
MAML3
SEMA5B
TCF7L2
ZFHX3
TMPRSS2
APOE
HP
SLC44A4
HLA-DQA2
NOTCH4
CDKN2B
CDKN2A
IL23R
REL
TERT
IRF5
TNPO3
IKZF3
GSDMA
GSDMB
TOMM40
ABO
SH2B3
ATXN2
RBM6
SULT1A1
CHRNA5
BSND
CELSR2
TRAIP
C5ORF67
LPA
NPIPB8
FTO
APOC1

Supplementary Table 2. *C. elegans* genes associated with health, listing all genes from Tables 4+5.

hpa-1
hpa-2
let-23
plc-1
itr-1
ahr-1
C15H9.7
iglr-1
tsp-3
rcn-1
unc-36
INS-1
PDK-1
HSF-1
SKN-1
DAF-2
DAF-16
SOD-3
HSP-12.3
TPH-1
AKT-2
MEV-1
NHR-8
SIR-2.1
ETS-7
AGE-1
MEK-1
EAT-2
OSR-1
UNC-43
SEK-1

Supplementary Table 3. *C. elegans* genes associated with health, listing genes based on wormbase gene expression data.

hop-1
frk-1
itr-1
jun-1
sad-1
unc-43
nhx-2
hlh-2
rab-11.1
mlk-1
pept-1
pig-1
rig-6
let-767
egl-44
abts-1
elo-2
daf-22
pak-2
stn-1
mig-10
unc-68
unc-52
C35E7.10
nhr-8
gsp-4
elo-6
gsp-3
C18H7.4
ilys-3
C34F11.5
elo-5
elo-4
bub-3
unc-5
pgp-5
rhgf-2
hbl-1
ima-2
gsto-1
acly-1
mtm-6
mrg-1
rbr-2
acox-1
apl-1
lev-11
cat-4
acox-5
glr-2
ceh-44
unc-13
haf-4
ddr-1
eps-8
phy-2
ZC376.2
sor-3

Supplementary Table_D1: List of diseases and functional terms associated with the miRNAs enriched as regulators of the largest human healthspan pathway.

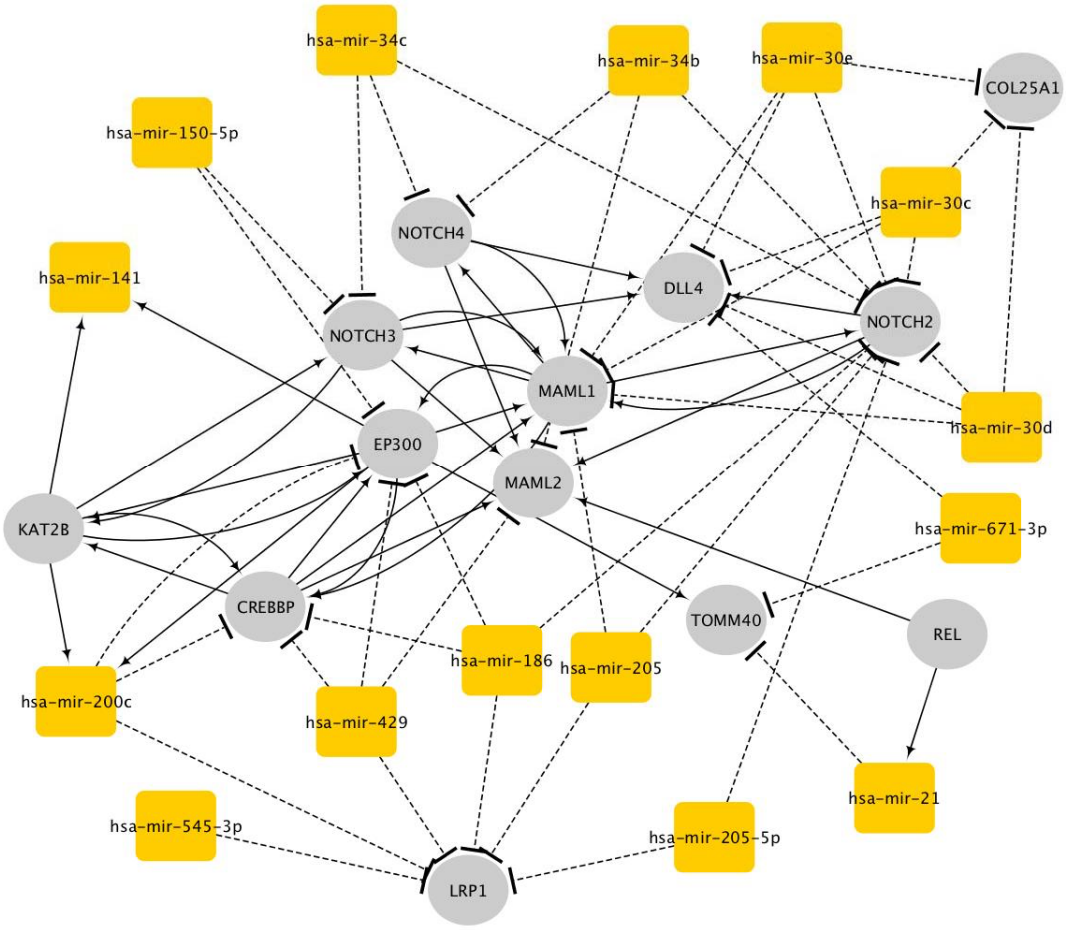
Associated Terms	miRNAs	Adjusted P-val
Epithelial-mesenchymal transition	hsa-mir-200c, hsa-mir-141, hsa-mir-429, hsa-mir-205, hsa-mir-30c, hsa-mir-30d, hsa-mir-30e, hsa-mir-21	0.0007
Nephrosclerosis	hsa-mir-141, hsa-mir-429, hsa-mir-205	0.0003
Kidney Neoplasms	hsa-mir-21, hsa-mir-200c, hsa-mir-141	0.0005
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

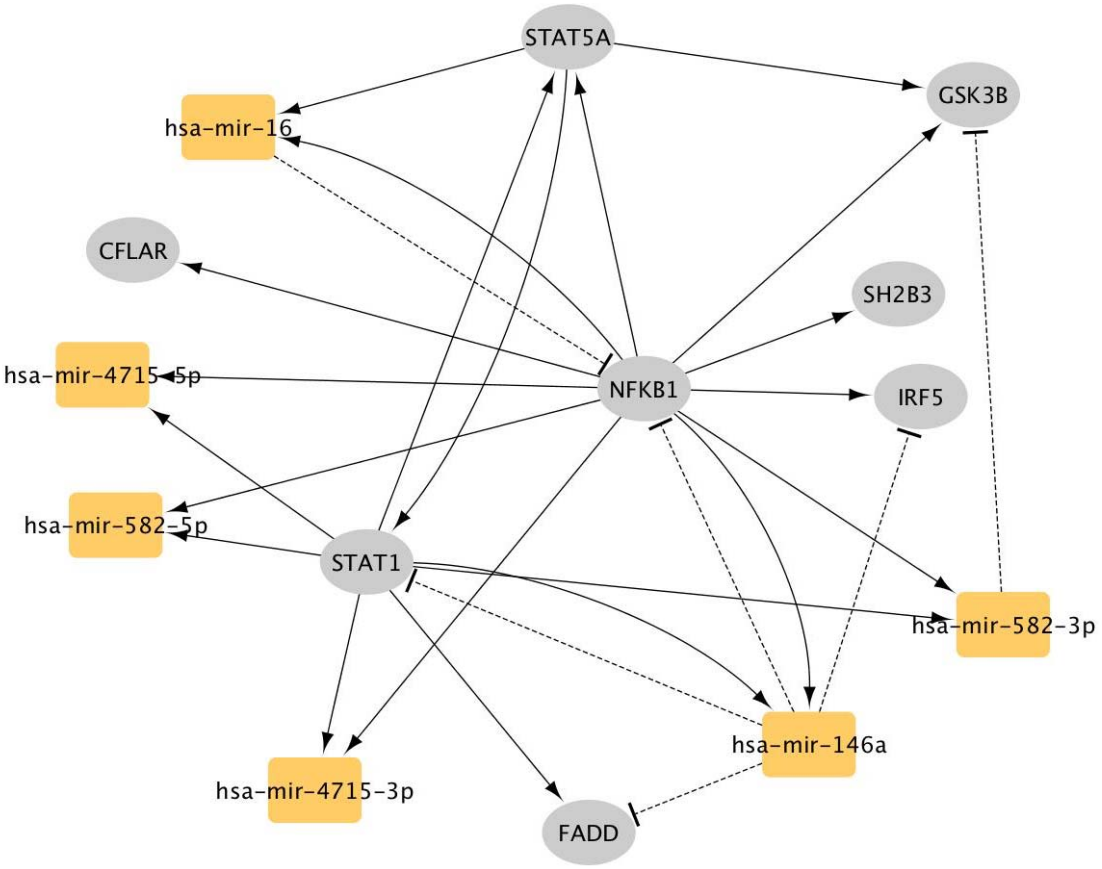
Supplementary Table_D2: List of diseases associated with the miRNAs enriched as regulators of the second-largest human healthspan pathway.

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

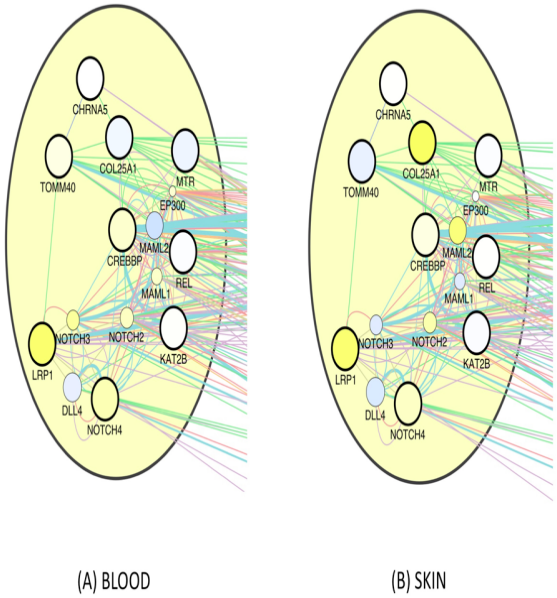
Supplementary Fig._D1: The regulatory interactions between the largest human healthspan pathway and the corresponding enriched miRNAs. The network was constructed using the TFmiR webserver and visualized using Cytoscape.



Supplementary Fig._D2: The regulatory interactions between the second-largest human healthspan pathway and the corresponding enriched miRNAs. The network was constructed using the TFmiR webserver and visualized using Cytoscape.

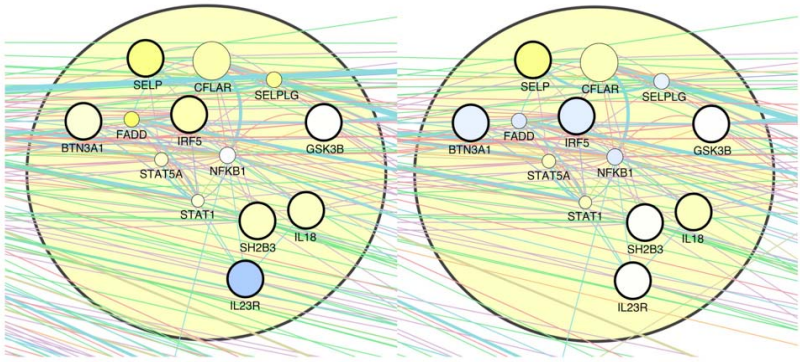


Supplementary Fig._E1: Comparison of expression patterns in two aging tissues, largest human healthspan pathway. Differentially expressed genes corresponding to blood (A) and skin (B) samples, contrasting young with old tissue, available from GEO (accessions GSE103232 and GSE75337), mapped to the human healthspan pathway of Fig._A1. Color conventions as in the Fig._A1.



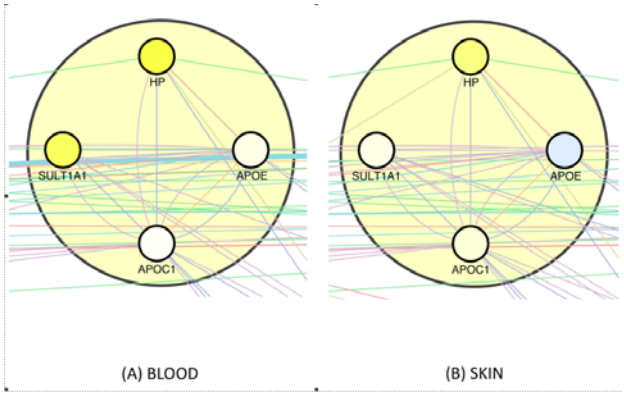
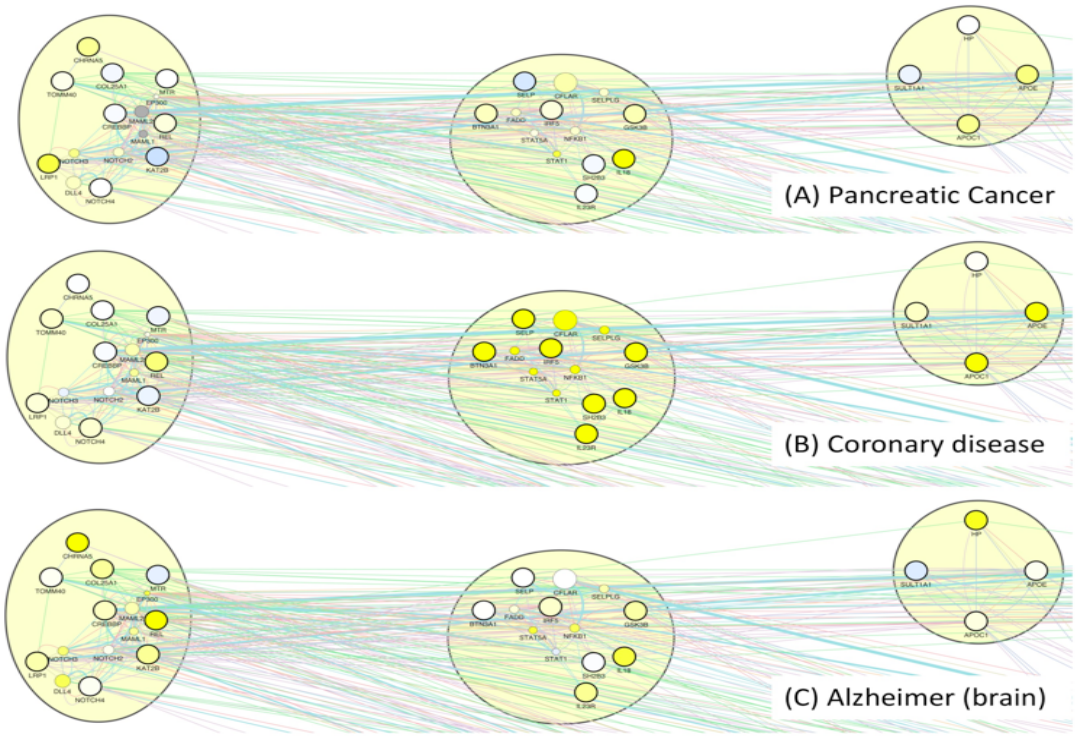
(A) BLOOD (B) SKIN

Supplementary Fig._E2: Comparison of expression patterns in two aging tissues, second-largest human healthspan pathway. See Supplementary Fig._E1 for further explanation.



(A) BLOOD (B) SKIN

Supplementary Fig._E3: Comparison of expression patterns in two aging tissues, third healthspan pathway. See Supplementary Fig._E1 for further explanation.



Supplementary Fig._E4: Comparison of expression patterns in two disease-affected tissues, top 3 healthspan pathways. Differentially expressed genes corresponding to datasets for pancreas cancer (A), coronary disease (B), and Alzheimer brain (C) samples, available from GEO (accessions GSE28735, GSE43292 and GSE15222 respectively), contrasting healthy with diseased tissue, mapped to the human healthspan pathway of Fig._A1. Color conventions as in the Fig._A1.