Compression of sickspan by interventions that steepen the survival curve

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Abstract

Longevity research aims to enhance the period of healthy life, known as the healthspan, while minimizing the duration of disability and morbidity, known as the sickspan. Various interventions extend lifespan in model organisms, but it is not known which of these are likely to compress the relative sickspan. Here, we present a theory that predicts which interventions effectively compress the sickspan. The theory is based on the shape of the survival curve - the fraction of organisms surviving as a function of age. Interventions that lengthen mean lifespan while preserving the shape of the survival curve, known as scaling, are hypothesized to extend the sickspan proportionally, without compressing it. Such interventions include caloric restriction and most other known longevity interventions. Conversely, a small subset of interventions that extend lifespan and alter the shape of the survival curve, particularly by steepening it, are predicted to compress the sickspan. We provide intuitive explanations for this theory and demonstrate its origin from the saturated removal model, a recent mathematical aging model calibrated in mice. We discuss specific longevity interventions in mice that steepen the survival curves, including senolytics, ketogenic diet, and agents that reduce glucose spikes and protect blood vessels, as potential candidates for compressing the sickspan. This hypothesis offers insights into the potential strategies for achieving compressed morbidity and extending healthspan.

Introduction

The lifespan of an individual typically begins with a period of health, called the healthspan, followed by a period of morbidity and disability called the sickspan [1–3]. Sickspan imposes substantial burden on human quality of life and is a chief and growing driver of economic expenditure across societies [4–6].

Understanding what sets lifespan, and how to extend it, has been a central goal of longevity research. There are several possible scenarios of lifespan extension regarding sickspan. In principle, an intervention could extend the sickspan exclusively (Fig. 1a). Alternatively, both healthspan and sickspan could be simply ‘stretched’ in proportion to lifespan. Finally, as advanced by James Fries [7] and emphasized in the field of gerontology, one could ideally aim to

extend healthspan and compress sickspan (Fig 1a) [1–3]. Optimal aging trajectories restrict morbidity to a brief period before death [3,7].

Research has identified diverse interventions that can extend lifespan in model organisms including baker's yeast, nematodes, flies and mice [8–12]. These include dietary restriction [13,14], perturbations of the IGF1 pathway, mTOR inhibition by drugs such as rapamycin [15,16], senolytic treatments [17–19] , *in vivo* partial reprogramming [20,21], along with several additional classes of drugs [22].

These experimental longevity interventions in general delay morbidity and reduce age-related decline in multiple physiological systems. However, whether they compress the sickspan in absolute or relative terms has not generally been reported. Data from *Caenorhabditis elegans* suggests that many interventions that extend lifespan extend sickspan proportionally [23,24] , and thus do not compress the sickspan in absolute or relative terms. A similar effect has been seen in medflies [25] .

It is thus of interest to identify which longevity interventions are likely to selectively compress sickspan, and which instead have the unintended consequence of extending it. Identifying factors affecting the relative healthspan and sickspan in model organisms would improve our basic understanding of the biology of aging and age-related morbidity, and inform the search for socially beneficial anti-aging therapies.

Here we present a hypothesis that can predict which longevity interventions compress sickspan. The hypothesis is based on the survival curve, the fraction of organisms surviving to a given age [26]. Many dietary and environmental longevity interventions that lengthen median lifespan preserve the shape of this curve, a phenomenon called scaling (Figure 1b-d) [23,27–29]. We predict that such interventions extend sickspan proportionally. In contrast, a small number of interventions in mice extend median lifespan and steepen the survival curve, making its shape more rectangular. We predict that these interventions compress the sickspan.

Here we provide intuitive arguments for this theory, and show that it arises naturally from a recent mathematical model of aging called the saturated removal model that was calibrated in mice [29–32]. We also discuss mice longevity interventions that steepen the survival curve as candidate interventions to compress sickspan, and discuss their potential mechanisms.

Results

Interventions that scale the survival curve also scale sickspan in the saturating-removal model

Many genetic, nutritional and pharmacological interventions have been identified that extend lifespan in mice and other organisms [8,9]. The effect of these interventions is classically measured using the survival curve [26], which reports the fraction of the study group surviving to a given age. The age of 50% survival is called the median lifespan.

By definition, longevity-enhancing interventions shift the survival curve to greater ages. Yet they often preserve its underlying shape, a phenomenon called scaling [23,27,28]. Interventions are said

to be scaling if their survival curves collapse onto the wild type survival curve when age is normalized by the median lifespan. Scaling was discovered by Strousturp and colleagues in *C. elegans* [27], and found in longevity interventions in budding yeast [28]. For example, Fig. 1b,c shows how median lifespan increases in *C. elegans* at low temperatures, but survival curves scale to a good approximation. Caloric restriction also extends lifespan [13,14,33], as reviewed by Fotana, Partridge and Longo [14]. But when we reanalyze the data and normalize age by median lifespan we find that the survival curves are similar in shape (Fig. 1d, statistical analysis presented below).

Interventions that extend lifespan and scale the survival curve may be considered intuitively to slow down the overall pace of aging. We hypothesize that they should accordingly slow down the processes responsible for age-related morbidity and functional decline. The upshot is a scaling of the sickspan and healthspan. Relative sickspan, that is the sickspan divided by lifespan, should remain unchanged by the intervention.

To test this intuition quantitatively we use the saturated-removal mathematical model for aging [29,31,32]. This model describes the dynamics of damage that is causal for morbidity and mortality. The model is agnostic to the molecular identity of this driving damage. Its mathematical form was deduced from dynamic data on mouse senescent cells, and its predictions were tested experimentally in mice [29]. The model was further tested by comparing it to diverse quantitative datasets on aging [29,31,32].

In the model, the driving form of damage is denoted X. It rises with age, and fluctuates around this rising trend on a timescale that is fast compared to the lifespan. The equation for X accounts for production, removal and noise: dX/dt = production - removal + noise.

The driving damage X is produced in the model by damage-producing units that accumulate linearly with age (Fig. 2a). The rate of production is therefore proportional to age, namely production = ηt. The driving damage is removed by repair processes that saturate at high damage levels. This is modeled as Michaelis-Menten-like saturation, i.e., removal = $\beta X/(\kappa+X)$, where β is the maximal removal rate and κ the halfway saturation point. The noise is modeled as white noise with amplitude σ. The SR model equation is shown in Fig. 2a.

Death is modeled to occur when driving damage X crosses a mortality threshold Xc. Age-related morbidity is modeled to occur when X crosses a morbidity threshold Xd. Since morbidity occurs before death, one has $Xd<Xc$.

Simulating the model shows that each individual has a rising stochastic trajectory of driving damage X (Fig. 2b). Each individual therefore crosses the death threshold Xc at a different time. The risk of death in the model rises exponentially with age and saturates at very old ages - this is the Gompertz law found in many organisms including humans. The survival curves are sigmoidal in shape (well described by Weibull distributions) and resemble empirical survival curves [31].

The beginning of the sickspan in each individual is defined by crossing the morbidity threshold Xd (Fig. 2b). The probability of crossing this threshold rises exponentially with age until it slows at very old ages. This simple model captures the incidence of hundreds of different age-related diseases as shown by Katzir et al [31].

A striking feature of the SR model is its ability to provide near-perfect scaling of survival curves [29]. Changes in the rate of damage production, namely the parameter η, shift median survival but

preserve the shape of the curve (Fig. 2c,d). When age is normalized by the median lifespan, the perturbed survival curve overlaps almost precisely with the unperturbed curve. Thus, changing production rate results in near-perfect scaling.

Crucially for our discussion, changing the production rate η also causes almost perfect scaling of the disease-free survival curve (Fig. 2c,d). Thus, changes in η cause scaling of the mortality curve, and scaling of the lifespan and sickspan. Relative sickspan remains almost unchanged - and in fact increases slightly in longevity interventions that affect damage production rate η.

Interventions that steepen the survival curve compress the relative sick span in the saturated removal model

The same model can be used to explore the effects of changing the other parameters. Changing any of the other model parameters, such as removal rate β, affects not only lifespan but also the shape of the curve (Fig. 2e).

In particular, increasing removal rate β increases lifespan and makes the survival curve steeper (Fig. 2e, left panel). Increasing β also makes the disease-free survival curve more steep (Fig. 2e, right panel). Importantly, the increase in lifespan occurs mostly by extending healthspan, and not sickspan. Thus, the relative sickspan is reduced by such interventions (Fig. 2e,g-h).

The same applies to raising the thresholds for mortality and morbidity Xc and Xd. Lifespan is increased mildly and steepness is increased strongly. There is a strong effect on relative sickspan: raising the thresholds strongly reduces both absolute and relative sickspan (Fig. 2f-h).

The reason that these parameters all affect steepness is that the shape of the survival curve in the model is essentially determined by a single dimensionless parameter $\beta Xc/\sigma$. Any perturbation that affects these parameters should have a non-scaling effect on the sickspan.

Note that production rate η does not appear in this dimensionless group, it affects lifespan but not the shape of the curve and hence changes in η preserve scaling.

In summary, longevity interventions can have differential effects on relative sickspan depending on which parameters they affect. Changes in damage production rate η slightly increase absolute and relative sickspan. In contrast, increases in damage removal or thresholds prolong lifespan and reduce relative sickspan- they lengthen lifespan primarily by lengthening the healthspan.

Several interventions in mice are predicted to compress sickspan

Next, we compare our prediction with published experimental data. Several experimental groups have developed the methods to directly measure frailty and sickspan in mice [35–37], but to our knowledge none have yet published individual-level sickspan or longitudinal frailty index of mice cohorts under longevity interventions [37,38]. Thus we do not yet have data on sickspan in mice.

In contrast, individual-level lifespan of mice under longevity interventions are routinely reported in the literature via survival curves. Thus we analyzed longevity interventions in mice from the literature and tested whether they show scaling or instead change the steepness of the survival

curve. Steepness was defined as the median lifespan divided by the interquartile range (IQR) of lifespan [26]. We tested the significance of steepening by bootstrapping with multiple-hypothesis-testing corrections (Methods).

We analyzed the 42 nutritional and pharmacological interventions in the National Institute on Aging (NIA) Interventions Testing Program (ITP) [39] (Fig. 3a, Fig. S2, S3). These are stringent tests repeated in three sites with genetically heterogeneous mice. In addition to the NIA ITP database, we also analyzed intervention experiments reported in 32 additional publications, including those with transgenic mice (Fig. 3b, Fig. S4).

Among the 65 interventions (42 in NIA ITP, 26 outside of NIA ITP) analyzed, most did not show steepening effects. We identified 6 classes of interventions that showed significant lifespan increase with steepening (Fig. 3a-b). Only 1 intervention showed lifespan increase with significantly reduced steepness.

The steepening intervention classes are senolytic treatment, ketogenic diet, VEGF overexpression, antioxidants such as nordihydroguaiaretic acid [45] (NDGA) and green tea extract [46] (GTE), and in males only, 17α-estradiol [42,47,48] and hypoglycemic agents such as acarbose [41,47,48] and canagliflozin [44] (Fig. 3c-h).

Senolytic treatments showed life extension using a genetic approach to ablate cells that express p16 [19,49]. Transgenic production of VEGF showed a 45.2±0.3% life extension and 147.6±2.3% increased steepness for females [40] (Fig. 3b, Fig. S4). Cyclic ketogenic diet in two separate studies [43,50], and a ketogenic dietary supplement [51] showed evidence of life extension and steepening. In the NIA ITP dataset, acarbose [41] and 17α-estradiol [42] showed a dose dependent effect, with both lifespan and steepness increasing with dose.

Additional interventions showed steepening with only mild lifespan extension, such as green tea extract (females) and nordihydroguaiaretic acid [45] (males) in the NIA ITP data (Fig. 3a). The antioxidant taurine has a mild steepening effect (Fig. S4). High dosage of rapamycin (42ppm in females) causes steepening but is different from other rapamycin data points which show approximate scaling (Fig. 3a, Fig. S4, see Methods for statistical test of scaling).

The steepening interventions may constitute promising targets for future measurements of mice sickspan.

One prediction of the present approach concerns combinations of interventions. Combining a perturbation that scales with one that steepens should show synergy with a steep survival curve in which lifespan is extended beyond either individual intervention. This prediction is supported by NIA ITP data on a combination of acarbose (steepening in males) and rapamycin (scaling) administered at 9 months. Longevity was enhanced compared to acarbose or rapamycin alone, and steepness was comparable to the acarbose mono-treatment, as predicted (Fig. 3a, Fig. S5, S6).

Discussion

We present the hypothesis that longevity interventions that steepen the survival curves reduce relative sickspan. In contrast, interventions that scale the survival curve change healthspan and

sickspan proportionally as they extend lifespan, and thus do not compress relative sickspan. We show that these effects hold in the saturated removal mathematical model of aging. The model indicates that interventions that affect the production of the driving damage cause approximate scaling and mild extension of sickspan, whereas those that increase removal of the driving damage cause steepening and compress sickspan. We discuss interventions in mice which steepen the survival curve and are thus predicted to be candidates to shorten relative sickspan.

One can intuitively argue for this hypothesis without need for a mathematical model. Many of the longevity interventions that scale the survival curve seem to generally slow the tempo of aging processes. If one assumes that age-related morbidity is caused by many of the same factors that cause mortality, it makes sense that healthspan and sickspan should also scale by the same proportions as lifespan. Experiments on *C. elegans* support this proposition: lifespan-extending interventions that scale survival curves also preserve relative sickspan, defined by the period of lack of motility at old age [23]. There exist longevity mutants in *C. elegans* that change the steepness of the survival curve, such as nuo-6 mutations, but they have not been tested for sickspan.

In contrast, longevity interventions that steepen the survival curve may be argued to affect processes more proximal and specific to aging-related decline than its overall tempo or the rate of living. They make the mortality curve more rectangular and thus the time of death less variable between individuals. This can be considered to reduce the proportion of frail individuals that normally die at ages younger than the median lifespan. The interventions seem to make the whole population more robust (longer lived) and at the same time more homogenous in terms of frailty. An extreme steepening means that almost all individuals reach the maximal lifespan. In humans, individuals close to the maximal lifespan seem to have a relatively short sickspan [52,53]. Thus it is plausible that relative sickspan is shortened by steepening interventions.

To obtain a quantitative account of how steepness may result in compressed sickspan we used the staurating removal (SR) model. This model was developed and calibrated based on data in mice and adjusted to human timescales[29]. The SR model assumes a form of damage *X* that drives morbidity and mortality, which rises with age in a fluctuating way. The model is agnostic to the nature of this damage, which could be different in different species. The model includes production of driving damage X at a rate that rises linearly with age - corresponding to accumulation of damage-producing units. It also assumes that removal of the damage *X* occurs by mechanisms that saturate at high damage.

The SR model accounts for many quantitative features of aging across organisms, including human disease incidence [31], mouse heterochronic parabiosis [30], the Gompertz hazard curve [29] and rapid transitions between hazard curves upon certain midlife interventions [29]. The SR model was recently found to describe damage fluctuations in bacterial starvation[32].

The SR model shows that any perturbation that increases the steepness of survival curves also compresses the sickspan, whereas any perturbation that scales the survival curve also scales the sickspan. More specifically, changing the rate of production of the damage that drives aging leads to approximate scaling of mortality and sickspan. Raising the specific removal rate of the driving damage extends lifespan with steepening, and compresses the sickspan. The greatest compression, albeit with modest lifespan extension, is seen with perturbations that raise the thresholds for mortality and morbidity.

Interventions that steepen the survival curve offer clues as to the damage that drives aging-related phenotypes

Aging research has made progress in understanding molecular and cellular processes that contribute to aging. This progress is not yet matched by theoretical frameworks that integrate these empirical findings [54]. The present theoretical framework and hypothesis offers a potential tool to interpret diverse longevity interventions - scaling points to effects on damage production, and steepening to effects on removal or thresholds.

The experimental interventions that steepen the survival curve offer clues as to the damage that drives aging-related phenotypes. These steepening interventions extend median lifespan more than they extend maximal lifespan (eg. 90th percentile of lifespan).

Senolytics extend lifespan and steepen the survival curve, as noted by Kowald and Kirkwood [49] who analyzed data from two types of senolytic drugs and from genetic ablation of p16 positive cells. Thus, in mice, senescent cells may play the role of the driving damage *X* in the SR model, as suggested by Karin et al [29]. The effects of senescent cells include secretion of inflammatory signals and signals that slow stem cell renewal, as well as signals that affect the ECM [55]. Senescent cells also stimulate other cells to adopt a senescent phenotype in a paracrine manner. These signals occur both systemically and locally in aged mammals; the inflammatory secretory profile of senescent cells contains factors known to contribute to many if not all age-related pathology [56–58]. Ablation of senescent cells delays or ameliorates a range of age-related diseases in mice models [17,19,59].

An intriguing class of steepening interventions includes cyclic ketogenic diet [43,50], acarbose [41] and canagliflozin [44]. These three interventions share a common mechanism: they all lower glucose spikes after a meal. Ketogenic diet is low on carbohydrates. The drug acarbose inhibits an enzyme that releases glucose from complex carbohydrates in the gut. The diabetes drug canagliflozin inhibits the glucose transporter in the gut as well as kidney glucose reabsorption. All three interventions extend median lifespan in male but not female mice.

One of the main tissues damaged by glucose spikes is the vasculature. Damage to blood vessels is a major pathology in diabetes, causing cardiovascular and renal disease. Endothelial cells are damaged by high glucose in several ways including reactive oxygen species, glycation of proteins and activation of signaling pathways such as PKC [60]. Intermittent high glucose causes senescence in endothelial cells [61] .

One hypothesis is that damaged microvasculature reduces access of immune cells to the senescent cells in tissues. Interventions that protect microvasculature may thus enhance the ‘roads’ for immune clearance, and hence increase the removal rate beta. This can explain their steepening effect on the survival curves.

The idea of vascular protection as a mechanism for the steepening interventions is consistent with a VEGF longevity intervention which increases lifespan by 40% and, in females, steepens the survival curve [40]. VEGF promotes vascular repair, and can also slow age-related loss of microvasculature, increasing access of immune cells to senescent cells and assisting the removal of senescent cells, increasing removal rate β.

The vascular hypothesis is also supported by the captopril intervention, which increases steepness with a mild longevity gain. Captopril is an angiotensin-converting enzyme (ACE)

inhibitor. It decreases hormones that narrows the blood vessels, so it helps to relax the veins and arteries and improve functioning of the vasculature.

The non-feminizing estradiol, 17□α□estradiol, steepens survival curves and extends lifespan even when administered late in life in aging male mice [62]; it has no longevity effect in females. The longevity effects of this estradiol are reported to depend on testicular hormones. It is not clear how this estradiol ties into the mechanism of the other steepening interventions, although it is plausible that it has protective roles on tissues [63,64] and thus may reduce the load on the removal mechanisms and hence raise the removal parameter β.

Rapamycin treatment also extends life when started at middle age [65–67,48,68], but does not generally steepen the survival curve except at the old-age tail of the survival curve (Fig. S7, S8). It may thus have a major effect that scales survival, such as mTOR inhibition that slows translation, slowing the rate of living. Notably, rapamycin at high doses shows a steepening effect in females, particularly on the part of the survival curve that corresponds to very old ages. It may thus have a second effect in older individuals.

Additional interventions that cause steepening but with milder longevity effects include antioxidants and anti-inflammatory agents (NDGA [45], green tea extract [46]) (Fig. S9). In the SR model, strong steepening with mild lifespan extension characterizes coordinated increases in the mortality and morbidity threshold *Xc* and *Xd*. One may hypothesize that these thresholds are affected by tolerance to inflammatory damage and reactive oxygen species.

Interventions that scale the survival curve are predicted not to compress sickspan - instead they are suggested to extend sickspan by the same proportion that they extend lifespan. These interventions include caloric restriction. Caloric restriction is thought to lower the rate of living, and has pleiotropic effects beyond its effects on longevity such as reducing body weight, reducing fertility and increasing frailty [69].

It would be interesting to see whether emerging interventions such as in vivo partial epigenetic reprogramming scale or not. Data from initial studies does not show significant steepening [20,21] (Fig. S10), although further research is warranted.

A note of caution in interpreting steepness of survival curves is that heterogeneity in the population would affect steepness. Thus, studies in model organisms with well-controlled conditions and genetic backgrounds are essential to test the present approach.

Summary

We presented a hypothesis that longevity interventions that steepen the survival curve have the potential to compress relative sickspan. Conversely, interventions that do not change the shape of the survival curve, namely interventions that show scaling, are predicted to extend both lifespan and healthspan proportionally and do not compress relative sickspan. The hypothesis is supported by mathematical modeling using the saturated removal (SR) model of aging. We discuss various interventions in mice that steepen survival curves, such as senolytics, ketogenic diet, acarbose, and canagliflozin, and suggest their potential mechanisms of action. This study provides insights into the relationship between survival curves, longevity interventions, and relative sickspan, contributing to our understanding of strategies to improve health in aging individuals.

Methods

Saturated removal (SR) model simulations

For our baseline, we simulated the SR model using parameters calibrated in mice [29]: η=2.3x10^{-4}/day^2, β=0.15 /day, κ=0.5, σ=0.16 /day, Xd=12, Xc=17, and then rescaled the time units by 35 fold to approximate human lifespan. This choice originates from the parameters calibrated for mice in [29], with η reduced by a factor 35 to obtain a median human lifespan of 83 years. The Xd value was set to 12, which is the lower end of the estimated thresholds for age-related diseases [31]. It results in a mean sickspan of 12.3y, consistent with reported age achieved without major chronic diseases in the United States [70]. We simulated interventions by varying an individual parameter η or β, or by simultaneously changing Xc and Xd by the same factor. Simulation used Mathematica 12.3.1.

Collection of survival curves from the literature

We collected survival curves from existing publications, including those conducted by NIA ITP, and those independent of it.

For experiments conducted by NIA ITP, we downloaded lifespans of individual mice from all experimental and control cohorts (C2004 - C2017) directly from the Mouse Phenome Database (https://phenome.jax.org/projects/ITP1, June 2023). Experiments with different drug concentrations and different initiation age and regimes of exposure are analyzed and reported separately as different interventions. Data from all 3 sites of NIA ITP were always pooled together. NIA ITP used UM-HET3 mice, a heterogenous stock produced by a four-way cross.

For experiments independent of NIA ITP, we downloaded the PDFs of the relevant publications, and used WebPlotDigitizer (https://apps.automeris.io/wpd/) to digitize the survival curves. We also recorded the genetic background and the sample sizes of control and treated cohorts. From these survival curves, we inferred the time of deaths of individual mice constituting the data. We excluded all publications using mice strains considered to be disease models that have specific deleterious genes. In contrast to NIA ITP, all but one study used inbred strains or hybrids involving 2-way or 3-way crosses among inbred strains.

Evidence of steepening

We calculated steepness from the survival curves defined as the median lifespan divided by the interquartile range (IQR), that is $Age_{50}/(Age_{75}-Age_{25})$, where Age_x the age at which x% survive. This definition of steepness is exactly the inverse of relative IQR previously studied by Carnes et al [26].

We implemented 2 statistical tests to evaluate the effects of each pair of treated and control cohorts. The first test is based on quartiles, and it evaluates whether the treated group is significantly different from the control group in either steepness or longevity (quartiles test). The second test evaluates whether the treated and control group have scaling survival curves, i.e. whether they have the same distribution of lifespan after rescaling (AFT+KS test).

To implement the quartile test, we bootstrapped the death times with returns. For every mice cohort (control and interventions groups form 2 independent cohorts), we resampled with replacement, 100 times the recorded death times and censorship status. Based on these bootstrapped samples, we then estimated the joint distributions of median lifespan and steepness for both control μ_c=(ln(L_c), ln(S_c)) and intervention cohorts μ_i=(ln(L_i), ln(S_i)) by 2D normal distributions $N_c(\mu_c, \Sigma_c)$ and $N_i(\mu_i, \Sigma_i)$. We then tested the null hypothesis that the control and intervention groups have the same longevity and steepness (H_0: L_i/L_c=1 and S_i/S_c=1). Because $\mu_i - \mu_c \sim N_i(\mu_i-\mu_c, \Sigma_i+\Sigma_c)$, H_0 is equivalent to that the Mahalanobis distance between control group (ln(L_c), ln(S_c)) and intervention group (ln(L_i), ln(S_i)) is 0. Thus, for every intervention, we calculated z-scores for longevity and steepness effects using Cholesky whitening transformations and tested H_0 by applying a one-sided chi-square test to the square sum of the z-scores.

For intervention experiments conducted by NIA ITP, we apply a p-value threshold adjusted for multiple comparisons using the Benjamini-Hochberg method. For α level of 0.05, the adjusted p-value threshold is 0.0113. For intervention experiments conducted independent of NIA ITP, due to the varying data quality and statistical power, we conducted Benjamini-Hochberg adjustment separately from those in NIA ITP, resulting in an adjusted p-value threshold of 0.0214 (α=0.05).

The second test (AFT+KS) tests whether the control and treated groups have the same shape of lifespan distribution after rescaling, and is based on a Kolgomorov-Smirnov (KS) test modified to accommodate right censored data [71]. The rescaling is based on the accelerated failure time model (AFT), and implemented using Python package lifelines (version 0.27.7). Stroustrup et al [27] evaluated the power of this combined testing strategy (AFT+KS) using simulated data. We use this test to classify NIA ITP treatments into scaling and non-scaling categories. In this case, one should be equally mindful of Type I and Type II errors, and thus we applied a balanced p-value threshold of α=0.10. For treatments independent of NIA ITP, due to the varying sample sizes and statistical powers, we use an effect size treathold to classify steepening vs non-steepening treatments. The AFT+KS test is nonetheless performed on these interventions and its results are combined with the quartile test to determine statistical significance (Fig. S4).

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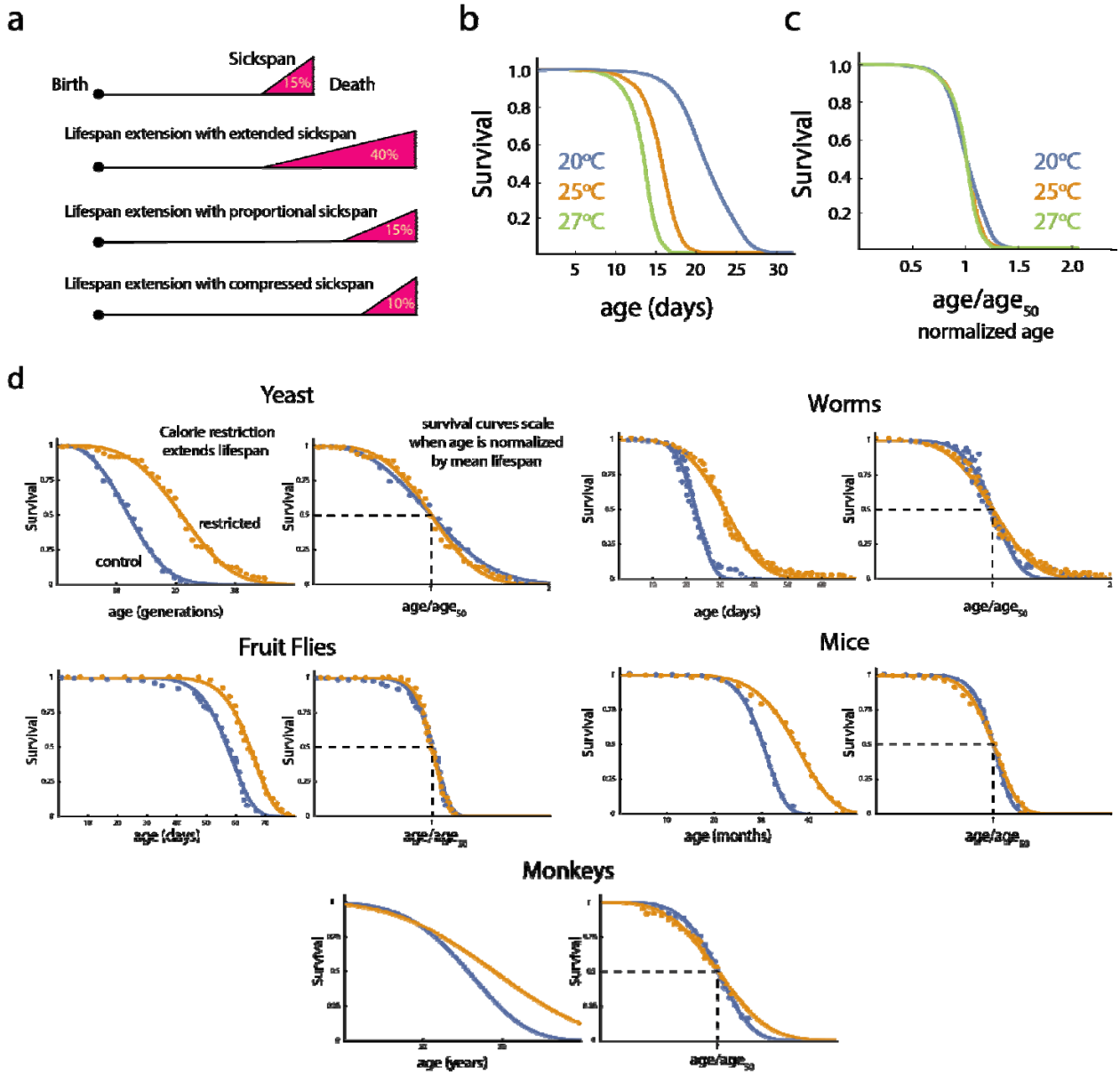


Figure 1. Many interventions that extend lifespan show scaling in their survival curves. **a**, Lifespan extension can lead to an extended period of morbidity called sickspan; it can also lead to a proportional increase in sickspan, or to a compression of relative sickspan [3]. Schematics inspired from reference [3,4] **b**, Survival curves in *C. elegans* show increased median lifespan at lower temperatures. **c**, The survival curves in (**b**) scale, i.e. the survival curves collapse onto the same curve when age is normalized by median lifespan (adapted from reference [27]). **d**, Caloric restriction shows approximate scaling in different model organisms. Each panel shows the survival curves plotted versus age or versus age normalized by median lifespan, with data in dots and a Weibull curve fit in a thin line. Data adapted from reference [34].

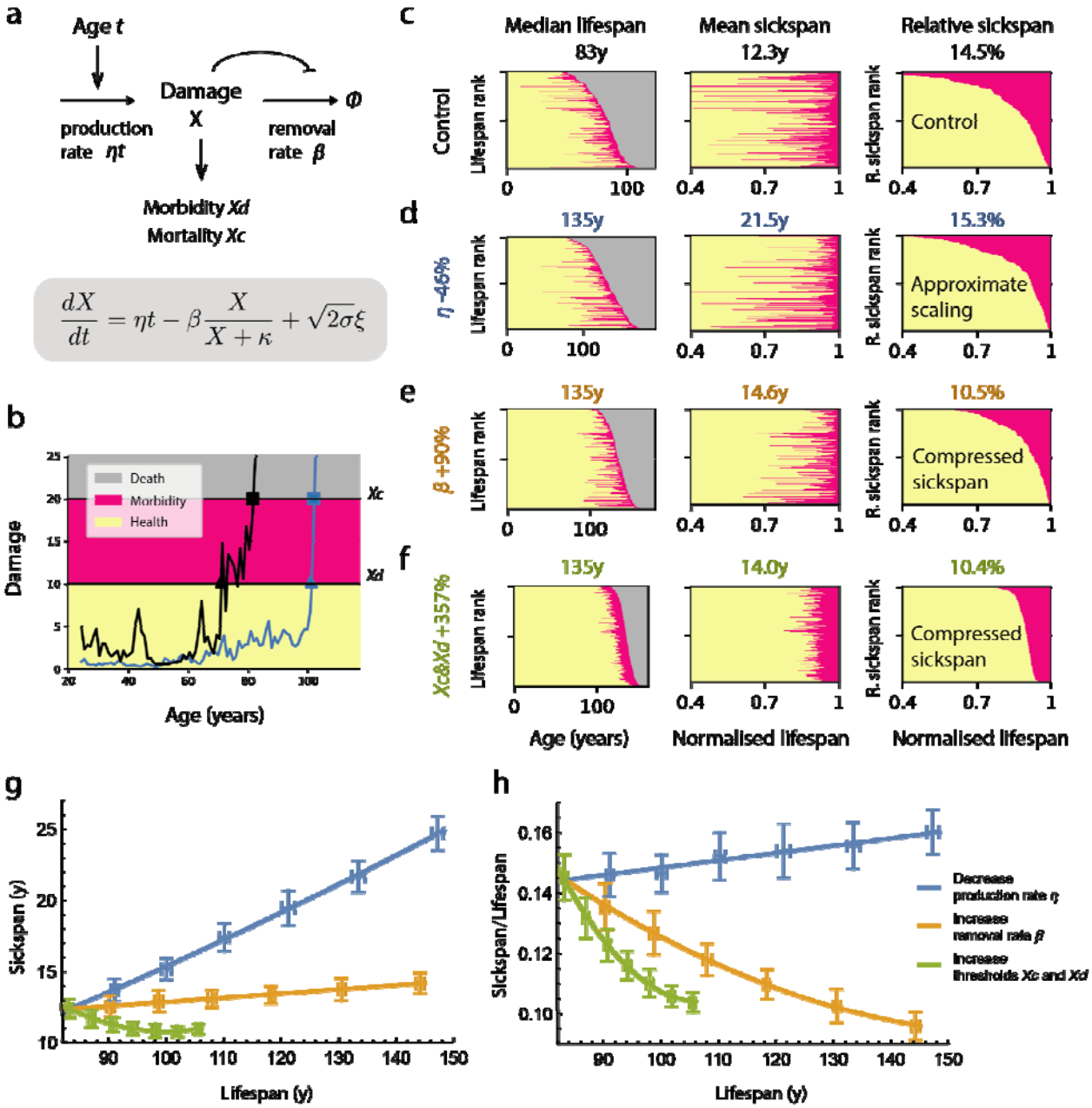


Figure 2. Interventions that scale survival curves also scale the sickspan, whereas interventions that steepen survival curves compress relative sickspan. a, Schematic of the staurating removal (SR) model and model equation. **b**, Trajectories of damage *X* for two simulated individuals, crossing the thresholds for morbidity and mortality at different times. **c**-**f**, Distributions of lifespans, healthspans and sickspans in SR model, using baseline (**c**) parameters and under hypothetical longevity interventions (**d**-**f**) targeting different model parameters. All interventions raise median lifespan by the same extent. Each row shows simulations of 200 individuals shown in three ways in 3 columns: left, ranked by lifespan versus age (survival curve); middle, ranked by lifespan (same as left column) versus age normalized by individual lifespan to show relative healthspan and sickspan; right, individuals re-ranked by relative sickspan. Red indicates the sickspan. Numbers above the axes indicate, from left to right, median lifespan, mean sickspan, mean relative sickspan. **c**, Baseline parameters (see Methods). **d**, Simulations with damage production rate *η* lowered by 46%. **e**, Simulations with removal rate higher by 90%. **f**, Simulations with both thresholds for morbidity *Xd* and mortality *Xc* raised by a factor of 3.57. **g**-**h**, Effects on absolute and relative sickspan by hypothetical longevity interventions targeting different model parameters. Absolute (**g**) and relative (**h)** sickspan are shown as functions of median lifespan. See Fig. S1 for absolute and relative sickpan shown as functions of parameter changes.

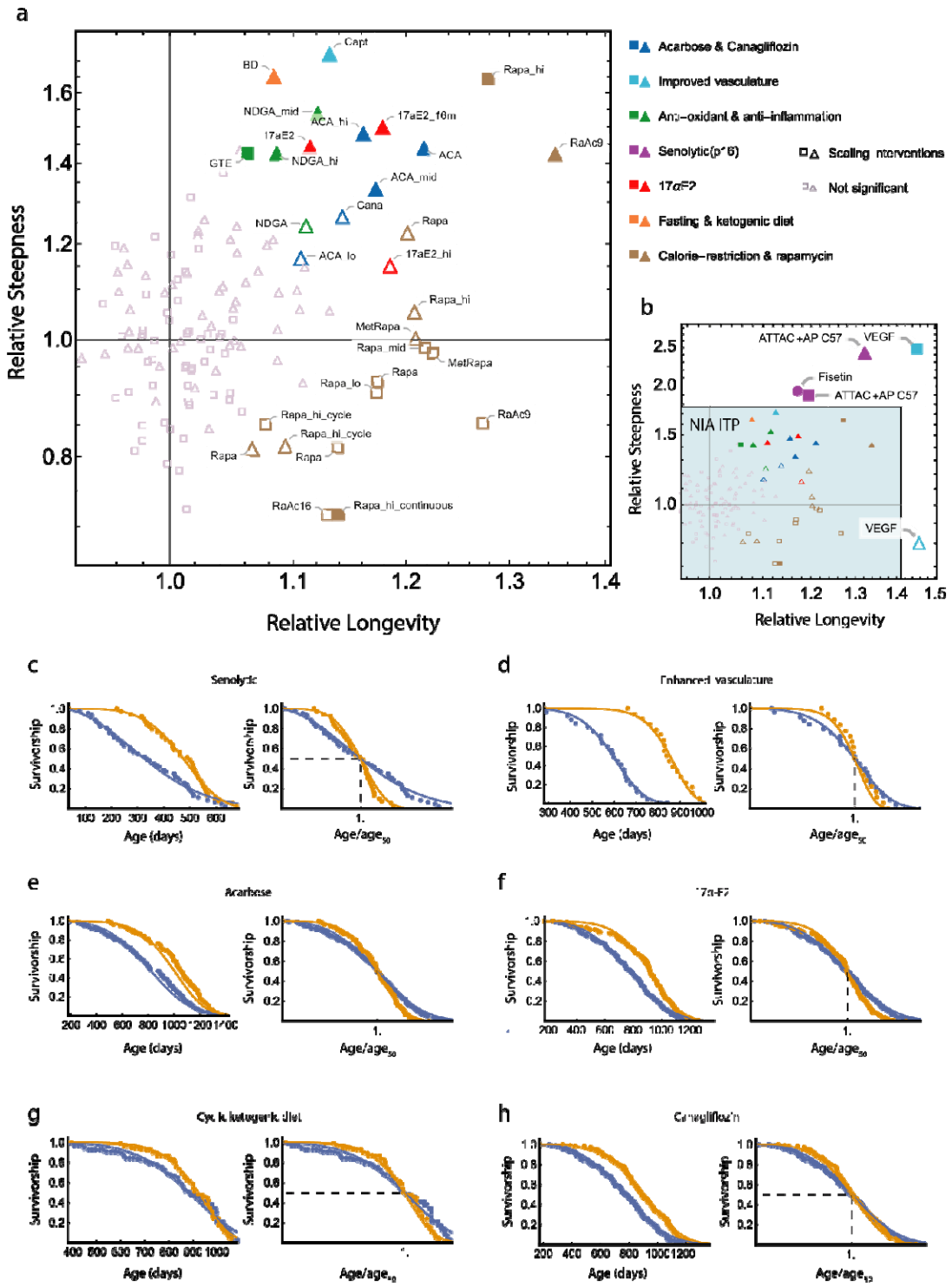
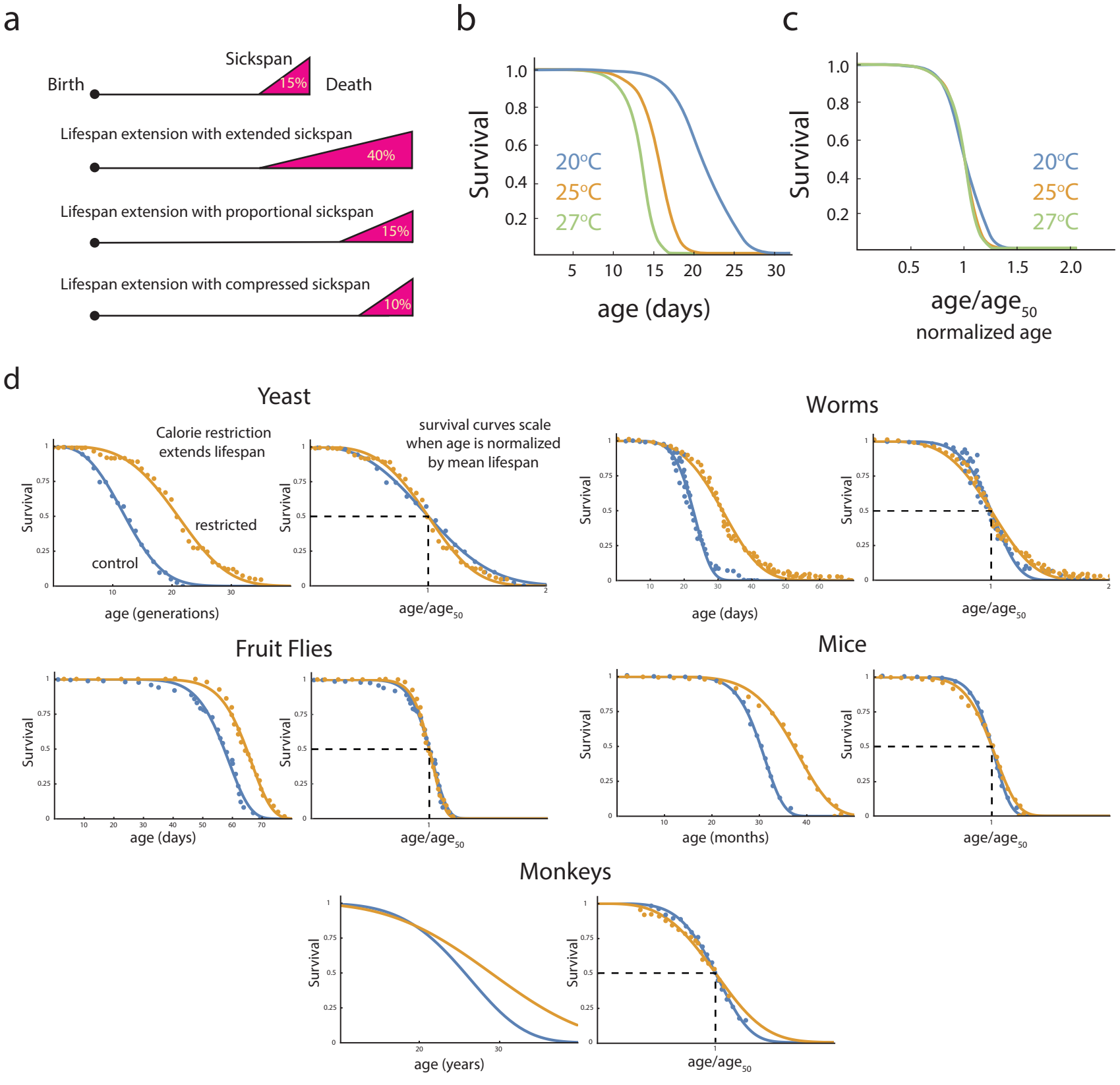
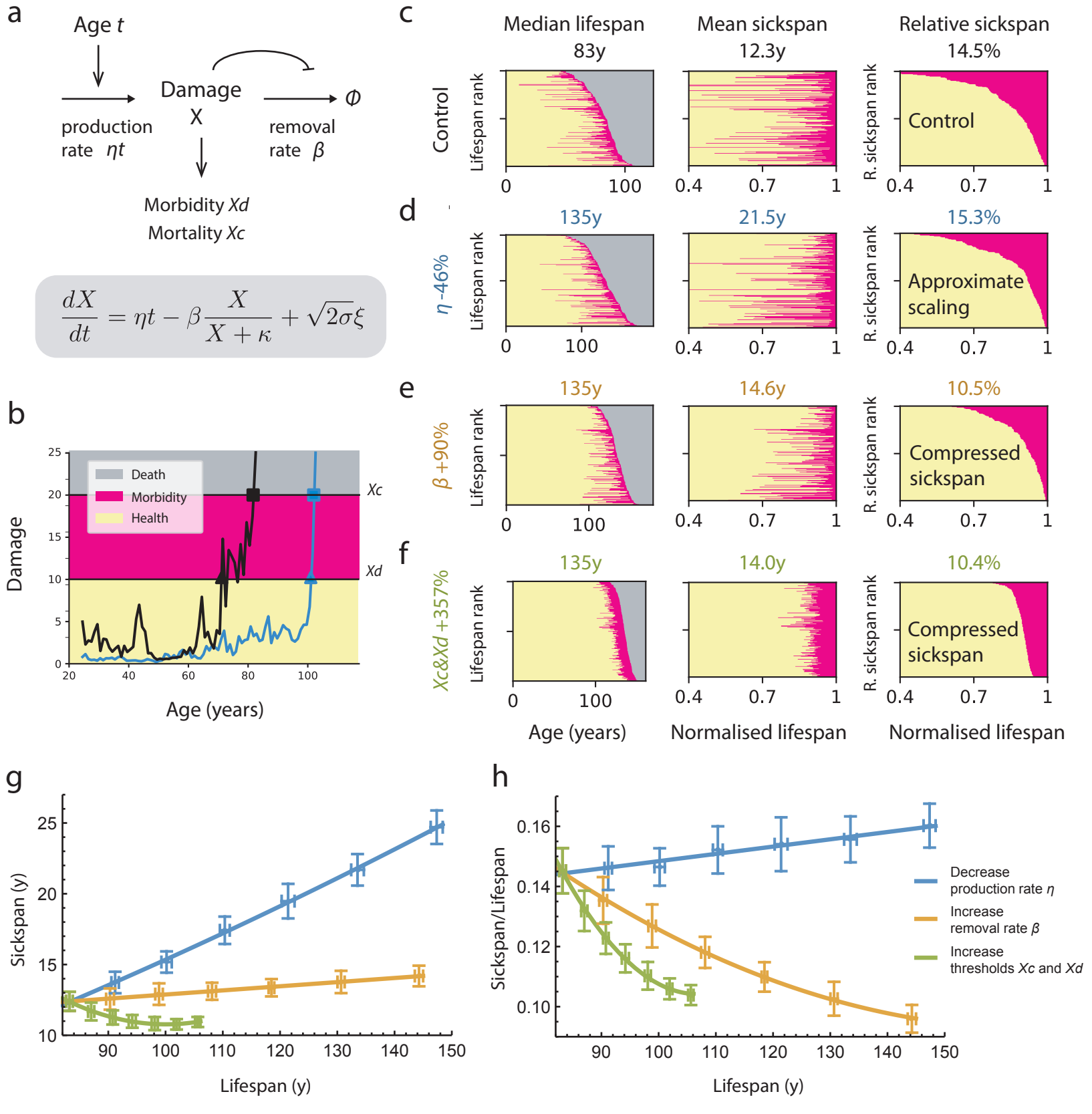
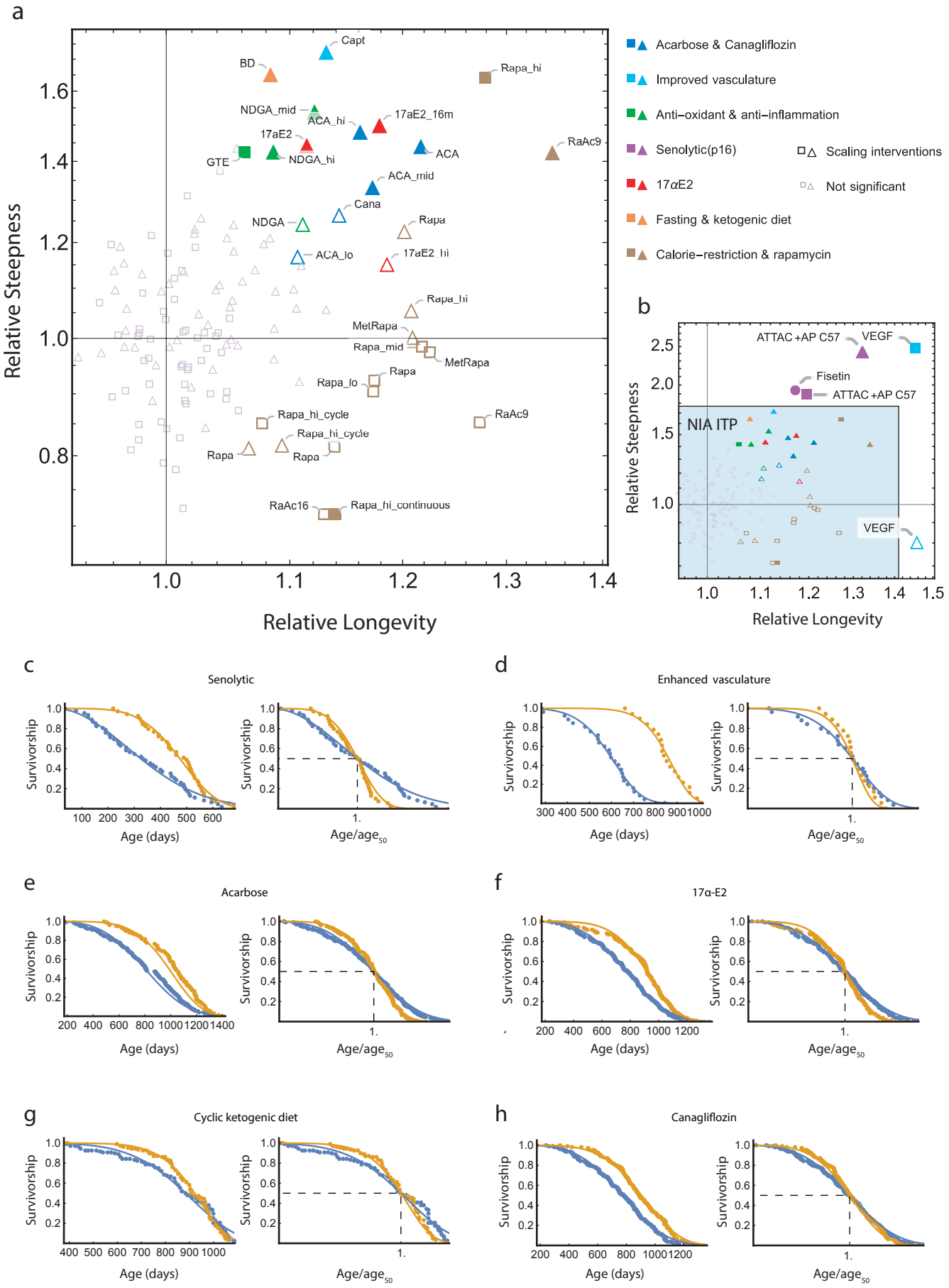
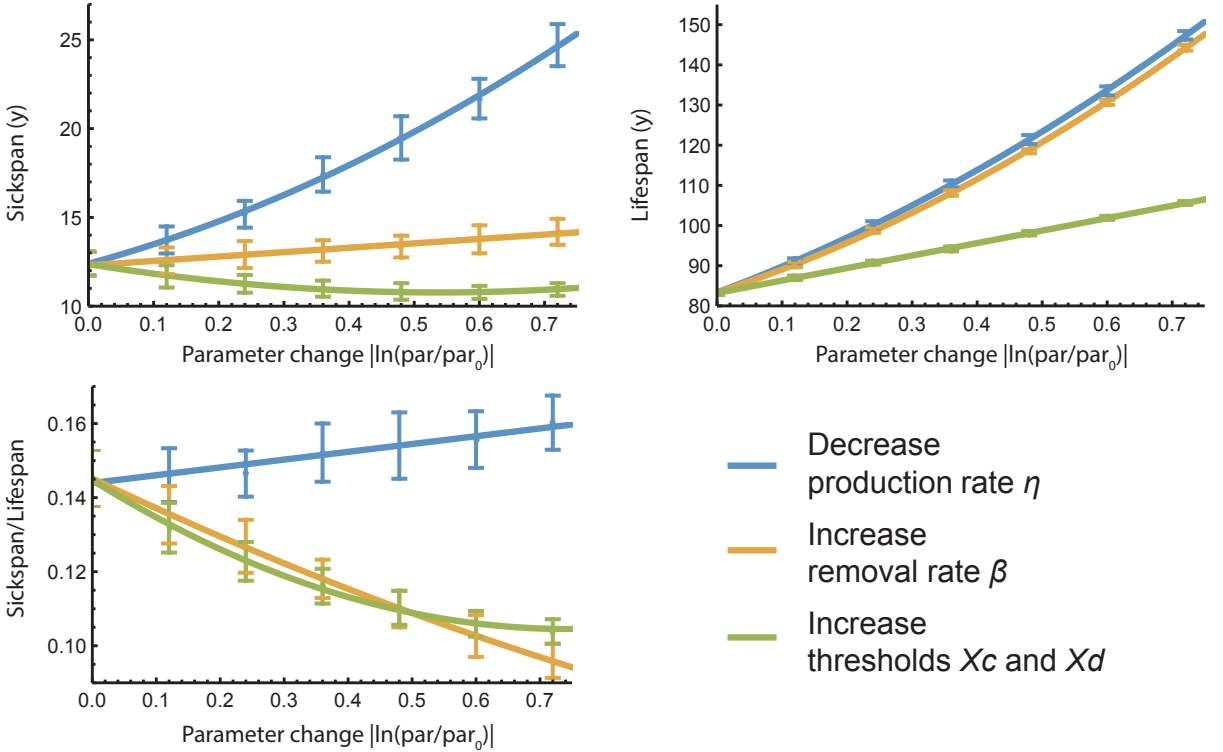

a
Relative Steepness
Relative Longevity
Acarbose & Canagliflozin
Improved vasculature
Anti-oxidant & anti-inflammation
Senolytic(p16)
17αE2
Fasting & ketogenic diet
Calorie-restriction & rapamycin
Scaling intervention
Not significant
Capt
BD
Rapa_hi
NDGA_mid
ACA_hi
17aE2_10m
17aE2
GTE
NDGA_hi
ACA
RaAc9
ACA_mid
Cana
NDGA
Rapa
ACA_lo
17aE2_hi
Rapa_hi
MetRapa
Rapa_mid
MetRapa
Rapa
Rapa_lo
Rapa_hi_cycle
RaAc9
Rapa_hi_cycle
Rapa
Rapa
RaAc16
Rapa_hi_continuous
b
ATTAC+AP C57
VEGF
Fisetin
ATTAC+AP C57
NIA ITP
VEGF
Relative Steepness
Relative Longevity
c
Senolytic
d
Enhanced vasculature
e
Acarbose
f
17α-E2
g
Cyclic ketogenic diet
h
Canagliflozin
Survivorship
Age (days)
Age/age50

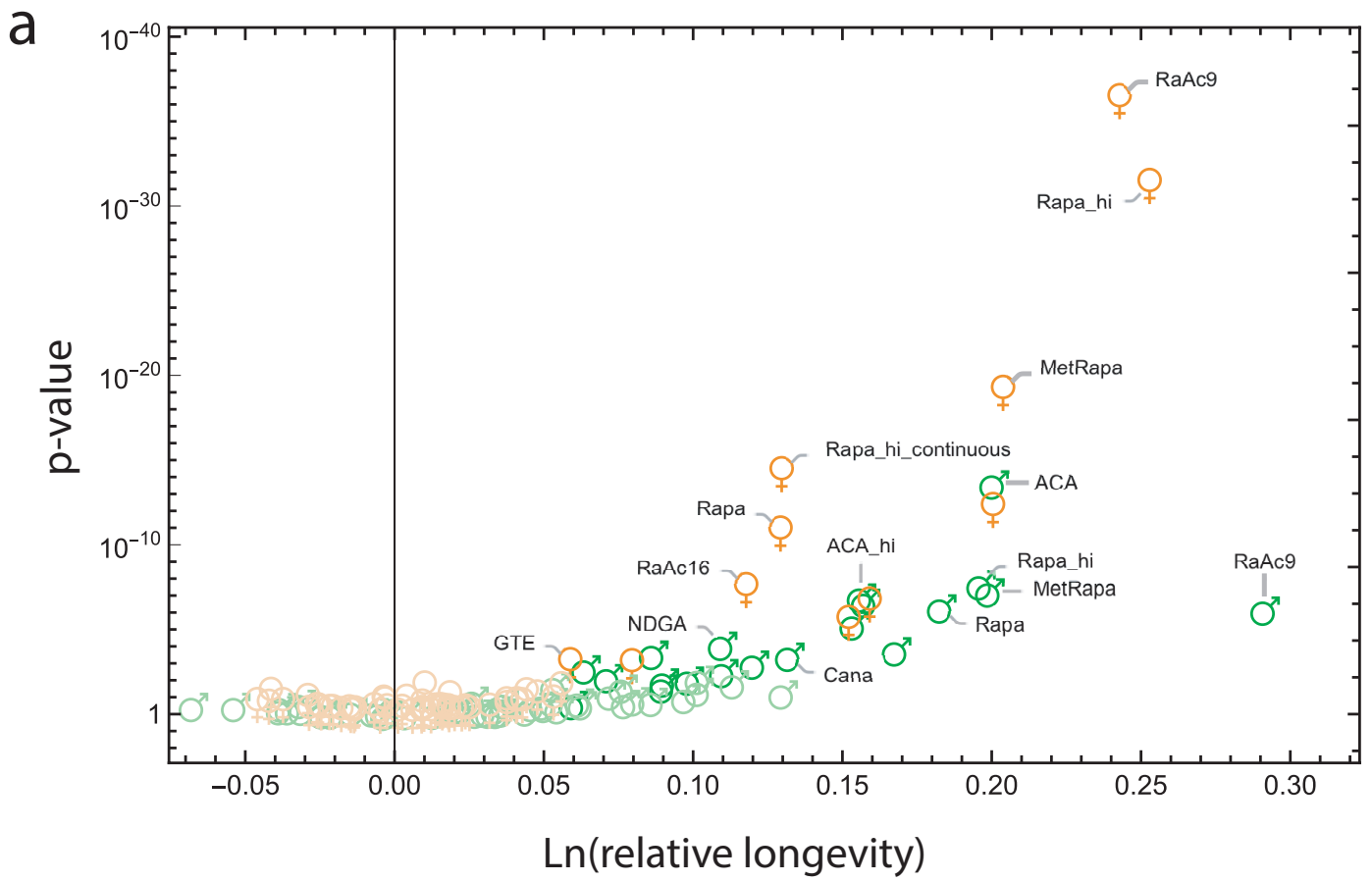
Figure 3. Lifespan extension interventions in mice that steepen the survival curve. a, Longevity and steepness relative to control in all interventions from the NIA Interventions Testing Program (NIA ITP) [39]. Square and triangle symbols denote interventions for female and male mice cohorts respectively. Small and faint symbols indicate interventions that are not statistically significant (see Methods for details of the test, α=0.05 with Benjamini-Hochberg correction for multiple comparisons). Filled and empty symbols indicate scaling and non-scaling interventions respectively (based on AFT+KS test strategy evaluated by Stroustrup et al [27], see Methods). See Supp. Data 1 for statistical test results. **b**, Relative longevity and steepness of selected intervention in the literature outside of the NIA ITP, including those based on transgenic mice. Due to the varying sample size and statistical power of the studies analyzed, only interventions that are without counterparts in NIA ITP are shown. See Fig. S4 and Supp. Data 2 for all non-NIA-ITP interventions analyzed. Graphic symbols and legend are the same as those in (**a**). Round symbol denotes a study that only reported mixed gender cohorts. Effect sizes are not directly comparable between the shown interventions and those in NIA ITP because of the differences in controls. **c**-**h**, Survival curves and rescaled survival curves of steepening interventions (orange) and their respective control (blue). **c**, Senolytic treatment (genetic ablation of p16 expressing cells) [19] in mice from both sexes starting at 12 months of age. **d**, Transgenic VEGF female mice with mildly increased systemic VEGF level via induced production by hepatocytes [40]**. e**, Acarbose (2500 ppm) in male mice starting at age of 8 months [41]. **f**, Non-feminizing estradiol 17aE2 in male mice (14.4 ppm) starting from age of 16 months [42]. **g**, Cyclic ketogenic diet consisting of alternating weeks of ketogenic and control diets in male mice starting at 12 months of age [43]. **h**, Glucose transporter inhibitor canagliflozin [44] at dose 180 ppm in male mice starting from age of 7 months [44].

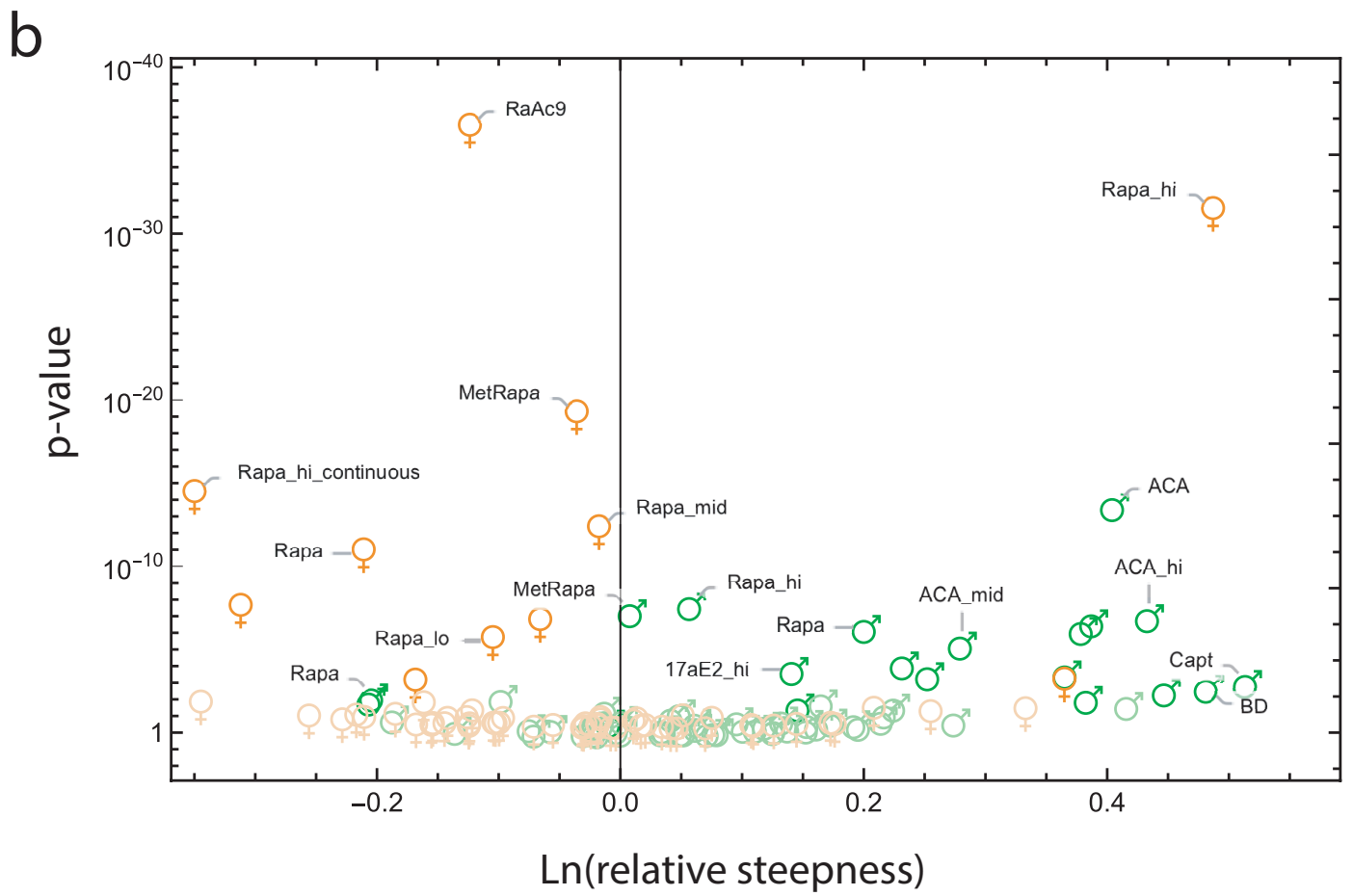

a
Sickspan
Birth
15%
Death
Lifespan extension with extended sickspan
40%
Lifespan extension with proportional sickspan
15%
Lifespan extension with compressed sickspan
10%
b
Survival
20°C
25°C
27°C
age (days)
c
Survival
20°C
25°C
27°C
age/age50
normalized age
d
Yeast
Calorie restriction extends lifespan
restricted
control
survival curves scale when age is normalized by mean lifespan
Survival
age (generations)
age/age50
Worms
Survival
age (days)
age/age50
Fruit Flies
Survival
age (days)
age/age50
Mice
Survival
age (months)
age/age50
Monkeys
Survival
age (years)
age/age50

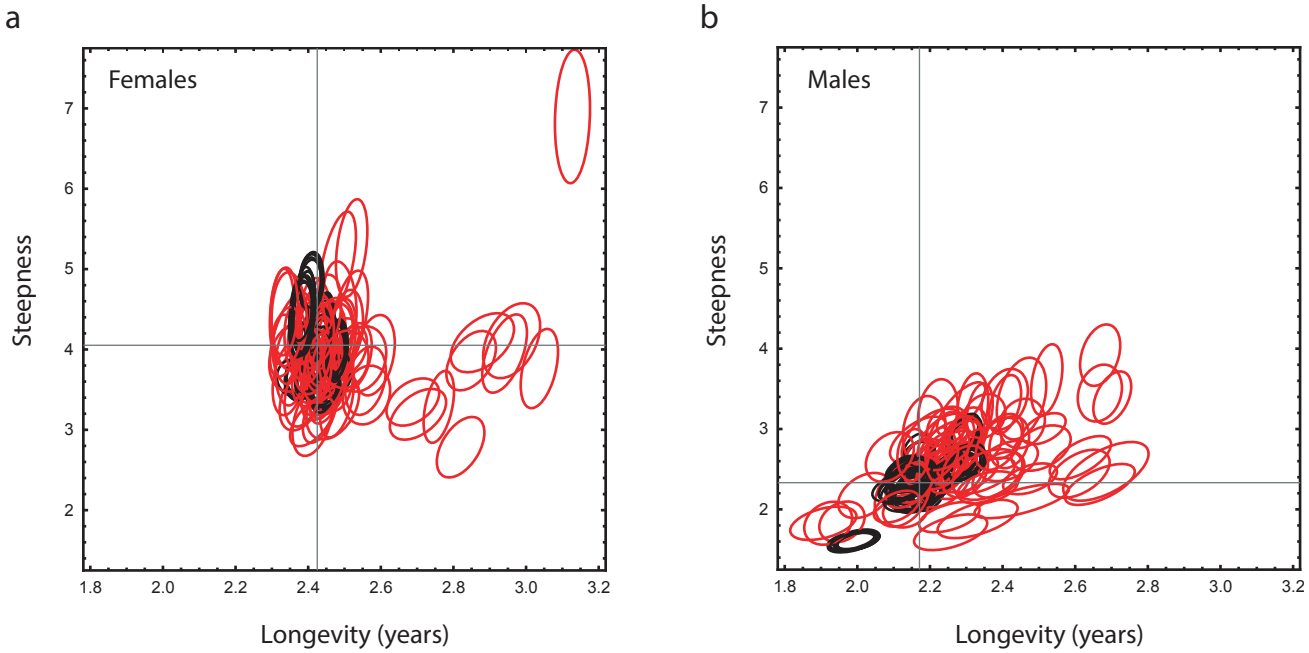

a
Age t
Damage
X
Φ
production rate ηt
removal rate β
Morbidity Xd
Mortality Xc
$$\frac{dX}{dt} = \eta t - \beta \frac{X}{X+\kappa} + \sqrt{2\sigma}\xi$$
b
Death
Morbidity
Health
Xc
Xd
Damage
Age (years)
c
Median lifespan
83y
Mean sickspan
12.3y
Relative sickspan
14.5%
Control
Lifespan rank
R. sickspan rank
d
135y
21.5y
15.3%
η -46%
Approximate scaling
e
135y
14.6y
10.5%
β +90%
Compressed sickspan
f
135y
14.0y
10.4%
Xc&Xd +357%
Compressed sickspan
Age (years)
Normalised lifespan
Normalised lifespan
g
Sickspan (y)
Lifespan (y)
h
Sickspan/Lifespan
Lifespan (y)
Decrease production rate η
Increase removal rate β
Increase thresholds Xc and Xd

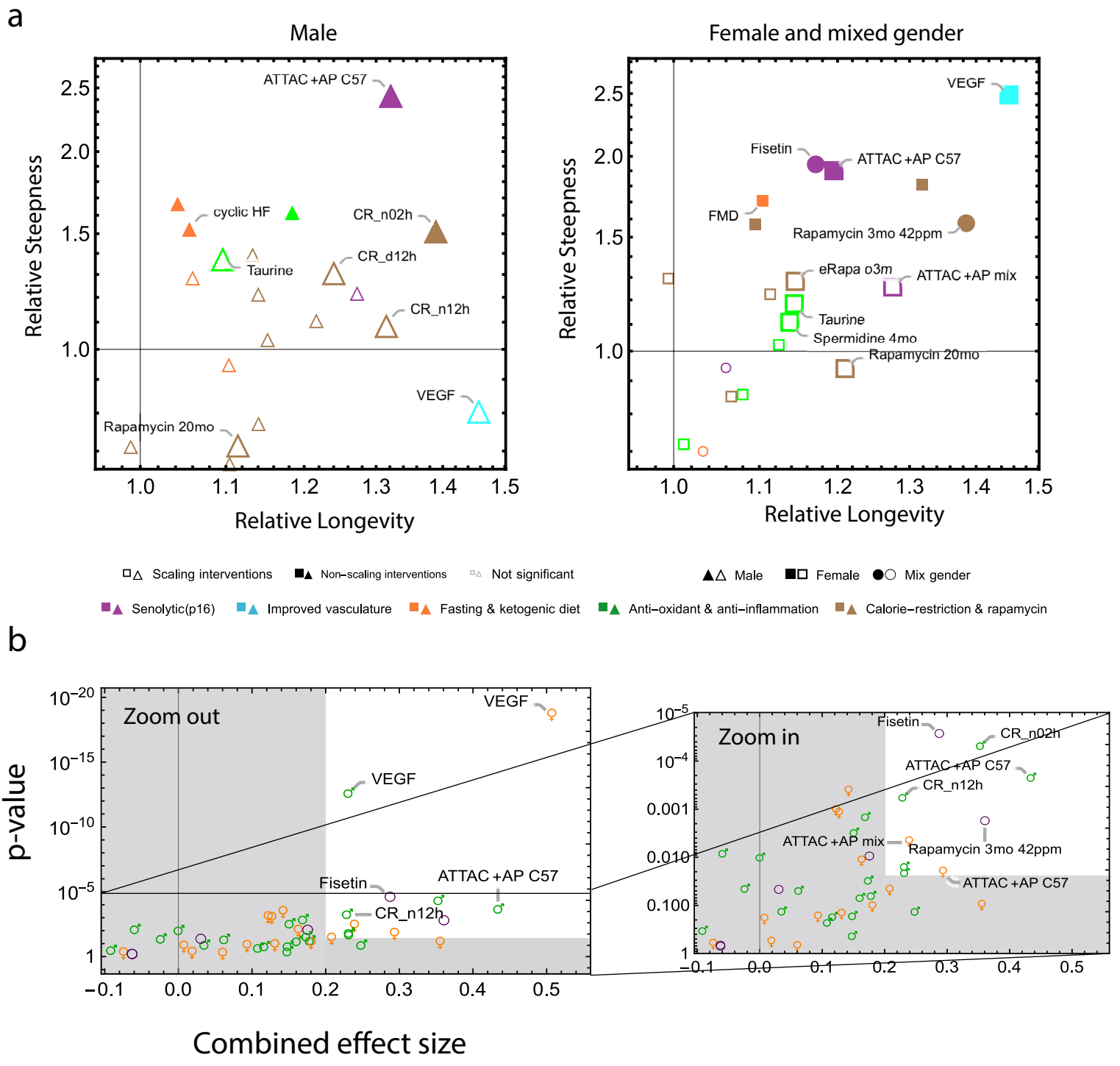

a
Relative Steepness
Relative Longevity
Capt
BD
Rapa_hi
NDGA_mid
ACA_hi
17aE2_16m
17aE2
GTE
NDGA_hi
ACA
RaAc9
ACA_mid
Cana
NDGA
Rapa
ACA_lo
17aE2_hi
Rapa_hi
MetRapa
Rapa_mid
MetRapa
Rapa
Rapa_lo
Rapa_hi_cycle
RaAc9
Rapa_hi_cycle
Rapa
Rapa
RaAc16
Rapa_hi_continuous
Acarbose & Canagliflozin
Improved vasculature
Anti–oxidant & anti–inflammation
Senolytic(p16)
17αE2
Fasting & ketogenic diet
Calorie–restriction & rapamycin
Scaling interventions
Not significant
b
ATTAC +AP C57
VEGF
Fisetin
ATTAC +AP C57
NIA ITP
VEGF
Relative Steepness
Relative Longevity
c
Senolytic
d
Enhanced vasculature
e
Acarbose
f
17α-E2
g
Cyclic ketogenic diet
h
Canagliflozin
Survivorship
Age (days)
Age/age50

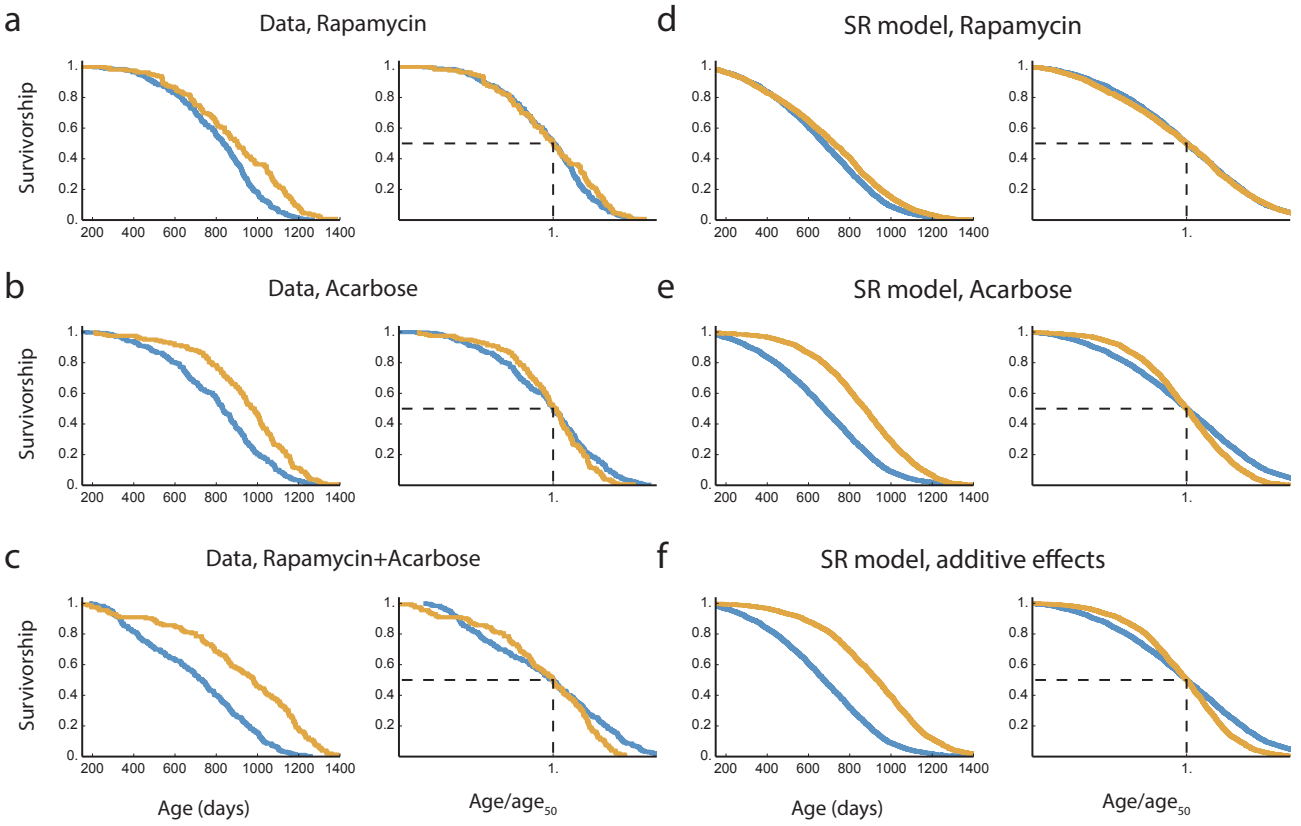

Sickspan (y)
25
20
15
10
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
Parameter change |ln(par/par0)|
Lifespan (y)
150
140
130
120
110
100
90
80
Parameter change |ln(par/par0)|
Sickspan/Lifespan
0.16
0.14
0.12
0.10
Parameter change |ln(par/par0)|
Decrease production rate η
Increase removal rate β
Increase thresholds Xc and Xd

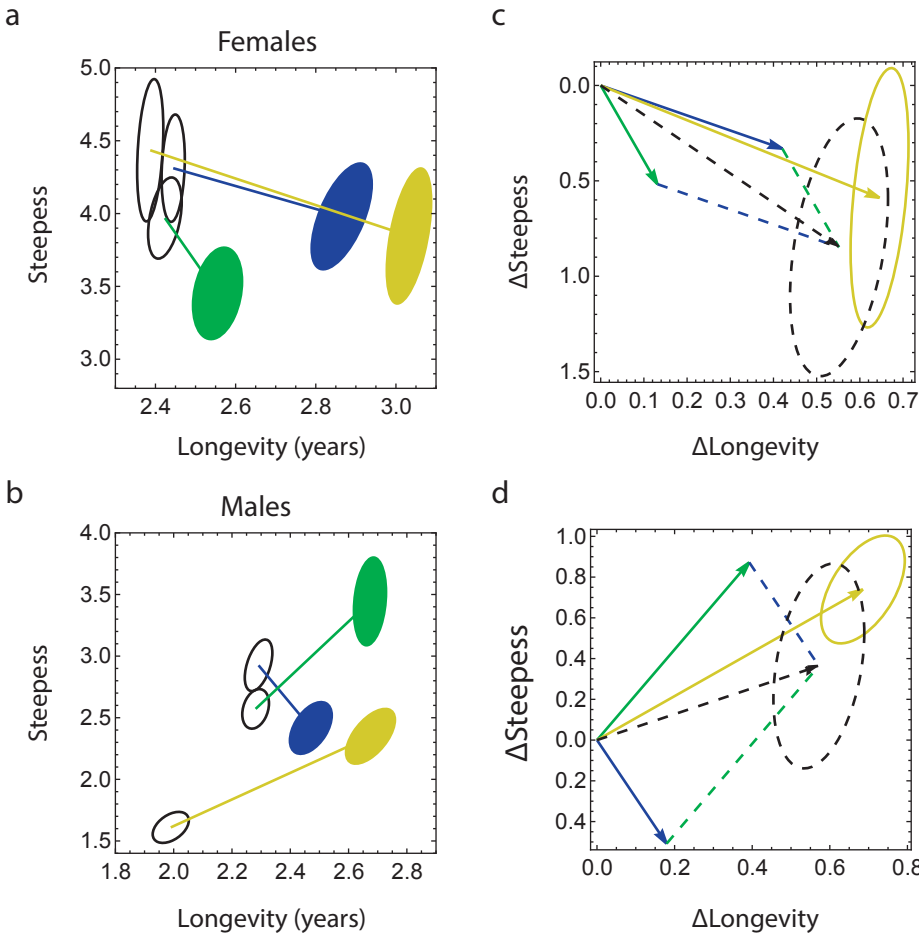

a
p-value
Ln(relative longevity)
RaAc9
Rapa_hi
MetRapa
Rapa_hi_continuous
ACA
Rapa
ACA_hi
RaAc16
Rapa_hi
MetRapa
RaAc9
NDGA
Rapa
GTE
Cana


b
p-value
Ln(relative steepness)
RaAc9
Rapa_hi
MetRapa
Rapa_hi_continuous
ACA
Rapa_mid
Rapa
ACA_hi
MetRapa
Rapa_hi
ACA_mid
Rapa
Rapa_lo
17aE2_hi
Capt
Rapa
BD

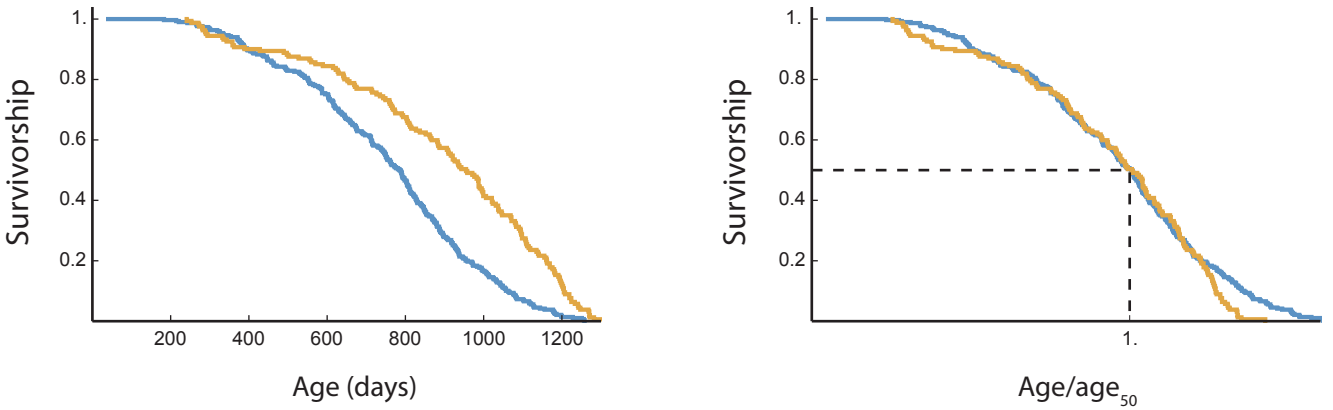

a
Females
Steepness
Longevity (years)
b
Males
Steepness
Longevity (years)


a
Male
Female and mixed gender
Relative Steepness
Relative Longevity
ATTAC +AP C57
cyclic HF
CR_n02h
Taurine
CR_d12h
CR_n12h
VEGF
Rapamycin 20mo
Fisetin
FMD
Rapamycin 3mo 42ppm
eRapa o3m
ATTAC +AP mix
Spermidine 4mo
Scaling interventions
Non–scaling interventions
Not significant
Male
Female
Mix gender
Senolytic(p16)
Improved vasculature
Fasting & ketogenic diet
Anti–oxidant & anti–inflammation
Calorie–restriction & rapamycin
b
Zoom out
Zoom in
p-value
Combined effect size

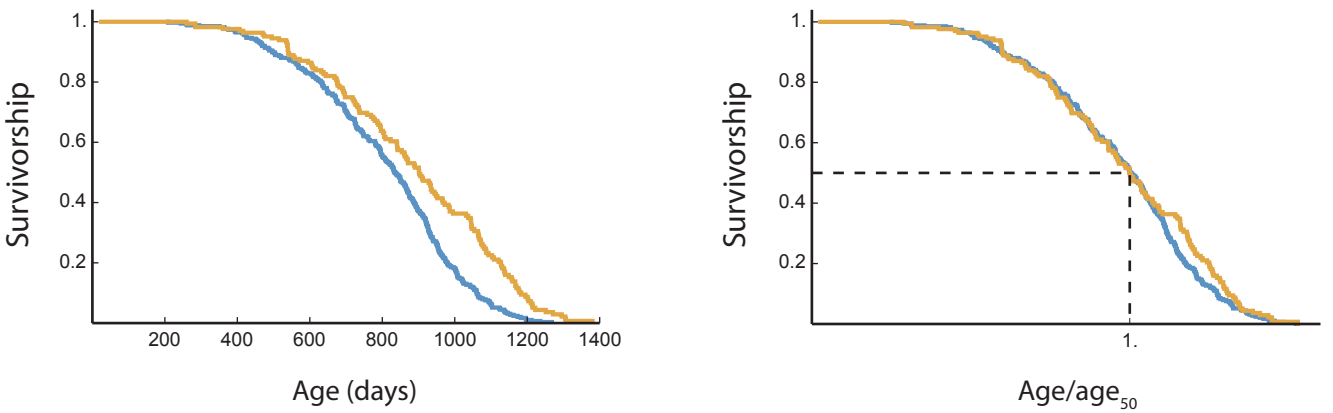

a
Data, Rapamycin
b
Data, Acarbose
c
Data, Rapamycin+Acarbose
d
SR model, Rapamycin
e
SR model, Acarbose
f
SR model, additive effects
Survivorship
Age (days)
Age/age50
1.
0.8
0.6
0.4
0.2
0.
200
400
600
800
1000
1200
1400


a
Females
Steepess
Longevity (years)
5.0
4.5
4.0
3.5
3.0
2.4
2.6
2.8
3.0
c
ΔSteepess
ΔLongevity
0.0
0.5
1.0
1.5
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7
b
Males
Steepess
Longevity (years)
4.0
3.5
3.0
2.5
2.0
1.5
1.8 2.0 2.2 2.4 2.6 2.8
d
ΔSteepess
ΔLongevity
1.0
0.8
0.6
0.4
0.2
0.0
0.2
0.4
0.0 0.2 0.4 0.6 0.8

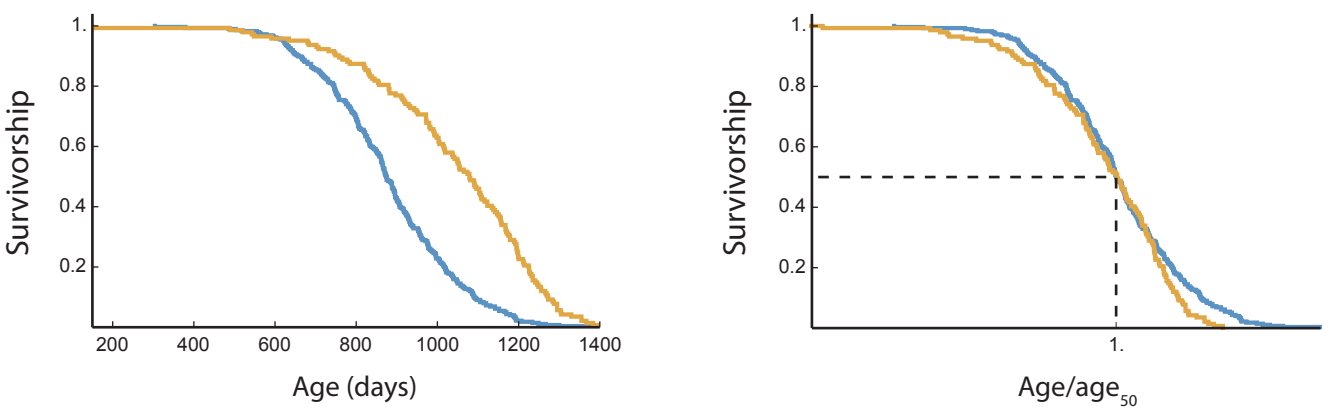
Female, rapamycin, 14ppm



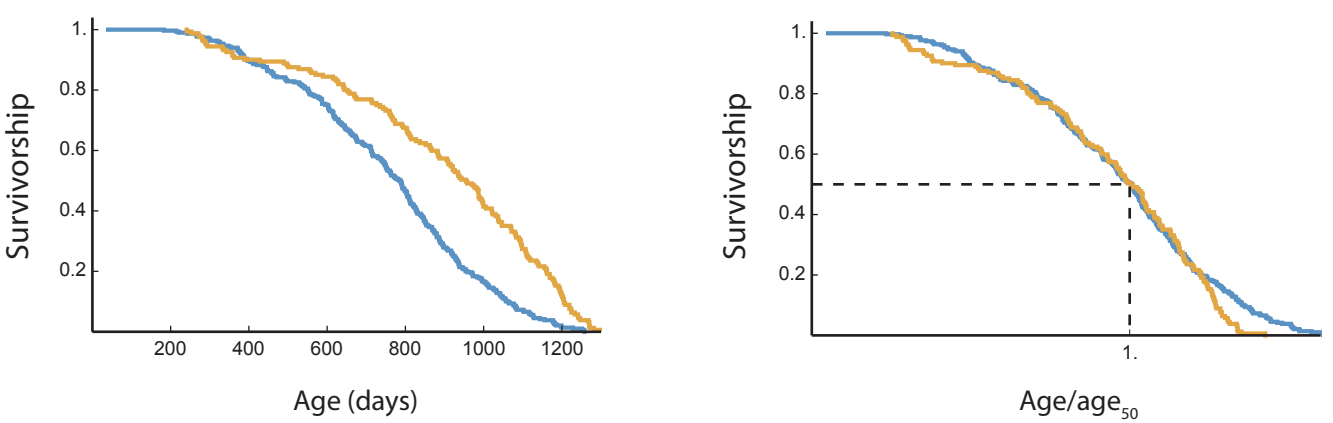
Male, rapamycin, 14ppm

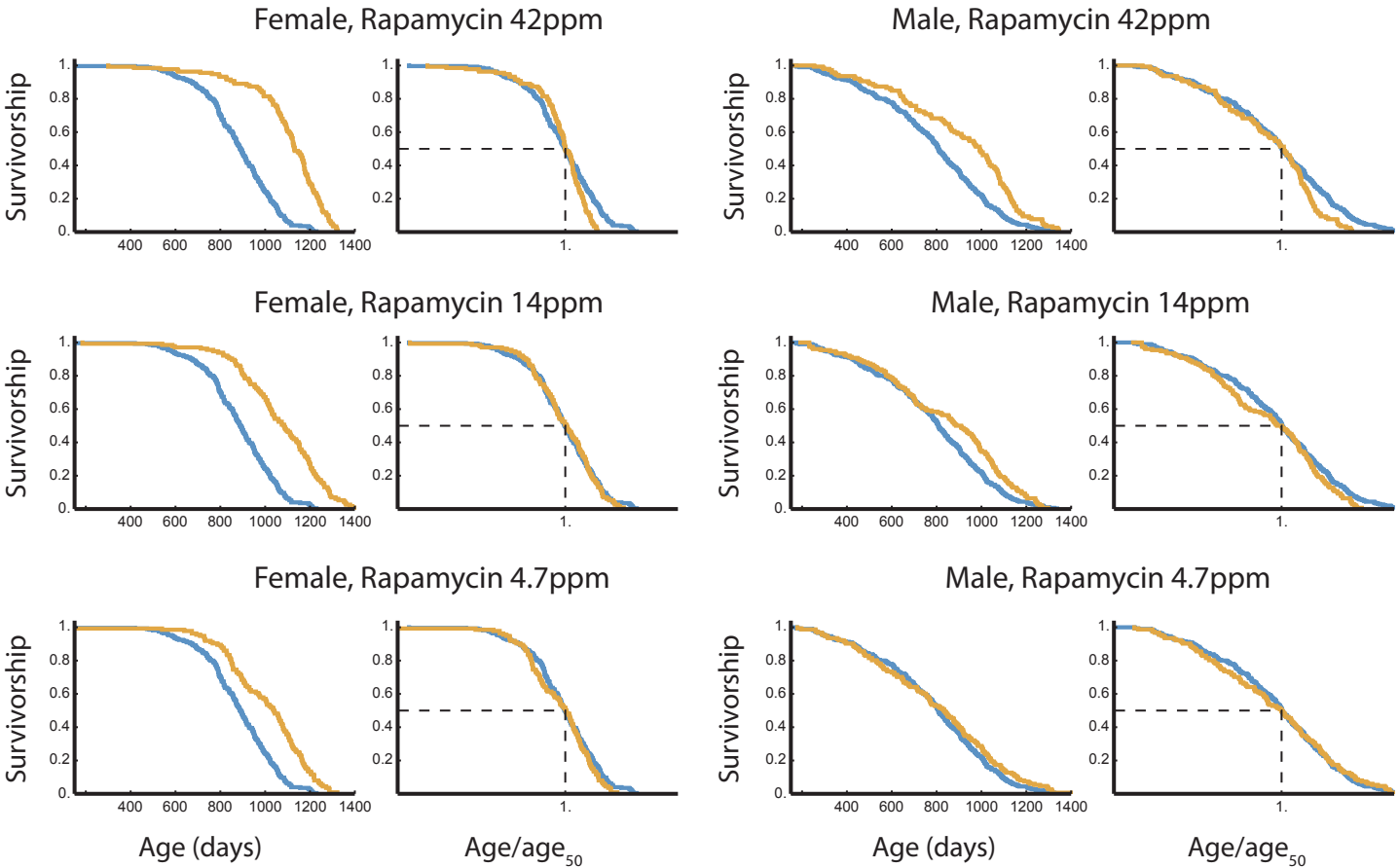


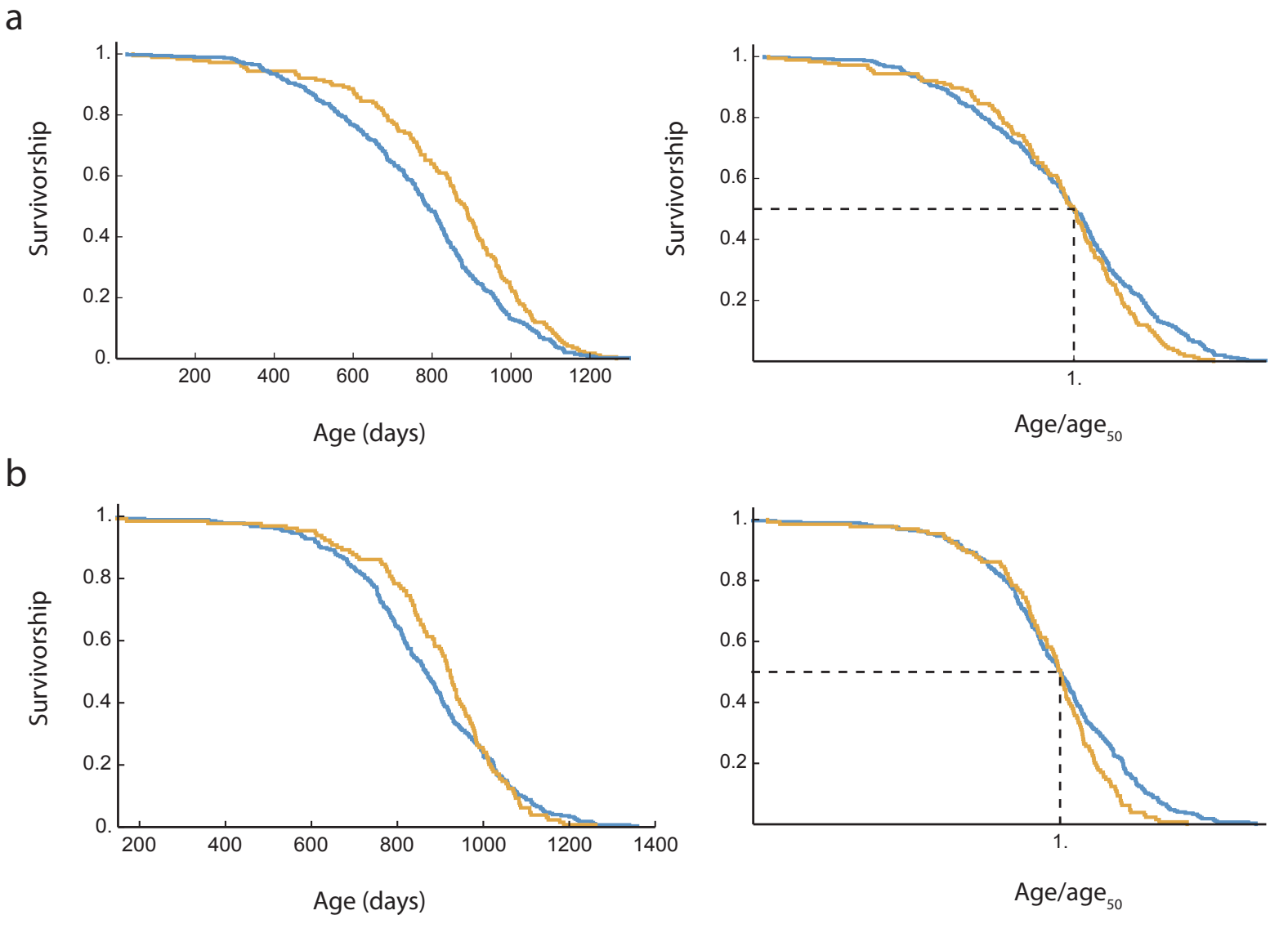
Female, metformin + rapamycin, 1000ppm +14ppm



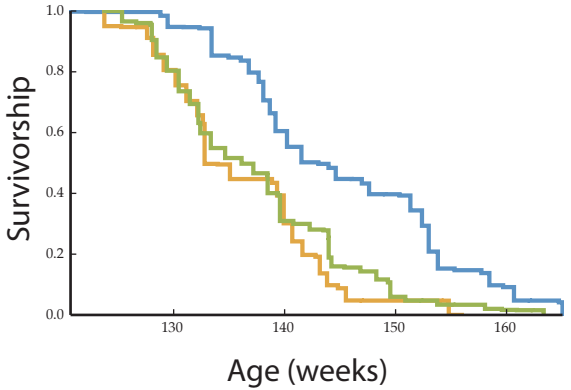
Male, metformin + rapamycin, 1000ppm +14ppm

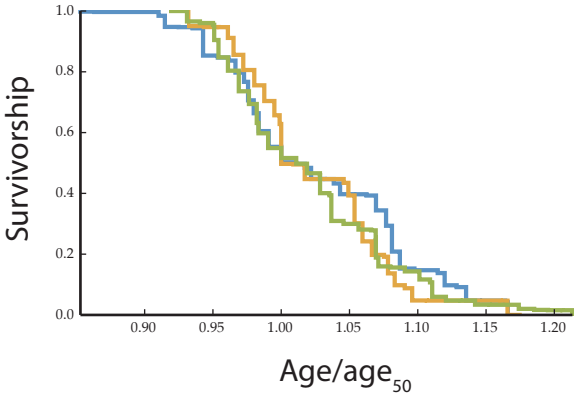



Female, Rapamycin 42ppm
Male, Rapamycin 42ppm
Female, Rapamycin 14ppm
Male, Rapamycin 14ppm
Female, Rapamycin 4.7ppm
Male, Rapamycin 4.7ppm
Survivorship
1.
0.8
0.6
0.4
0.2
0.
400
600
800
1000
1200
1400
1.
Age (days)
Age/age50


a
Survivorship
1.
0.8
0.6
0.4
0.2
0.
200
400
600
800
1000
1200
Age (days)
Survivorship
1.
0.8
0.6
0.4
0.2
1.
Age/age50
b
Survivorship
1.
0.8
0.6
0.4
0.2
0.
200
400
600
800
1000
1200
1400
Age (days)
1.
0.8
0.6
0.4
0.2
1.
Age/age50

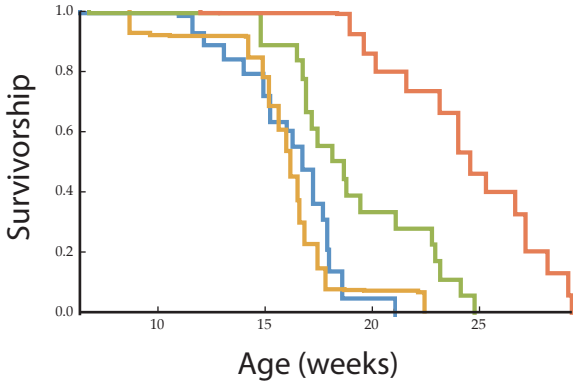
a

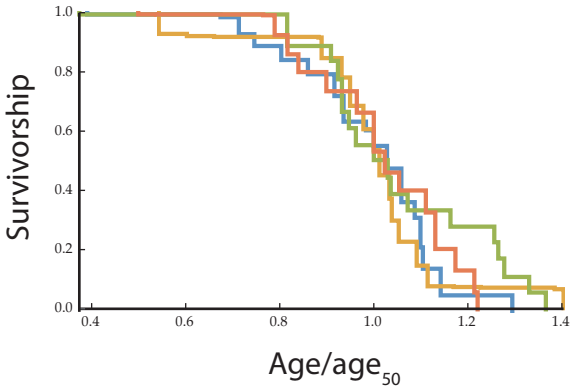




TRE- OSK_20
Control_Dox_20
Jax _Historical_33

b





- Dox_LAKI
+Dox_LAKI
- Dox_LAKI_4F
+Dox_LAKI_4F