Caloric restriction increases the lifespan of mice

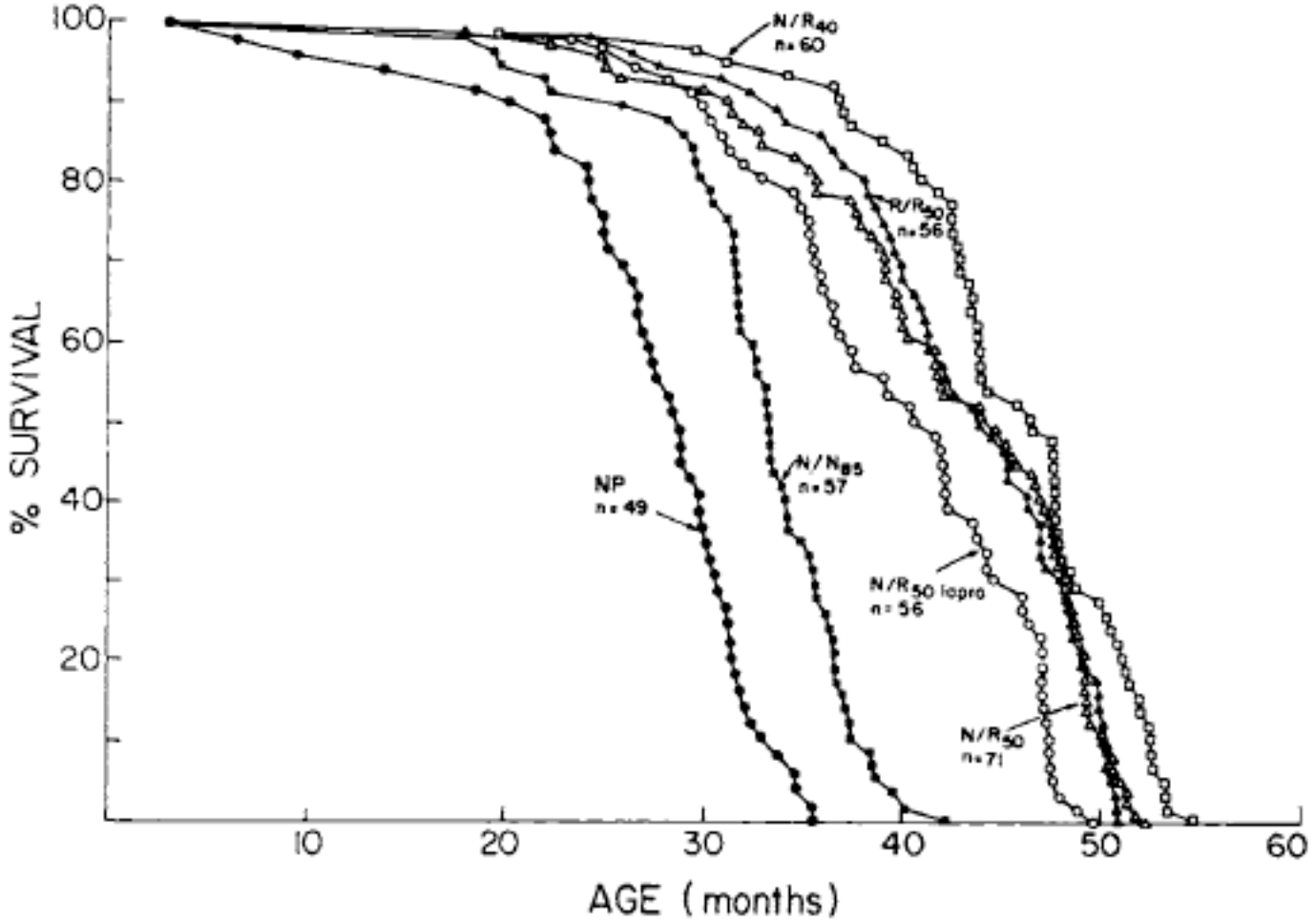


Fig. 2 Influences of diet on survival. Each symbol represents an individual mouse. Diet groups, see fig. 1 or text.

alive in each group at the indicated age. Diet groups: NP, fed nonpurified diet (reference group); N/N_{85}, fed normally before and after weaning, postweaning fed diet 1 at ~85 kcal/wk (25% less than ad libitum) (controls); N/R_{50}, fed normally before weaning, after weaning fed a diet enriched in vitamins and minerals (diet 2) because of their restricted intake (~50 kcal/wk, fed about every other day); R/R_{50}, restricted in feeding levels before and after weaning; $N/R_{50lopro}$, restricted after weaning to ~50 kcal/wk with a decrease in protein content with age; N/R_{40}, restricted after weaning to ~40 kcal/wk of diet 2, schedule as for R_{50}.

Dietary Restriction (DR), also called caloric restriction (CR), extends the lifespan not only of mice as shown in the previous slide but also of yeast, nematode worms, and fruit flies.

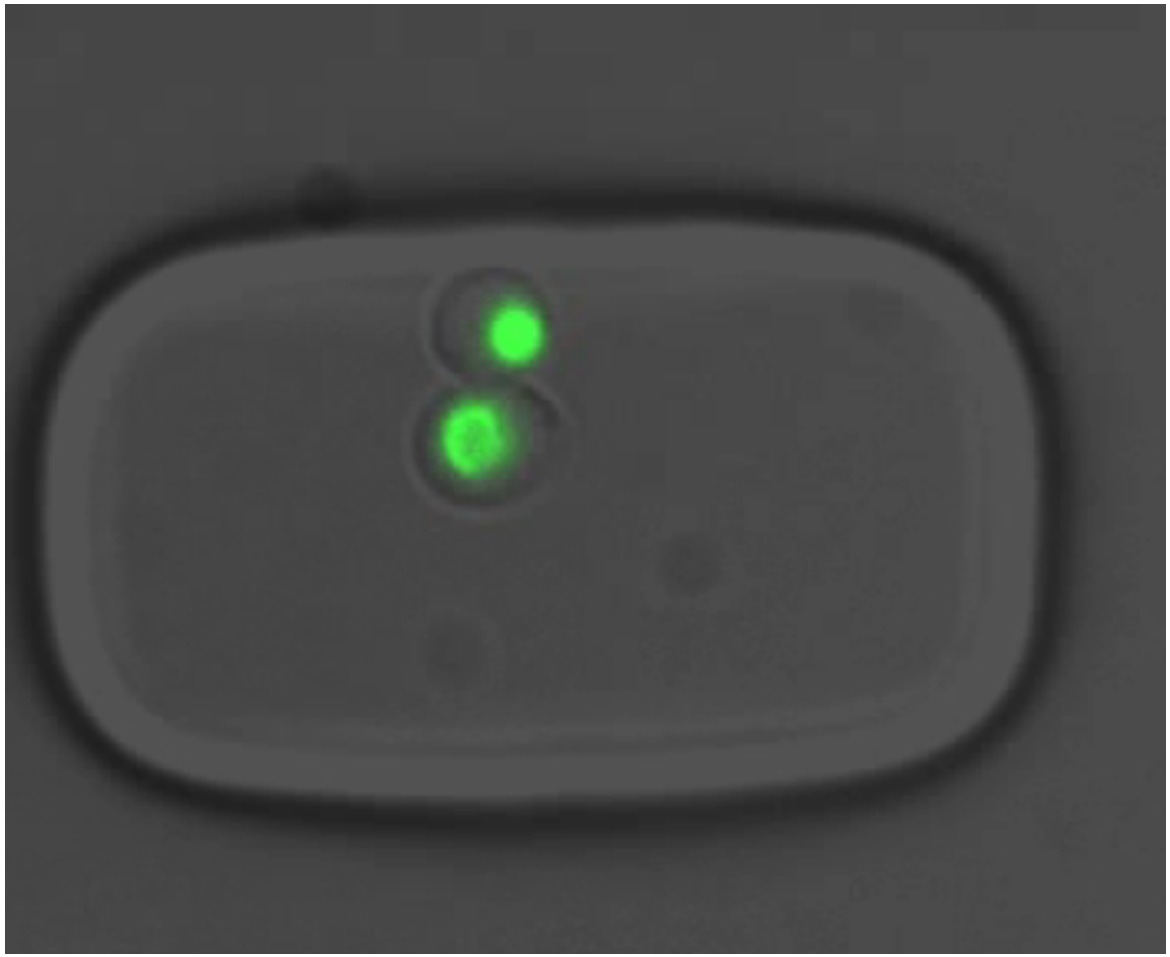
What is the molecular underpinning to the CR extension of lifespan?

Perhaps surprisingly, there appears to be some common molecular themes that run across the eukaryotic evolutionary spectrum …. But there's still lots that isn't known and controversy about the roles of some of the players.

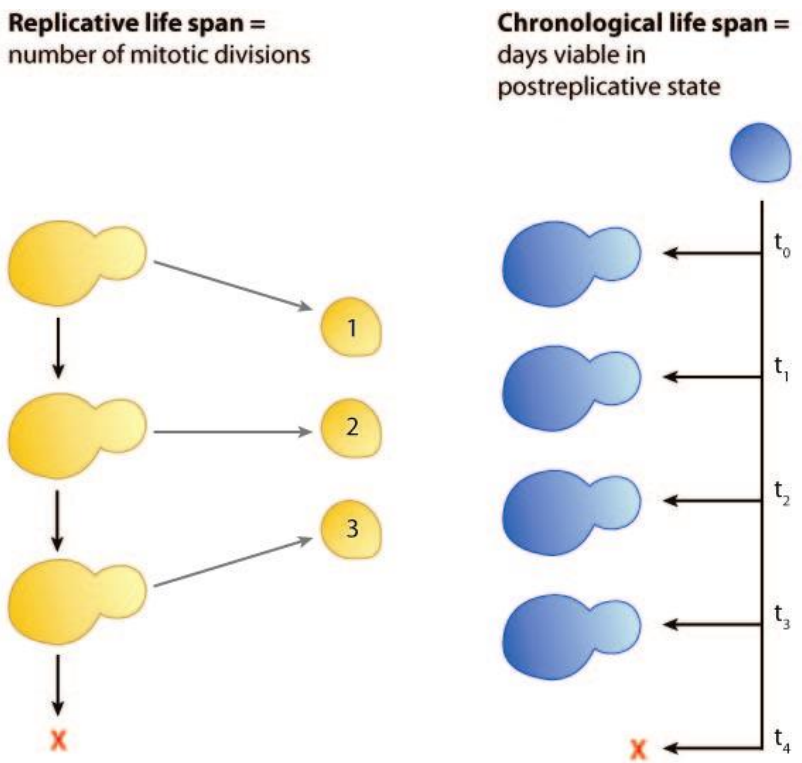
Yeast?

Does yeast age? Can yeast tell us anything about what is commonly perceived as phenomenon associated with multi-cellular organisms?

Skeptics abound



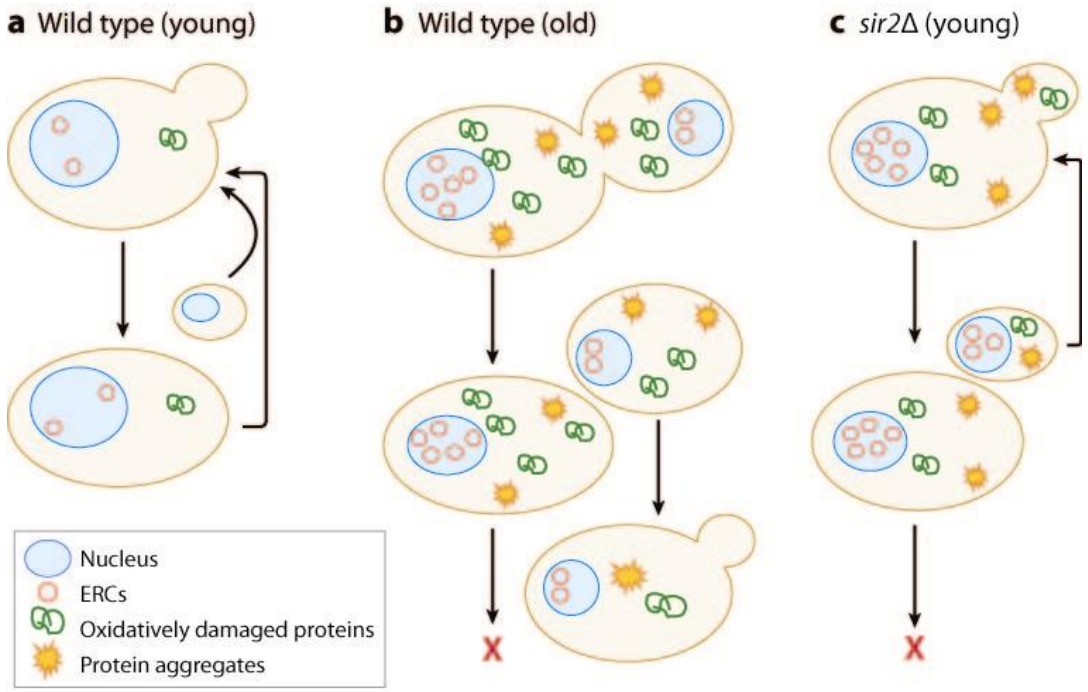
Replicative and chronological aging



Steinkraus KA, et al. 2008.
Annu. Rev. Cell Dev. Biol. 24:29–54

Annual Reviews

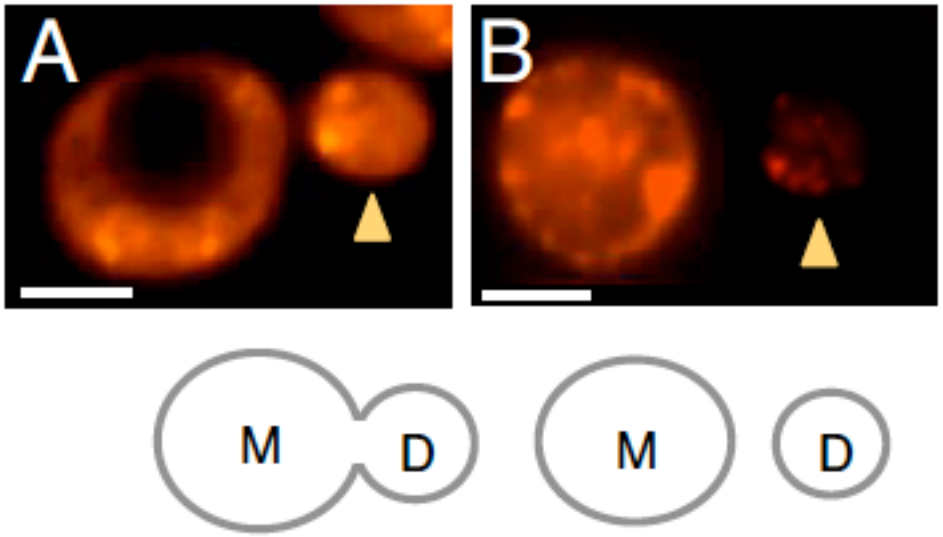
Old vs Young



Steinkraus KA, et al. 2008.
Annu. Rev. Cell Dev. Biol. 24:29–54

Annual Reviews

ROS accumulates in mother cells



Werner Syndrome

Yeast Werner syndrome gene, *SGS1,* is necessary for lifespan

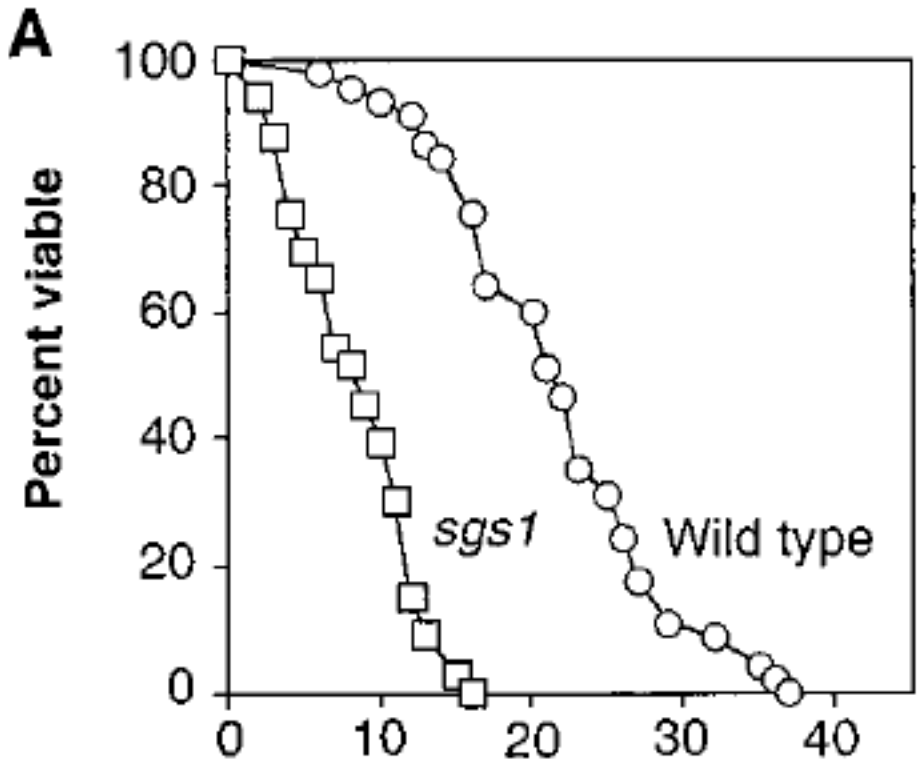
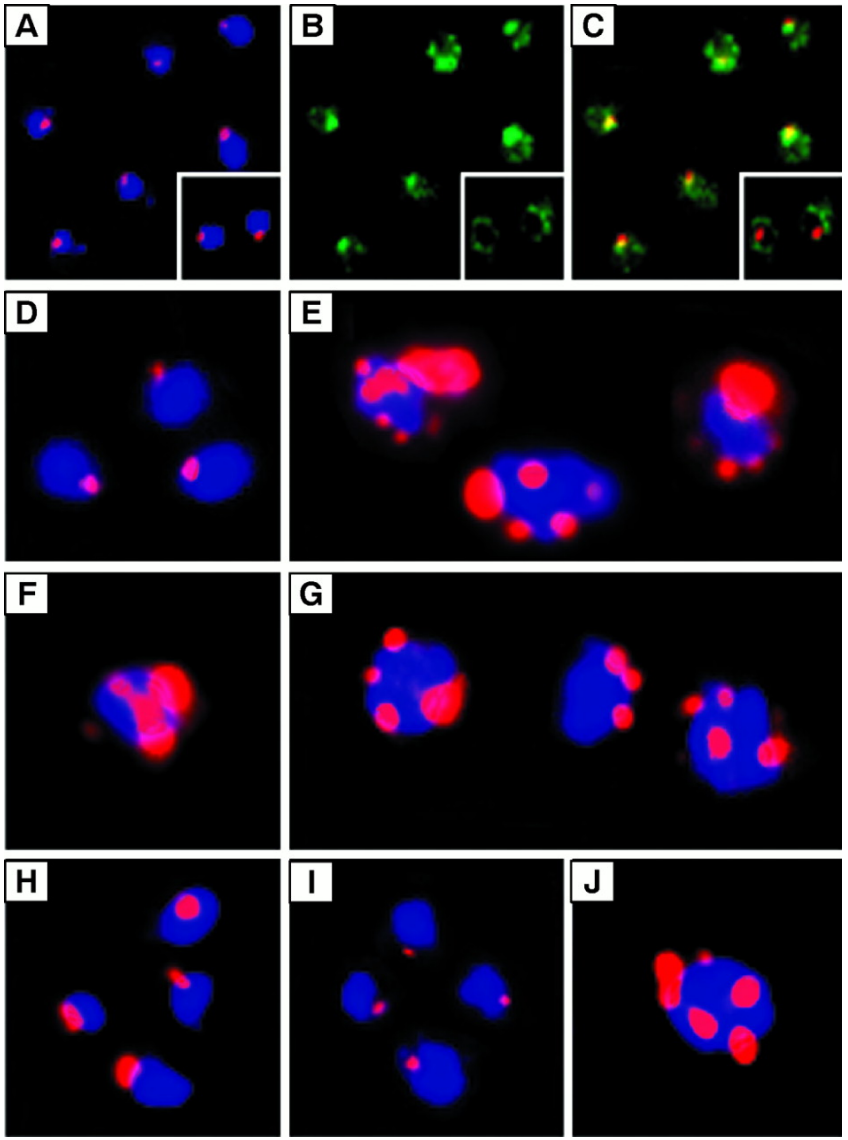


Figure 3 Nucleolar localization of Sgs1p and nucleolar fragmentation in old cells.

Red = nucleolus
Blue = DNA
Green = Sgs1

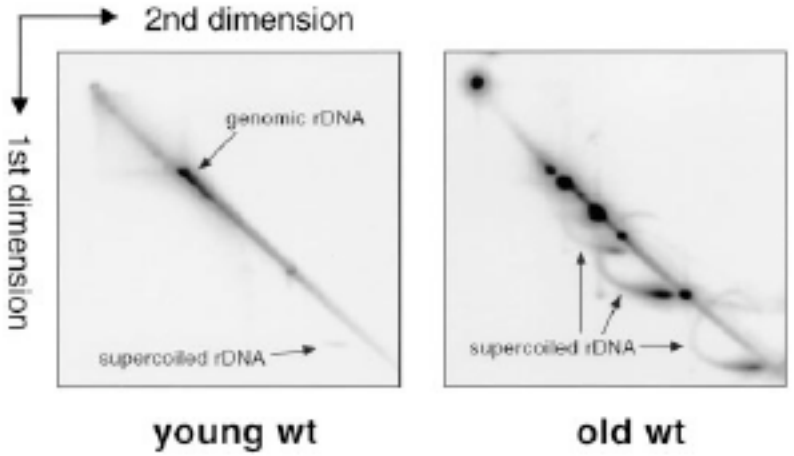
D = young wt
E = old *sgs1*
F = old wt



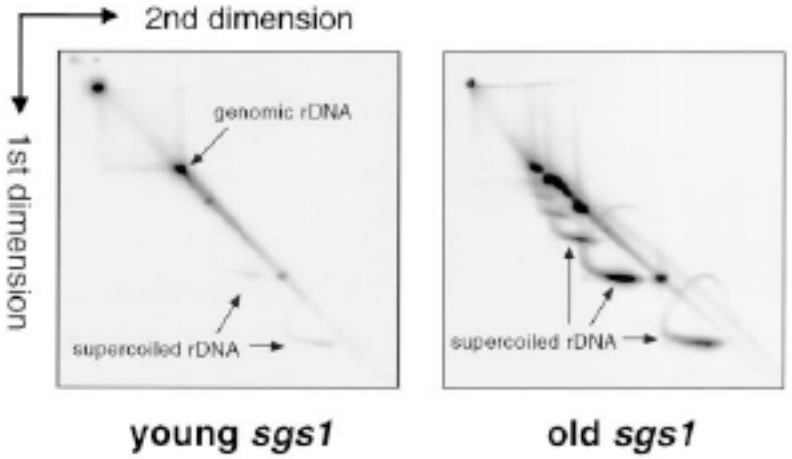
D A Sinclair et al. Science 1997;277:1313-1316

Nucleolar fragmentation reflects formation of rDNA circles in old cells

Old = 20 cell divisions

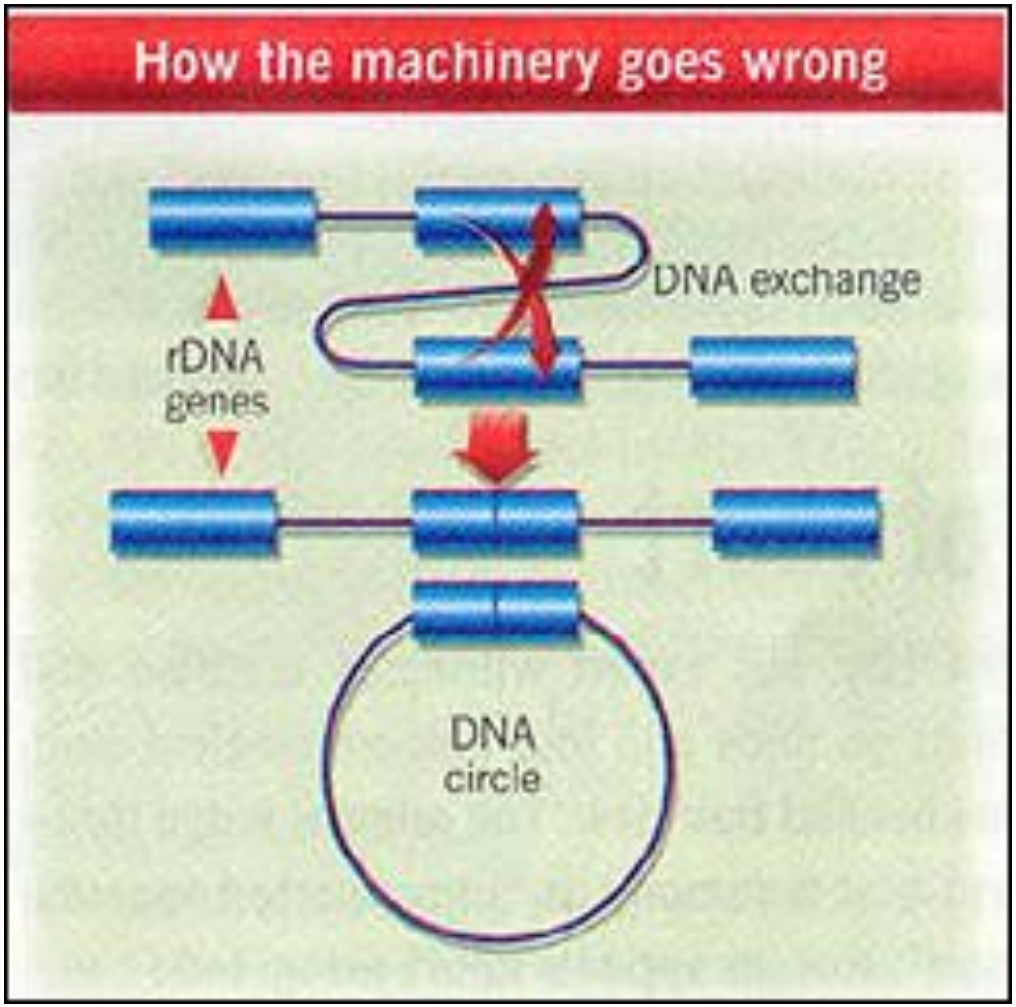


Old = 9 cell divisions

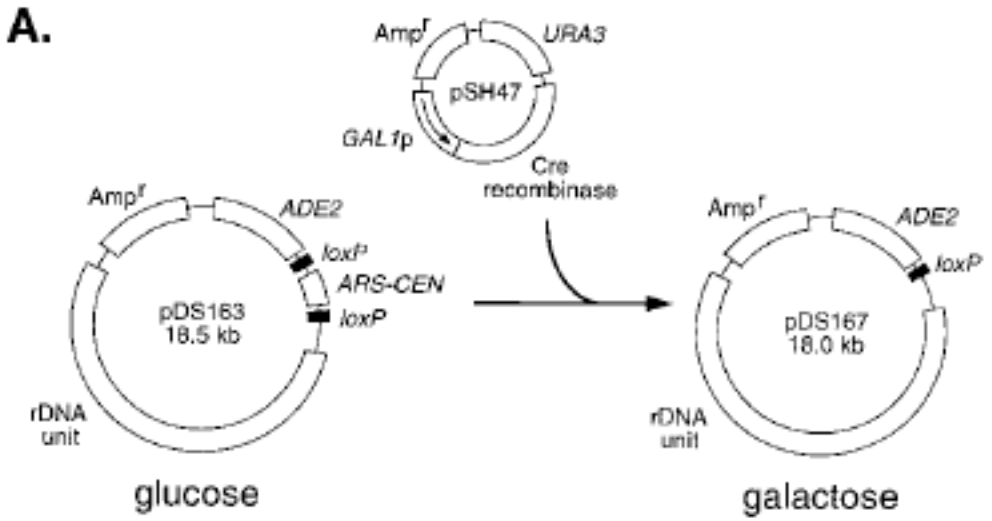


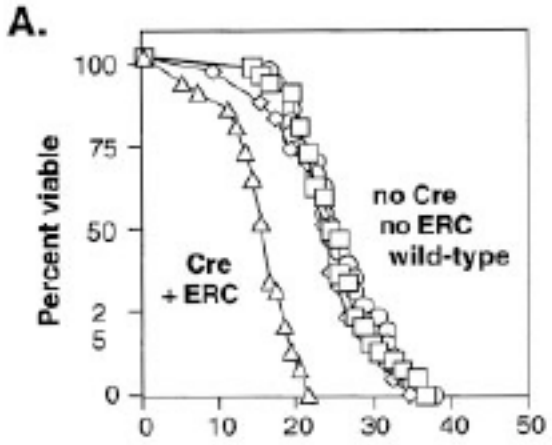
rDNA circles

rDNA is a tandem array of ~200 copies. Recombination between copies can excise circles.



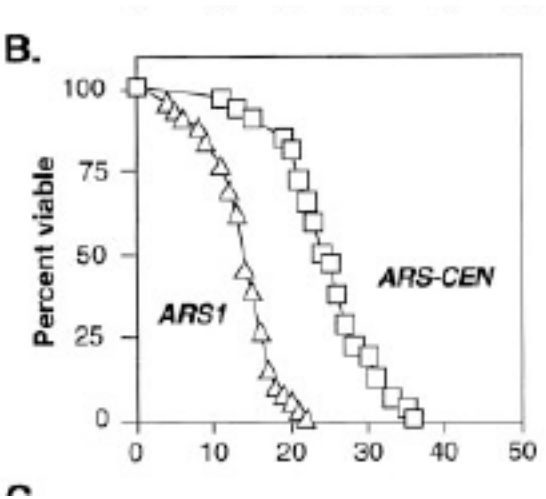
Premature production of rDNA circles can cause aging ….





… but it is not just rDNA circles that can cause aging

ARS1 = high copy number plasmid



ARS1-CEN = low copy number plasmid

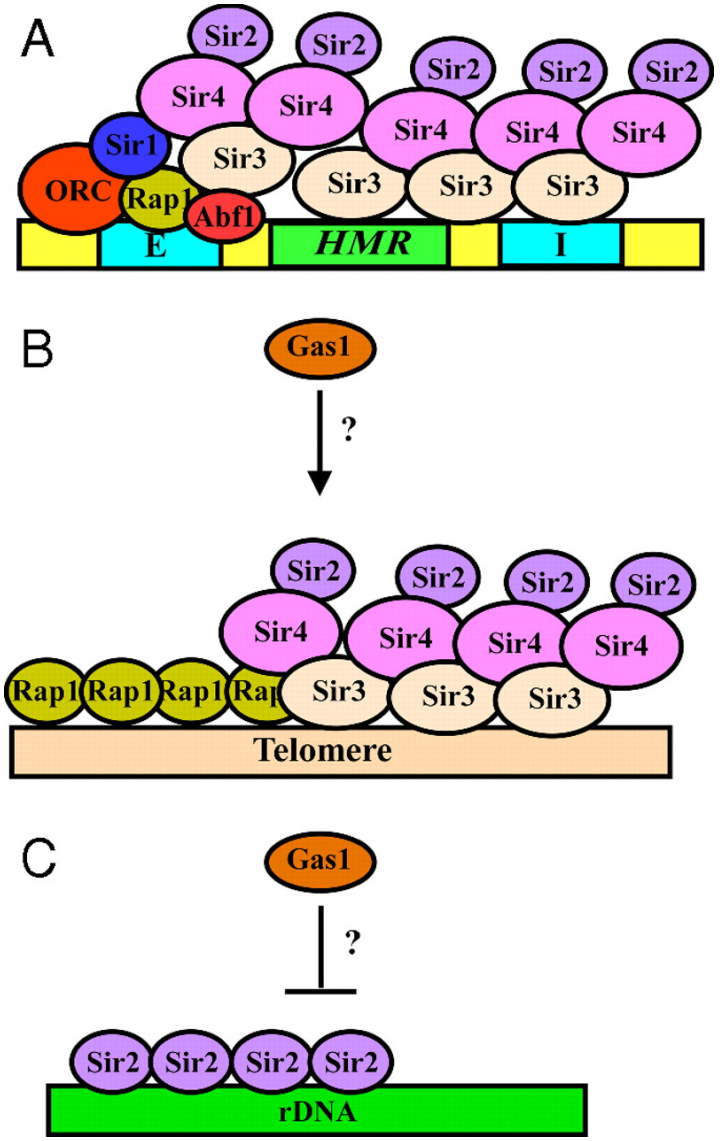
Sir proteins mediate silent chromatin state

At telomeres

At silent mating type loci

At rDNA

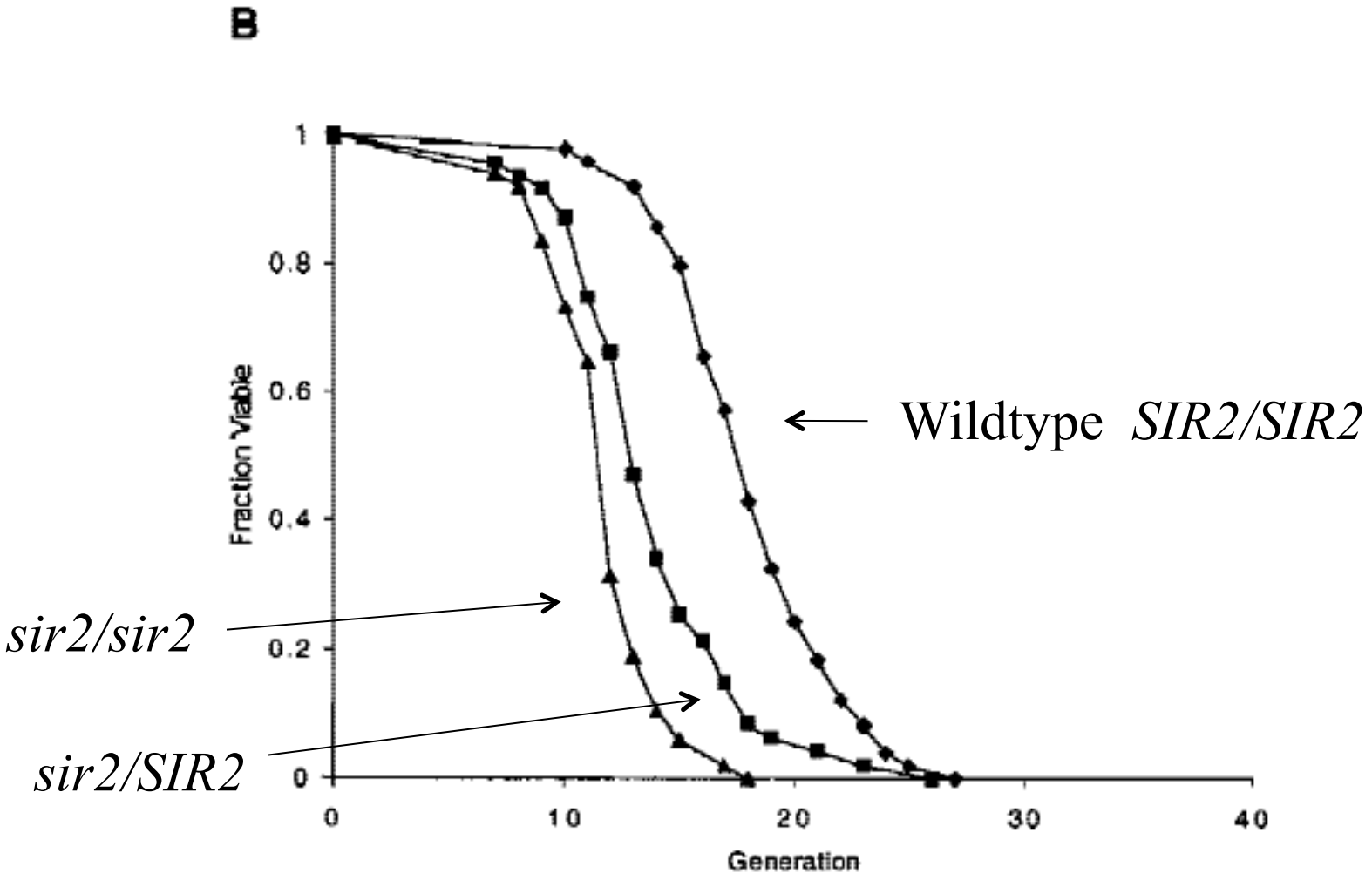
Role of Gas1 in transcriptional silencing at the three silent chromatin loci in budding yeast.



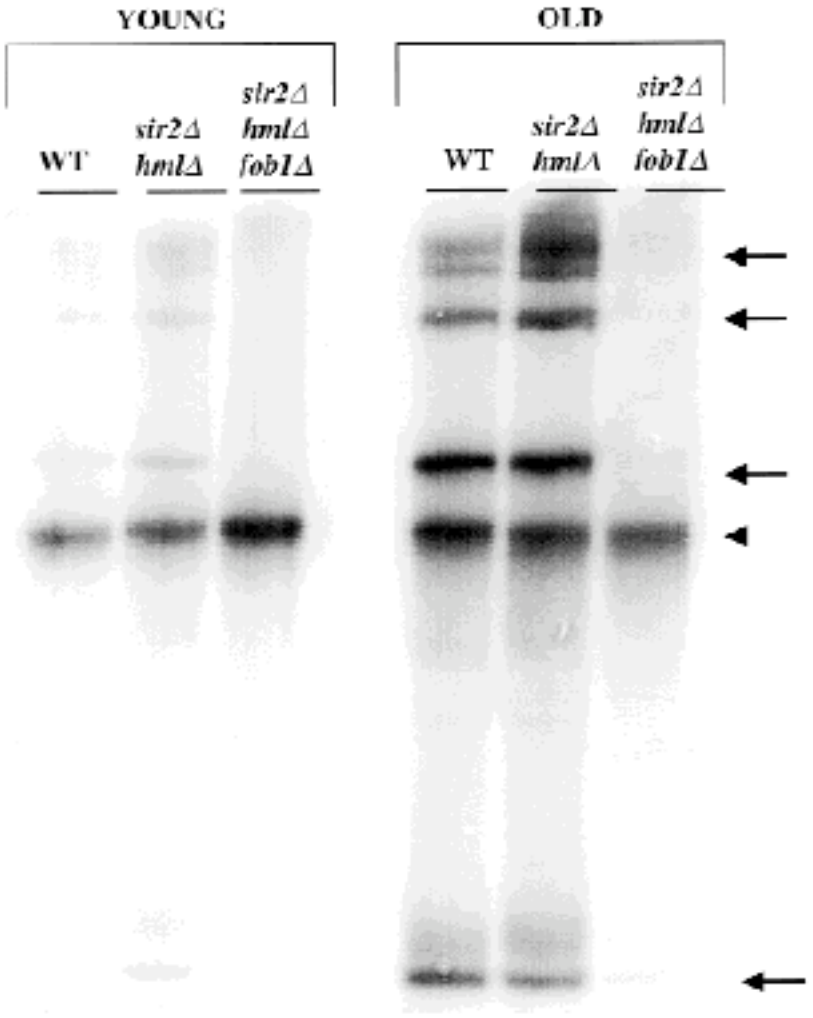
Burgess R J et al. PNAS 2009;106:10879-10880

Sir2 is required for longevity in diploids

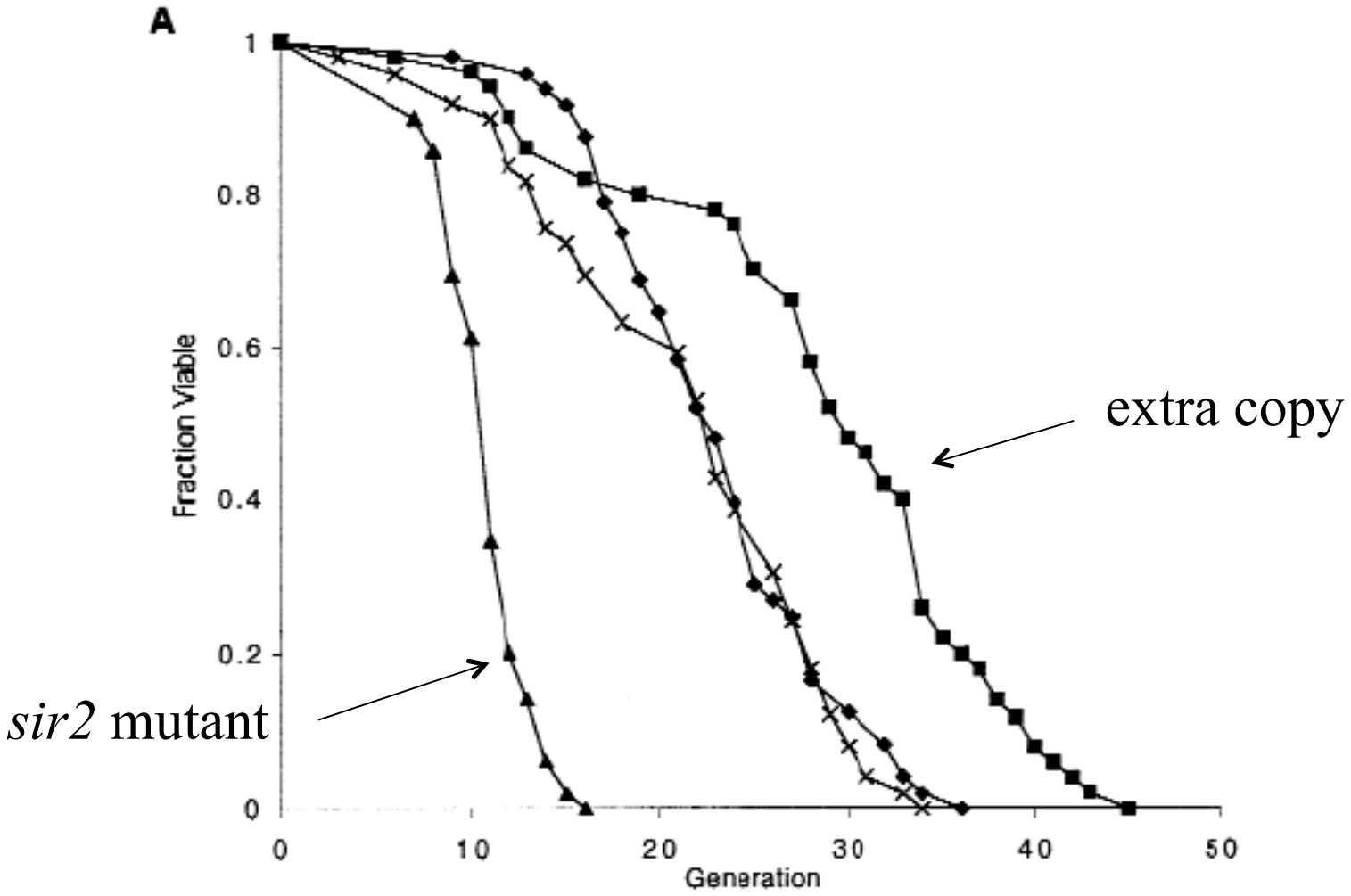
(and in haploids, too, but data not shown)



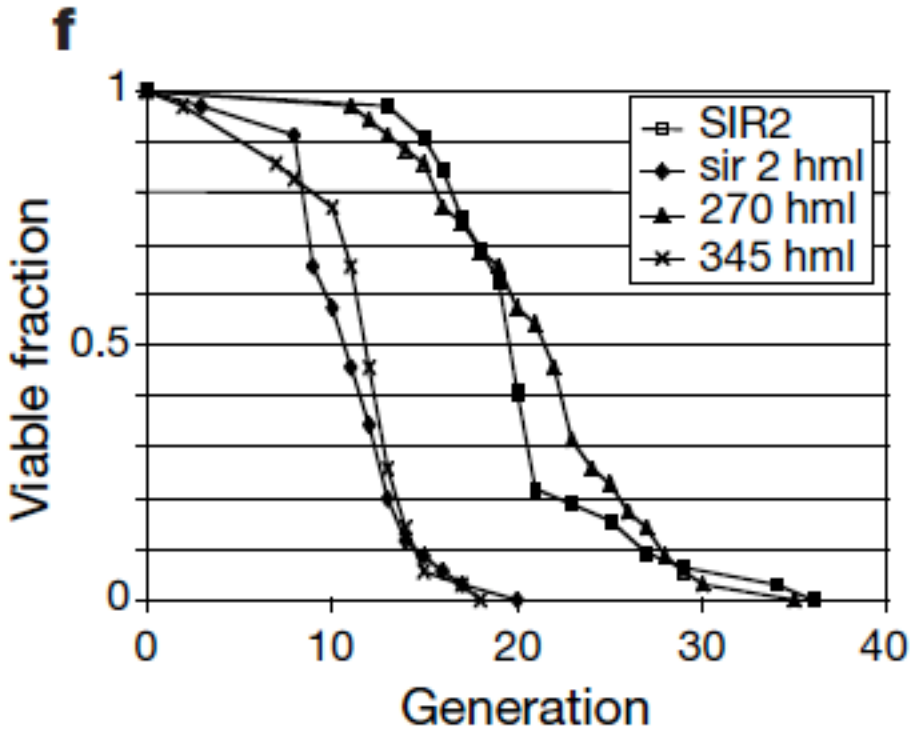
sir2 mutants accumulate rDNA circles



An extra copy of *SIR2* extends lifespan

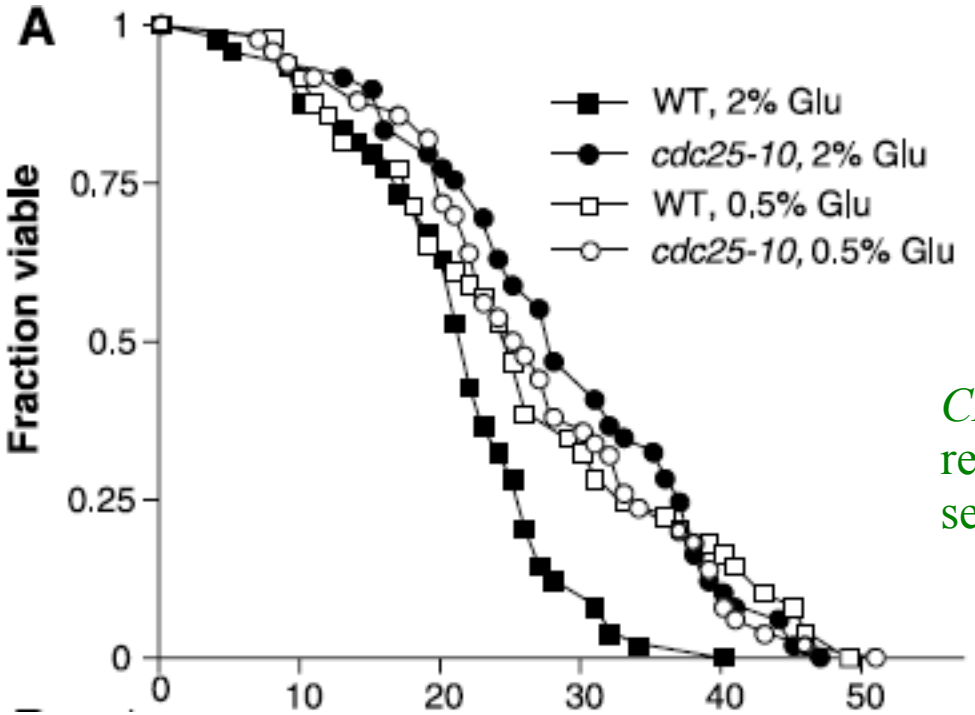


Sir2 is an NAD-dependent histone deacetylase. The deacetylase is required for longevity



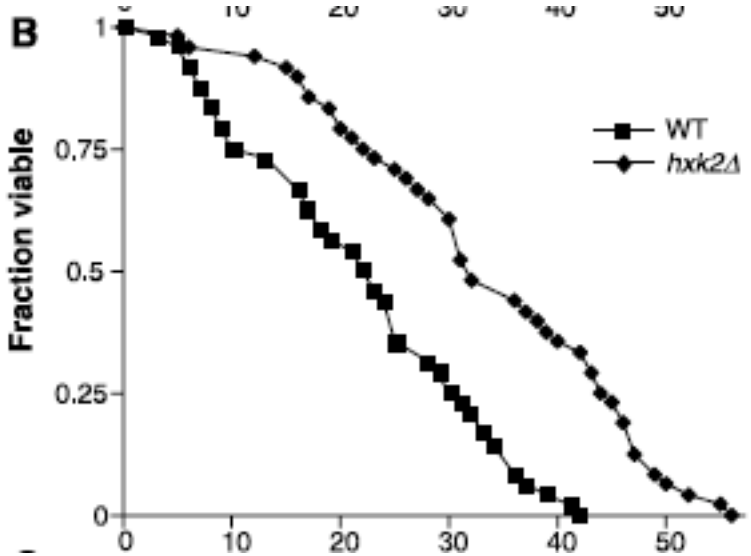
345 is a point mutant that abolishes deacetylase activity; 270 does not

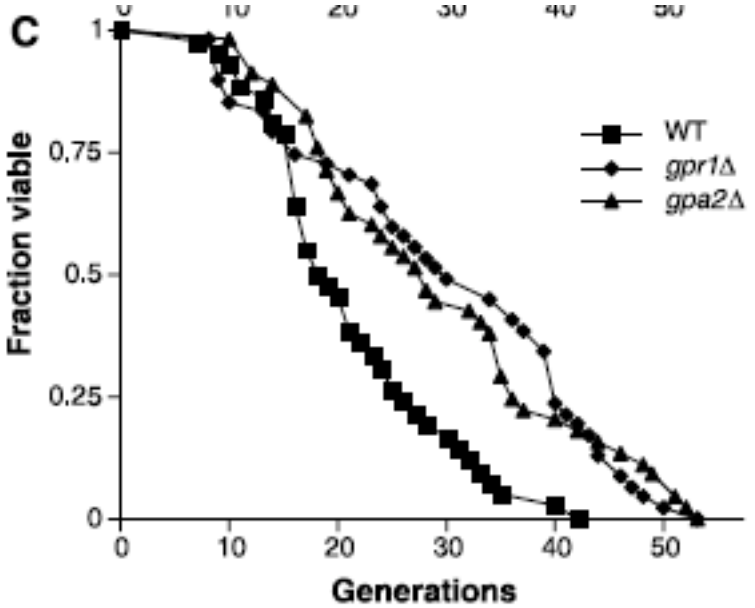
Yeast lifespan increases under CR conditions …



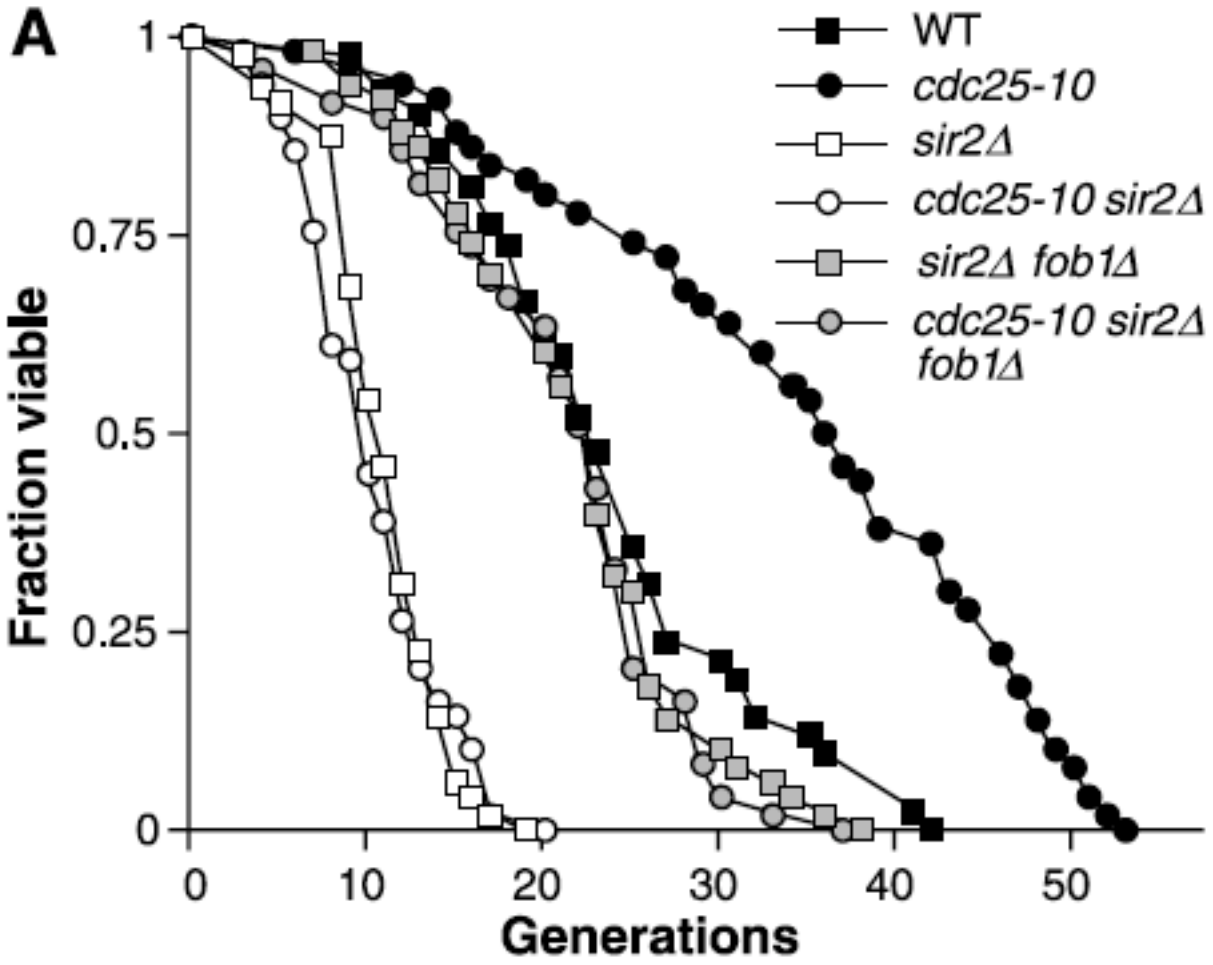
CDC25, *GPR1*, and *GPA2* - regulators of Ras nutrient sensing pathway

HXK2 - hexokinase





…. and the increase appears to require Sir2



Moreover, CR appears to affect the cellular NAD/NADH ratio

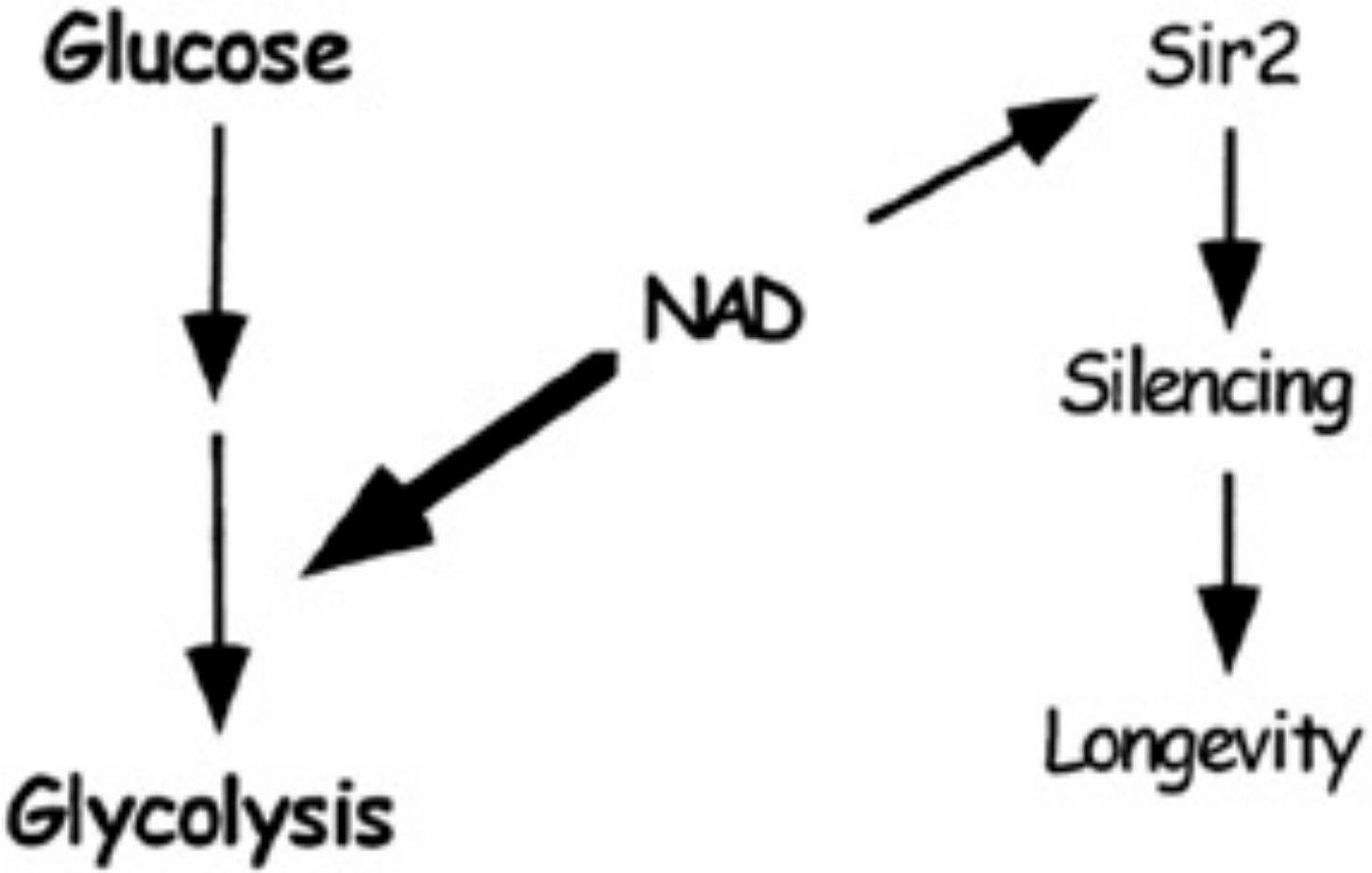
Table 1. *Intracellular concentrations of NAD and NADH*

Normal condition (mM)			Calorie restriction (mM)			
NAD	NADH	NAD/NADH ratio	NAD	NADH	NAD/NADH ratio	References
2.14[a,b]	—	—	—	—	—	Smith et al. 2000
1.14[a,c]	—	—	—	—	—	Ashrafi et al. 2000
2–3	—	—	2–3	—	—	Lin et al. 2001
2[a,d]	0.78[a,d]	2.56	—	—	—	Anderson et al. 2002
1.26 ± 0.06[e]	0.85 ± 0.13	1.48	1.19 ± 0.08	0.39 ± 0.11	3.05	This study

So, maybe we can posit a grand unifying theory:

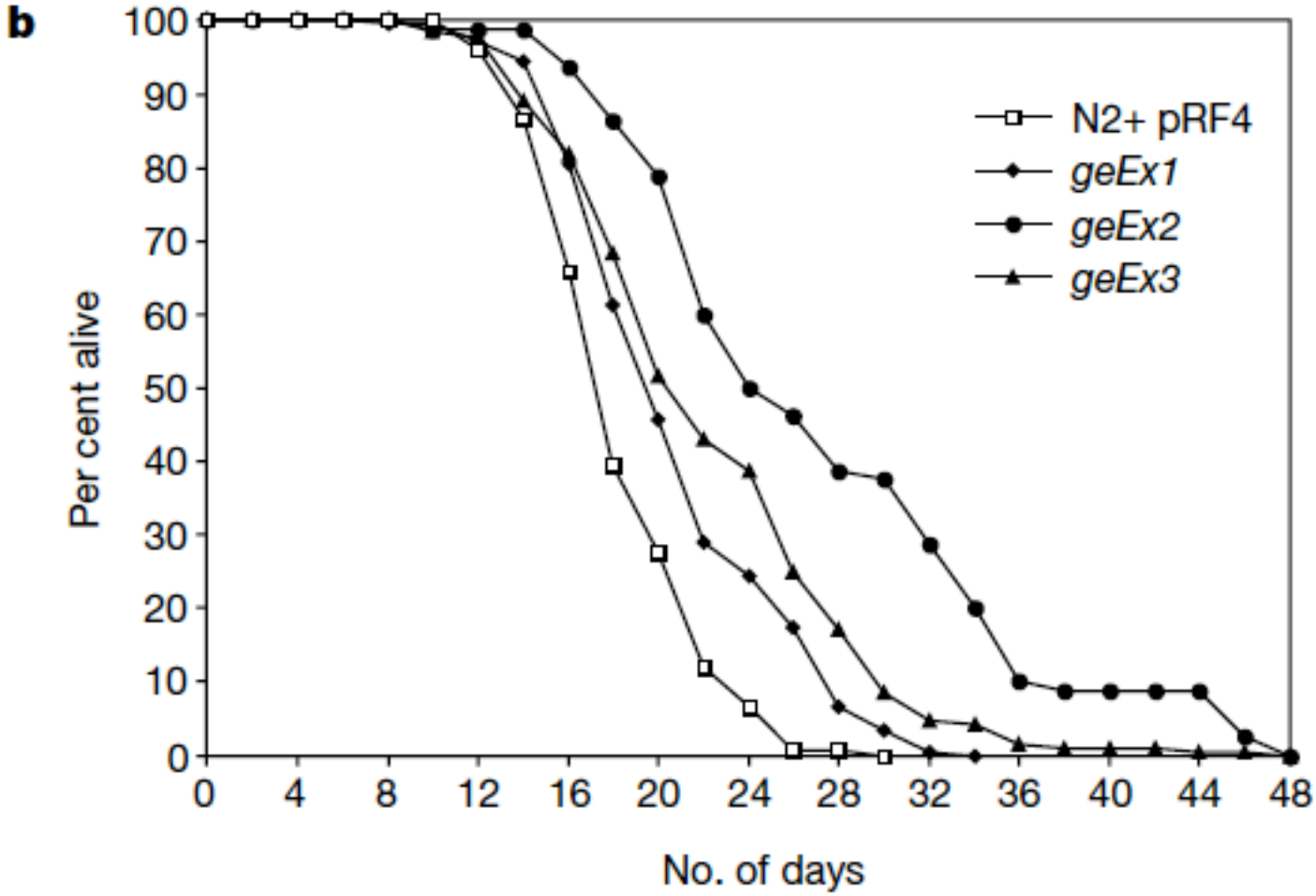
CR affects lifespan by controlling the activity of Sir2

NAD provides an appealing connection between diet and Sir2

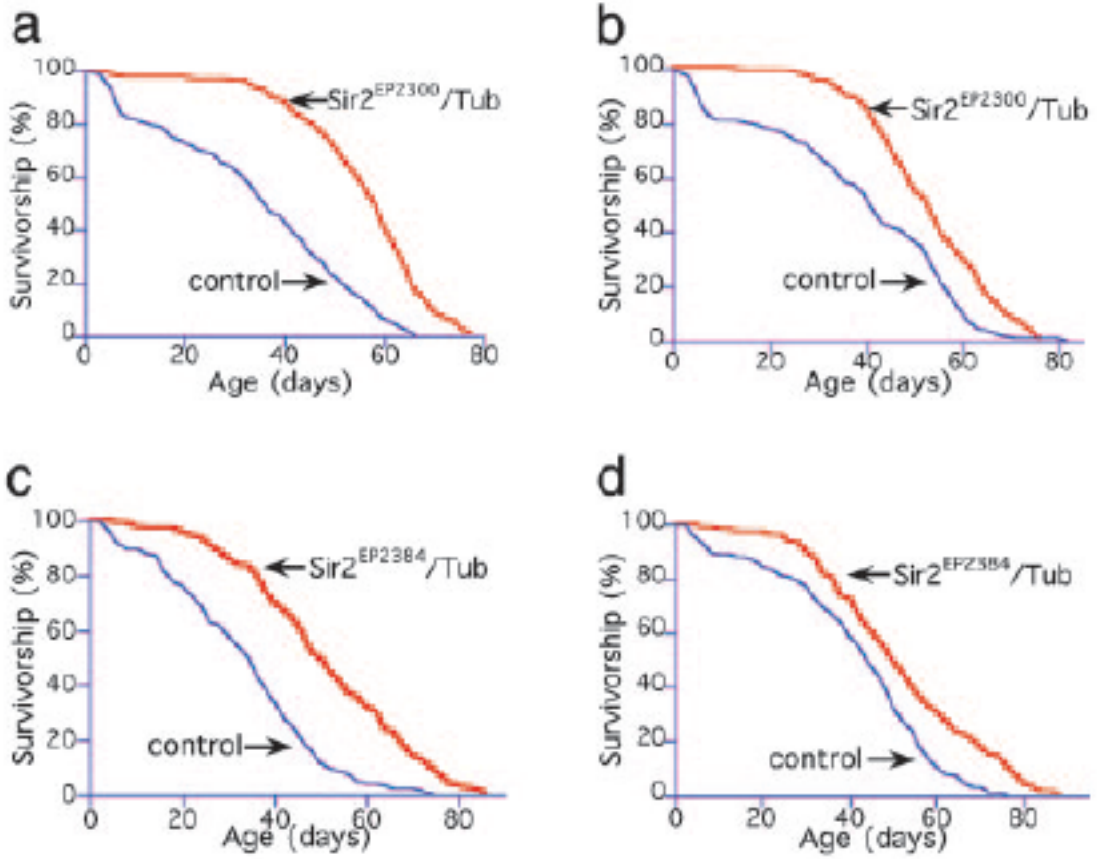


Sir2 may be involved in lifespan control in nematodes

Nematodes have 4 Sir2-like genes. Overexpression of the worm Sir2 gene that most resembles the yeast *SIR2* gene extends lifespan

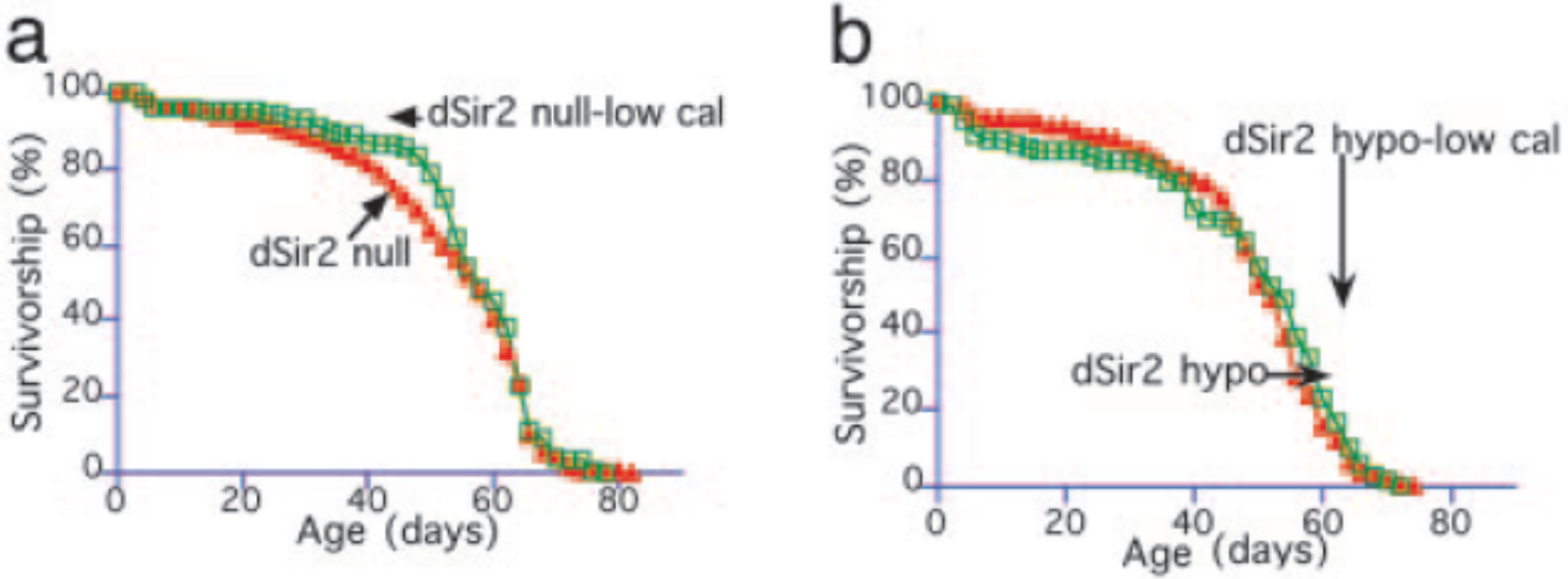


Sir2 and CR are connected to lifespan in Drosophila

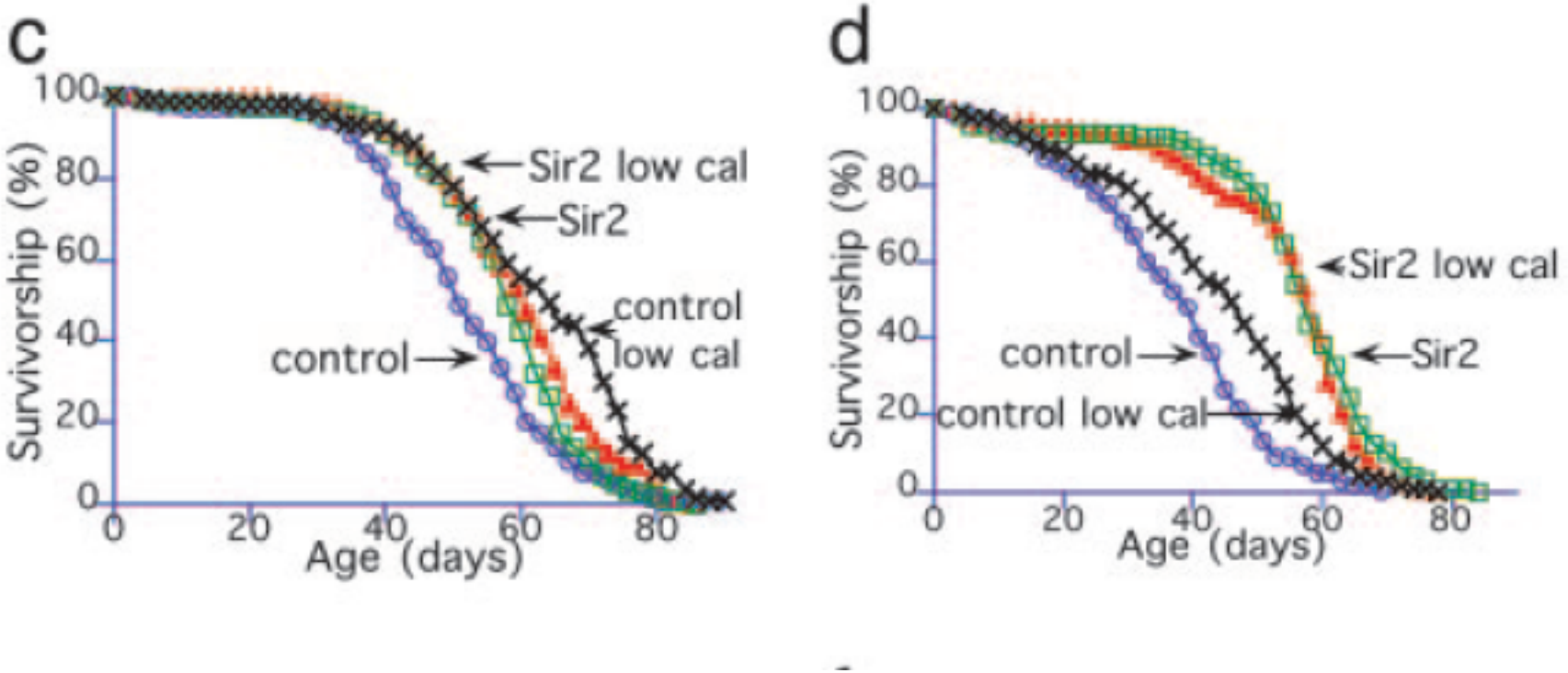


Conclusion: overexpression extends lifespan

Fly Sir2 is required for CR-mediated lifespan expansion



And CR does not lengthen the extended lifespan conferred by Sir2 overexpression



Thus it appears that Sir2-like proteins are involved in lifespan control in a variety of organisms

And Sir2 may have a connection to CR

But there is controversy in the yeast aging research community

Is there indeed a connection?

Are there more players?

Why is there controversy?

Why is there controversy?

That's the way science is and progresses.
No single paper or small group of papers can tell the whole story.

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Technical difficulties – different strains, different protocols, etc.

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egos

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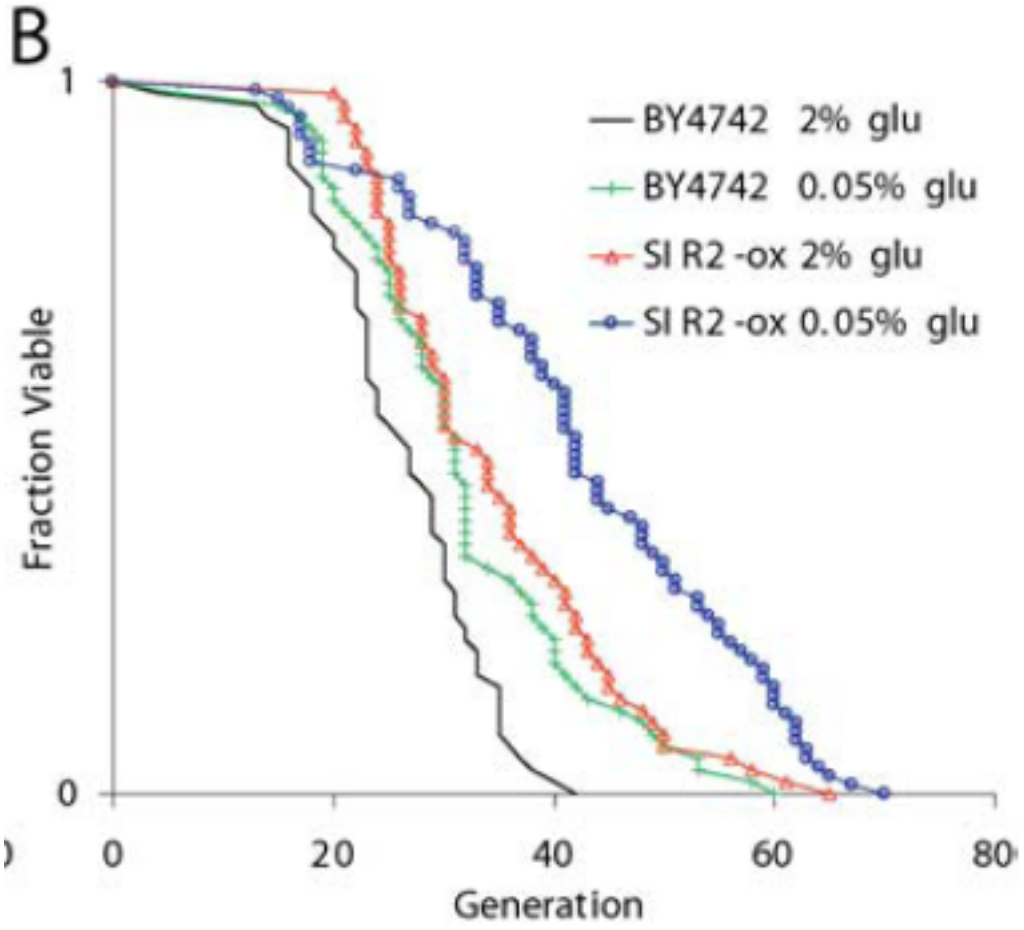
No single paper or small group of papers can tell the whole story.

Technical difficulties – different strains, different protocols, etc.

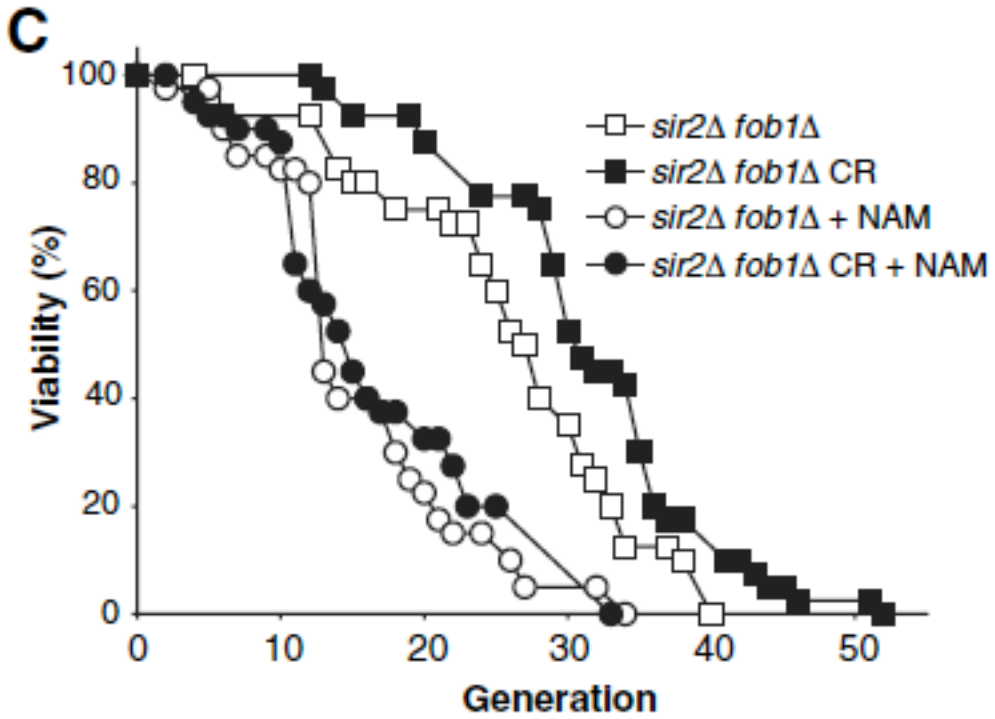
egos

money

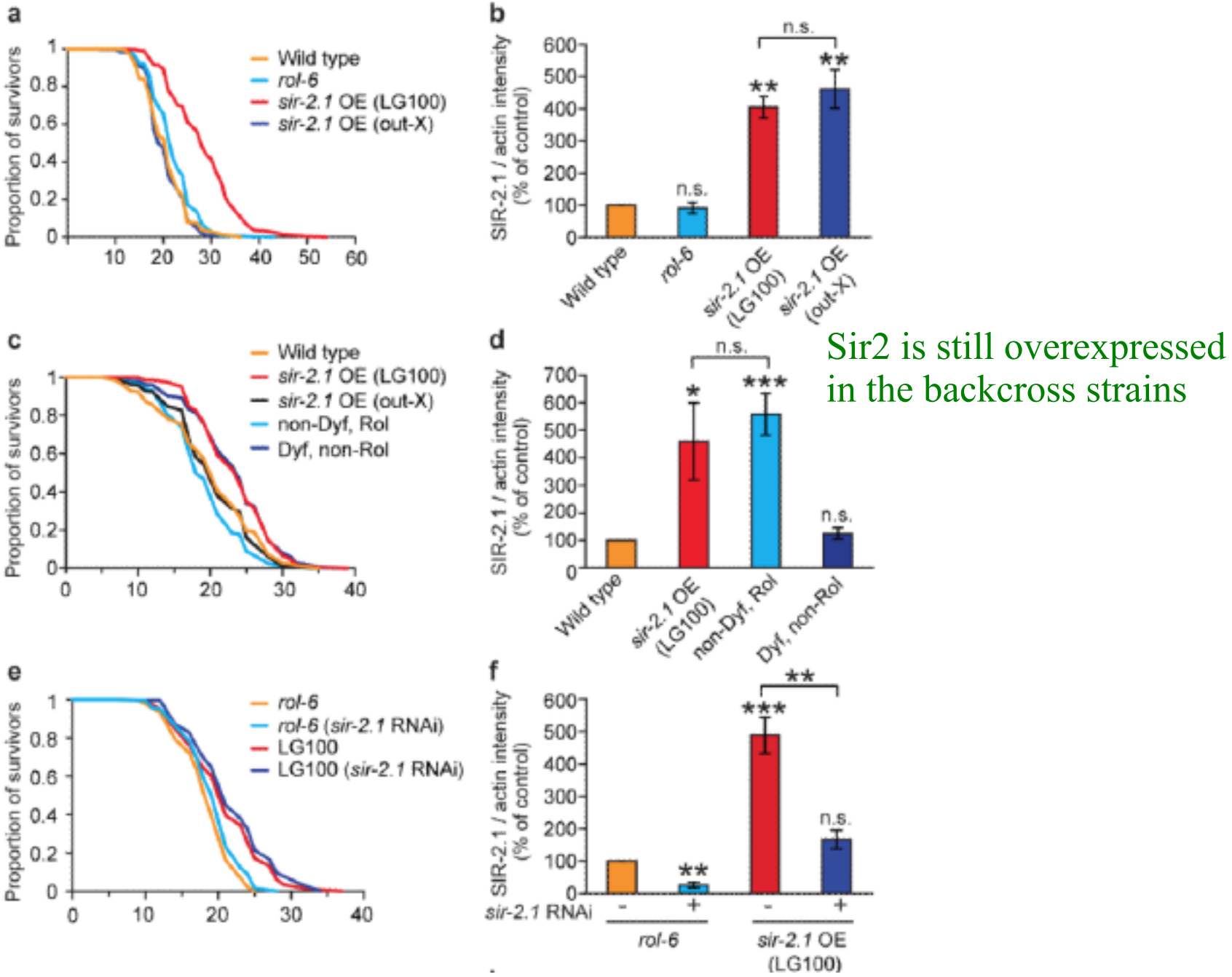
CR extends lifespan in Sir2-overexpressing strains



CR extends lifespan in *sir2* mutant strains

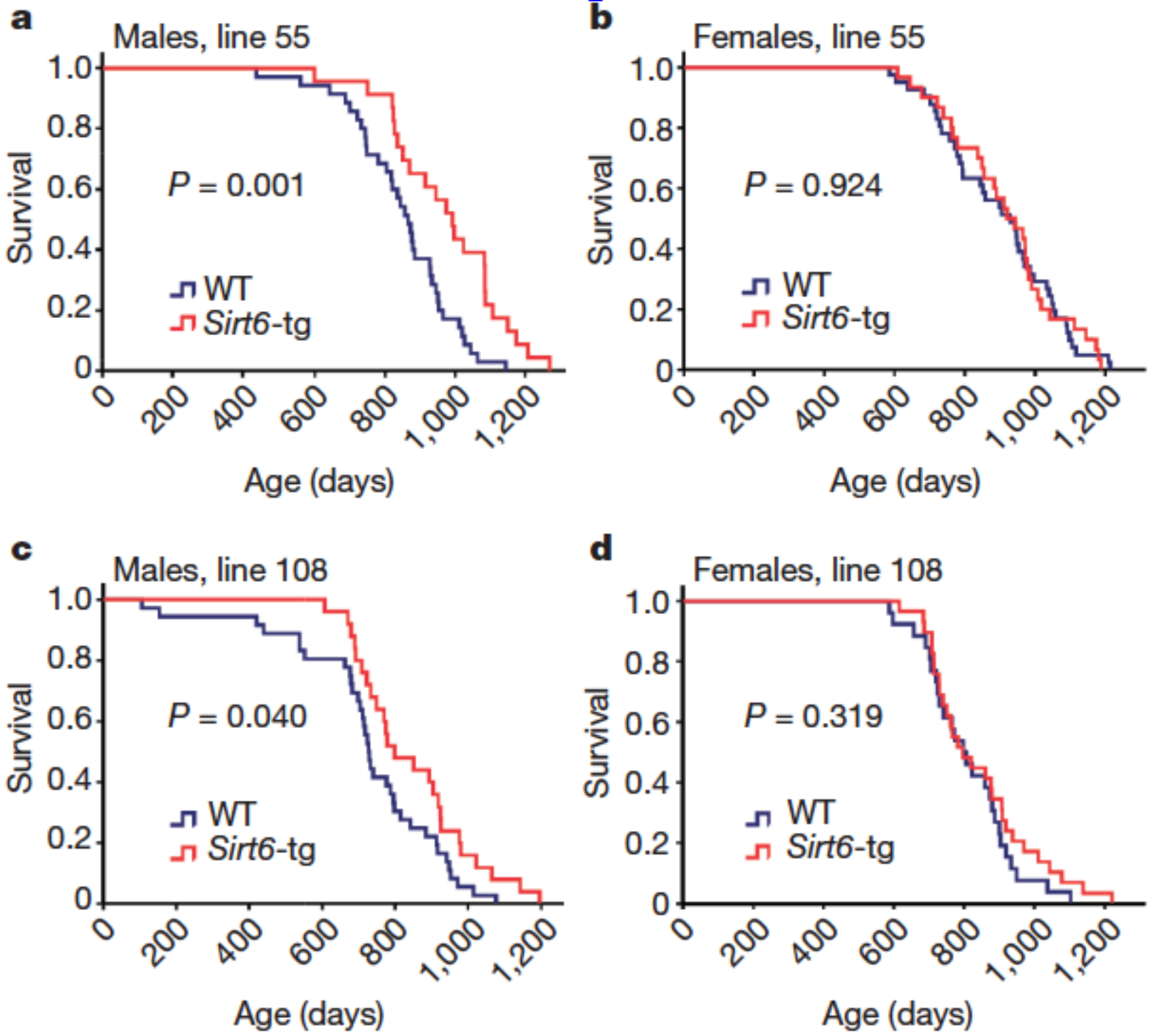


Outcrossing removes Sirt2 affect on *C. elegans* lifespan



Sir2 is still overexpressed in the backcross strains

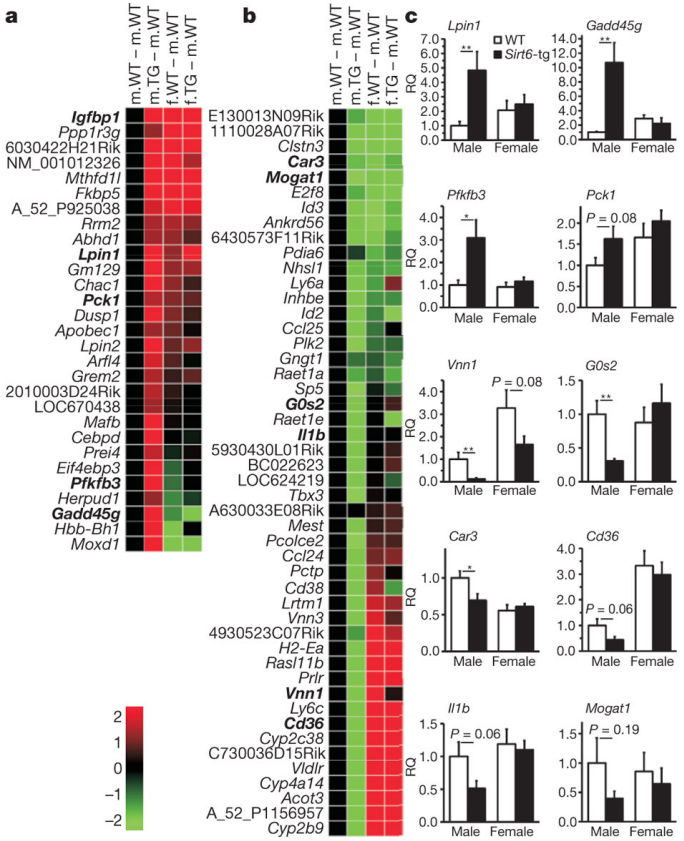
Sirt6 extends lifespan in male mice



Expression profile of differentially expressed genes in male *Sirt6*-transgenic mice.

A number of the affected genes are involved in metabolism and are also affected under CR.

Affected genes include a couple in the insulin-like growth factor pathway.



Y Kanfi *et al*. *Nature* **000**, 1-4 (2012) doi:10.1038/nature10815

On to worms

Worms have a quiescent state, analogous to spores in bacteria and fungi, that is induced in L1 larvae under various stress conditions. This quiescent state is called the dauer state and larvae in this state are called dauer larvae.

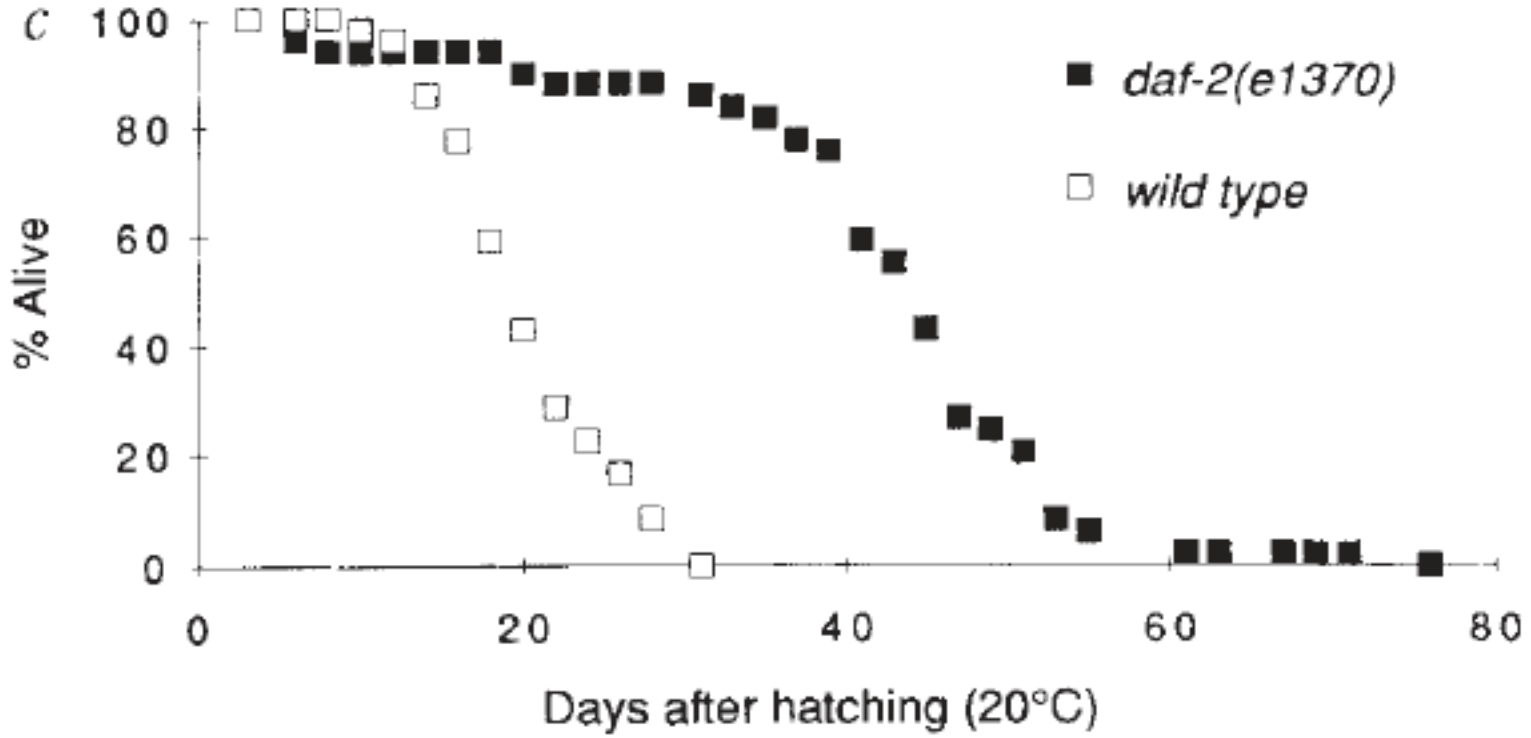
Many of the genes that were found to affect lifespan were first found in screens for mutants defective for dauer formation and are called *daf* genes.

The primary lifespan pathway in worms is an insulin-like growth factor (IGF) pathway

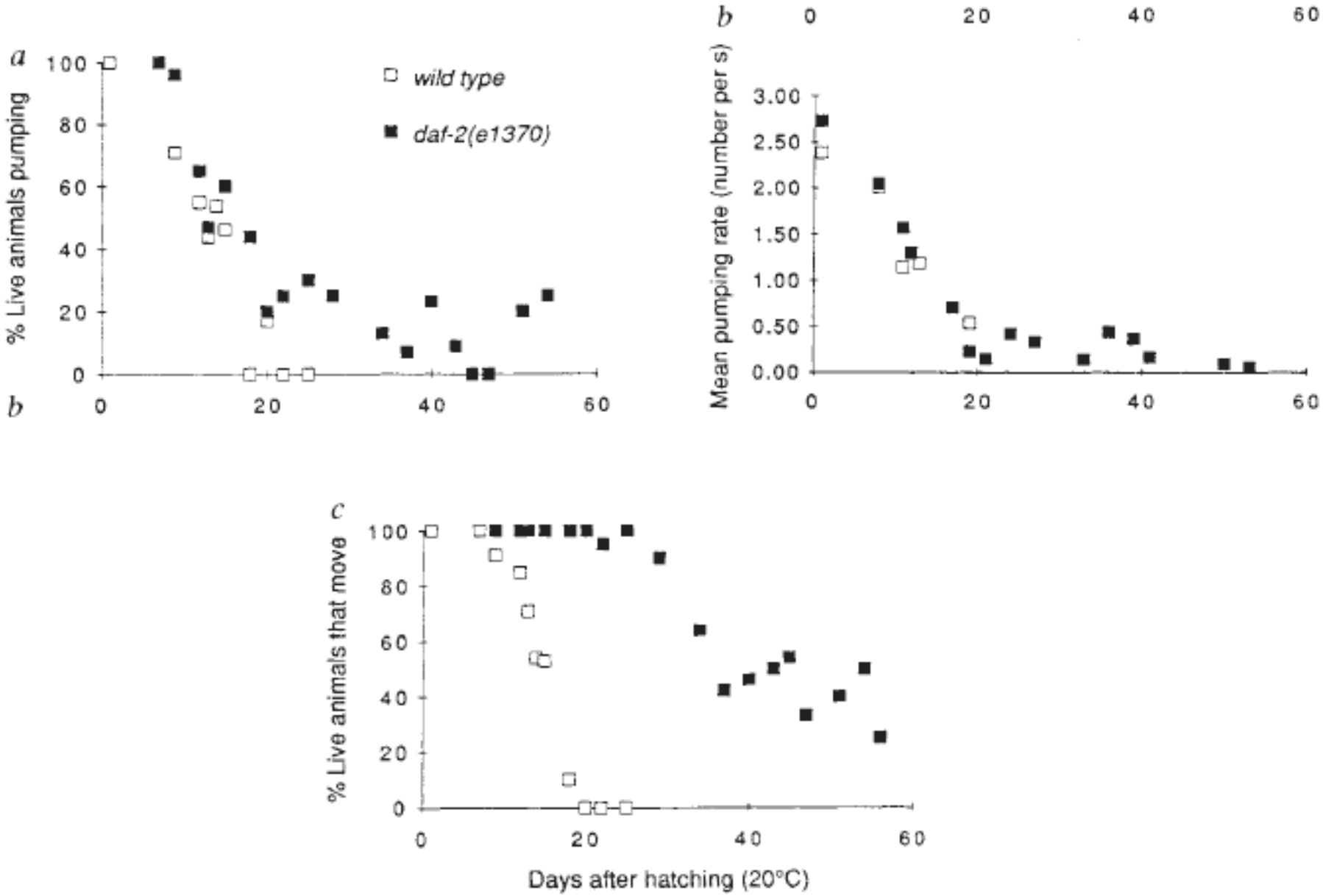
The order of gene function in the pathway was deduced from double mutant phenotypes using pairs of gene mutations that confer different phenotypes.

The molecular functions of the genes was learned by cloning, DNA sequencing, and comparing the deduced protein sequences to protein sequence databases.

daf-2 mutants have increased lifespans

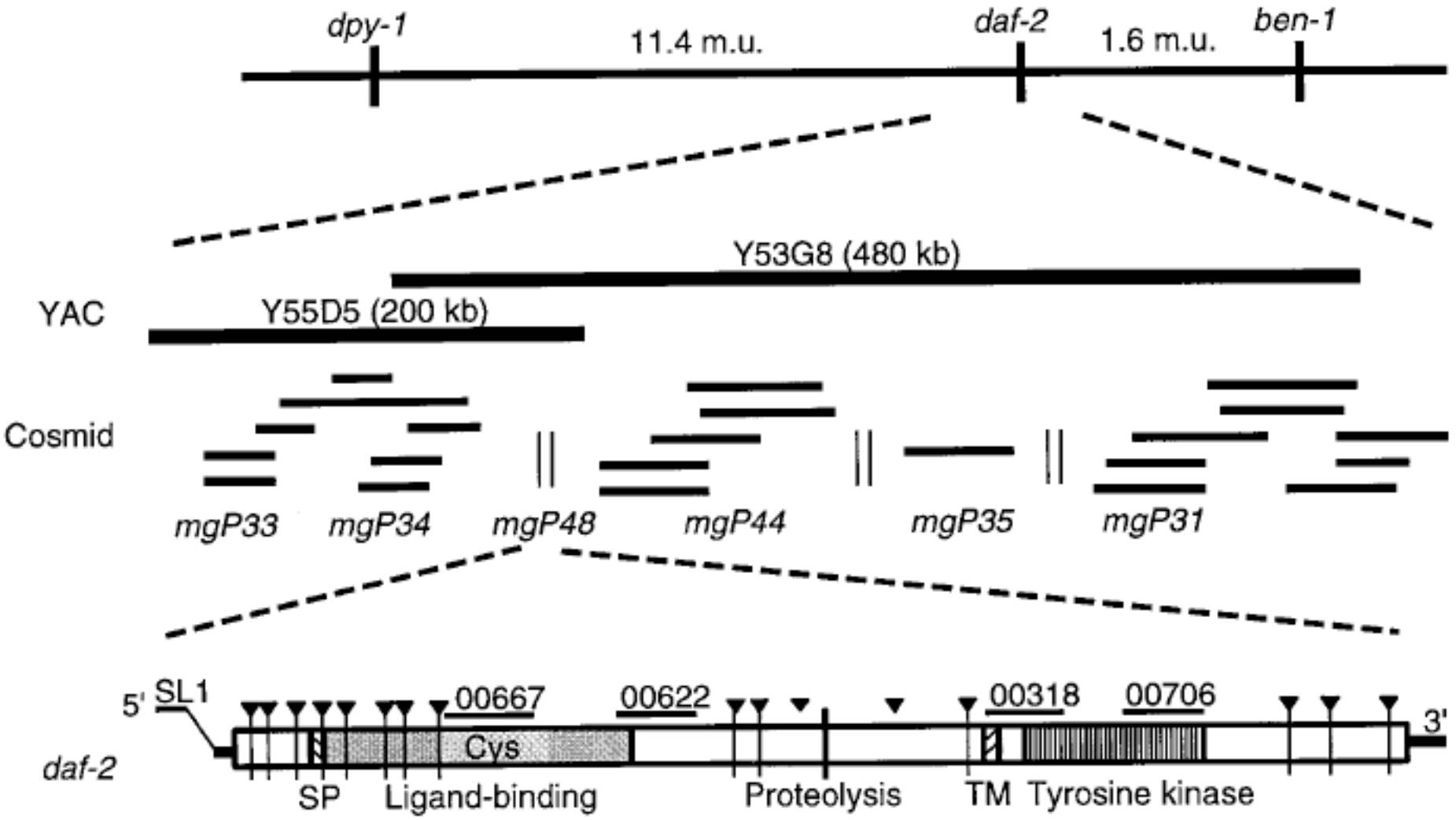


daf-2 mutants have normal behavior



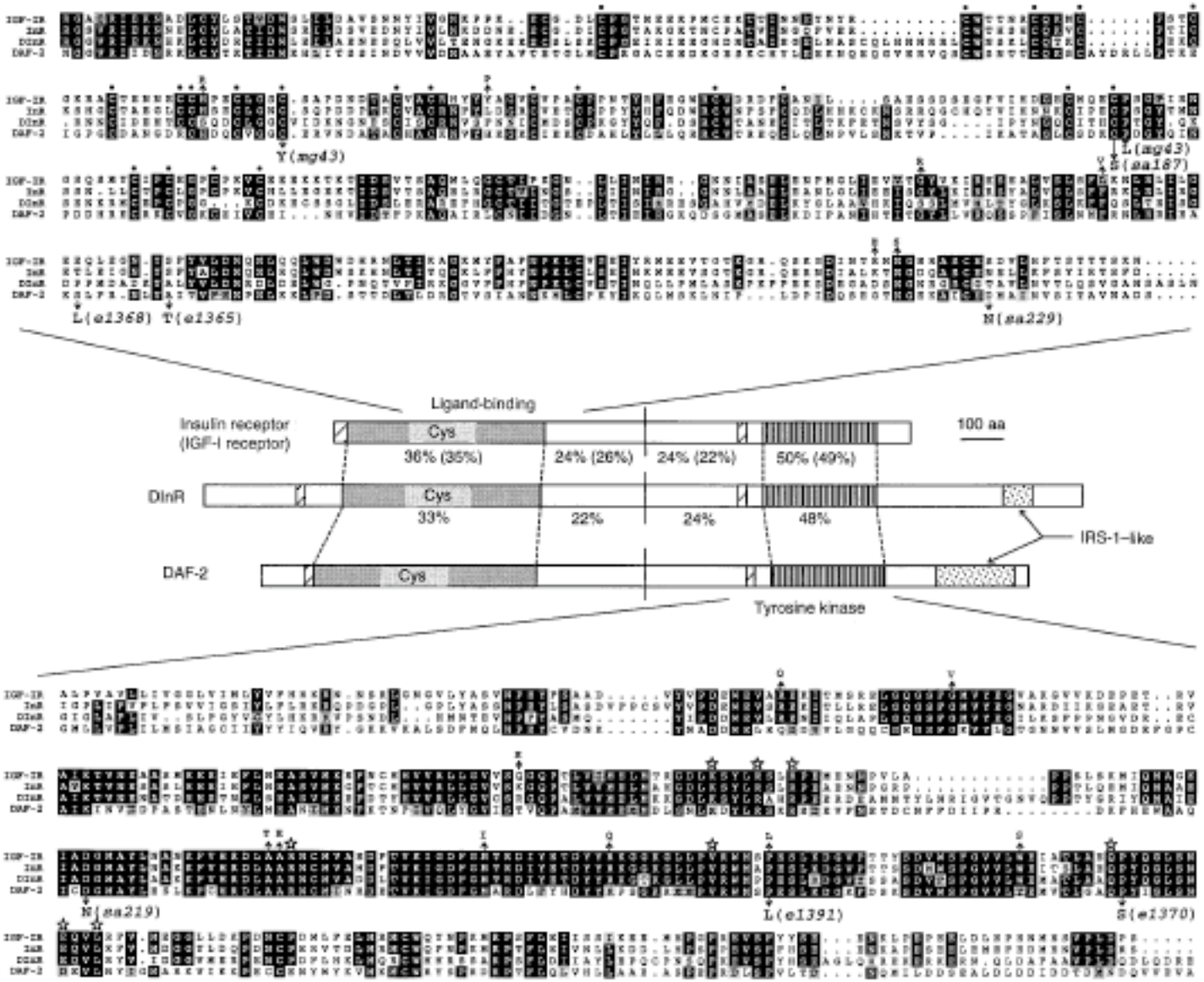
daf-2 encodes an insulin receptor-like protein

mapping

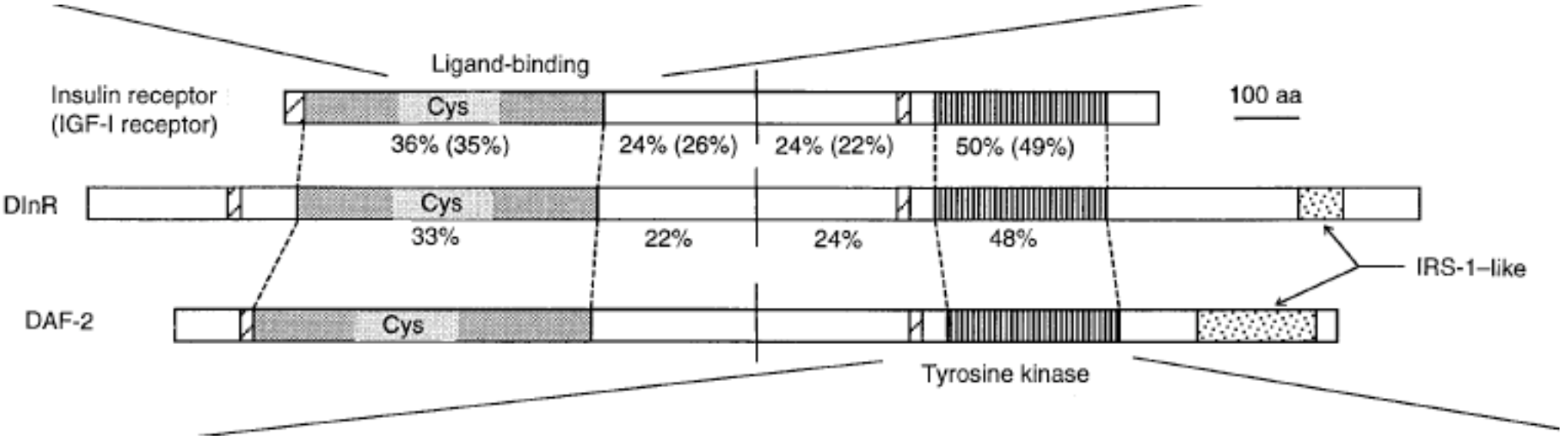


daf-2 encodes an insulin receptor-like protein

protein sequence comparisons



daf-2 encodes an insulin receptor-like protein



A second gene, *pdk-1*

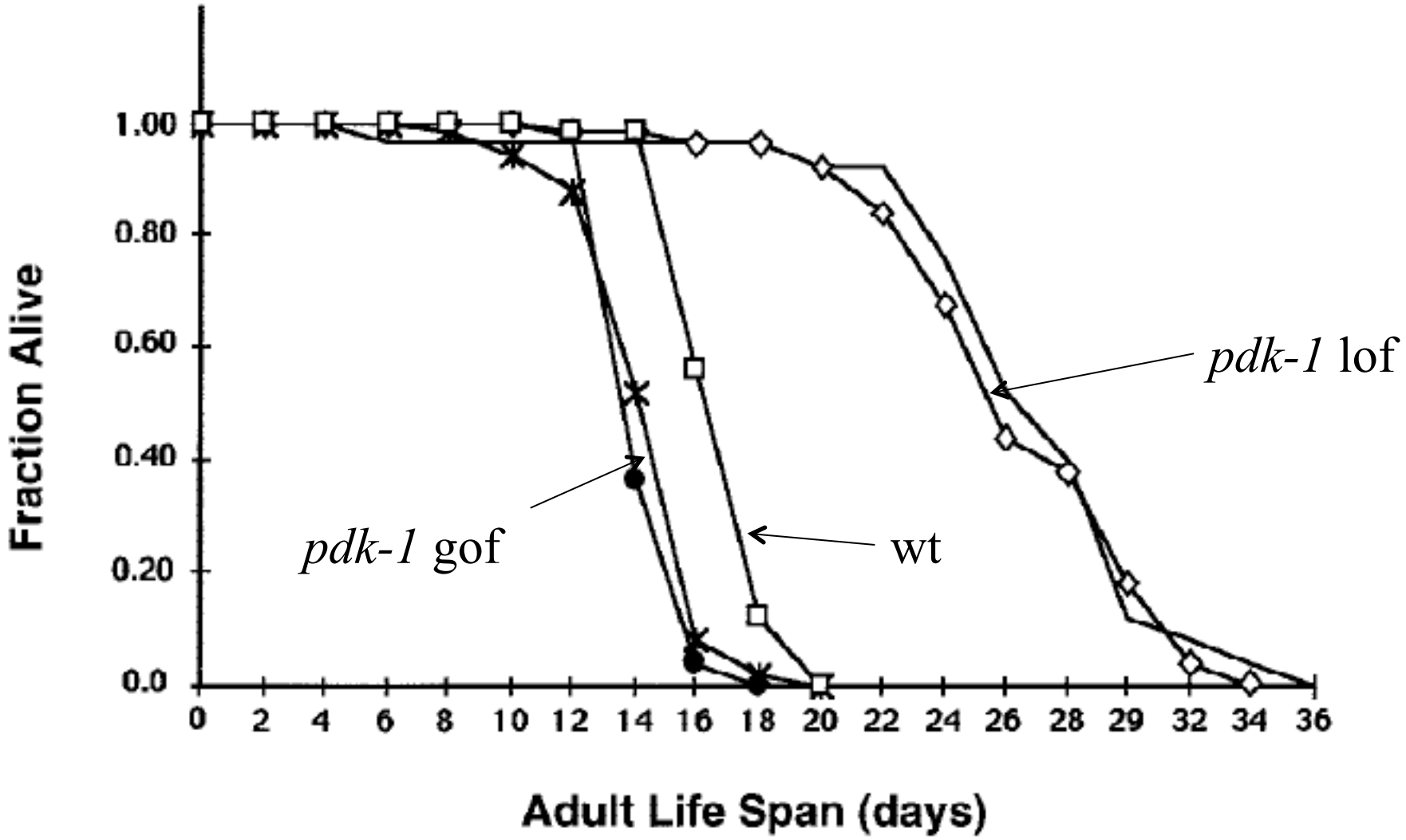
New mutations were isolated that affected dauer formation and lifespan

Recessive mutations caused constitutive dauer formation at 27 degrees

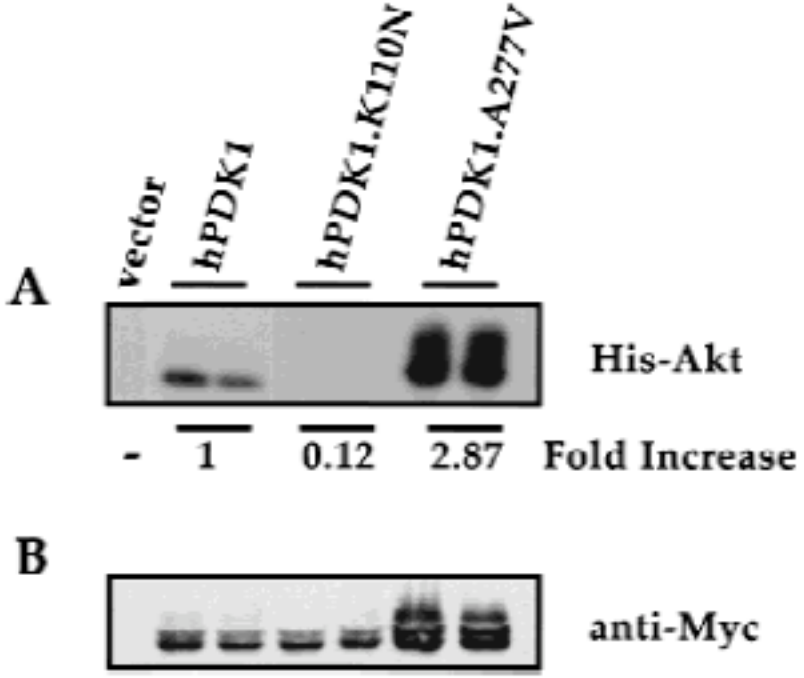
These mutations mapped near to a dominant mutation that blocked dauer formation

Both the dominant and recessive mutations proved to be alleles of the same gene, a gene that is a homolog of mammalian *PDK1*, which encodes a protein kinase.

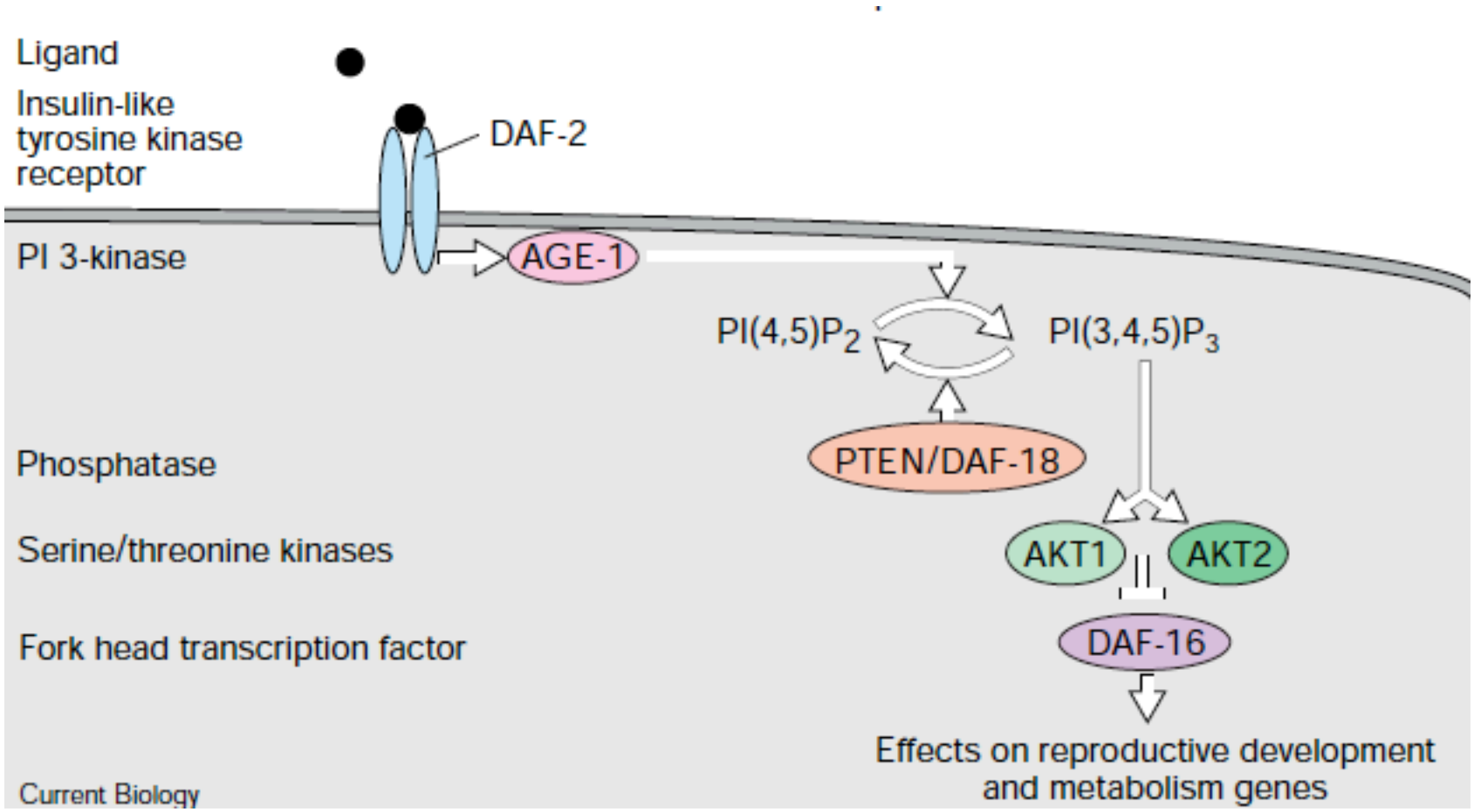
pdk-1 recessive mutations lengthen lifespan, dominant mutations shorten lifespan



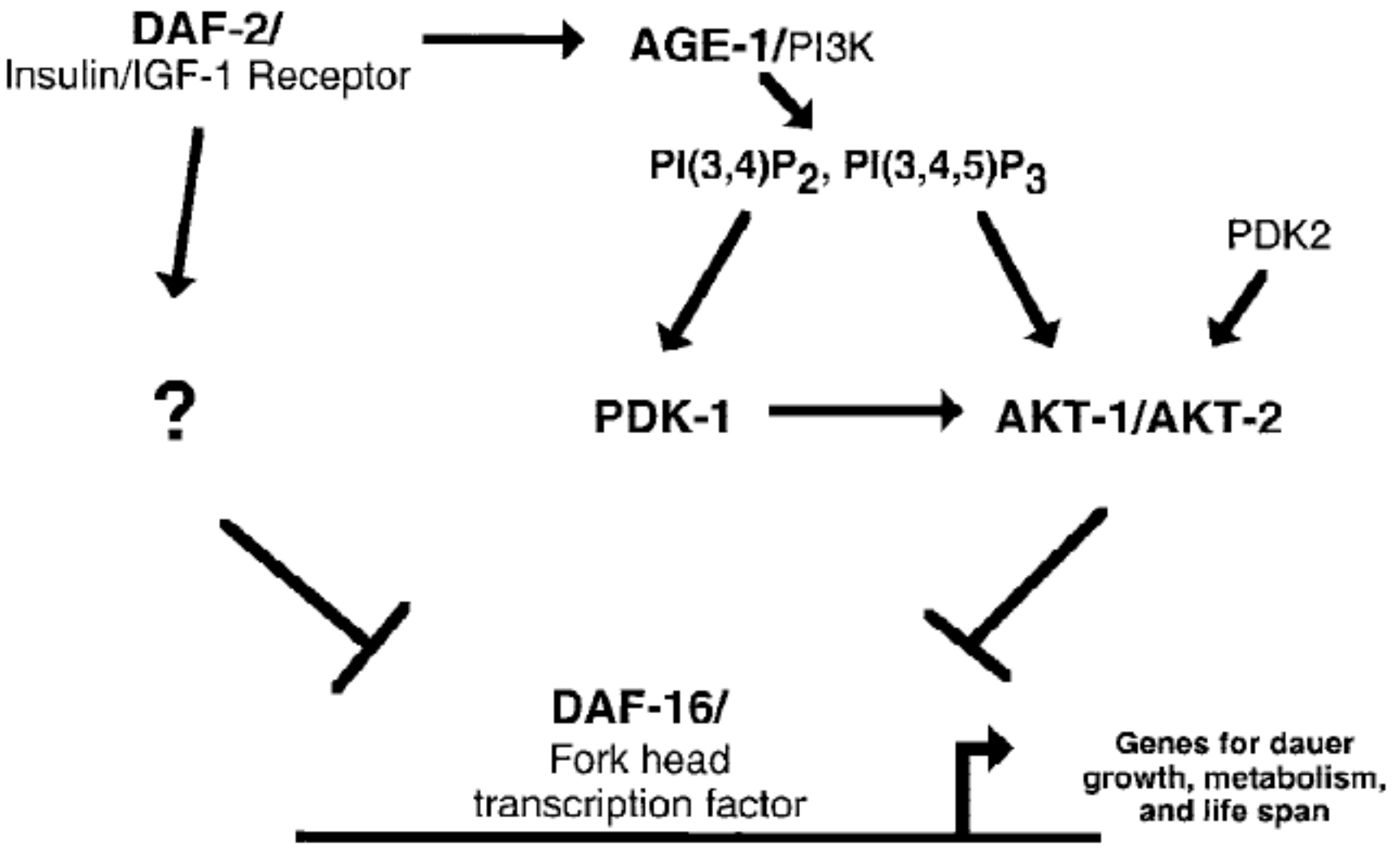
pdk-1 recessive mutations abolish kinase activity, dominant mutations increase kinase activity



Pathway model



Pathway model (with Pdk1 included)



What genes might execute lifespan control?

2 approaches

1. Genome-wide RNAi screen

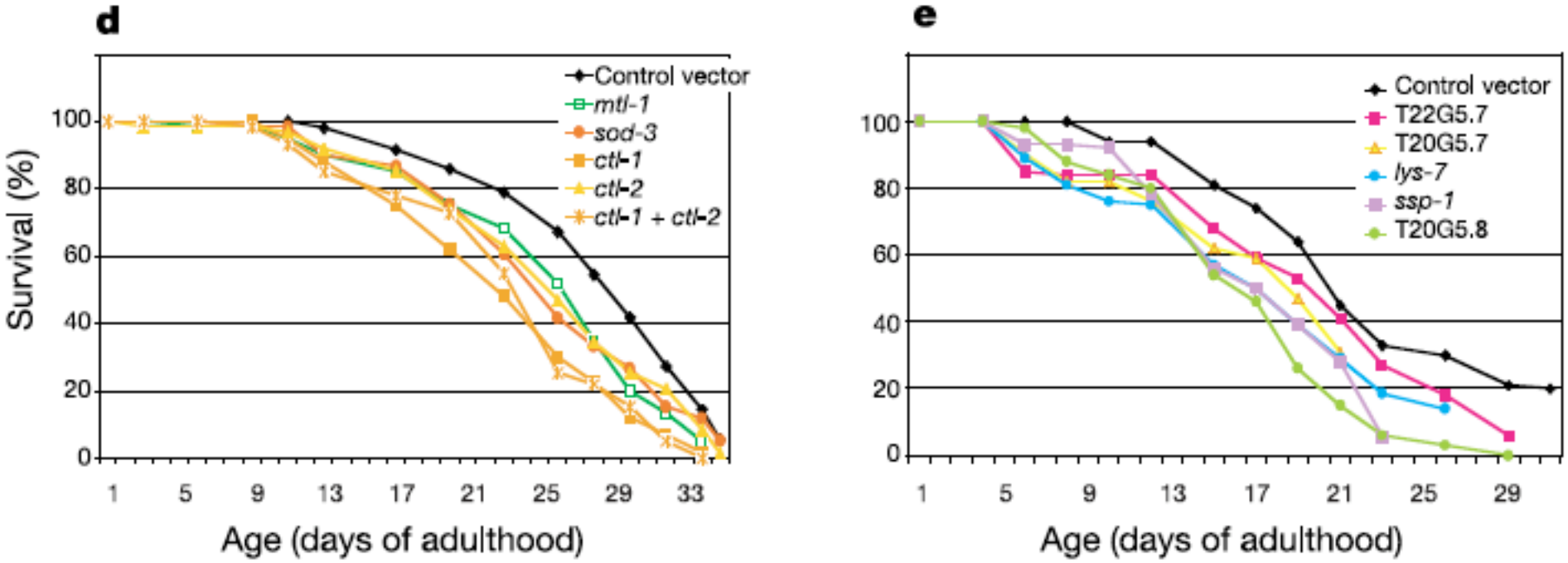
89 genes extend lifespan. Gene ontology in metabolism, signaling, protein turnover. None has as strong a phenotype as the insulin pathway signaling mutants – perhaps that's what one would expect.

Some genes appear to function in the insulin pathway, some in the Sir2 pathway, and still others appear to be independent of both pathways.

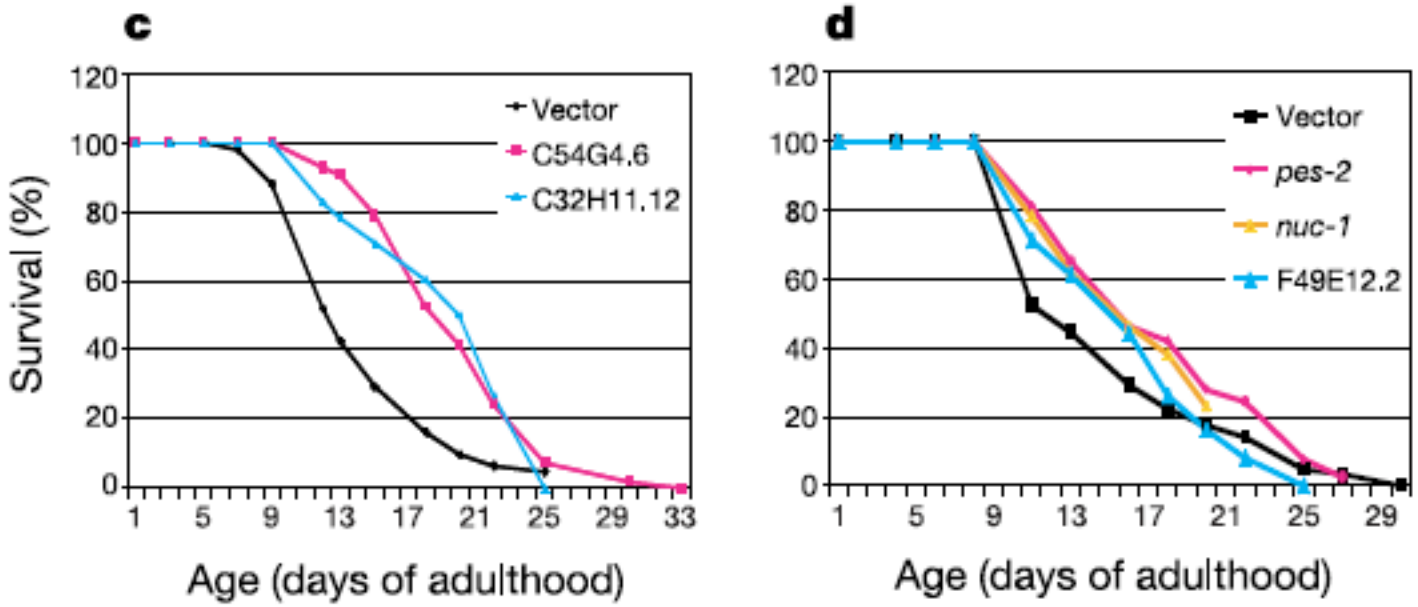
2. Microarray analysis

Concentrated on two clusters.

One cluster showed induced expression in *daf-2* mutants but repressed expression in *daf-16* mutants. These genes may extend lifespan. Indeed RNAi supports this. Genes involved in steroid synthesis, lipid synthesis, and metabolism

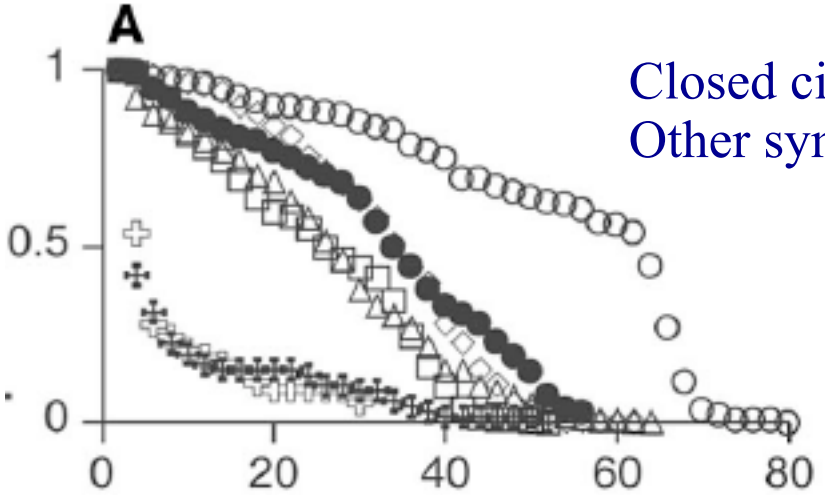


The other cluster showed the opposite pattern – reduced expression in *daf-2* mutants but increased expression in *daf-16* mutants. These genes may shorten lifespan. Indeed RNAi supports this. Many genes of unknown function.

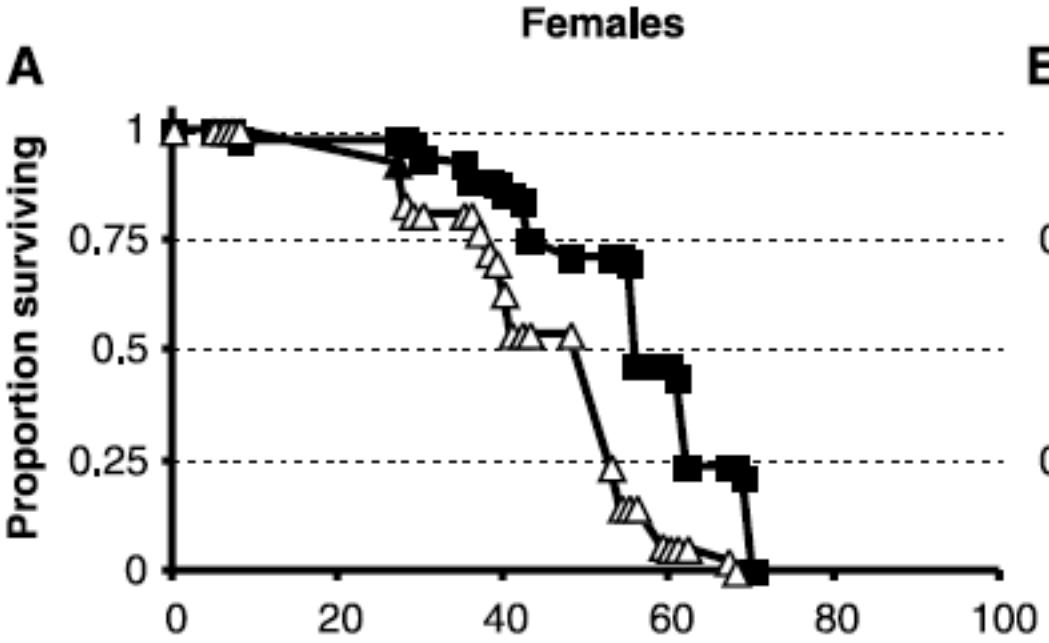


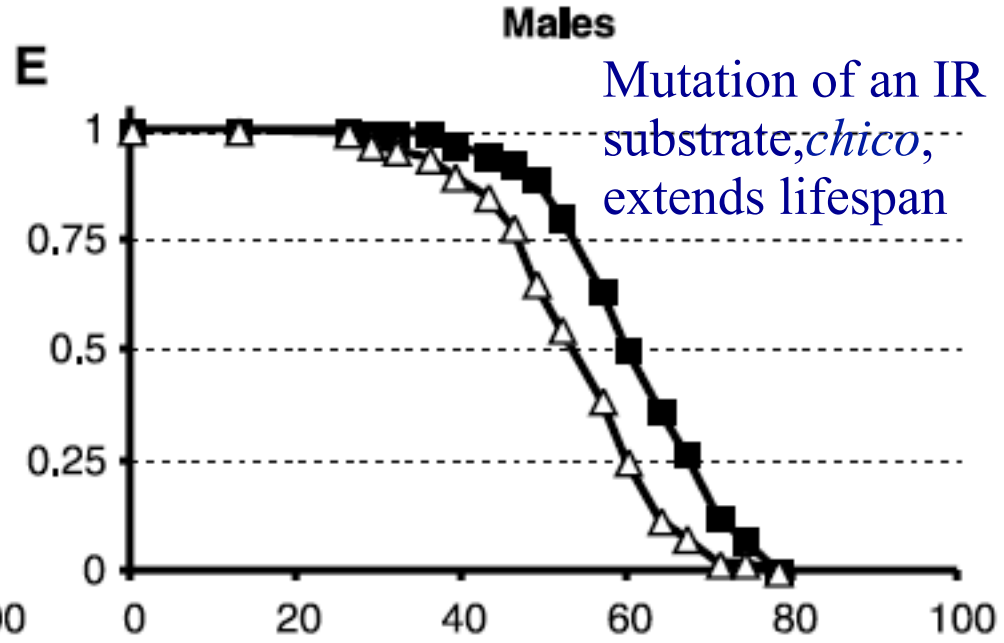
Insulin receptor is also involved in lifespan control in Drosophila and in mice

Drosophila



Closed circles, wt;
Other symbols, IR heteroalleles

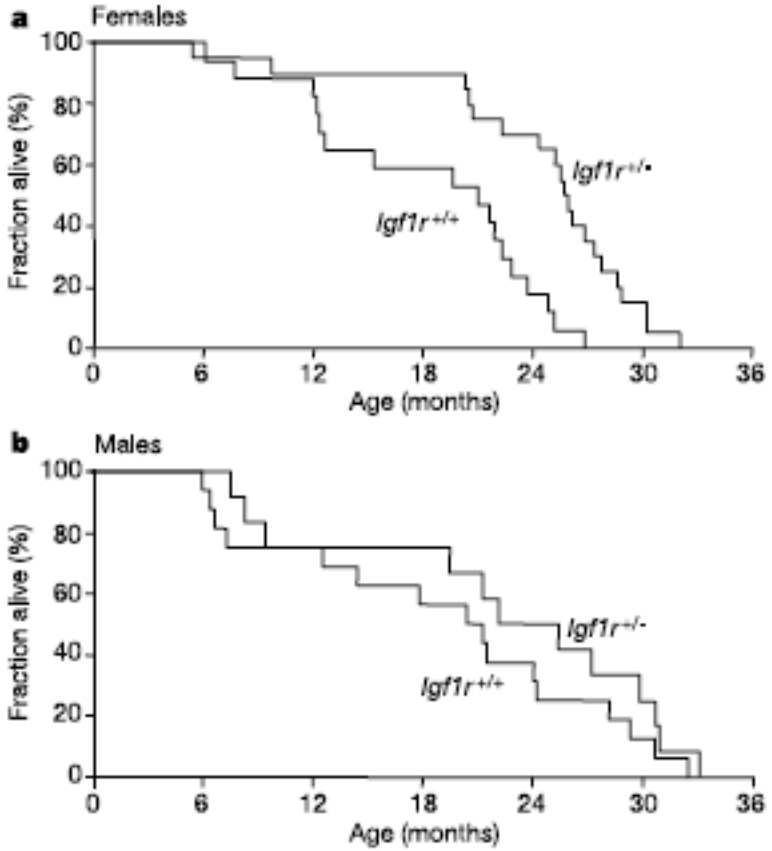




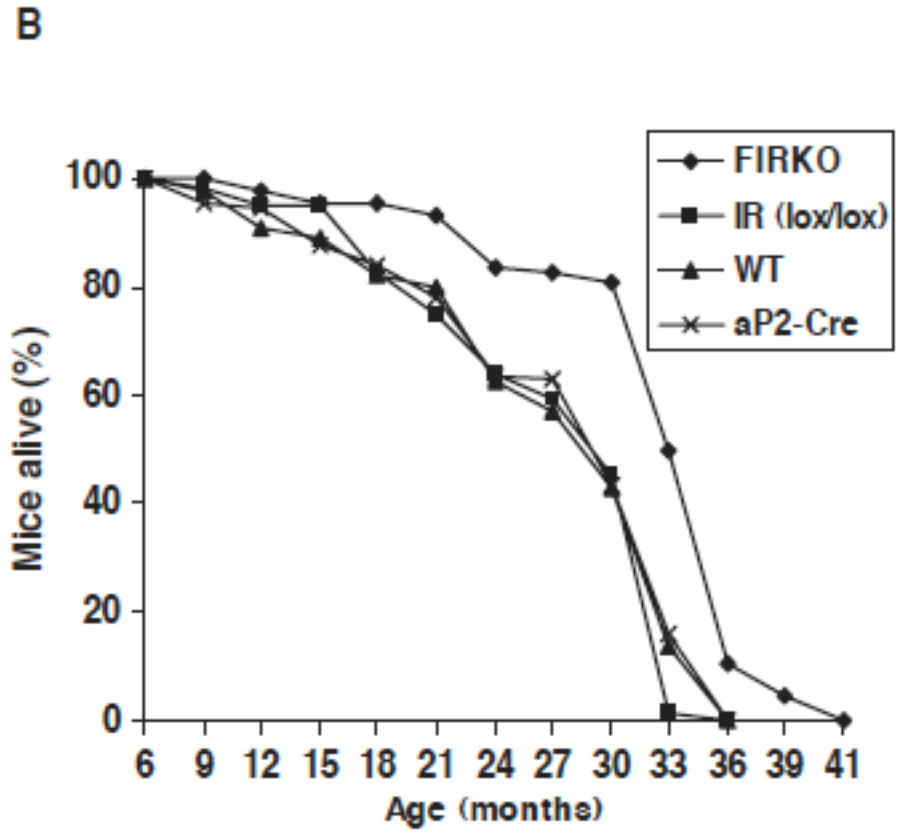
Mutation of an IR substrate, *chico*, extends lifespan

Mice

Knock-out heterozygotes



Adipose-specific homozygous knockout using *loxP* sites flanking exon 4 and Cre recombinase under control of an adipose-specific promoter



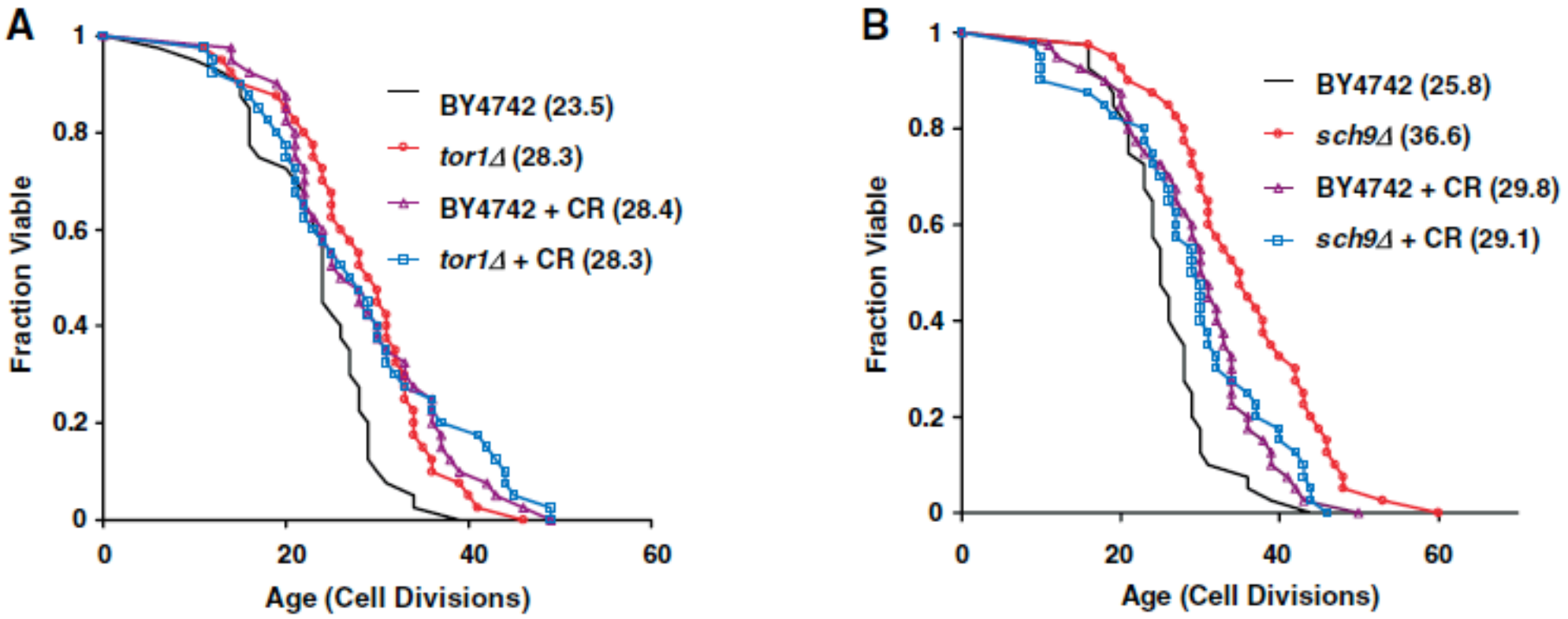
Result is context specific. Equivalent experiment done for liver results in diabetic mice

Back to yeast.
The beginning of an unbiased genetic approach.

Screen ~600 mutants from the deletion collection for increased replicative lifespan

Found 10 genes, including one involved in Sir2/ERC. Provides “proof of principle”.

Several genes in TOR pathway, a nutrient sensing pathway. One of these genes is SCH9, a gene related to AKT which functions in the insulin pathway of multicellular creatures


A
Fraction Viable
1
0.8
0.6
0.4
0.2
0
0
20
40
60
Age (Cell Divisions)
BY4742 (23.5)
tor1Δ (28.3)
BY4742 + CR (28.4)
tor1Δ + CR (28.3)
B
Fraction Viable
1
0.8
0.6
0.4
0.2
0
0
20
40
60
Age (Cell Divisions)
BY4742 (25.8)
sch9Δ (36.6)
BY4742 + CR (29.8)
sch9Δ + CR (29.1)

Integration of aging signals in yeast?

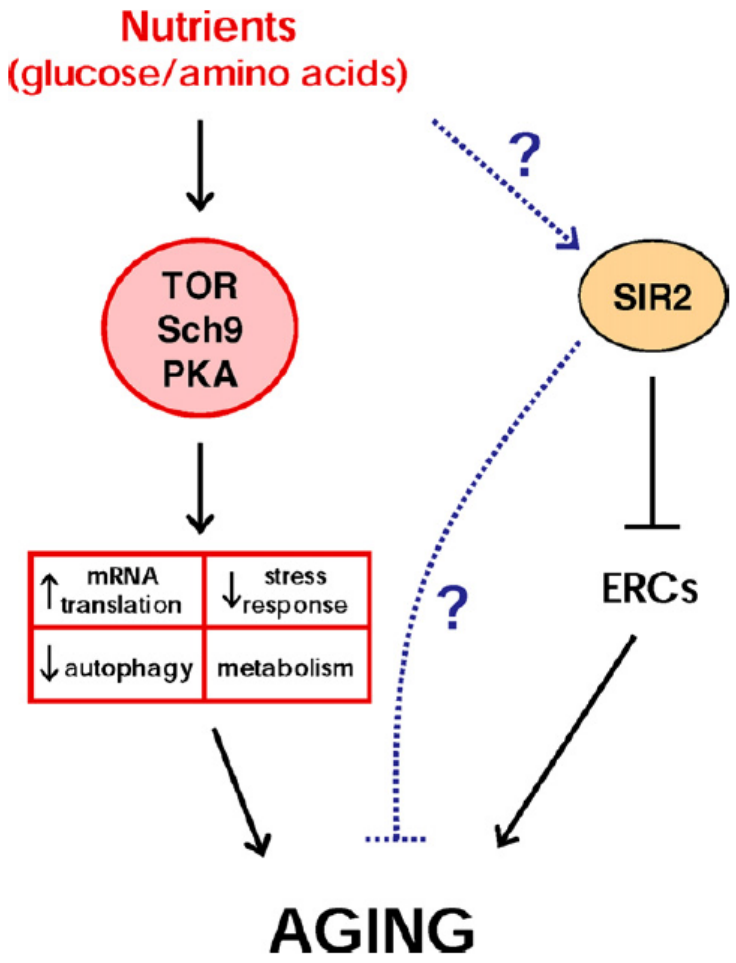


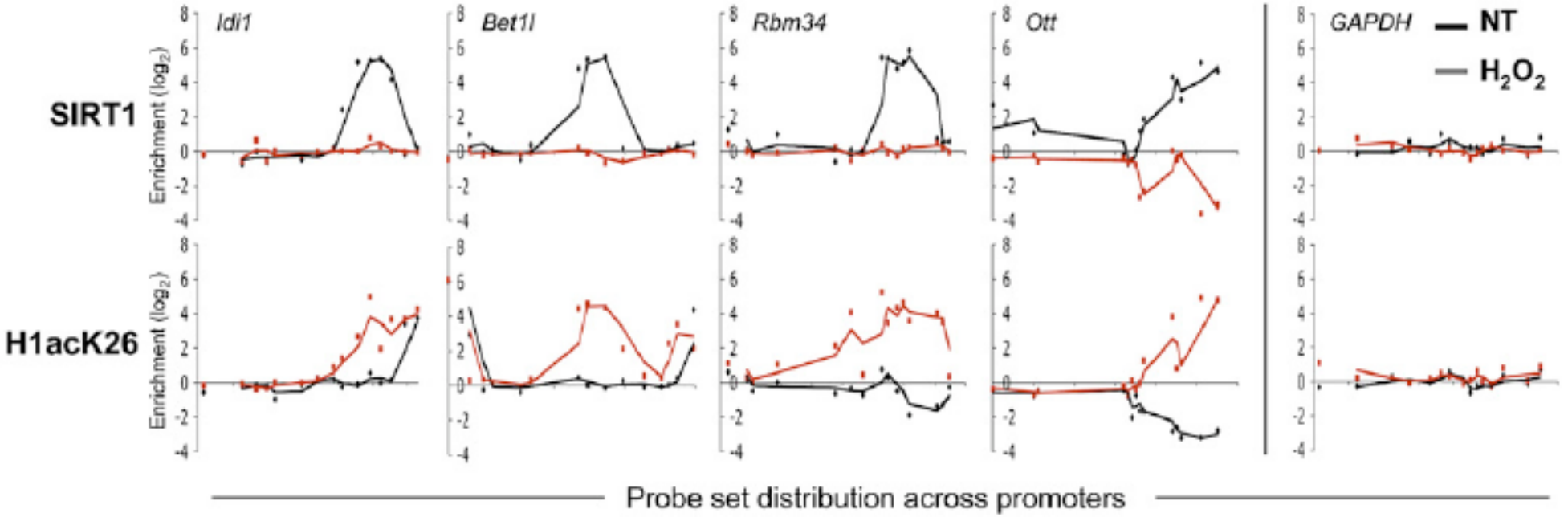
Table 1. Conserved longevity modifiers

Yeast gene	Worms	Flies	Mice	References
TOR1	*let-363*	*dTOR*	*mTOR*	Vellai *et al.* (2003), Kapahi *et al.* (2004), Kaeberlein *et al.* (2005a, c), Powers *et al.* (2006) and Lamming *et al.* (2012)
SCH9	*rsks-1*	*dS6K*	*S6K1*	Fabrizio *et al.* (2004), Kapahi *et al.* (2004), Hansen *et al.* (2007), Pan *et al.* (2007) and Selman *et al.* (2009)
SIR2	*sir-2.1**	*dSir2**		Kaeberlein *et al.* (1999), Tissenbaum & Guarente (2001), Rogina & Helfand (2004) and Burnett *et al.* (2011)
RPD3		*Rpd3*		Kim *et al.* (1999), Jiang *et al.* (2002) and Rogina *et al.* (2002)
DBP3	*B0511.6*			Curran & Ruvkun (2007) and Smith *et al.* (2008)
PMR1	*eat-6*			Lakowski & Hekimi (1998) and Smith *et al.* (2008)
YGR130C	*erm-1*			Curran & Ruvkun (2007) and Smith *et al.* (2008)
IDH1, IDH2	*F43G9.1*			Hamilton *et al.* (2005) and Smith *et al.* (2008)
TIF4631	*ifg-1*			Curran & Ruvkun (2007), Pan *et al.* (2007) and Smith *et al.* (2008)
TIF1, TIF2	*inf-1*			Curran & Ruvkun (2007) and Smith *et al.* (2008)
PKH2	*pdk-1*			Paradis *et al.* (1999) and Smith *et al.* (2008)
TIS11	*pos-1*			Curran & Ruvkun (2007) and Smith *et al.* (2008)
YPT6	*rab-10*			Hansen *et al.* (2005) and Smith *et al.* (2008)
RPL19A	*rpl-19*			Hansen *et al.* (2007) and Smith *et al.* (2008)
RPL6B	*rpl-6*			Hansen *et al.* (2007) and Smith *et al.* (2008)
RPL9A	*rpl-9*			Hansen *et al.* (2007) and Smith *et al.* (2008)
SAM1	*sams-3*			Curran & Ruvkun (2007) and Smith *et al.* (2008)
HSE1	*sem-5*			Curran & Ruvkun (2007) and Smith *et al.* (2008)
AFG3	*spg-7*			Curran & Ruvkun (2007), Smith *et al.* (2008) and Delaney *et al.* (2013a)
SPT4	*spt-4*			Hamilton *et al.* (2005) and Smith *et al.* (2008)
ALG12	*T27F7.3*			Curran & Ruvkun (2007) and Smith *et al.* (2008)
INP51, INP53	*unc-26*			Lakowski & Hekimi (1998) and Smith *et al.* (2008)
ADH1	*W09H1.5*			Hamilton *et al.* (2005) and Smith *et al.* (2008)
SIS2	*Y46H3C.6*			Hamilton *et al.* (2005) and Smith *et al.* (2008)
COX4	cco-1			Dillin *et al.* (2002) and Miceli *et al.* (2011)
Intervention	Worms	Flies	Rodents	References
Dietary restriction	X	X	X	McCay *et al.* (1935), Klass (1977), Lakowski & Hekimi (1998), Lin *et al.* (2000), Mair *et al.* (2003) and Kaeberlein *et al.* (2006)
Rapamycin	X	X	X	Medvedik *et al.* (2007), Harrison *et al.* (2009), Bjedov *et al.* (2010), Miller *et al.* (2011), Robida-Stubbs *et al.* (2012) and Wilkinson *et al.* (2012)
Resveratrol*	X*	X*		Howitz *et al.* (2003), Wood *et al.* (2004), Kaeberlein *et al.* (2005d) and Bass *et al.* (2007)
Spermidine	X	X		Eisenberg *et al.* (2009)
Heat shock	X	X		Lithgow *et al.* (1995), Khazaeli *et al.* (1997) and Shama *et al.* (1998)

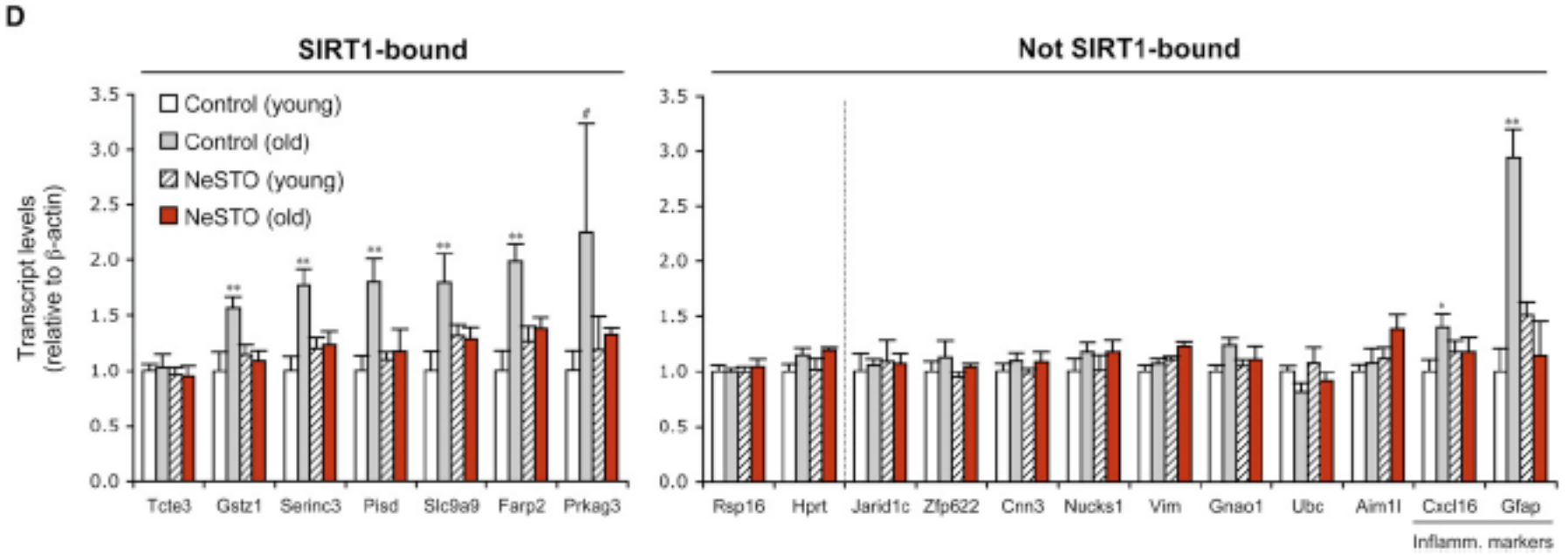
*Refers to data that have been questioned in subsequent peer-reviewed papers.

And, finally, back to Sir2

Mouse Sir2 (SIRT1), redistributes on chromatin during various sorts of stress and during aging



Transcriptional deregulation of SIRT1 target genes occurs during normal aging



NeSTO confers neocortex-specific SIRT1 overexpression