In format provided by Bonkowski & Sinclair (doi:10.1038/nrm.2016.93)

Supplementary information S1: Relevance of nematode and fruit fly sirtuins to longevity

Following the discovery of the budding yeast sirtuin Sir2p (silent information regulator 2) as a longevity factor, Sir2 homologs were tested in *Caenorhabditis elegans* (Sir2.1)[1] and *Drosophila melanogaster* (dSir2)[2] and their overexpression was reported to extend lifespan by 25-50%. Subsequently, the ability of sirtuins to extend lifespan was contradicted by a study that indicated the robustness of the lifespan extension in the original worm study was an artifact caused by a linked mutation[3]. The results from overexpression of fly dSir2 were also questioned[3]. An accompanying article declared these data the "final nail in the coffin" for the idea that sirtuins are involved in aging[4].

In the past few years however, new data have largely settled the debate. With regards to the nematode work, independent laboratories have shown that Sir2.1 overexpression extends longevity up to 10-25%[5-7], consistent with the original report[1]. It was also independently verified that Sir2.1 and Pnc1, an NAD salvage pathway gene that extends lifespan in yeast and flies, are both required for calorie restriction to extend lifespan[8], a finding consistent with a recent report that resveratrol and oxyresveratrol extend lifespan in a Sir2.1-dependent manner[9].

The initial fly work[2] was also repeated using an inducible "gene switch" system to temporally regulate the levels of dSir2 expression in adult flies and circumvent criticisms of the first paper[10]. When compared to genetically identical backcrossed controls, low and high levels of dSir2 overexpression (<2 fold and >5 fold, respectively) failed to extend longevity but moderate overexpression (2-5 fold) provided at least a 15% extension of longevity. This result is consistent with other work in species such as yeast and mice clearly demonstrating that high levels of Sir2p or SIRT1 are deleterious (reviewed in[11]).

Further support for fly Sir2 being a longevity gene has come from two recent reports. One showed that fat-body-specific overexpression of dSir2 extends fly lifespan, mimics calorie restriction and is necessary for the effects of calorie restriction[12, 13]. The other showed the effects of resveratrol on fly longevity are reproducible but possibly sexually dimorphic and dependent on upon nutrient composition[14]. Taken together, the weight of the evidence is that sirtuins are mediators of lifespan extension by calorie restriction, not only in yeast, but in nematodes and fruit flies (Table 1).

Reference List

1. Tissenbaum,H.A. & Guarente,L. Increased dosage of a sir-2 gene extends lifespan in Caenorhabditis elegans. *Nature* **410**, 227-230 (2001).

2. Rogina,B. & Helfand,S.L. Sir2 mediates longevity in the fly through a pathway related to calorie restriction. *Proc. Natl. Acad. Sci. U. S. A* **101**, 15998-16003 (2004).

3. Burnett,C. *et al.* Absence of effects of Sir2 overexpression on lifespan in C. elegans and Drosophila. *Nature* **477**, 482-485 (2011).

4. Lombard,D.B., Pletcher,S.D., Canto,C., & Auwerx,J. Ageing: longevity hits a roadblock. *Nature* **477**, 410-411 (2011).

5. Viswanathan,M. & Guarente,L. Regulation of Caenorhabditis elegans lifespan by sir-2.1 transgenes. *Nature* **477**, E1-E2 (2011).

6. Rizki,G. *et al.* The evolutionarily conserved longevity determinants HCF-1 and SIR-2.1/SIRT1 collaborate to regulate DAF-16/FOXO. *PLoS. Genet.* **7**, e1002235 (2011).

7. Schmeisser,K. *et al.* Role of sirtuins in lifespan regulation is linked to methylation of nicotinamide. *Nat. Chem. Biol.* **9**, 693-700 (2013).

8. Moroz,N. *et al.* Dietary restriction involves NAD(+) -dependent mechanisms and a shift toward oxidative metabolism. *Aging Cell* **13**, 1075-1085 (2014).

9. Lee,J., Kwon,G., Park,J., Kim,J.K., & Lim,Y.H. SIR-2.1-dependent lifespan extension of Caenorhabditis elegans by oxyresveratrol and resveratrol. *Exp. Biol. Med. (Maywood.)*(2016).

10. Whitaker,R. *et al.* Increased expression of Drosophila Sir2 extends life span in a dose-dependent manner. *Aging (Albany. NY)* **5**, 682-691 (2013).

11. Sinclair,D. & Verdin,E. The longevity of sirtuins. *Cell Rep.* **2**, 1473-1474 (2012).

12. Banerjee,K.K. *et al.* dSir2 in the adult fat body, but not in muscles, regulates life span in a diet-dependent manner. *Cell Rep.* **2**, 1485-1491 (2012).

13. Banerjee,K.K., Ayyub,C., Sengupta,S., & Kolthur-Seetharam,U. dSir2 deficiency in the fatbody, but not muscles, affects systemic insulin signaling, fat mobilization and starvation survival in flies. *Aging (Albany. NY)* **4**, 206-223 (2012).

14. Wang,C. *et al.* The effect of resveratrol on lifespan depends on both gender and dietary nutrient composition in Drosophila melanogaster. *Age (Dordr.)* **35**, 69-81 (2013).