J Biosci Vol. 43, No. 2, June 2018, pp. 243–247
DOI: 10.1007/s12038-018-9754-5

Brief communication

Loss of mitochondrial SIRT4 shortens lifespan and leads to a decline in physical activity

Sweta Parik, Sandipan Tewary, Champakali Ayyub and Ullas Kolthur-Seetharam*

Department of Biological Sciences, Tata Institute of Fundamental Research, Mumbai, India

**Corresponding author (Email, ullas@tifr.res.in)*

MS received 15 March 2018; accepted 27 March 2018; published online 21 April 2018

Mitochondrial mechanisms and pathways have recently emerged as critical determinants of organismal aging. While nuclear sirtuins have been shown to regulate aging, whether mitochondrial sirtuins do so is still unclear. Here, we report that mitochondrial *dSirt4* mediates organismal aging. We establish that absence of *dSirt4* leads to reduced lifespan independent of dietary inputs. Further by assaying locomotion, a key correlate of aging, we demonstrate that *dSirt4* null flies are severely physically impaired with a significant reduction in locomotion. In summary, we report that mitochondrial *dSirt4* is a key determinant of longevity and its loss leads to early aging.

Keywords. Activity; aging; diet; *Drosophila*; mitochondria; Sirt4

1. Introduction

Sirtuins are evolutionarily conserved NAD+ dependent enzymes, which impinge on cellular functions by deacylating several proteins (Chang and Guarente 2014). SIR2/SIRT1 and SIRT6 are essential determinants of organismal survival and lifespan (Rogina and Helfand 2004; Banerjee *et al.* 2012; Kanfi *et al.* 2012; Satoh *et al.* 2013). We have earlier demonstrated that *dSir2/Sirt1* positively regulates lifespan in a diet dependent manner, using control and *dSir2/Sirt1* perturbed flies, which were otherwise genetically identical (Banerjee *et al.* 2012).

Mitochondrial sirtuins have emerged as key regulators of several mitochondrial functions, which contribute to healthy aging and organismal survival (He *et al.* 2012). It should be noted that among mitochondrial sirtuins, SIRT4 is one of the least characterized across species. Others and we have demonstrated that SIRT4 is a negative regulator of catabolic processes and is crucial for maintaining cellular ATP (Nasrin *et al.* 2010; Ho *et al.* 2013). Although, SIRT4 null mice display enhanced fatty acid oxidation (Nasrin *et al.* 2010; Laurent *et al.* 2013a), there are no reports on long-term consequences of SIRT4 absence on lifespan.

2. Results

We sought to determine the role of SIRT4 in aging using *Drosophila melanogaster* as a model system. Phylogenetic analyses indicated that dSIRT4 is the fly orthologue of human SIRT4 (figure 1A). We also generated transgenic flies expressing Myc-tagged dSIRT4 and found it to be exclusively localized to the mitochondria like its mammalian counterpart (figure 1B), as has also been reported recently (Wood *et al.* 2018).

We generated backcrossed *dSirt4* mutants (hereafter denoted as $dSirt4^{-/-bck}$) to w^{1118} to compare longevity in otherwise genetically identical organisms (figure 1C). On standard cornmeal diet, $dSirt4^{-/-bck}$ flies had a significantly shorter lifespan when compared to controls (figure 1D and H). These findings are similar to those that are reported in a concurrent study from Stephan Helfand's group (Wood *et al.* 2018). Importantly, together our studies clearly demonstrate that mitochondrial Sirt4 is required for organismal longevity.

Relative ratios of sugar and yeast in diet rather than their concentration determine organismal physiology and longevity (Lee *et al.* 2008; Zhu *et al.* 2014). Others and we have earlier established that the nuclear SIRT1 is essential for mediating diet-dependent longevity (Rogina and Helfand 2004; Banerjee *et al.* 2012). The diet-dependence and the role of Sirt4 in aging are still unknown. In this context, on

Electronic supplementary material: The online version of this article (https://doi.org/10.1007/s12038-018-9754-5) contains supplementary material, which is available to authorized users.

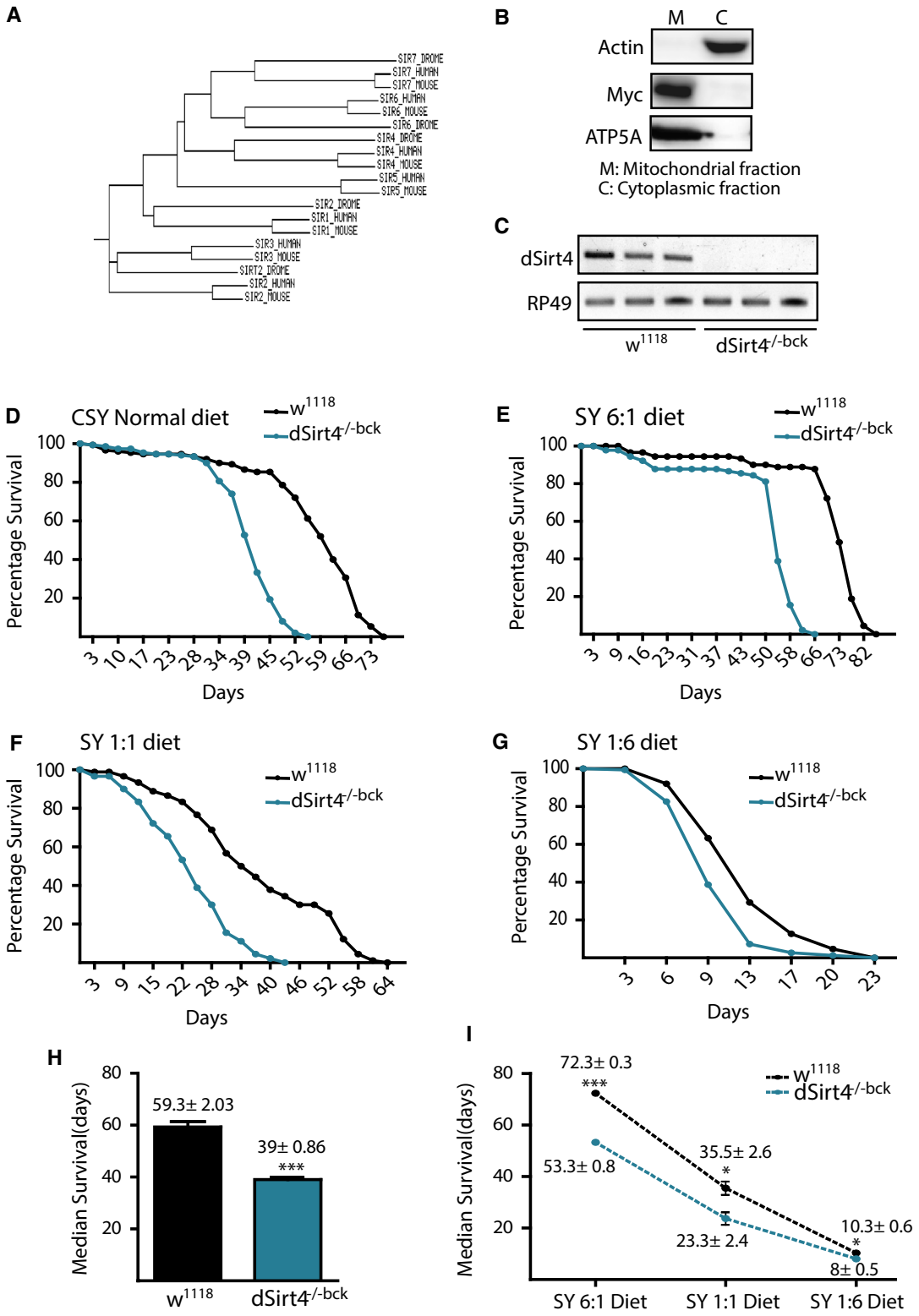

Figure 1. *dSirt4*$^{-/-bck}$ flies have reduced lifespan across dietary conditions: (**A**) Phylogeny of sirtuins (SIRT1-7) in the indicated species. (**B**) Mitochondrial localisation of dSIRT4-MYC in transgenic flies. (**C**) *dSirt4* mRNA expression in control and *dSirt4*$^{-/-bck}$ flies. (**D–H**) Lifespan of control and *dSirt4*$^{-/-bck}$ flies reared under. (**D**) Standard cornmeal diet (N=2, n=160), (**E**) S:Y 6:1 diet (N=2, n=160), (**F**) S:Y 1:1 diet (N=2, n=160) and (**G**) S:Y 1:6 diet (N=3, n=220). Median lifespan of control and *dSirt4*$^{-/-bck}$ under (**H**) standard diet and (**I**) across S:Y media.

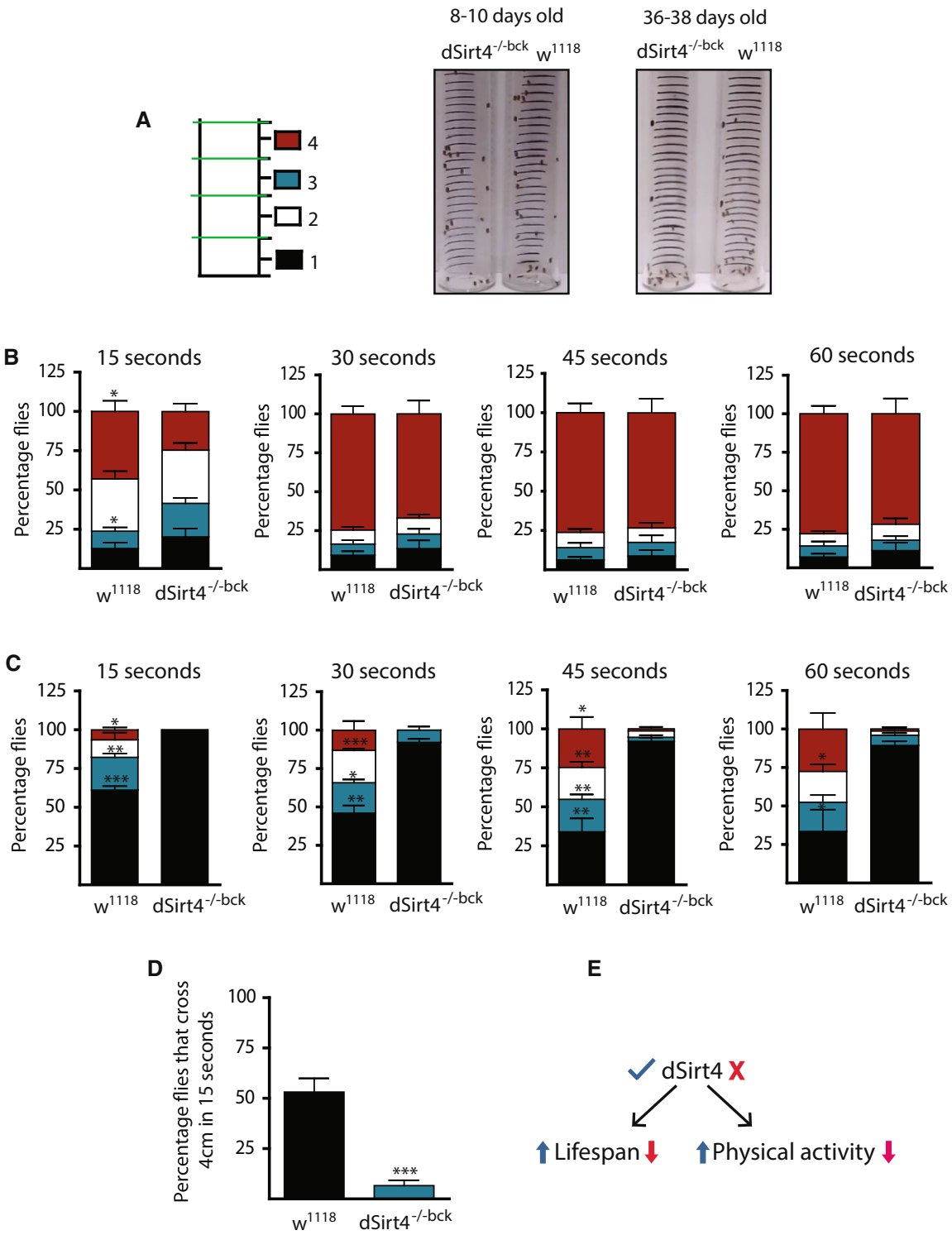

Figure 2. Aged *dSirt4*$^{-/-bck}$ flies have reduced physical activity: (**A**) Schematic and representative images from video for negative geotactic climbing assay and quantification. (**B-C**) Climbing activity in control and *dSirt4*$^{-/-bck}$ flies, which were (**B**) 8–10 days old (N=4, n=285) and (**C**) 36–38 days old (N=3, n=210). (**D**) Percentage of 36–38 days old control and *dSirt4*$^{-/-bck}$ flies that cross a distance of 4 cm in 15 s. (**E**) *dSirt4* regulates lifespan and physical activity.

assaying for survival under varying dietary regimes (sugar:yeast or S:Y diets), we found that *dSirt4*$^{-/-bck}$ flies had significantly shortened lifespan on all the diets (figure 1E–G). It is important to note that increasing yeast

concentrations led to early death in control flies as reported earlier (Lee *et al.* 2008). However, absence of *dSirt4* led to a further reduction in lifespan across diets (figure 1I). Importantly, our study provides a novel angle vis-à-vis the essentiality of Sirt4 in mediating organismal aging across dietary regimes.

To check if reduced lifespan was associated with decline in physical fitness, we assessed the activity of both control and *dSirt4*$^{-/-bck}$ flies by performing a climbing assay (figure 2A). Although, this aspect was studied in the concurrent report, overall activity was assessed in young flies, which does not indicate if there is a progressive decline as the organism ages. Control flies showed an age-dependent decrease in locomotion (figure 2B and C). In 8-10 days old flies, there was a minor lag in the climbing activity of *dSirt4*$^{-/-bck}$ flies at 15 seconds with a higher percentage of flies occupying region 2, as compared to controls (figure 2B).

Importantly, on assessing this at a later age we found a striking decrease in climbing activity in *dSirt4*$^{-/-bck}$ flies when compared to controls (figure 2C). The drastic decline was made apparent by the fact that most mutant flies occupied the base of the vial (region 1) and a significantly smaller percentage was seen at the top (regions 3 and 4). *dSirt4*$^{-/-bck}$ flies were found to be severely impaired in their ability to cross a threshold distance (figure 2D).

3. Discussion

Our study highlights the role of a mitochondrial sirtuin Sirt4 in regulating organismal longevity in *Drosophila melanogaster*. Until recently only the nuclear sirtuins, Sirt1 and Sirt6, were shown to play a deterministic role in extending lifespan across model systems (Rogina and Helfand 2004; Banerjee *et al.* 2012; Kanfi *et al.* 2012; Satoh *et al.* 2013). Given that mitochondria have been proposed to play critical roles in several biological processes including aging, our findings about the importance of Sirt4, a mitochondrial NAD-dependent deacylase, in regulating aging is significant.

Sirt4 has been one of the poorly characterized sirtuins. Recent studies have shown that it can have multiple NAD-dependent catalytic functions (Haigis *et al.* 2006; Ahuja *et al.* 2007; Du *et al.* 2009; Laurent *et al.* 2013b; Rauh *et al.* 2013; Mathias *et al.* 2014; Anderson *et al.* 2017; Pannek *et al.* 2017). However, the most robust deacylase activity for Sirt4 is yet to be unraveled. Although, Sirt4 has been knocked out in mice, there are no studies on its role in aging. Others and we have earlier demonstrated that it plays a critical role in metabolic and energy homeostasis (Nasrin *et al.* 2010; Ho *et al.* 2013). Therefore, it is not surprising to find that Sirt4 is a determinant of organismal aging. A concurrent study by Wood *et al.* also demonstrated its role in aging, which was also associated with metabolic dysregulation (Wood *et al.* 2018). However, it should be mentioned that most of these were assayed under one particular dietary regime. In this context, we now demonstrate that Sirt4 is necessary for determining organismal lifespan across dietary regimes. It is interesting to note that this is unlike Sirt1, which we have shown to be essential for mediating DR (dietary restriction) dependent lifespan extension, Further, although Sirt6 also extends lifespan, whether this effect is diet dependent is still unclear. This highlights that different sirtuins may be required for organismal survival in response to different metabolic states.

The progressive loss of muscle mass and hence strength, with age, termed 'sarcopenia' is often deemed responsible for weakness and impaired locomotion in aged individuals (Cruz-Jentoft *et al.* 2010). In *Drosophila*, while most tissues exhibit age-induced damage, several studies have shown that thoracic muscles, required for walking/climbing and flight, display the most severe defects (Demontis *et al.* 2013). In this regard, our results together with the findings from Wood *et al.* (Wood *et al.* 2018) clearly establish that a loss of Sirt4 impairs physical activity, which is exaggerated in older flies. The underlying mechanisms, which are downstream to Sirt4 vis-à-vis organismal survival and locomotor activity, need to be investigated in the future.

Taken together, our results demonstrate that *dSirt4*$^{-/-bck}$ flies have reduced climbing ability with a severe reduction in locomotion, which is enhanced in aged cohorts. It also clearly indicates that absence of *dSirt4* leads to a loss of physical activity akin to early aging. In conclusion, our novel findings illustrate that *dSirt4*, a mitochondrial sirtuin, is essential for organismal survival across diets and that its loss leads to accelerated aging.

4. Methods

Please refer to the supplementary information for detailed description.

Acknowledgements

We acknowledge Namrata Shukla, Eisha Shaw, Arpan Parichha and Kushal Banerjee for technical help. DAE-TIFR (India) (Grant Number 12P-0122) and Swarnajayanti Fellowship (DST, India) (Grant number DST/SJF/LSA-02/2012-13) to UKS is acknowledged.

References

Ahuja N, Schwer B, Carobbio S, Waltregny D, North BJ, Castronovo V, Maechler P and Verdin E 2007 Regulation of insulin secretion by SIRT4, a mitochondrial ADP-ribosyltransferase. *J. Biol. Chem.* **282** 33583–33592

Anderson KA, Huynh FK, Fisher-Wellman K, Stuart JD, Peterson BS, Douros JD, Wagner GR, Thompson JW, Madsen AS, Green MF, Sivley RM, Ilkayeva OR, Stevens RD, Backos DS, Capra

JA, Olsen CA, Campbell JE, Muoio DM, Grimsrud PA and Hirschey MD 2017 SIRT4 Is a Lysine Deacylase that Controls Leucine Metabolism and Insulin Secretion. *Cell Metab*. **25** 838–855 e815

Banerjee KK, Ayyub C, Sengupta S and Kolthur-Seetharam U 2012 dSir2 deficiency in the fatbody, but not muscles, affects systemic insulin signaling, fat mobilization and starvation survival in flies. *Aging (Albany NY)* https://doi.org/10.18632/aging.100435

Chang HC and Guarente L 2014 SIRT1 and other sirtuins in metabolism. *Trends Endocrinol. Metab*. **25** 138–145

Cruz-Jentoft AJ, Baeyens JP, Bauer JM, Boirie Y, Cederholm T, Landi F, Martin FC, Michel JP, Rolland Y, Schneider SM, Topinkova E, Vandewoude M, Zamboni M and European Working Group on Sarcopenia in Older P 2010 Sarcopenia: European consensus on definition and diagnosis: Report of the European Working Group on Sarcopenia in Older People. *Age Ageing* **39** 412–423

Demontis F, Piccirillo R, Goldberg AL and Perrimon N 2013 Mechanisms of skeletal muscle aging: insights from Drosophila and mammalian models. *Dis. Model Mech*. **6** 1339–1352

Du J, Jiang H and Lin H 2009 Investigating the ADP-ribosyltransferase activity of sirtuins with NAD analogues and 32P-NAD. *Biochemistry* **48** 2878–2890

Haigis MC, Mostoslavsky R, Haigis KM, Fahie K, Christodoulou DC, Murphy AJ, Valenzuela DM, Yancopoulos GD, Karow M, Blander G, Wolberger C, Prolla TA, Weindruch R, Alt FW and Guarente L 2006 SIRT4 inhibits glutamate dehydrogenase and opposes the effects of calorie restriction in pancreatic beta cells. *Cell* **126** 941–954

He W, Newman JC, Wang MZ, Ho L and Verdin E 2012 Mitochondrial sirtuins: regulators of protein acylation and metabolism. *Trends Endocrinol. Metab*. **23** 467–476

Ho L, Titus AS, Banerjee KK, George S, Lin W, Deota S, Saha AK, Nakamura K, Gut P, Verdin E and Kolthur-Seetharam U 2013 SIRT4 regulates ATP homeostasis and mediates a retrograde signaling via AMPK. *Aging (Albany NY)* **5** 835–849

Kanfi Y, Naiman S, Amir G, Peshti V, Zinman G, Nahum L, Bar-Joseph Z and Cohen HY 2012 The sirtuin SIRT6 regulates lifespan in male mice. *Nature* **483** 218–221

Laurent G, de Boer VC, Finley LW, Sweeney M, Lu H, Schug TT, Cen Y, Jeong SM, Li X, Sauve AA and Haigis MC 2013a SIRT4 represses peroxisome proliferator-activated receptor alpha activity to suppress hepatic fat oxidation. *Mol. Cell Biol*. **33** 4552–4561

Laurent G, German NJ, Saha AK, de Boer VC, Davies M, Koves TR, Dephoure N, Fischer F, Boanca G, Vaitheesvaran B, Lovitch SB, Sharpe AH, Kurland IJ, Steegborn C, Gygi SP, Muoio DM, Ruderman NB and Haigis MC 2013b SIRT4 coordinates the balance between lipid synthesis and catabolism by repressing malonyl CoA decarboxylase. *Mol. Cell* **50** 686–698

Lee KP, Simpson SJ, Clissold FJ, Brooks R, Ballard JW, Taylor PW, Soran N and Raubenheimer D 2008 Lifespan and reproduction in Drosophila: New insights from nutritional geometry. *Proc. Natl. Acad. Sci. USA* **105** 2498–2503

Mathias RA, Greco TM, Oberstein A, Budayeva HG, Chakrabarti R, Rowland EA, Kang Y, Shenk T and Cristea IM 2014 Sirtuin 4 is a lipoamidase regulating pyruvate dehydrogenase complex activity. *Cell* **159** 1615–1625

Nasrin N, Wu X, Fortier E, Feng Y, Bare OC, Chen S, Ren X, Wu Z, Streeper RS and Bordone L 2010 SIRT4 regulates fatty acid oxidation and mitochondrial gene expression in liver and muscle cells. *J. Biol. Chem*. **285** 31995–32002

Pannek M, Simic Z, Fuszard M, Meleshin M, Rotili D, Mai A, Schutkowski M and Steegborn C 2017 Crystal structures of the mitochondrial deacylase Sirtuin 4 reveal isoform-specific acyl recognition and regulation features. *Nat. Commun*. **8** 1513

Rauh D, Fischer F, Gertz M, Lakshminarasimhan M, Bergbrede T, Aladini F, Kambach C, Becker CF, Zerweck J, Schutkowski M and Steegborn C 2013 An acetylome peptide microarray reveals specificities and deacetylation substrates for all human sirtuin isoforms. *Nat. Commun*. **4** 2327

Rogina B and Helfand SL 2004 Sir2 mediates longevity in the fly through a pathway related to calorie restriction. *Proc. Natl. Acad. Sci. USA* **101** 15998–16003

Satoh A, Brace CS, Rensing N, Cliften P, Wozniak DF, Herzog ED, Yamada KA and Imai S 2013 Sirt1 extends life span and delays aging in mice through the regulation of Nk2 homeobox 1 in the DMH and LH. *Cell Metab*. **18** 416–430

Wood JG, Schwer B, Wickremesinghe PC, Hartnett DA, Burhenn L, Garcia M, Li M, Verdin E and Helfand SL 2018 Sirt4 is a mitochondrial regulator of metabolism and lifespan in Drosophila melanogaster. *Proc. Natl. Acad. Sci*. **115** 1564–1569

Zhu CT, Ingelmo P and Rand DM 2014 GxGxE for lifespan in Drosophila: mitochondrial, nuclear, and dietary interactions that modify longevity. *PLoS Genet*. **10** e1004354

Corresponding editor: B Jagadeeshwar Rao