

Absence of effects of Sir2 overexpression on lifespan in *C. elegans* and *Drosophila*

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Overexpression of sirtuins (NAD⁺-dependent protein deacetylases) has been reported to increase lifespan in budding yeast (*Saccharomyces cerevisiae*), *Caenorhabditis elegans* and *Drosophila melanogaster*^{1–3}. Studies of the effects of genes on ageing are vulnerable to confounding effects of genetic background⁴. Here we re-examined the reported effects of sirtuin overexpression on ageing and found that standardization of genetic background and the use of appropriate controls abolished the apparent effects in both *C. elegans* and *Drosophila*. In *C. elegans*, outcrossing of a line with high-level *sir-2.1* overexpression¹ abrogated the longevity increase, but did not abrogate *sir-2.1* overexpression. Instead, longevity co-segregated with a second-site mutation affecting sensory neurons. Outcrossing of a line with low-copy-number *sir-2.1* overexpression² also abrogated longevity. A *Drosophila* strain with ubiquitous overexpression of *dSir2* using the UAS-GAL4 system was long-lived relative to wild-type controls, as previously reported³, but was not long-lived relative to the appropriate transgenic controls, and nor was a new line with stronger overexpression of *dSir2*. These findings underscore the importance of controlling for genetic background and for the mutagenic effects of transgene insertions in studies of genetic effects on lifespan. The life-extending effect of dietary restriction on ageing in *Drosophila* has also been reported to be *dSir2* dependent³. We found that dietary restriction increased fly lifespan independently of *dSir2*. Our findings do not rule out a role for sirtuins in determination of metazoan lifespan, but they do cast doubt on the robustness of the previously reported effects of sirtuins on lifespan in *C. elegans* and *Drosophila*.

The role of sirtuins in ageing was discovered in budding yeast, where overexpression of *SIR2* increases replicative lifespan⁵. It was then reported that elevated sirtuin levels increase lifespan in the nematode *C. elegans*^{1,2,6} and the fruitfly *Drosophila*³, indicating an evolutionarily ancient role of sirtuins in longevity assurance⁷. Dietary restriction (reduced food intake short of starvation) extends lifespan in organisms ranging from yeast to mammals⁸, and initial studies indicated that dietary restriction increases lifespan by activating sirtuins in yeast⁹, *C. elegans*¹⁰ and *Drosophila*³. Pharmacological activation of sirtuins has therefore been widely promulgated as a potential means to mimic dietary restriction and slow ageing in humans¹¹. However, several aspects of the role of sirtuins in ageing have proved controversial¹². Subsequent studies have indicated that sirtuins do not mediate the effects of dietary restriction on ageing, at least in budding yeast and *C. elegans*^{13,14}. The plant-derived polyphenol resveratrol and other compounds have been reported to activate sirtuins and extend lifespan^{15,16}, but more recent findings have challenged both effects^{17–20}. We therefore re-examined the effects of sirtuin overexpression on lifespan in *C. elegans* and *Drosophila*. In particular, we wished to

exclude the possibility that the increased longevity observed in strains with overexpression of sirtuin genes is caused by differences in genetic background, or by the mutagenic effects of transgene insertion, which frequently confound studies of the genetics of ageing⁴.

We first examined a high-copy-number *sir-2.1* transgenic *C. elegans* strain (LG100) carrying the integrated transgene array *gelIn3* [*sir-2.1 rol-6(su1006)*] (ref. 1). As expected, this strain was long-lived (Fig. 1a and Supplementary Table 1). However, outcrossing (×5) of *gelIn3* to wild type (N2) abrogated the increase in longevity (Fig. 1a and Supplementary Table 1) without affecting SIR-2.1 protein levels (Fig. 1b). This loss of longevity upon outcrossing was verified by an independent research team (Supplementary Table 2).

LG100 showed a neuronal dye-filling (Dyf) defect²¹ that did not segregate with the transgene upon outcrossing (Supplementary Fig. 2a). Dyf mutants often show extended lifespan²². To determine whether the longevity of LG100 might be attributable to a *dyf* mutation, we derived from this strain three Dyf, non-Rol lines (lacking *gelIn3*) and three non-Dyf, Rol lines (carrying *gelIn3*). Dyf, non-Rol lines were long-lived and showed wild-type SIR-2.1 protein levels (Fig. 1c, d and Supplementary Table 3). Non-Dyf, Rol lines showed elevated SIR-2.1 protein levels but had wild-type lifespans. Dyf mutant longevity seemed to be partially dependent on *daf-16* (Supplementary Fig. 2b), as seen previously for other Dyf mutants²². The co-segregation of longevity with this *dyf* mutation, but not with *gelIn3*, was previously noted by another research team (S. S. Lee, personal communication). Furthermore, knockdown of *sir-2.1* expression in LG100 using RNA-mediated interference did not suppress longevity, despite lowering SIR-2.1 protein to wild-type levels (Fig. 1e, f and Supplementary Table 4). Taken together, these results indicate that the longevity of LG100 is attributable to an unidentified *dyf* mutation (or possibly another mutation closely linked to the *dyf* locus), and that high-level overexpression of *sir-2.1* is not sufficient to increase lifespan in these strains.

A low-copy-number transgenic strain (NL3909) overexpressing *sir-2.1* (ref. 7) is also long-lived². We confirmed the increased lifespan of NL3909 (*pkIs1642* [*sir-2.1 unc-119*] *unc-119(ed3)*) relative to the control strain NL3908 (*pkIs1641* [*unc-119*] *unc-119(ed3)*) (Fig. 1g and Supplementary Table 5). We also observed an apparent increase in SIR-2.1 protein levels in NL3909 relative to NL3908 (Fig. 1h). Outcrossing (×6) of NL3909 once again abrogated longevity (Fig. 1g and Supplementary Table 5) without affecting SIR-2.1 protein levels (Fig. 1h and Supplementary Fig. 1c). This effect of outcrossing was independently verified (Supplementary Table 6). Thus, the longevity of NL3909 also seems to be attributable to effects of genetic background rather than to *pkIs1642*.

The duplication *mDp4* includes the *sir-2.1* locus, and the *mDp4*-containing strain DR1786 is long-lived¹. We found that DR1786 is

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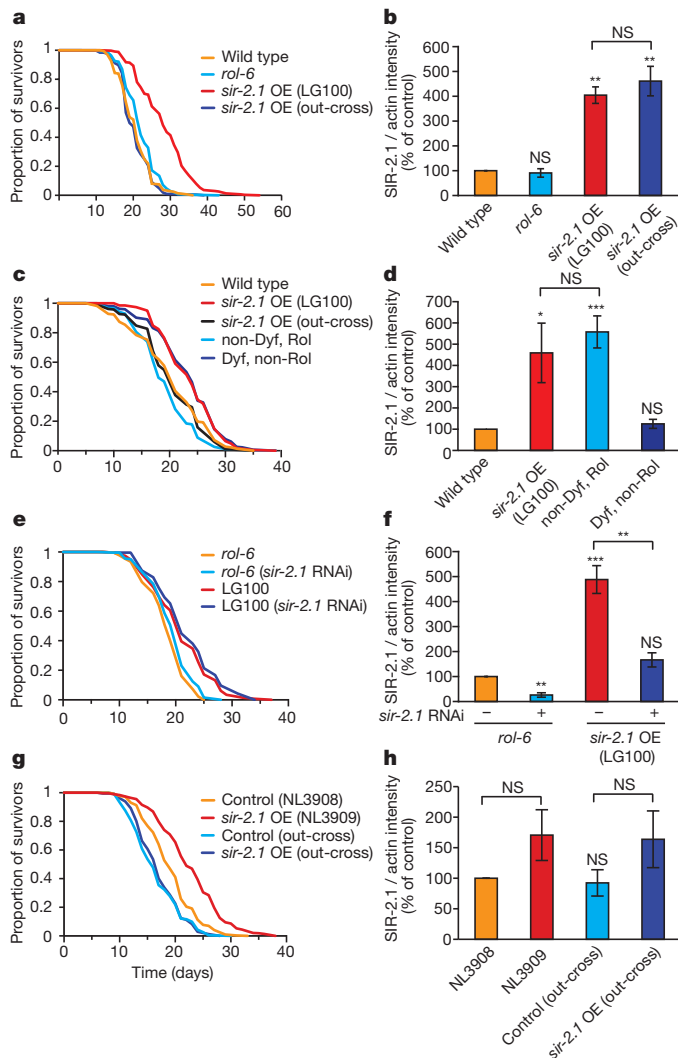


Figure 1 | Longevity of LG100 and NL3909 is not attributable to *sir-2.1* overexpression in *C. elegans*. **a, b,** Outcrossing of LG100 removes lifespan extension without affecting SIR-2.1 protein levels. Data in **b** are derived from western blots (mean of three trials, each using an independent protein preparation). A representative western blot is shown in Supplementary Fig. 1a. Quantitative reverse transcriptase PCR showed that *sir-2.1* mRNA is also elevated in both strains (data not shown). **c,** LG100-derived *Dyf*, non-*Rol* segregant lines are long-lived whereas non-*Dyf*, *Rol* lines are not. **d,** Non-*Dyf* *Rol* segregant lines have elevated SIR-2.1 levels, whereas *Dyf*, non-*Rol* lines do not. **e, f,** *sir-2.1* RNAi does not suppress LG100 longevity, but reduces SIR-2.1 protein levels. **g, h,** Outcrossing of NL3909 removes lifespan extension without affecting SIR-2.1 protein levels. See Supplementary Tables 1–5 for lifespan statistics for **a, c, e** and **g**, respectively. OE, overexpression. All error bars represent s.e.m. *, $0.01 < P < 0.05$; **, $0.001 < P < 0.01$; ***, $P < 0.001$; NS, not significant; Student's *t*-test (two-tailed). One remaining possibility is that the outcrossed *sir-2.1* strains both contain second-site mutations that suppress longevity effects. However, *daf-2* RNAi strongly induced longevity in both strains (data not shown), arguing against the presence of a general suppressor of longevity in each case.

indeed long-lived, and also shows elevated *sir-2.1* expression. However, longevity was not suppressed by *sir-2.1* RNA interference (RNAi) (Supplementary Fig. 3 and Supplementary Table 7) indicating causation by factors other than *sir-2.1*, either on *mDp4* or elsewhere in the genome.

In *Drosophila*, overexpression of *dSir2* reportedly increases lifespan relative to wild-type controls³. Overexpression was achieved using the GAL4-UAS binary system²³, with the largest increases in lifespan being produced by the combination of EP-UAS-*dSir2* (*dSir2*^{EP2300}) with a ubiquitously expressed tubulin-GAL4 driver. We outcrossed these

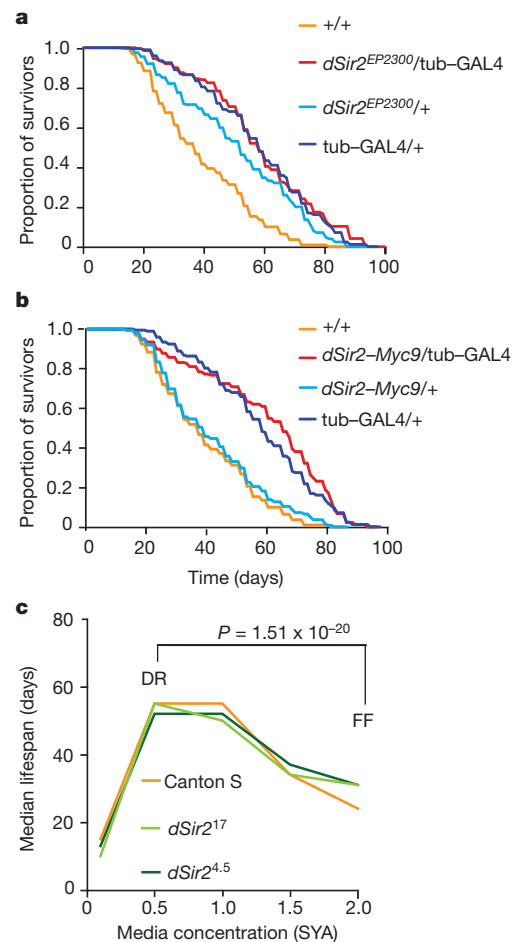


Figure 2 | Absence of effects of *dSir2* on lifespan in *Drosophila*. All lines were outcrossed into *w*^{Dah} (+/+). **a,** Lifespan in flies overexpressing *dSir2*^{EP2300} driven via tubulin-GAL4 (tub-GAL4) is longer than in the wild type, but not longer than in the tubulin-GAL4/+ genetic control. Median lifespans: +/+, 39 days; *dSir2*^{EP2300}/tubulin-GAL4, 59 days; *dSir2*^{EP2300}/+, 53 days, tubulin-GAL4/+, 60 days. $P = 0.0006$ for comparison of *dSir2*^{EP2300}/tubulin-GAL4 versus *dSir2*^{EP2300}/+; $P = 0.9295$ for *dSir2*^{EP2300}/tubulin-GAL4 versus tubulin-GAL4/+; $P < 0.0001$ for *dSir2*^{EP2300}/tubulin-GAL4 versus +/+. **b,** Lifespan in flies overexpressing *dSir2-Myc9* is longer than in wild type, but not longer than in the tubulin-GAL4 control. Median lifespans: +/+, 39 days; *dSir2-Myc9*/tubulin-GAL4, 67 days; *dSir2-Myc9*/+, 41 days; tubulin-GAL4/+, 60 days. *dSir2-Myc9*/tubulin-GAL4 versus *dSir2-Myc9*/+, $P = 0.0001$; *dSir2-Myc9*/tubulin-GAL4 versus tubulin-GAL4/+, $P = 0.1354$; *dSir2-Myc9*/tubulin-GAL4 versus +/+, $P < 0.0001$. All comparisons were made using log-rank tests, $n = 200$. **c,** The effect of dietary restriction on *Drosophila* lifespan is not *dSir2*-dependent. Flies were assayed over five concentrations of SYA media and data are presented as the median lifespan on each food concentration. All lines were outcrossed into Canton S (+/+). P values confirm that all flies respond normally to dietary restriction when median lifespans are compared for dietary restriction (DR) versus fully-fed (FF) conditions³⁰.

two transgenes ($\times 6$) into the control white Dahomey (*w*^{Dah}) background. When assayed on a medium similar to that used in the original study, EP-UAS-*dSir2*/tubulin-GAL4 flies were longer-lived than wild-type controls, as previously reported³ (Fig. 2a). However, they did not live longer than the tubulin-GAL4/+ control flies (Fig. 2a). This implies that lifespan extension is due to transgene-linked genetic effects other than the overexpression of *dSir2*. Lifespan was assayed on a range of food media (see Methods for details) to test for nutrient dependence of any effect. However, in no case were EP-UAS-*dSir2*/tubulin-GAL4 flies longer-lived than one or both transgenic controls (Supplementary Fig. 4).

The lack of an observable effect on lifespan could reflect the relatively modest increase in *dSir2* expression in EP-UAS-*dSir2*/tubulin-GAL4

flies, both in terms of messenger RNA levels (Supplementary Fig. 5) and protein levels (increased by 35% relative to wild type; Supplementary Fig. 6). We therefore created lines with a higher level of overexpression of *dSir2* (UAS-*dSir2-Myc9/tubulin-GAL4*). Here, *dSir2* mRNA and protein levels were robustly increased relative to wild type (an increase of 318% relative to wild-type protein levels; Supplementary Figs 5 and 6). We examined recombinant protein raised in *Escherichia coli* to check that the presence of the Myc tag did not interfere with dSir2 histone deacetylase activity, as measured by deacetylation of the fluorophore-containing p53 substrate (Fluor de Lys) or of native acetylated histone H4 substrates, and it did not (Supplementary Fig. 7). We also found that dSir2 histone deacetylase activity was unaffected by addition of resveratrol in either assay (Supplementary Fig. 7). We saw no increase in lifespan in UAS-*dSir2-Myc/tubulin-GAL4* flies relative to *tubulin-GAL4/+* controls, either on a food medium similar to that used in the original study (Fig. 2b), or relative to either control on a range of other media (Supplementary Fig. 4b, c, f). An independent research team also saw no increase in lifespan in UAS-*dSir2-Myc9/tubulin-GAL4* flies (Supplementary Fig. 8). These results indicate that the previously observed longevity of EP-UAS-*dSir2/tubulin-GAL4* flies was not attributable to elevated expression of *dSir2*, and that stronger, ubiquitous overexpression of *dSir2* also does not extend fly lifespan.

The role of sirtuins in the extension of lifespan by dietary restriction in yeast and *C. elegans* is controversial, with several groups reporting that sirtuins are not required for lifespan extension via dietary restriction in both organisms⁸. In *Drosophila*, it was reported that dietary restriction does not increase lifespan in *dSir2* deletion-mutant flies³. We tested this too, using the deletion alleles *dSir2*^{4,5} (tested previously³) and *dSir2*¹⁷. We first outcrossed these alleles (Supplementary Fig. 9a) into the Canton S wild type (see Methods), which was used in the previous dietary-restriction study³. We then checked the effect of each allele on *dSir2* gene expression. The allele *dSir2*¹⁷ abrogated *dSir2* mRNA, indicating that this is a null allele. By contrast, *dSir2*^{4,5}, which contains a relatively small deletion at the 5' end of the gene, did not reduce *dSir2* mRNA levels (Supplementary Fig. 9b, c).

To reassess the role of *dSir2* in dietary restriction in *Drosophila*, we compared lifespans of wild-type (Canton S), *dSir2*^{4,5} and *dSir2*¹⁷ homozygotes. All genotypes responded similarly and normally to dietary restriction in trials conducted by two independent research teams (Fig. 2c and Supplementary Fig. 10), hence the effect of dietary restriction on lifespan did not require *dSir2*.

In this study, we were unable to verify the effect of sirtuin overexpression on lifespan in either *C. elegans* or *Drosophila*. Increased lifespan was seen in two *C. elegans* lines with elevated *sir-2.1* expression, derived from independent studies, as previously reported, but in each case this was abrogated by outcrossing. Overexpression of *sir-2.1* does exert effects on traits other than lifespan. For example, *geIn3* is neuroprotective in a worm model of neuron dysfunction in Huntington's disease²⁴ and, notably, this effect is not attributable to the *dyf* mutation (Supplementary Fig. 11). Moreover, both NL3909 and its outcrossed derivative are thermotolerant (M. Somogyvári and C. Söti, unpublished data). In *Drosophila*, lines overexpressing *dSir2* were longer-lived than wild-type controls, as previously reported, but they were not longer-lived than lines containing the appropriate transgenic controls. The fact that all transgenic lines were longer-lived than the Dahomey wild type into which they had been outcrossed could reflect heterosis in the vicinity of the transgene inserts, or a mutagenic effect of the GAL4 insert.

Lifespan was not increased either by overexpression of *sir-2.1* from its own promoter in *C. elegans*, or by ubiquitous overexpression of *dSir2* from a heterologous promoter in *Drosophila*. Our findings call into question the robustness of earlier reports of a role for sirtuins in longevity assurance on the basis of overexpression in *C. elegans* and *Drosophila*, and also the role of *dSir2* in the response to dietary restriction in *Drosophila*. However, sirtuins can affect lifespan in animals

under certain conditions: *C. elegans daf-2(e1370)* mutants are hypersensitive to genetic effects on lifespan²⁵, and in these mutants, deletion of *sir-2.1* reproducibly increases lifespan⁶ (Supplementary Fig. 12).

Our finding that resveratrol does not activate the histone deacetylase activity of dSir2 using a native histone H4 peptide is consistent with earlier findings using yeast SIR2 and mammalian SirT1 (refs 17, 18). Resveratrol increased *Drosophila* lifespan in one study²⁶ but not in another²⁷. In principle, this could reflect sensitivity of resveratrol effects to subtle differences in culture conditions. If this were the case, our findings would indicate that such effects are not attributable to direct activation of dSir2 by resveratrol.

METHODS SUMMARY

Nematode strains and maintenance. Nematodes were maintained on nematode-growth-medium agar at 20 °C, with *E. coli* OP50 bacteria as a food source. Nematode strains used included: wild type (N2), GA707 *wuEx166 [rol-6(su1006)] [rol-6 control]*, LG100 *geIn3 [sir-2.1 rol-6(su1006)] dyf-2(wu250)*, NL3909 *pkIs1642 [sir-2.1 unc-119] unc-119(ed3)* and the control strain NL3908 *pkIs1641 [unc-119] unc-119(ed3)*.

Nematode lifespan measurements. These were performed as previously described²⁸, at 20 °C. To prevent progeny production, 5-fluoro-2'-deoxyuridine (FUDr) was added to seeded plates, to a final concentration of 10, 40 or 50 µM. Before testing the effects of RNAi on lifespan, worms were kept for two generations on the RNAi bacteria. The statistical significance of effects on lifespan was estimated using the log-rank test, performed using JMP, Version 7 (SAS Institute).

Drosophila stocks and maintenance. *Tubulin-GAL4* and *dSir2*^{EP2300} were obtained from the Bloomington Stock Center. The *dSir2-Myc2* and *dSir2-Myc9* lines were generated by germline transformation into strain *w*⁰⁴. The *dSir2*^{4,5}/*SM6B*, *dSir2*¹⁷/*Cyo* and Canton S lines were gifts from S. Pletcher, J. Rine and S. Helfand. All lines were outcrossed at least six times into the relevant controls. Experiments were performed at 25 °C on a 12h:12h light:dark cycle at constant humidity.

Drosophila lifespan assays. Flies were bred at standard density, allowed to mate for 48 h after emerging, then sorted into ten females per vial. Vials were changed every 48 h, and deaths per vial were scored until all flies were dead. In overexpression studies, *n* = 200. In *dSir2*-mutant studies, *n* = 100. For statistical methodology, see earlier.

dSir2 deacetylation assays. We used both the SirT1 Fluorimetric Drug Discovery Kit (Enzo Life Sciences) and an HPLC-based acetyl-histone-H4 deacetylation assay²⁹. *dSir2* and *dSir2-Myc* were cloned into pET SUMO (Invitrogen) and proteins were purified on HisPur cobalt spin columns (Thermo Scientific).

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

Received 27 October 2010; accepted 10 June 2011.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We thank A. Gartner for providing an antibody against *C. elegans* SIR-2.1; D. Chen, P. Kapahi, S. Pletcher and D. Skorupa for providing data; S. S. Lee for permission to cite unpublished results; S. Helfand and J. Rine for providing fly strains; W. Mair for performing preliminary trials and R. Baumeister for useful discussion. Some nematode strains were provided by the *Caenorhabditis* Genetics Center, which is funded by the National Institutes of Health National Center for Research Resources. We acknowledge funding from the European Union (FP6-036894 to C.B., D.G., L.P. and S.V. and FP6-518230 to D.G. and C.S.), the Hungarian Science Foundation and Norway Grants (NNF-78794 to C.S.), INSERM and ANR, Paris (R.V., A.-M.O. and C.N.), the National Institutes of Health (CA129132 to A.B., R01AG031108 to M.K. and T32AG000057 to G.L.S.) and the Wellcome Trust (Strategic Award to C.A., F.C., D.G., L.P. and M.R.). C.S. is a Bolyai Research Scholar of the Hungarian Academy of Sciences and M.K. is an Ellison Medical Foundation New Scholar in Aging.

Author Contributions The project was conceived by D.G. and L.P. and the experiments were designed by A.B., C.B., F.C., D.G., K.H., M.K., J.J.M., C.N., L.P., C.S. and S.V. The experiments were performed and analysed by C.A., DA, C.B., F.C., J.J.M., M.G., M.H., A.-M.O., M.D.P., M.R., G.L.S., M.S., G.V., R.P.V.-M., S.V. and V.L. The manuscript was written by C.B., F.C., D.G., L.P. and S.V.

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METHODS

Nematode strains and maintenance. *Caenorhabditis elegans* were cultured under standard monoxenic conditions^{31,32}. Strains used included N2 (wild type), GA707 *wuEx166 [rol-6(su1006)]*, HT1593 *unc-119(ed3)*, LG100 *geln3 [sir-2.1 rol-6(su1006)] dyf-?(wu250)*, NL3908 *pkIs1641 [unc-119] unc-119(ed3)* and NL3909 *pkIs1642 [sir-2.1 unc-119] unc-119(ed3)*.

Outcrossing of nematode strains. LG100 was outcrossed with N2 and the Rol trait was used to detect the presence of *gln3*. NL3908 and NL3909 were outcrossed using HT1593 *unc-119(ed3)*. Rescue of Unc (uncoordinated movement) was used to detect the presence of the transgene array.

Isolation of Dyf, non-Rol and non-Dyf, Rol lines. LG100 was crossed with N2 and lines were established from individual F₂ animals with Dyf, non-Rol or non-Dyf, Rol phenotypes. The Dyf phenotype was identified by staining with the dye 1,1'-dioctadecyl-3,3,3'-tetramethylindocarbocyanine perchlorate (DiI) and looking for absence of dye uptake into the amphid and phasmid neurons. Non-Dyf, Rol F₂ animals that were heterozygous for the *geln3* transgene array (the *rol-6* marker is dominant) were identified by the presence of non-Rol animals in the F₃, and were excluded.

RNAi in *C. elegans*. Animals were fed *E. coli* containing the HT115 vector, either with or without a portion of the *sir-2.1* gene cloned into it. The *sir-2.1* feeding strain was obtained from the Ahringer RNAi library³³. Worms were maintained on RNAi feeding strains for two generations before lifespan measurements. One day before starting measurements, FUDr was applied to seeded plates at 10 μ M to prevent progeny production.

Analysis of SIR-2.1 protein levels in *C. elegans*. Protein was prepared from synchronous nematode cultures (L4 larvae and young adults) raised on *E. coli* OP50 or RNAi bacteria for two generations. Western blots were performed with anti-actin monoclonal antibodies (Santa-Cruz Biotechnology) and an anti-SIR-2.1 polyclonal antibody (provided by A. Gartner³⁴). For all assays, 3–5 replicate worm cultures were used.

Neuroprotection assays in *C. elegans*. To test for sirtuin-mediated protection from expanded polyglutamines (polyQs), we crossed GA919 (*geln3* dissociated from *dyf-?(wu250)*) to strains carrying integrated polyQ arrays. These polyQ strains co-express the first 57 amino acids of human huntingtin with either 19 or 128 Gln residues fused to cyan fluorescent protein and expressed from the *mec-3* promoter, and YFP expressed from the *mec-7* promoter in touch-receptor neurons²⁴. The response to touch at the tail was tested as previously described²⁴. Three trials were performed and 150–200 animals were tested per genotype.

Lifespan analysis in *C. elegans*. Lifespans of synchronized population cohorts were measured as previously described²⁸. FUDr was applied to the plates at 10, 40 or 50 μ M (see Supplementary Tables). Lifespan experiments were performed at 20 °C. A small proportion of animals were censored, usually due to uterine rupture, which mainly occurred at mid-adulthood (~day 9–11).

Statistical analysis of *C. elegans* data. The statistical significance of effects on lifespan was estimated using the log-rank test, performed using JMP, Version 7 (SAS Institute).

***Drosophila* stocks and maintenance.** *Tubulin-GAL4* and *dSir2^{EP2300}* lines were obtained from the Bloomington Stock Center. *dSir2-Myc2* and *dSir2-Myc9* lines were generated by germline transformation. These were outcrossed into white Dahomey (*w^{Dah}*). The strains *dSir2^{4.5}/SM6B* (ref. 35) and *dSir2¹⁷/Cyo* (ref. 36), provided by S. Pletcher and J. Rine, were outcrossed into Canton S. All lines were outcrossed at least six times. The presence of the deletion was detected by PCR using the following primers: 149F (5'-AGATATGACATAAGGCAGTGGC-3'), 1427R (5'-TCCCGTTAGCACAAATGATCTTC-3') and 3909R (5'-GAAGGCGG TAGCAATGG TGACAA-3'). Flies were maintained at 25 °C on a 12 h:12 h light:dark cycle at constant humidity.

Myc-tagged *dSir2*. The Myc tag was added to RE27621 (Riken) using standard techniques and cloned into pUASP. The construct was microinjected into *w⁰⁴* and the transformant lines *dSir2-Myc2* and *dSir2-Myc9* were recovered. Primers were: Sir5'R2 (5'-CAAGAATTCCAACGAGAATTTTACACAGGTCGTGTG-3'), Sir3'Xba (5'-ATC GAGTCTAGACACTGCTGCTAACTGTCCTGGAGG-3') and MYC3'Xba (5'-GAGCT ATCTAGAGGATCCGAGGAGCAGAAGCTGATC-3').

Lifespan assays in *Drosophila*. Flies were bred at standard density (~300 flies per 200-ml bottle), allowed to mate for 48 h after emerging (once mated) and then

sorted into ten females per vial (experiments performed at University College London) or 35 per vial on 15% SYA (experiments performed at University of Michigan). Vials were changed every 48 h and deaths per vial were scored until all flies were dead. The numbers of flies used in lifespan assays were: overexpression studies, $n \approx 200$ (UCL) or $n \approx 350$ (U. Michigan); dietary-restriction studies, $n = 100$. For the overexpression studies, the fly-food recipes were as follows: SYA (100 g yeast, 50 g sugar, 15 g agar, 30 ml nipagin and, in most trials, 3 ml propionic acid per litre of food); ASG (20 g yeast, 85 g sugar, 10 g agar and 60 g maize per litre of food); ASG¹ (31 g yeast, 124 g sugar, 9 g agar, 53 g cornmeal and 25 ml nipagin per litre of food); 15% SYA (150 g yeast, 150 g sugar, 21 g agar and 15 ml tegosept). For the dietary-restriction trials, the food dilutions used were as follows: 15 g agar, 30 ml nipagin, 3 ml propionic acid, with yeast and sugar both altered to final concentrations of 10 g, 50 g, 100 g, 150 g or 200 g per litre of food. All food was prepared as previously described²⁷.

Genetic crosses in *Drosophila*. *Tubulin-GAL4/TM3* males were crossed to *dSir2^{EP2300}*, *dSir2-Myc2* or *dSir2-Myc9* virgin females, and *dSir2^{EP2300}/+*; *tubulin-GAL4/+*, *dSir2-Myc2/+*; *tubulin-GAL4/+* or *dSir2-Myc9/+*; and *tubulin-GAL4/+* females were selected from the progeny. For the controls, *tubulin-GAL4/TM3* males or *dSir2^{EP2300}*, *dSir2-Myc2* or *dSir2-Myc9* virgin females were crossed to *w^{Dah}* and *dSir2^{EP2300}/+*, *tubulin-GAL4/+*, *dSir2-Myc2/+* or *dSir2-Myc9/+* females were selected from the progeny.

Quantitative reverse transcriptase PCR in *Drosophila*. RNA was extracted from ten females at 10 days of age using standard techniques and transcribed into cDNA. Four biological replicates were run per genotype, each in triplicate. Samples were normalized to either actin5C or ribosomal protein 49 (RP49). Primers used were: Sir2-4 5'-GCTCTCCACCGTTGTCTGAGGGCC-3' (ref. 3), Sir2-5 5'-GGCGCAGCTGTGTGCGATGAG-3' (ref. 3), Actin5CF 5'-CAC ACCAAATCTTACAAAATGTGTGA-3', ActinCR 5'-AATCCGGCCTTGACATG-3', RP49F 5'-ATGACCATCCGCCAGCATCAGG-3' and RP49R 5'-ATCTCGCCAGTAAACG-3'.

Analysis of *dSir2* protein levels. Protein was extracted from 30 females at 7 days of age. Western blots were performed using antibodies c-myc 9E10 (Santa Cruz Biotechnology), p2E2 (Developmental Studies Hybridoma Bank) and tubulin (Sigma).

***dSir2* deacetylation assays.** Sequences encoding *dSir2* (RE27621) and *dSir2-Myc* were cloned into pET SUMO (Invitrogen) and proteins were purified on HisPur cobalt spin columns (Thermo Scientific). For the Fluor de Lys assay, using the SirT1 Fluorimetric Drug Discovery Kit (Enzo Life Sciences), results presented are the mean \pm s.e.m. of three biological replicates. In each biological replicate, samples were run in triplicate. Final concentrations were: resveratrol and suramin, 0.2 mM; NAD⁺ 0.1 mM. Deacetylation of native acetyl-histone-H4 peptide was monitored by HPLC²⁹. Deacetylation of histone H4 amino-terminal peptide (SGRGKGGKGLGKGGGA(acetyl-K)RHRC) (Biomatik) was carried out using 500 μ M NAD⁺, 100 mM Tris-HCl (pH 8.0), 150 mM NaCl, 0.5 mM dithiothreitol and 0.05% Triton X-100, and monitored by HPLC (Agilent 1100) with an ACE C8-300 150 \times 3.0 mm column. The elution profiles were analysed using Chemstation for LC 3D software.

Statistical analysis of *Drosophila* data. Survivorships and the response to dietary restriction were compared using the log-rank test and analyses were performed using JMP, Version 7 (SAS Institute).

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