LETTER

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# The sirtuin SIRT6 regulates lifespan in male mice

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**The significant increase in human lifespan during the past century confronts us with great medical challenges. To meet these challenges, the mechanisms that determine healthy ageing must be understood and controlled. Sirtuins are highly conserved deacetylases that have been shown to regulate lifespan in yeast, nematodes and fruitflies[1]. However, the role of sirtuins in regulating worm and fly lifespan has recently become controversial[2]. Moreover, the role of the seven mammalian sirtuins, SIRT1 to SIRT7 (homologues of the yeast sirtuin Sir2), in regulating lifespan is unclear[3]. Here we show that male, but not female, transgenic mice overexpressing *Sirt6* (ref. 4) have a significantly longer lifespan than wild-type mice. Gene expression analysis revealed significant differences between male *Sirt6*-transgenic mice and male wild-type mice: transgenic males displayed lower serum levels of insulin-like growth factor 1 (IGF1), higher levels of IGF-binding protein 1 and altered phosphorylation levels of major components of IGF1 signalling, a key pathway in the regulation of lifespan[5]. This study shows the regulation of mammalian lifespan by a sirtuin family member and has important therapeutic implications for age-related diseases.**

Sirtuins are highly conserved $NAD^+$-dependent deacetylases that have been shown to regulate lifespan in several organisms. Increasing the sirtuin level through genetic manipulation extends the lifespan of yeast, nematodes and flies[1]. Yet, despite many publications supporting a pro-longevity role for sirtuins, there has been recent debate about the direct role of *Caenorhabditis elegans* and *Drosophila melanogaster* SIR-2 in ageing and lifespan extension in response to calorie restriction (also known as dietary restriction)[2,6,7]. Some mammalian sirtuins have been shown to regulate age-related diseases, but mice that overexpress SIRT1 have the same lifespan as control, wild-type (WT), mice[8]. Thus, the role of SIRT1 and other mammalian sirtuins in regulating mammalian lifespan is unclear[3].

Several key findings support a potential role for SIRT6 in regulating mammalian lifespan. SIRT6-deficient mice are small and have severe metabolic defects, and by 2–3 weeks of age, they develop abnormalities that are usually associated with ageing[9]. In addition, SIRT6 regulates nuclear factor-κB signalling, which controls ageing-associated changes in gene expression[10]. Recently, we showed that SIRT6 levels increase in rats that are fed a calorie-restricted diet[11], and transgenic mice that overexpress exogenous mouse SIRT6 (*Sirt6*-transgenic mice; also known as MOSES mice)[4] are protected against the physiological damage caused by diet-induced obesity, including triglyceride and low-density-lipoprotein-associated cholesterol accumulation in the serum, increased body fat and reduced glucose tolerance. In normal animals, these metabolic defects become apparent by middle age, whereas their appearance is delayed in animals fed a calorie-restricted diet. Thus, in this study we sought to determine whether *Sirt6*-transgenic mice remain healthy for longer and have a longer lifespan than wild-type mice.

The lifespan of *Sirt6*-transgenic mice was examined in comparison to their control littermates. *Sirt6*-transgenic mice were produced on a segregating stock containing equal contributions from C57BL/6J and BALB/cOlaHsd mouse strains, both of which are considered to be long lived[12]. The study was carried out on 245 mice (119 males and 126 females) from two transgenic lines (line 55 and line 108) generated from two separate founders. Log-rank test analysis showed significant differences in the survival curves between male WT and male transgenic mice, but not between female WT and female transgenic mice, for both lines (Fig. 1a–d and Supplementary Table 1). Relative to male WT littermates, the median lifespan of male *Sirt6*-transgenic mice increased by 14.5% and 9.9%, and the mean lifespan increased by 14.8% and 16.9%, for line 55 and 108, respectively (log-rank test, $\chi^2 = 10.529$, d.f. = 1 and $P = 0.001$ for line 55; and $\chi^2 = 4.225$, d.f. = 1 and $P = 0.040$ for line 108). In female *Sirt6*-transgenic mice, no significant increase in median or mean lifespan was found relative to female WT littermates for either line (log-rank test, $\chi^2 = 0.009$,

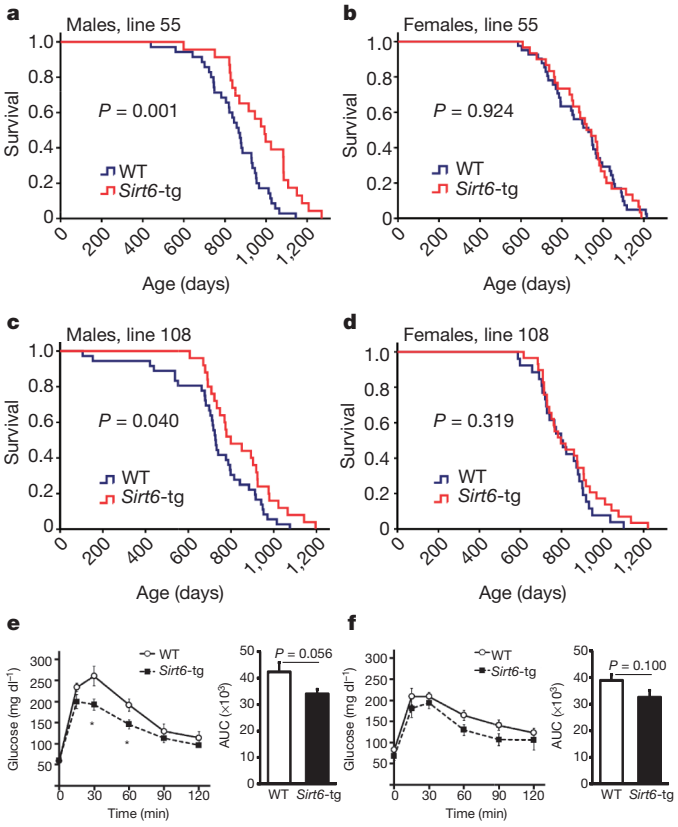


**Figure 1 | Extended lifespan of male *Sirt6*-transgenic mice.** Kaplan–Meier survival curves for male and female WT and *Sirt6*-transgenic (*Sirt6*-tg) mice from two transgenic lines, line 55 (**a**, **b**) and line 108 (**c**, **d**). *P* values were derived from log-rank calculations. Glucose tolerance testing was carried out in WT and *Sirt6*-transgenic males (**e**) and females (**f**) at 19 months (572–577 days) of age (males, $n = 6$ per genotype; females, $n = 4$ per genotype). The area under the curve (AUC) for each glucose tolerance test is shown on the right (**e**, **f**; *y* axis values shown are the AUC divided by 1,000 ). The values shown are mean ± s.e.m. *, $P < 0.05$ (two-tailed *t*-test).

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d.f. = 1 and $P = 0.924$ for line 55; and $\chi^2 = 0.993$, d.f. = 1 and $P = 0.319$ for line 108). Relative to WT littermates, the maximum lifespan of transgenic males (that is, the mean lifespan of the oldest 10% of a cohort to die) increased by 15.8% and 13.1% for line 55 and 108, respectively. Comparison of the maximum lifespan of WT and *Sirt6*-transgenic mice using the quantile regression approach at the ninetieth percentile[13] showed a significant difference between males in one line only ($P = 0.03$ and $P = 0.11$ for line 55 and 108, respectively) and no difference for females ($P = 0.45$ and $P = 0.67$ for line 55 and 108, respectively). Cox regression analysis (using the stepwise backward, Wald method) with the recruitment date, parental identity, gender, genotype and mouse line as main effects and line-by-genotype as the interaction variable showed an additive effect of genotype and line (Supplementary Table 2). However, there was no interaction between mouse line and genotype ($P = 0.693$), indicating that SIRT6 overexpression had an equivalent effect on the mortality of both lines. In summary, our data show that SIRT6 overexpression increased the longevity of males but not females.

SIRT6 has been shown to regulate genomic stability and metabolism[4,9], two important contributors to longevity. Loss of genomic stability is known to be an important aspect of cancer. Post-mortem gross and microscopic examination of the WT and transgenic mice revealed malignant tumours in a variety of organs, with the highest incidence of tumours in all mice being in the lungs. No significant differences in tumour spectrum or incidence were found between WT and transgenic mice (Supplementary Table 3). Similarly, pathological analysis revealed no differences between WT and transgenic males in the incidence of non-neoplastic findings (for example, diffuse mesangial sclerosis and pulmonary emphysema) or age-related pathologies (for example, femoral osteoporosis, basal ganglia calcification and adrenal cortical hyperplasia) (data not shown). Interestingly, the median lifespan of *Sirt6*-transgenic mice with lung tumours showed a trend towards being longer (by 11.7%) than that of WT mice with lung tumours. Therefore, the hypothesis that the effect of SIRT6 on lung cancer has a role in SIRT6's pro-longevity effect cannot be entirely excluded. However, given the proportion of mice with lung tumours in each genotype, a protective role of SIRT6 against lung cancer is likely to contribute only partially to the pro-longevity effect (Supplementary Information). Thus, further studies are required to evaluate the contribution of SIRT6 to age-sensitive traits, in addition to its effect on lung cancer.

The protective role of SIRT6 against metabolic disorders that are induced by a high-fat diet[4] suggests that SIRT6 might positively affect age-associated metabolic disorders, such as declining insulin sensitivity and impaired glucose tolerance. No significant differences in glucose metabolism were found between young (4–7 month old) WT and *Sirt6*-transgenic mice (data not shown). However, an intraperitoneal glucose tolerance test showed that old *Sirt6*-transgenic mice (19 months old, the maximum age of WT mice before a considerable proportion of the litter died) displayed a trend towards improved glucose homeostasis compared with WT mice of the same age (Fig. 1e, f). An analysis of variance (ANOVA) test for the area under the curve (AUC) values of the glucose tolerance tests indicated no sex-specific effect but showed a significant effect of genotype ($P = 0.016$). Therefore, although SIRT6 overexpression had a positive effect on glucose homeostasis in old mice, this finding cannot explain the sexual dimorphism in longevity.

To understand further the mechanisms of the gender-specific lifespan extension in *Sirt6*-transgenic mice, we used whole genome microarray analysis to examine differential gene expression in the livers of animals of both sexes (Supplementary Table 4). In agreement with the sexual dimorphism in liver gene expression[14], differential expression analysis using Significance Analysis of Microarrays (SAM) software[15] showed that the most extensive gene expression differences occurred between genders (Supplementary Table 5). Notably, significant differences were also found between *Sirt6*-transgenic and WT males, but the differences between *Sirt6*-transgenic and WT females were minor (Supplementary Table 5). ANOVA analysis uncovered a subset of genes whose expression differed significantly between genotypes and that were gender-specific (Supplementary Table 5). Gene Ontology (GO) functional analysis showed that the differentially expressed gene set between *Sirt6*-transgenic males and WT males is significantly enriched for categories related to metabolism and cellular responses (Supplementary Table 6). We next compared this differentially expressed gene set with the set of genes that was differentially expressed between male and female WT mice. This analysis revealed a significant similarity between the two gene sets. Of the differentially expressed genes in *Sirt6*-transgenic males, 50% (41 of 82) were also differentially expressed between male and female WT mice ($P = 0$) (Fig. 2a, b and Supplementary Table 5).

To confirm the microarray results, 11 of the differentially expressed genes in male *Sirt6*-transgenic mice were selected for validation by quantitative PCR. The expression pattern of all of these 11 genes confirmed the microarray data (Figs 2c and 3c). Moreover, to examine whether the transcriptional changes due to SIRT6 are mouse-line-specific, the expression of several of these genes was followed in another transgenic line, and the same pattern of transcriptional changes was observed (Supplementary Fig. 1). Calorie restriction and starvation[16–19] have previously been shown to have a similar effect to SIRT6 overexpression on the transcription of several genes (30% of the differentially expressed genes in male *Sirt6*-transgenic mice showed a similar expression pattern in male mice fed a calorie-restricted diet[16]). For

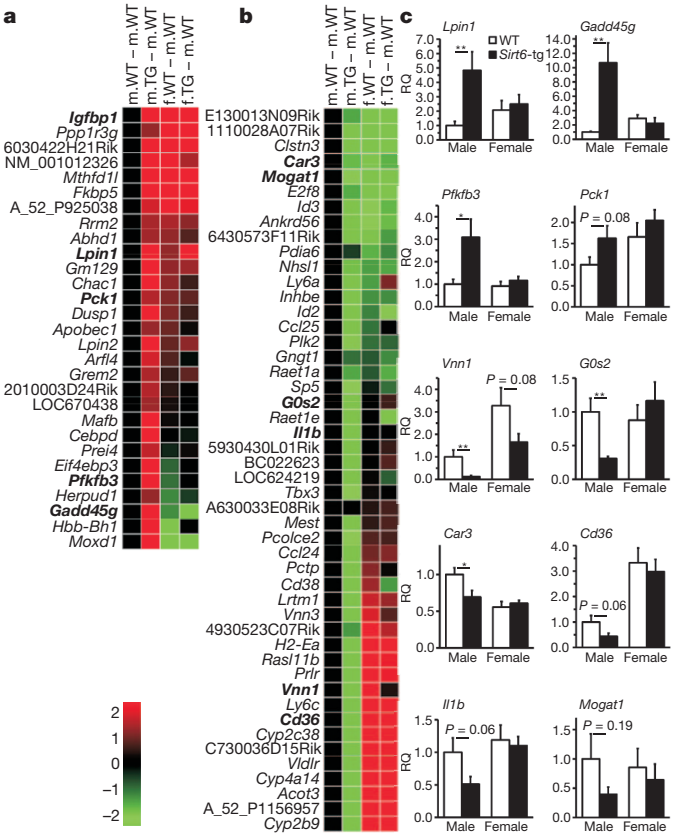


**Figure 2 | Expression profile of differentially expressed genes in male *Sirt6*-transgenic mice. a**, **b**, Heat maps displaying the significantly upregulated (red) and downregulated (green) genes in *Sirt6*-transgenic males (m.TG) compared with WT males (m.WT). The expression profile of these genes in WT females (f.WT) or *Sirt6*-transgenic females (f.TG) compared with WT males is also illustrated. Statistical analysis was performed using all 24 arrays. The quantitative-PCR-validated genes are shown in bold. **c**, The relative expression levels of hepatic genes were confirmed by quantitative PCR in 20 male and 20 female mice. The values shown are mean ± s.e.m. *, $P < 0.05$; **, $P < 0.01$; $n = 10$ per group. RQ, relative quantification.

example, the upregulated genes *Lpin1*, *Lpin2*, *Gadd45g*, *Fkbp5*, *Dusp1* and *Cebpd* and the downregulated genes *Vnn1*, *Vnn3*, *Pctp*, *Vldlr*, *Car3* and *G0s2* in the expression profile of male *Sirt6*-transgenic mice are also differentially expressed in the livers of mice fed a calorie-restricted diet[16–18].

A key factor in the regulation of lifespan is the IGF1 signalling pathway. Worms and flies with a mutated insulin/IGF1 receptor and mice that are heterozygous for the IGF1 receptor have an extended lifespan[5]. Moreover, rodents fed a calorie-restricted diet have lower IGF1 levels early in life than rodents fed a normal chow diet, and many rodent genetic models with a prolonged lifespan have lower levels of serum IGF1 or IGF1 signalling than do control groups[5,20]. Although no difference was found between WT and *Sirt6*-transgenic females, young transgenic males (6 months old) had lower serum IGF1 levels than WT male littermates (Fig. 3a), and these IGF1 levels in *Sirt6*-transgenic males were similar to those in all females. This significant difference in IGF1 levels between young transgenic and WT males was sustained until 19 months of age (Fig. 3b). In line with this finding, one of the genes that was highly upregulated in *Sirt6*-transgenic males, to the same levels as in WT or *Sirt6*-transgenic females, was the gene encoding IGF-binding protein 1 (IGFBP1) (Fig. 3c). IGFBP1 is thought to be the main short-term modulator of IGF1 bioavailability[21]. Calorie restriction increases the expression of IGFBP1 (ref. 17), and high levels of IGFBP1 correlate with protection against metabolic disorders[22]. No change was found in the expression of gene encoding other IGF1-binding proteins, such as IGFBP3 and acid-labile subunit (ALS; also known as IGFALS) (Fig. 3c).

To follow the changes in IGF1 signalling, components of this pathway were analysed in the three main metabolic tissues: liver, white adipose tissue (WAT) and muscle. Analyses included the phosphorylation levels of AKT activation sites (Thr 308 and Ser 473), FOXO1 (Thr 24) and FOXO3 (Thr 32). The most significantly decreased phosphorylation levels were observed in the perigonadal WAT of *Sirt6*-transgenic males in comparison to WT males (Fig. 3d–g and Supplementary Fig. 2a–d). The levels of phosphorylated AKT (on both activation sites), FOXO1 and FOXO3 in WAT were lower in the transgenic mice (Fig. 3e, f). Therefore, we further explored this pathway in WAT and found that the phosphorylation levels of the IGF1 receptor (Tyr 1135) and S6 (Ser 235/236) were lower in *Sirt6*-transgenic males than in the WT male littermates (Fig. 3d, g). Importantly, no significant change in the phosphorylation levels of these markers was observed in female mice (Fig. 3d–g and Supplementary Fig. 2a–d). Moreover, the decrease in the phosphorylation levels of AKT and FOXO proteins in male *Sirt6*-transgenic mice is in agreement with previous reports that show that lifespan is positively regulated by changes in IGF1 signalling in the whole organism, or specifically in the fat tissues, of nematodes and fruitflies[5,23].

There is much doubt about whether mammalian sirtuins regulate lifespan[3,5,8]. Moreover, in the fly and nematode, a recent study challenged the role of sirtuins in regulating lifespan, claiming that the increased

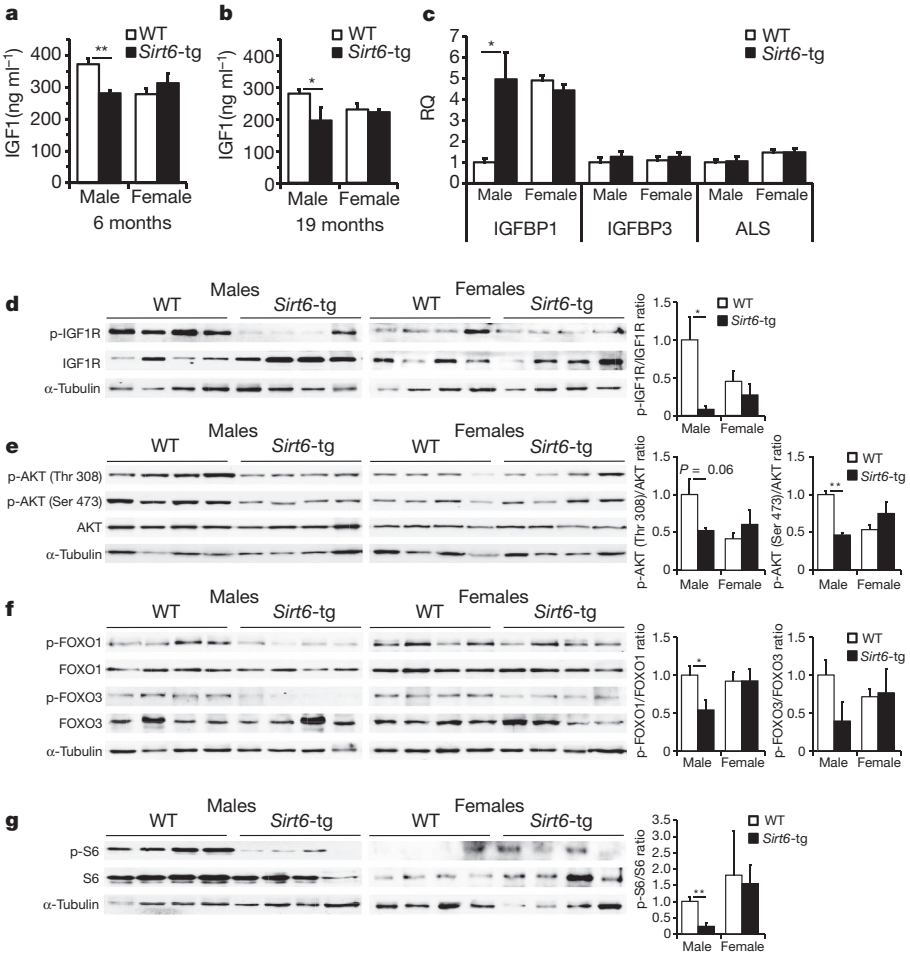


**Figure 3 | Alterations in the IGF1–AKT pathway in *Sirt6*-transgenic males.** **a**, **b**, Serum IGF1 levels in male and female WT and *Sirt6*-transgenic mice at 6 months (**a**) and 19 months (**b**) of age ($n = 4$–7). **c**, The relative expression of hepatic *Igfbp1*, *Igfbp3* and *Als* measured by quantitative PCR ($n = 4$–7). **d**–**g**, The phosphorylation levels of the IGF1 receptor (IGF1R) at Tyr 1135 (**d**), AKT at both the Thr 308 and Ser 473 activation sites (**e**), FOXO1 at Thr 24 and FOXO3 at Thr 32 (**f**), and S6 at Ser 235/236 (**g**) in perigonadal WAT ($n = 4$ mice per genotype). All mice were killed at the same time of day. The phosphorylated to unphosphorylated protein ratios, as determined by densitometry, are shown on the right. **a**–**g**, The values shown are the mean ± s.e.m. *, $P < 0.05$; **, $P < 0.01$.

longevity observed in strains with SIR-2 overexpression is caused by differences in genetic background or by mutagenic effects of transgene insertion[2]. To address potential complications owing to strain-specific effects and integration sites, we used a segregating background with equal contributions from the C57BL/6J and BALB/cOlaHsd mouse strains and studied two separate lines. Indeed, we showed that SIRT6 extends male lifespan regardless of the integration site (Supplementary Fig. 3) and in two control lines with different lifespans. Here, we reveal a role for the mammalian sirtuin SIRT6 in regulating lifespan. SIRT6 overexpression extends lifespan only in males, potentially by reducing IGF1 signalling specifically in WAT. Mice with a fat-specific insulin receptor gene knockout have been shown to have an increased mean lifespan of similar magnitude to the male transgenic mice in our study[23], demonstrating the central role of fat in regulating lifespan. Most genetic modifications of the IGF1 or insulin signalling pathway affect the lifespan of both genders or show a stronger effect in females. Yet here the effect of SIRT6 on IGF1 signalling was male specific. Therefore, further research is required to determine whether the effects of SIRT6 are blocked in females rather than enhanced in males. Taken together, our findings suggest that SIRT6 is an important regulator of mammalian longevity and indicate the feasibility of manipulating SIRT6 levels to treat age-related diseases.

## METHODS SUMMARY

*Sirt6*-transgenic mice on the CB6F1 background, containing equal contributions from C57BL/6J and BALB/cOlaHsd mouse strains, were generated as described previously[4], and the glucose tolerance tests and lifespan analyses were performed as described previously[24–26]. Tissues were taken after natural death, fixed in formaldehyde for histopathological analysis, embedded in paraffin, sectioned, and stained with haematoxylin and eosin. Quantitative PCR was performed using ABsolute Blue SYBR Green on a StepOnePlus instrument. Microarray sample labelling and hybridization were performed as previously described[4], and data were normalized using the program dChip. Differentially expressed genes were identified using SAM and defined as those with a $q$ value of $<10.0\%$ and a minimum of a 1.5 fold change.

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

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**Author Contributions** H.Y.C. designed experiments, analysed data and contributed to writing the paper. Y.K. designed and performed experiments, analysed data and contributed to writing the paper. S.N. designed and performed experiments and contributed to writing the paper. G.A. performed the histopathological analysis. V.P. and L.N. performed experiments. G.Z. and Z.B.-J. developed analytical tools, analysed data and contributed to writing the paper. S.N and G.A. contributed equally to this work.

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