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Ribosomal protein S6 kinase 1 signaling regulates mammalian lifespan

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Abstract

Caloric restriction (CR) protects against aging and disease but the mechanisms by which this affects mammalian lifespan are unclear. We show in mice that deletion of the nutrient-responsive mTOR (mammalian target of rapamycin) signaling pathway component ribosomal S6 protein kinase 1 (S6K1) led to increased lifespan and resistance to age-related pathologies such as bone, immune and motor dysfunction and loss of insulin sensitivity. Deletion of *S6K1* induced gene expression patterns similar to those seen in CR or with pharmacological activation of adenosine monophosphate (AMP)-activated protein kinase (AMPK), a conserved regulator of the metabolic response to CR. Our results demonstrate that S6K1 influences healthy mammalian lifespan, and suggest therapeutic manipulation of S6K1 and AMPK might mimic CR and provide broad protection against diseases of aging.

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Genetic studies in *S. cerevisiae*, *C. elegans* and *D. melanogaster* implicate several mechanisms in the regulation of lifespan. These include the insulin and insulin-like growth factor 1 (IGF-1) signaling (IIS) and mammalian target of rapamycin (mTOR) pathways which both activate the downstream effector ribosomal protein S6 kinase 1 (S6K1) (1, 2). Although the role of these pathways in mammalian aging is less clear, there is mounting evidence that IIS regulates lifespan in mice (1). Global deletion of one allele of the IGF1 receptor (*Igf1r*), adipose-specific deletion of the insulin receptor (*Insr*), global deletion of insulin receptor substrate protein 1 (*Irs1*) or neuron-specific deletion of *Irs2* all increase mouse lifespan (1). Lifespan-extending mutations in the somatotropic axis also appear to work through attenuated IIS (3). *Igf1r* has also been implicated as a modulator of human longevity (4). However, the action of downstream effectors of IIS or mTOR signaling in mammalian longevity is not fully understood.

S6K1 transduces anabolic signals that indicate nutritional status to regulate cell size and growth and metabolism through various mechanisms (5). These include effects on the translational machinery and on cellular energy levels through the activity of adenosine monophosphate (AMP)-activated protein kinase (AMPK) (6, 7). Furthermore, S6K1 serine phosphorylates IRS1 and IRS2 thereby decreasing insulin signaling (5). Given the key role of S6K1 in IIS and mTOR signaling, and the regulation of aging in lower organisms by mTOR, S6K, and their downstream effectors (2) we used log rank testing to evaluate differences in lifespan of wild-type (WT) and *S6K1*$^{-/-}$ littermate mice on a C57BL/6 background (8). Data for both sexes combined showed median lifespan in *S6K1*$^{-/-}$ mice increased by 80 days (from 862 to 942 days) or 9% relative to that of WT mice ($X^2 = 10.52$, $p < 0.001$) (Fig. 1A and Table 1). Maximum lifespan (mean lifespan of the oldest 10% within a cohort) was also increased (1077±16 and 1175±24 days, $p < 0.01$ for WT and *S6K1*$^{-/-}$ mice, respectively). Analysis of each sex separately showed that median lifespan in female *S6K1*$^{-/-}$ mice was increased, by 153 days (from 829 to 982 days) or 19% relative to that of WT mice ($X^2 = 11.07$, $p < 0.001$) (Fig. 1B and Table 1). Female maximum lifespan was also increased (Table 1). In contrast, deletion of *S6K1* in male mice had no effect on median ($X^2 = 0.34$, $p > 0.05$) (Fig. 1C and Table 1) or maximum lifespan (Table 1). Similar gender effects on lifespan have been reported in other long-lived IIS mouse mutants (8, 9). Cox regression analysis of pooled male and female lifespan data revealed no effect of recruitment date, parental identity or gender but that of genotype was significant (table S1). Therefore deletion of *S6K1* increases longevity in female mice.

Female *S6K1*$^{-/-}$ mice also showed improvements in a number of age-sensitive biomarkers of aging. In forced motor activity on a rotating rod (rotarod) assay to assess motor and neurological function, 600-day old female *S6K1*$^{-/-}$ mice performed better than WT littermates (Fig. 2A). Performance in open field testing to analyse general activity and exploratory drive was also enhanced (Fig. 2, B and C). An increase in abundance of memory T cells and a reduced number of naïve T cells are seen in mice with age and the extent of these changes may be inversely correlated with longevity (10). Female *S6K1*$^{-/-}$ mice at 600 days of age had significantly fewer memory and more naïve T cells than did WT mice (Fig. 2D), although male mice also displayed this phenotype (data not shown). Micro-computed tomography scanning of tibia from 600 day old female *S6K1*$^{-/-}$ mice revealed attenuation of the normal age-dependent loss of cancellous bone volume seen in C57BL/6 mice (11) (Fig.

2, E and F). However, there was no difference in the incidence of macroscopic tumors in *S6K1*$^{-/-}$ and WT animals (data not shown).

Young, high fat diet-fed male *S6K1*$^{-/-}$ mice (12) display increased insulin sensitivity and reduced adiposity relative to those of WT mice, phenotypes also seen in WT mice under caloric restriction (CR), an evolutionary conserved environmental manipulation that extends lifespan (13). Insulin sensitivity (assessed by the updated homeostasis model, HOMA2) was significantly greater in 600-day old female *S6K1*$^{-/-}$ mice than in WT animals (Fig. 2G) and glucose tolerance was improved (Fig. 2H), in contrast to the impaired glucose tolerance seen in young animals (fig S1A). Fat mass and plasma leptin levels were lower in old female *S6K1*$^{-/-}$ mice (Fig. 2, I and J) despite increased food intake (fig. S1B). Core temperature and resting metabolic rate (with general linear modelling to account for body mass differences) were not significantly different (fig. S1, C and D). Although *S6K1*$^{-/-}$ mice were smaller than their littermates throughout their lives (Fig. 2K), endocrinologically they did not resemble long-lived pituitary dwarfs (14) as their total circulating IGF-1, pituitary growth hormone, thyroid-stimulating hormone and prolactin concentrations were normal (Fig. 2, L and M, and fig. S1, E and F). Male *S6K1*$^{-/-}$ mice at 600 days of age had normal fasting and fed glucose levels and reduced fat mass (data not shown).

We compared the effect of *S6K1* deletion on genome-wide hepatic gene expression in 600-day old female mice to transcriptional changes induced by long-term CR (15). The 500 gene categories most overrepresented among genes with altered expression in *S6K1*$^{-/-}$ mice showed a highly significant overlap with categories over-represented among CR-regulated genes ($p=3.25*10^{-42}$ and $p=1.15*10^{-19}$ for up- and down-regulated categories respectively, Fisher's exact test) (fig. S2A). Hepatic transcript profiles in long-lived *Irs1*$^{-/-}$ mice (8) were also similar (fig. S2B; $p=1.60*10^{-21}$ and $p=8.61*10^{-20}$ for up- and down-regulated categories, respectively). Furthermore, we observed significant correlations in the directions of transcriptional changes associated with highly significant functional categories ($p<10^{-4}$, two-tailed) in both comparisons (fig. S2, A and B). This is consistent with the existence of common mechanisms underlying the effects of S6K1, CR and IIS on aging.

We examined transcription of individual genes in liver, skeletal muscle and white adipose tissue (WAT) in 600-day old female *S6K1*$^{-/-}$ and WT mice, looking for genes previously associated with longevity (tables S2, A and B, S3, A and B and S4, A and B). Significant crosstalk exists between peroxisome proliferator-activated receptor gamma, coactivator 1 alpha (PGC-1α), AMPK and NAD-dependent deacetylase sirtuin-1 (SIRT1) signaling which may be critical to cellular energy metabolism and perhaps aging (16). Increased expression of genes associated with these pathways was observed in liver (*Ppargc1a, Ppargc1b, Foxo1, Foxo3a, Cpt1b, Pdk4, Glut1, Cyc*) and muscle (*Ppargc1a, Ppara, Foxo1, Foxo3a, Pdk4, Glut1, Sirt1, Ucp3*) of *S6K1*$^{-/-}$ mice. Adipose tissue is a key tissue in longevity assurance in *C. elegans*, *Drosophila melanogaster* and mice (17). In WAT of *S6K1*$^{-/-}$ mice fewer PGC-1α regulated genes (*Foxo3a, Pdk4, Nampt, Angptl4*) showed increased expression compared to changes seen in liver and muscle, but there was also increased expression of the α2 catalytic and β1 regulatory subunits of AMPK (Log2 fold change =1.7, $p=2.88*10^{-6}$ and 1.2, $p=4.95*10^{-5}$ respectively, Cyber-T analysis). AMPK activity is increased in WAT, muscle and liver of *S6K1*$^{-/-}$ mice (7). Moreover, comparison of gene expression patterns in muscle

of *S6K1*$^{-/-}$ mice with those of mice treated with the AMPK activator aminoimidazole carboxamide ribonucleotide (AICAR) (18) revealed a strong overlap between gene categories that showed increased expression, including those associated with PPAR signaling and lipid metabolism (fig. S2C). We confirmed enhanced AMPK activation by AICAR in isolated hepatocytes from *S6K1*$^{-/-}$mice (Fig. 3A).

In *C. elegans* AMPK mediates the effects on lifespan of one particular form of CR (19) and perturbing IIS (20, 21), raising the possibility that the longevity of *S6K1*$^{-/-}$ mice results from increased AMPK activity. To test this, we studied long-lived *C. elegans rsks-1(ok1255)* null mutants, which lack the single worm S6K1 homolog. *rsks-1* mutants showed increased phosphorylation of the worm AMPK catalytic subunit AAK-2 (Fig. 3B), consistent with increased AMPK activity. These findings imply that in worms, as in mice, loss of S6K1 increases AMPK activity. *rsks-1* mutants are long lived (Fig. 3C, and table S5), with reduced and delayed fecundity and, like *S6K1*$^{-/-}$ mice, reduced body size (Fig. 3, D to F, and table S5), characteristics which could be attributed to reduced nutrient availability or possibly reduced overall translation (22). To test the role of AMPK in mediating the effects of *rsks-1* on longevity, we generated mutants lacking both *rsks-1* and *aak-2*. The *aak-2(ok524)* null allele fully suppressed *rsks-1* mutant longevity (Fig. 3C, and table S5). This effect is likely to be specific since several other modes of *C. elegans* longevity are not *aak-2* dependent (21). Moreover, *aak-2(ok524)* also suppressed the fecundity and body size defects of *rsks-1* mutants (Fig. 3, D to F). This also suggests that these defects do not reflect reduced overall translation; in fact, in muscle cells from growth-deficient S6K1-null mouse protein synthesis is reportedly not reduced (23). Taken together, these results imply that increased AMPK activity may contribute to the longevity of both *C. elegans* and mice lacking *S6K1*.

Our studies indicate that S6K1 signaling influences mammalian lifespan and age-related pathology. S6K1 is regulated in response to nutrient and hormonal signals and may thus participate in the response to CR. mTOR and AMPK are amenable to pharmacological intervention (24, 25). It might be possible to develop drug treatments that manipulate S6K1 and AMPK to achieve improved overall health in later life. Indeed short-term rapamycin treatment reduces adiposity in mice (26) and metformin treatment extends lifespan in short-lived mice (27). Furthermore, very recently it has been demonstrated that rapamycin treatment initiated late in life extends lifespan in mice (28). Our results suggest that this may occur via inhibition of S6K1 and together these studies indicate the feasibility of manipulating mTOR/S6K1 signalling in the treatment of aging-related disease.

Supporting Online Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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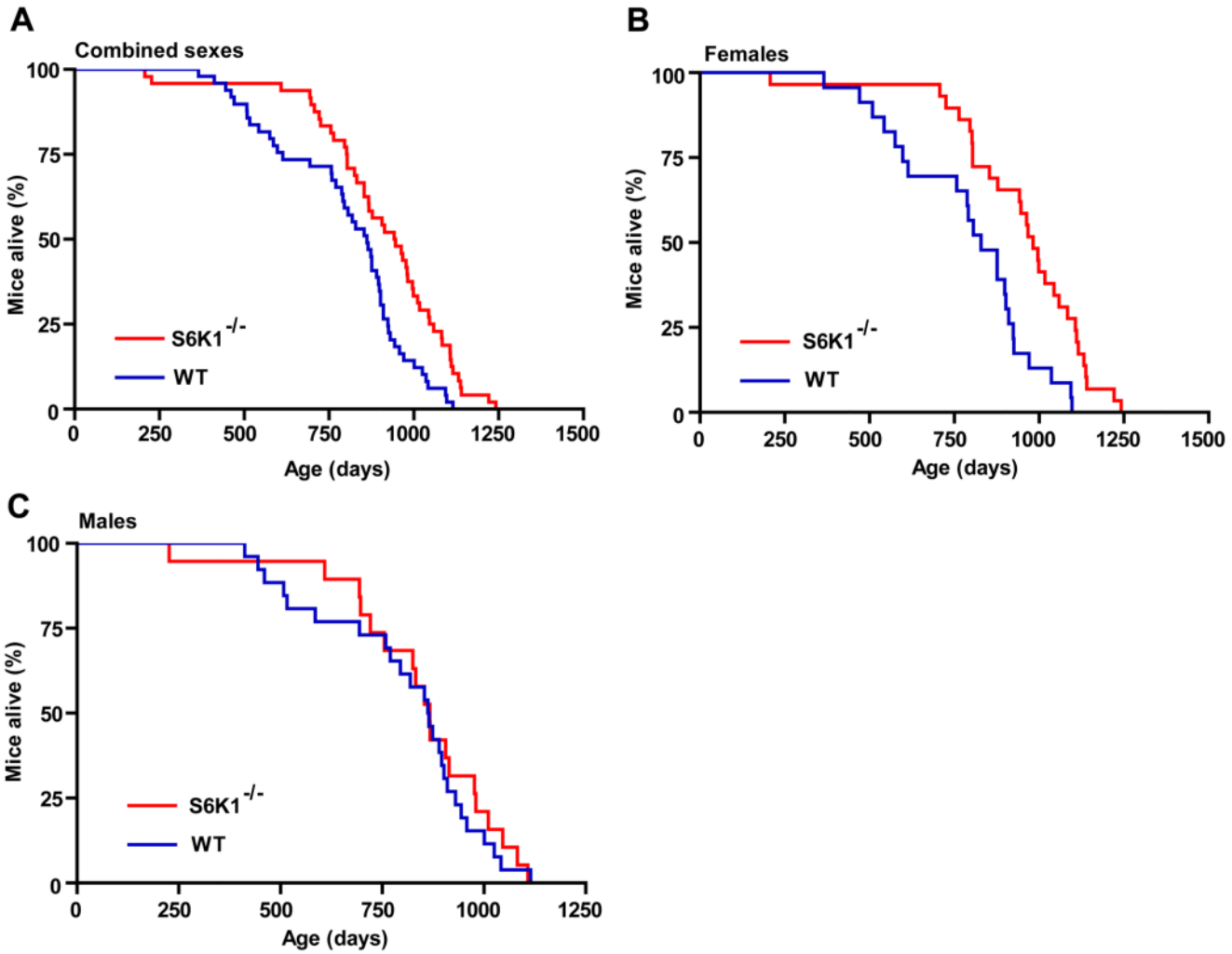

Fig. 1.

Extended lifespan of mice with deletion of *S6K1*$^{-/-}$. (**A**) Kaplan-Meier survival curves for combined male and female wild-type (WT) and *S6K1*$^{-/-}$ mice show a significant (Log-rank X^2=10.52, p<0.001) lifespan extension in *S6K1*$^{-/-}$ mice (n= 49 for WT mice and n= 48 for *S6K1*$^{-/-}$ mice). (**B**) Lifespan extension was observed in female S6K1$^{-/-}$ (X^2=11.07, p<0.001; n= 23 for WT mice and n= 29 for *S6K1*$^{-/-}$ mice). (**C**) Kaplan-Meier survival curve for male wild-type (WT) and *S6K1*$^{-/-}$ mice shows no significant increase in lifespan in *S6K1*$^{-/-}$ mice (Log-rank X^2=0.34, p>0.05; n = 26 for WT mice and n = 19 for *S6K1*$^{-/-}$ mice).

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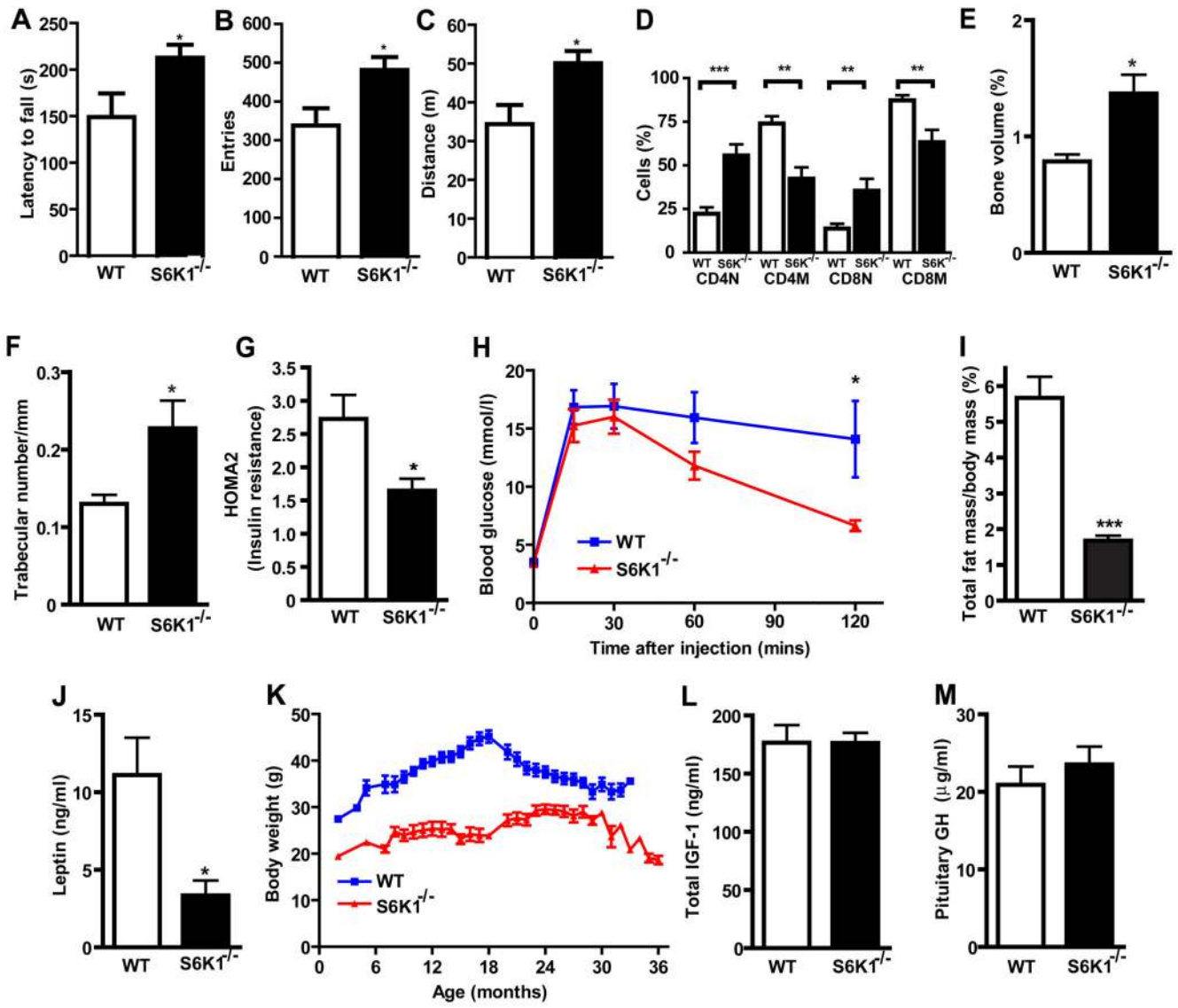

Fig. 2.

Age-related pathology and physiological characteristics of 600 day old female *S6K1*$^{-/-}$ mice. (**A**) *S6K1*$^{-/-}$ mice had improved rotarod performance. (**B** and **C**) Increased general activity and exploratory drive was observed in *S6K1*$^{-/-}$ mice. (**D**) Abundance of memory and naïve T cells in WT and *S6K1*$^{-/-}$ mice. (**E** and **F**) Bone volume and trabecular number in WT and *S6K1*$^{-/-}$ mice. (**G** and **H**) Insulin sensitivity and glucose tolerance of WT and S6K1$^{-/-}$ mice. (**I**) S6K1$^{-/-}$ mice were lean and (**J**) had reduced plasma leptin levels. (**K**) Body mass ($p<$ 0.01 at all time-points), total circulating IGF-1 (**L**) and pituitary GH concentrations in WT and S6K1$^{-/-}$ mice (**M**). Values are mean ± s.e.m. Asterisks indicate statistical difference compared to WT mice using two-tailed t-tests, * p<0.05, ** p<0.01 and *** p<0.001. n= 6-8 per genotype.

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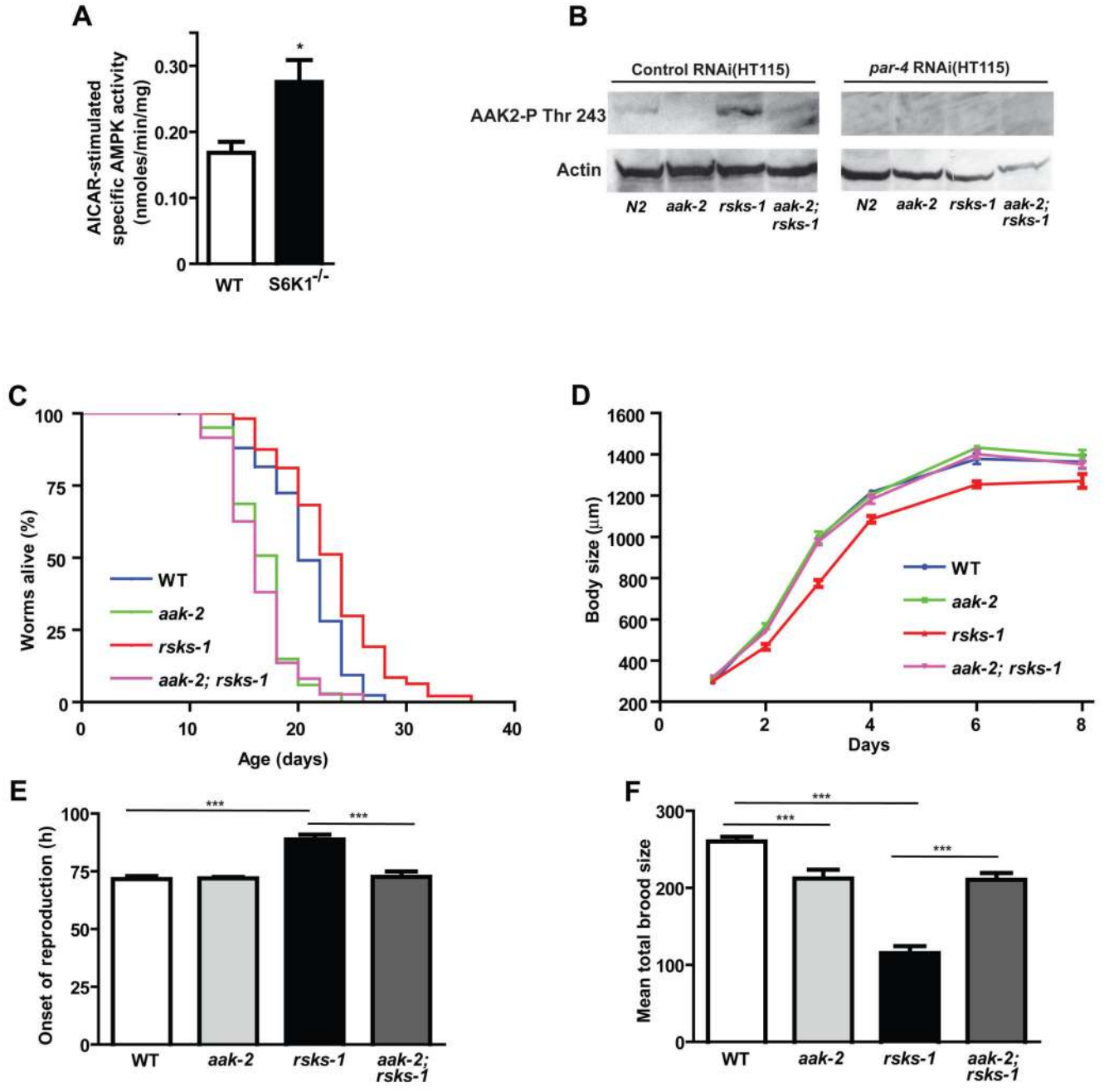

Fig. 3.

Enhanced AMPK activation by AICAR of *S6K1*$^{-/-}$ hepatocytes, increased AAK-2 phosphorylation in *rsks-1(ok1255)* mutants and effects of loss of *aak-2(ok524)* on longevity and physiology. (**A**) AICAR-stimulated AMPKα2 activity in isolated hepatocytes from *S6K1*$^{-/-}$ mice. (**B**) Phosphorylation of AAK-2 Thr243 in *rsks-1(ok1255)* null mutants subjected, or not, to RNAi for *par-4*, the worm LKB kinase which effects this phosphorylation (**C**) Lifespan of *rsks-1(ok1255)* nulls with mutation of *aak-2(ok524)*. (**D** to **F**) Body length, onset of reproductive function and brood size phenotypes in *rsks-1(ok1255)* mutants with or without *aak-2(ok524)* mutation. In (**D**) *rsks-1(ok1255)* is significantly

different ($p < 0.001$; one-way ANOVA) from all other groups from day 2 onwards but *rsks-1(ok1255);aak-2* is not significantly different from WT or *aak-2*. (**A**) to (**C**) show data from one representative experiment, and (**D** to **F**) show combined data from three similar independent experiments. Values (**A** and **D** to **F**) denote mean ± s.e.m. In (**A**), n = 3, and in (**D**), n > 8 for each strain and time point. For (**E**) and (**F**) n > 20 for each group. Asterisks indicate statistical differences using two-tailed t-tests, * $p < 0.05$, *** $p < 0.001$.

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Table 1

Comparative survival characteristics of *S6K1*$^{-/-}$ and WT mice.

Genotype	Median	Mean ± s.e.m.	Min-Max	Oldest 10%	Youngest 10%	N
			Combined sex			
WT	862	796±28	365-1115	1077±16	431±19	49
***S6K1*$^{-/-}$**	942	907±30	207-1242	1175±24	487±111	48
			Female			
WT	829	789±42	365-1097	1096±2	418±53	23
***S6K1*$^{-/-}$**	982	950±38	207-1242	1201±31	546±170	29
			Male			
WT	862	801±39	412-1115	1061±28	439±14	26
***S6K1*$^{-/-}$**	867	841±47	227-1108	1095±13	418±191	19

Lifespan is reported in days (±s.e.m., where appropriate) for wild-type (WT) and *S6K1*$^{-/-}$ mice. Oldest (youngest) 10% are the mean lifespan of the longest (or shortest) living 10% of animals within a genotype. N= Sample size.