

Pyruvate imbalance mediates metabolic reprogramming and mimics lifespan extension by dietary restriction in *Caenorhabditis elegans*

Laurent Mouchiroud,¹ Laurent Molin,¹ Prasad Kasturi,^{1,*} Mohamed N. Triba,^{2,†} Marc Emmanuel Dumas,² Marieangela C. Wilson,³ Andrew P. Halestrap,³ Damien Roussel,⁴ Ingrid Masse,^{1,†} Nicolas Dalli  re,¹ Laurent S  galat,[†] Marc Billaud¹ and Florence Solari¹

¹UMR5201, CNRS, Universit   de Lyon, Centre L  on B  rard, 28 Rue Laennec, Lyon 69373, France

²Centre Europ  en de RMN    Tr  s Hauts Champs, CNRS FRE 3008, 5 rue de la Doua, Villeurbanne 69100, France

³The Department of Biochemistry and The Bristol Heart Institute, School of Medical Sciences, University of Bristol, University Walk, Bristol, BS8 1TD, UK

⁴UMR5123, CNRS, Universit   de Lyon, 43 boulevard du 11 novembre 1918, Villeurbanne Cedex 69622, France

Summary

Dietary restriction (DR) is the most universal intervention known to extend animal lifespan. DR also prevents tumor development in mammals, and this effect requires the tumor suppressor PTEN. However, the metabolic and cellular processes that underly the beneficial effects of DR are poorly understood. We identified *slcf-1* in an RNAi screen for genes that extend *Caenorhabditis elegans* lifespan in a *PTEN/daf-18*-dependent manner. We showed that *slcf-1* mutation, which increases average lifespan by 40%, mimics DR in worms fed *ad libitum*. An NMR-based metabolomic characterization of *slcf-1* mutants revealed lower lipid levels compared to wild-type animals, as expected for dietary-restricted animals, but also higher pyruvate content. Epistasis experiments and metabolic measurements support a model in which the long lifespan of *slcf-1* mutants relies on increased mitochondrial pyruvate metabolism coupled to an adaptive response to oxidative stress.

This response requires DAF-18/PTEN and the previously identified DR effectors PHA-4/FOXA, HSF-1/HSF1, SIR-2.1/SIRT-1, and AMPK/AAK-2. Overall, our data show that pyruvate homeostasis plays a central role in lifespan control in *C. elegans* and that the beneficial effects of DR results from a hormetic mechanism involving the mitochondria. Analysis of the SLCF-1 protein sequence predicts that *slcf-1* encodes a plasma membrane transporter belonging to the conserved monocarboxylate transporter family. These findings suggest that inhibition of this transporter homolog in mammals might also promote a DR response.

Key words: *Caenorhabditis elegans*; dietary restriction; hormesis; PTEN/*daf-18*; pyruvate.

Introduction

A number of studies have provided evidence that metabolism and aging are tightly linked. Initial evidence came from the observation that mutations reducing the activity of the insulin/IGF-1 receptor pathway (IIR) could double the average lifespan of the nematode *Caenorhabditis elegans* (Kenyon *et al.*, 1993). Subsequently, the role of IIR in lifespan regulation was shown to be conserved in higher organisms such as mice (Bluher *et al.*, 2003; Holzenberger *et al.*, 2003). Further investigations revealed that IIR functions primarily in the nervous system to control *C. elegans* lifespan (Wolkow *et al.*, 2002) and this was also recently established in mammals (Kappeler *et al.*, 2008). Another important, non-genetic, intervention that increases lifespan throughout animal kingdom is dietary restriction (DR), the reduction in caloric intake without malnutrition (Masoro, 2005). It has been shown that DR not only increases lifespan but also delays the onset of age-related diseases such as cancer (Maeda *et al.*, 1985; Lane *et al.*, 1999; Mattson & Wan, 2005; Colman *et al.*, 2009), and the oncosuppressive PTEN protein has been recently identified as a mediator of tumor inhibition by DR (Kalanany & Sabatini, 2009).

A number of observations have suggested that DR does not result simply from passive metabolic changes but rather exerts its effects through specific signaling pathway, although the molecular mechanisms involved are only starting to emerge. In *C. elegans*, mutations of genes encoding the transcription factors FOXA/PHA-4 (Panowski *et al.*, 2007), Nrf2/SKN-1 (Bishop & Guarente, 2007), FOXO/DAF-16 (Hsu *et al.*, 2003; Greer *et al.*, 2007), HSF1/HSF-1 (Steinkraus *et al.*, 2008), the energy sensor AMP-activated kinase AAK-2 (Apfeld *et al.*, 2004; Greer *et al.*, 2007), and the NAD-dependent deacetylase SIRT/SIR-2.1

Correspondence

Florence Solari, UMR5201, CNRS, Universit   de Lyon, Centre L  on B  rard, 28 Rue Laennec, Lyon, 69373, France. Tel.: (33) 4 69 16 66 24; fax: (33) 4 69 16 66 60; e-mail: solari@lyon.fnclcc.fr

Marc Billaud, UMR5201, CNRS, Universit   de Lyon, Centre L  on B  rard, 28 Rue Laennec, Lyon, 69373, France. Tel.: +(33) 4 69 16 66 24; fax: +(33) 4 69 16 66 60; e-mail: billaud@lyon.fnclcc.fr

[†]Present address: Department of Cellular Biochemistry, Max Planck Institute of Biochemistry, Am Klopferspitz 18, D-82152 Martinsried, Germany.

^{*}Present address: Universit   de Lyon, CNRS, UMR5534, 43 boulevard du 11 novembre 1918, Villeurbanne Cedex, 69622, France.

Accepted for publication 22 September 2010

(Wang & Tissenbaum, 2006) have all been shown to suppress lifespan extension induced by different DR protocols. It has been proposed, based on the identity of their target genes, that these transcription factors increase the lifespan of worms by improving their resistance to oxidative stress. However, the metabolic and cellular mechanisms responsible for the activation of these effectors remained unknown.

Here, we describe a novel *C. elegans* longevity factor, SLCF-1, whose inactivation increased lifespan in worms fed *ad libitum*, in a manner that mimicked that induced by DR. Characterization of *slcf-1* mutants provides new insights into the effects of diet on aging. First, the extension of lifespan by DR is generally associated with a strong reduction in fertility (Partridge *et al.*, 2005). However, our study shows that DR beneficial effects on lifespan can be obtained with minor effect on fecundity. Second, our work identifies PTEN/DAF-18 as a conserved effector of DR from *C. elegans* to mammals. Third, our data highlight the function of previously identified DR effectors in the adaptive response to a hormetic stress, which is essential for DR-mediated lifespan extension. Finally, our data establish a key role for pyruvate homeostasis in the response to DR.

Results and discussion

slcf-1 mutation increases lifespan and delays aging in *C. elegans*

We identified the clone F59F5.1 in an RNAi screen designed to isolate lifespan-extending genes that function with *PTEN/daf-18* (Masse *et al.*, 2008). Inhibition of F59F5.1 expression increased average lifespan by 40% (Table 1 and Fig. 1A). F59F5.1 encodes a predicted multipass transmembrane protein that is similar to proteins of the SLC16 monocarboxylate transporter (MCT). This family includes 14 members but substrates have only been assigned for six members. Four (MCT1–MCT4) transport monocarboxylates such as L-lactate and pyruvate while MCT8 transports thyroid hormone and MCT10 aromatic acids.

The overall amino acid identity between paralogous SLC16 family members tends to be rather low ($\leq 30\%$) (Halestrap & Meredith, 2004) but all family members share the same predicted topology: N-terminal and C-terminal cytoplasmic domains that flank two groups of six transmembrane domains that are separated by a variably sized cytoplasmic loop as shown in Fig. 1B. In agreement with this prediction, when F59F5.1 cDNA was introduced into mammalian cells or in *Xenopus* oocytes, it was expressed as a 40-KD protein that localized at the cellular membrane (Fig. S1A,B and data not shown). F59F5.1 was thus named SLCF-1 for SoLute Carrier Family-1.

Nine putative transporters homologous to mammalian SLC16 family members have been identified in *C. elegans* as reported in Wormbase (<http://www.wormbase.org>, NCBI KOG 2504). Only one member of this family, *gem-1*, has been functionally characterized so far and has been shown to play a role in gonadal cell division (Kemp *et al.*, 2009). To investigate whether any

other member of the *C. elegans* SLC16 family might be involved in longevity, lifespan was assessed after individual RNAi inactivation of each of the nine SLC16 family members. Under these conditions, only inactivation of *slcf-1* extended lifespan (Table S1 and Fig. S2).

To investigate the role of SLCF-1 in longevity further, we obtained *slcf-1(tm2258)* mutants from the Japanese national bioresource project. The *tm2258* allele results from a 665-bp deletion that generates an early stop codon at nucleotide 702. This should delete the six C-terminal transmembrane domains and thus *tm2258* is likely to represent a null mutant (Fig. 1B).

Like animals fed with the F59F5.1 RNAi clone, *slcf-1(tm2258)* mutants exhibited a longer lifespan than wild-type worms (Table 1 and Fig. 1C) and also showed a delay in the accumulation of lipofuscin autofluorescence and muscle fiber disorganization, two hallmarks of physiological aging in *C. elegans* (Fig. S3).

In worms carrying a transgene that rescued the *slcf-1(tm2258)* mutant lifespan phenotype (see Experimental procedures and Fig. S4), SLCF-1::GFP expression was restricted to the basolateral membrane of intestinal cells during larval development throughout adulthood (Fig. 1D).

We next asked whether SLCF-1 regulates lifespan primarily through an effect on the intestine. To do this, we performed RNAi in a *sid-1* mutant background. *sid-1* mutants have been shown to allow gene inactivation by RNA feeding that is limited to the intestine, because SID-1 is required for systemic spreading of dsRNA, while SID-2 is needed for intestinal uptake at the apical membrane (Winston *et al.*, 2007). Intestine-specific RNAi for *slcf-1* mimicked the extension of lifespan observed when *slcf-1* was inactivated in a systemic manner (Fig. 1E).

Overall, our results demonstrate that *slcf-1* inactivation increases longevity and delays aging, most probably by acting in both a cell autonomous and a cell nonautonomous manner.

slcf-1 inactivation mimics dietary restriction in worms fed *ad libitum*

We noticed that *slcf-1(tm2258)* mutants show a clear phenotype similar to calorie-restricted animals. Studies on DR were initiated in *C. elegans* by the characterization of *eat-2(ad465)* mutants that are partially starved because *eat-2* encodes a beta subunit of the nicotinic acetylcholine receptor which controls rates of pharyngeal pumping (Lakowski & Hekimi, 1998). However, pharyngeal pumping of *slcf-1(tm2258)* mutants was similar to wild-type (Fig. S5). Also, as with DR, *slcf-1* inactivation extended the lifespan of mutants for the insulin receptor/*daf-2* (Lakowski & Hekimi, 1998) (Fig. 2A and Table 1).

To explore further the hypothesis that *slcf-1* mutation may mimic a dietary-restricted state, we tested whether *slcf-1* was required for lifespan extension by DR. Various protocols have been developed to reduce food intake in *C. elegans*. In agreement with previous reports, decreasing food availability by bacteria dilution on solid medium (SDR, (Greer *et al.*, 2007)), in liquid culture (Mair *et al.*, 2009) or by bacterial deprivation (DD,

Table 1 Summary of lifespan experiments

Genotype and culture conditions	RNAi	Mean lifespan \pm SE (days)	Median lifespan (days)	P-values against control	P-values against specific group	No. death/censored (no. trial)
Wild-type	Control	17.4 \pm 0.2	16			620/580 (15)
<i>rrf-3(pk1426)</i>	<i>slcf-1</i>	23.1 \pm 0.2	24	$< 10^{-3}$		689/511 (15)
	Control	17.4 \pm 0.7	17			70/90 (2)
<i>rrf-3(pk1426); daf-18(mg198)</i>	<i>slcf-1</i>	23.3 \pm 0.6	25	$< 10^{-3}$		81/79 (2)
	Empty vector	13.2 \pm 0.3	13	$< 10^{-3}$		75/85 (2)
Wild-type	<i>slcf-1</i>	14.2 \pm 0.2	15	$< 10^{-3}$		118/42 (2)
	Control	16.9 \pm 0.5	16			147/93 (3)
<i>slcf-1(tm2258)</i>		21.9 \pm 0.5	22	$< 10^{-3}$		146/94 (3)
Wild-type	Control	15.5 \pm 0.5	14			104/36 (2)
	<i>slcf-1</i>	19.8 \pm 0.7	18	$< 10^{-3}$		97/43 (2)
<i>sid-1(qt2)</i>	Control	15.2 \pm 0.6	13	ns		93/47 (2)
	<i>slcf-1</i>	18.7 \pm 0.7	16	$< 10^{-3}$	$< 10^{-3a}$	94/46 (2)
Wild-type	Control	17.2 \pm 0.4	15			119/121 (3 ^b)
	<i>slcf-1</i>	22.0 \pm 0.6	24	$< 10^{-3}$		135/125 (3 ⁺)
	<i>daf-2</i>	33.5 \pm 0.7	34	$< 10^{-3}$		146/64 (3 ⁺)
	<i>slcf-1/daf-2</i>	38.1 \pm 0.6	37	$< 10^{-3}$	$< 10^{-3b}$	147/93 (3 ⁺)
Wild-type 5.10 ¹⁰ bacteria per mL (AL)		20.0 \pm 0.4	21			124/56 (2)
Wild-type 5.10 ⁹		21.3 \pm 0.5	21	0.039		96/84 (2)
Wild-type 5.10 ⁸		22.4 \pm 0.4	23	$< 10^{-3}$		103/77 (2)
Wild-type 5.10 ⁷		22.5 \pm 0.5	23	$< 10^{-3}$		91/89 (2)
Wild-type 5.10 ⁶		21.8 \pm 0.6	23	0.024		66/114 (2)
<i>slcf-1(tm2258)</i> 5.10 ¹⁰ (AL)		24.1 \pm 0.6	25	$< 10^{-3}$		95/85 (2)
<i>slcf-1(tm2258)</i> 5.10 ⁹		23.0 \pm 0.5	23		0.034 ^c	96/84 (2)
<i>slcf-1(tm2258)</i> 5.10 ⁸		20.6 \pm 0.6	21		$< 10^{-3c}$	90/90 (2)
<i>slcf-1(tm2258)</i> 5.10 ⁷		18.4 \pm 1.1	17		$< 10^{-3c}$	29/151 (2)
<i>slcf-1(tm2258)</i> 5.10 ⁶		19.4 \pm 0.8	19		$< 10^{-3c}$	43/137 (2)
Wild-type OD 1.5 (AL)		21.4 \pm 1.0	21			56/5 (1)
Wild-type OD 0.75		23.6 \pm 1.1	21	0.097		55/5 (1)
Wild-type OD 0.3		26.3 \pm 1.3	25	0.001		59/2 (1)
Wild-type OD 0.15		22.3 \pm 1.5	21	0.143		58/2 (1)
Wild-type OD 0		15.8 \pm 0.9	14	$< 10^{-3}$		59/3 (1)
<i>slcf-1(tm2258)</i> OD 1.5 (AL)		23.6 \pm 1.0	21	0.080		63/2 (1)
<i>slcf-1(tm2258)</i> OD 0.75		24.5 \pm 1.1	25		0.547 ^c	62/2 (1)
<i>slcf-1(tm2258)</i> OD 0.3		22.8 \pm 1.3	21		0.963 ^c	59/1 (1)
<i>slcf-1(tm2258)</i> OD 0.15		17.7 \pm 1.2	14		0.003 ^c	56/1 (1)
<i>slcf-1(tm2258)</i> OD 0		13.4 \pm 0.6	14		$< 10^{-3c}$	58/1 (1)
Wild-type AL		19.2 \pm 0.5	19			68/12 (1)
<i>slcf-1(tm2258)</i> AL		23.6 \pm 0.6	23	$< 10^{-3}$		86/14 (1)
Wild-type DD		32.6 \pm 2.2	33	$< 10^{-3}$		20/180 (1)
<i>slcf-1(tm2258)</i> DD		19.0 \pm 0.9	20	0.660	$< 10^{-3c}$	58/142 (1)
Wild-type		20.1 \pm 0.6	20			83/47 (2)
<i>slcf-1(tm2258)</i>		23.4 \pm 0.5	23	0.003		81/49 (2)
Wild-type + Pyruvate		22.9 \pm 0.6	22	0.001		81/49 (2)
<i>slcf-1(tm2258)</i> + Pyruvate		23.0 \pm 0.5	23	0.001	ns ^d	97/33 (2)
<i>daf-18(e1375)</i> 25°C		7.6 \pm 0.3	8			90/30 (2)
<i>daf-18(e1375)</i> + Pyruvate 25°C		8.5 \pm 0.2	9	ns		80/40 (2)
Wild-type	Control	16.6 \pm 0.5	16			89/71 (2)
	<i>slcf-1</i>	22.3 \pm 0.7	23	$< 10^{-3}$		94/66 (2)
	<i>pdh-1</i>	15.0 \pm 0.3	14	0.002		81/79 (2)
	<i>pdh-2</i>	15.3 \pm 0.3	14	0.009		115/45 (2)
	<i>pdhk-2</i>	19.8 \pm 0.7	18	$< 10^{-3}$		69/91 (2)
	<i>slcf-1/pdh-1</i>	15.9 \pm 0.4	14	0.185	$< 10^{-3d}$	87/73 (2)
	<i>slcf-1/pdh-2</i>	14.9 \pm 0.4	14	0.009	$< 10^{-3d}$	91/69 (2)
	<i>slcf-1/pdhk-2</i>	23.6 \pm 0.8	23	$< 10^{-3}$	0.244 ^d	81/79 (2)
Wild-type	Control	14.8 \pm 0.6	13			55/25 (1)
	<i>slcf-1</i>	18.7 \pm 0.9	18	$< 10^{-3}$		52/28 (1)
<i>isp-1(qm150)</i>	Control	22.9 \pm 1.6	21	$< 10^{-3}$		53/27 (1)

Table 1 (Continued)

Genotype and culture conditions	RNAi	Mean lifespan \pm SE (days)	Median lifespan (days)	P-values against control	P-values against specific group	No. death/censored (no. trial)
<i>slcf-1</i>	23.2 \pm 1.7	18	< 10 ⁻³	ns ^e	46/34 (1)	
Wild-type	Control	16.6 \pm 0.6	14			108/32 (2)
	<i>slcf-1</i>	20.9 \pm 0.7	21	< 10 ⁻³		101/39 (2)
<i>mev-1(kn1)</i>	Control	12.5 \pm 0.4	13	< 10 ⁻³		92/48 (2)
	<i>slcf-1</i>	13.7 \pm 0.5	13	< 10 ⁻³	0.036 ^f	94/46 (2)
Wild-type		17.8 \pm 0.5	18			116/44 (2)
<i>slcf-1(tm2258)</i>		26.2 \pm 0.8	27	< 10 ⁻³		92/68 (2)
Wild-type + NAC		14.8 \pm 0.5	13	0.001		109/51 (2)
<i>slcf-1(tm2258)</i> + NAC		16.2 \pm 0.7	13	0.416		93/67 (2)
Wild-type	Control	17.5 \pm 0.2	16			289/191 (6‡)
	<i>slcf-1</i>	22.4 \pm 0.3	23	< 10 ⁻³		319/161 (6‡)
	<i>pha-4</i>	17.9 \pm 0.6	17	0.370		72/88 (2‡)
	<i>aak-2</i>	14.6 \pm 0.4	14	< 10 ⁻³		136/104 (3‡)
	<i>hsf-1</i>	11.4 \pm 0.3	11	< 10 ⁻³		71/9 (1)
	<i>slcf-1/pha-4</i>	16.5 \pm 0.7	16	0.180	< 10 ^{-3d}	56/104 (2‡)
	<i>slcf-1/aak-2</i>	15.6 \pm 0.3	14	< 10 ⁻³	< 10 ^{-3d}	148/92 (3‡)
	<i>slcf-1/hsf-1</i>	11.0 \pm 0.3	11	< 10 ⁻³	< 10 ^{-3d}	67/13 (1)
Wild-type	Control	17.0 \pm 0.6	16			76/84 (2)
	<i>slcf-1</i>	20.6 \pm 0.6	21	< 10 ⁻³		103/57 (2)
<i>sir-2.1(ok434)</i>	Empty vector	16.9 \pm 0.4	18	ns		98/62 (2)
	<i>slcf-1</i>	16.8 \pm 0.3	16	ns	ns ^g	113/47 (2)

Summary of mean and median lifespans and statistical analysis (P-values) for lifespan experiments including all strains, RNAi feeding conditions and different treatments displayed in Figs 1A,C,E 2A–D, 3E–G, 4A,B, 5D and 6B–E. P-values from a log rank test comparing RNAi treatment population to the vector control or to specific groups (a versus *sid-1(qt2)* control, b versus *daf-2* RNAi and *daf-2(e1370)*, c versus *slcf-1(tm2258)* at the *ad libitum* (AL) food concentration, d versus *slcf-1* RNAi and *slcf-1(tm2258)*, e versus *isp-1(qm150)* control, f versus *mev-1(kn1)* control, g versus *sir-2.1(ok434)* control). DD, dietary deprived; NAC, N-acetyl cysteine.

P-values < 0.05 are considered statistically significant, demonstrating that the two lifespan populations are different. The total number of individuals scored is shown followed by the number of individuals censored because of bursting vulva or crawling off the agar.

†Pool of data using double RNAi and single RNAi in *daf-2(e1370)* mutants.

‡Pool of data from lifespan experiments using double RNAi and single RNAi in *slcf-1(tm2258)* mutants.

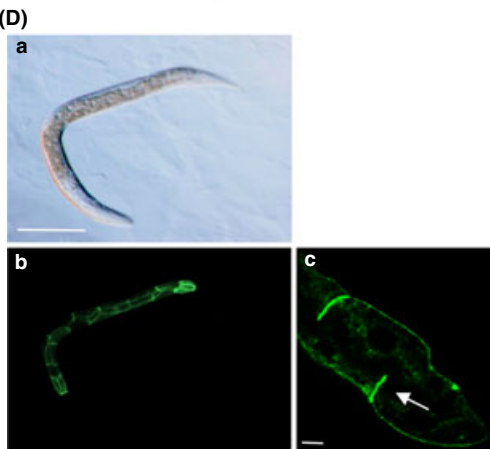
(Smith et al., 2008)) significantly increased the lifespan of wild-type worms (Fig. 2B–D and Table 1). However, *slcf-1(tm2258)* mutants were shorter lived than control when cultured at the bacterial concentration that maximizes lifespan (Fig. 2B–D), although at high food concentrations they remained long-lived (Fig. 2B–D). This is not unexpected because it would be predicted that limiting food intake in animals that are already in a DR state would starve them and be deleterious as previously reported for *eat-2* mutants under conditions of dietary restriction (Mair et al., 2009). Overall, these data suggest that lower SLCF-1 activity or expression mimics a DR response in worms fed *ad libitum*.

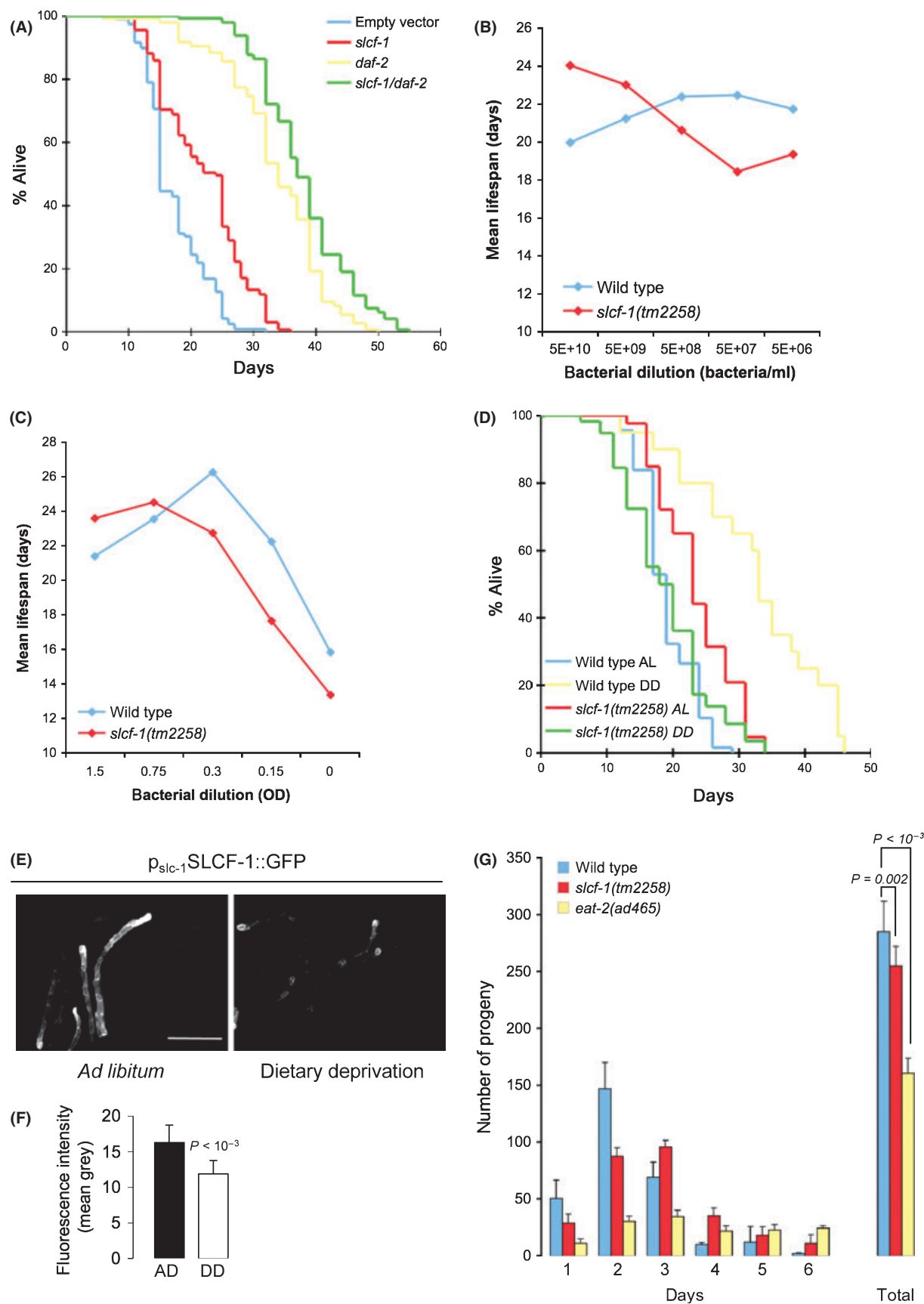
We next asked whether food intake may modulate SLCF-1::GFP expression. When worms were submitted to food restriction by dietary deprivation, bacterial dilution or *eat-2* mutation,

SLCF-1::GFP expression was significantly down-regulated (Figs 2E,F, and S6). This down-regulation took place, at least partly, at the transcriptional level because GFP expression under the control of the promoter only was also down-regulated (data not shown). These results show that SLCF-1::GFP expression depends on the nutritional status of worms, further demonstrating that inhibiting SLCF-1 activity or expression mimics at least some effects of the DR response.

Interestingly, while *slcf-1(tm2258)* mutants exhibit several features of calorie-restricted animals, they show only a minor reduction in progeny production compared to *eat-2* mutants (Fig. 2G). These data show that in *C. elegans*, the consequence of DR on longevity can be dissociated from its effect on fertility, similar to results recently reported in *Drosophila* (Grandison et al., 2009).

Fig. 1 Mutation of *slcf-1* gene delays aging. (A) Survival curves of *rff-3(pk1426)* and *rff-3(pk1426); daf-18(e1375)* mutants fed either control bacteria not expressing any dsRNA (HT115) or bacteria expressing dsRNA that targets *slcf-1* gene. (B) Multiple alignment of SLCF-1 with SLC16A7 (*Mus musculus*), SLC16A3 (*Gallus gallus*), SLC16A5 (*Homo sapiens*), SLC16A12 (*Danio rerio*) protein sequences with transmembrane domains (TM) highlighted (<http://www.tcd.org/analyze.php>). *tm2258* allele contains a deletion that introduces a stop codon (as indicated *) and thus removes the 6 TM underlined. (C) Survival curves of *slcf-1(tm2258)* mutants and wild-type animals. See Table 1 for additional data. (D) A rescuing SLCF-1::GFP fusion protein is expressed during all larval stages in the intestine, as illustrated here at the L4 stage by Normarski (a) and fluorescent (b) images. Scale bar: 100 μ m. Polarized expression is observed at the basolateral membrane of intestinal cells. Arrowhead: lumen. Scale bar: 30 μ m (c). (E) Survival curves of *sid-1(qt2)* mutants and wild-type animals fed either control bacteria not expressing any dsRNA (HT115) or bacteria expressing dsRNA that targets *slcf-1* gene. See Table 1 for additional data.





***slcf-1* mutation induces a metabolic shift that lowers lipids and increases pyruvate content**

To investigate the metabolic consequences of *slcf-1* inactivation further, *slcf-1* mutants were analyzed using whole-organism ^1H HR-MAS-NMR (Blaise et al., 2007, 2009). Untargeted metabolic profiling was performed and revealed major differences between *slcf-1(tm2258)* and wild-type populations (Fig. 3A). Lipid signals such as cholesterol, triglycerides, saturated and unsaturated fatty acids are significantly depleted in *slcf-1(tm2258)* mutants (Fig. 3B), as already reported in calorie-restricted mammals (Walford et al., 2002; Wang et al., 2007). This is probably as the result of enhanced lipid catabolism for energy production (Selman et al., 2006). Another metabolite showing major change was pyruvate. Although pyruvate levels varied between samples, they were significantly increased in *slcf-1(tm2258)* mutants compared to wild-type levels ($P = 0.005$), while lactate levels were similar (Fig. 3C,D).

To investigate the functional importance of pyruvate level for lifespan regulation, we tested whether feeding worms with pyruvate might be sufficient to modulate lifespan. In these experiments, dead rather than live bacteria were used to exclude an indirect effect resulting from pyruvate catabolism by bacteria. Worms fed with pyruvate showed an increased average lifespan by 14%, while *slcf-1* mutant lifespan was unchanged (Table 1 and Fig. 3E). Furthermore, *daf-18* mutation suppressed the lifespan increase induced by pyruvate (Table 1). These results highlighted the functional relevance of a higher pyruvate content for the increased longevity of *slcf-1* mutants.

To test whether pyruvate could be a substrate for SLCF-1, as it is for several mammalian SLC16A transporters, SLCF-1 was expressed in *Xenopus* oocytes. In those experimental conditions, SLCF-1 did not facilitate pyruvate transport across their plasma membranes (Fig. S1C). However, we cannot exclude that in a physiological context, the impact of *slcf-1* mutation on pyruvate level could be direct.

Pyruvate dehydrogenase activity is essential for lifespan extension by *slcf-1* mutation

Next, we investigated how pyruvate might affect lifespan extension in the *slcf-1* mutant. Pyruvate is produced from glucose by glycolysis and feeds the Krebs cycle via its conversion into acetyl-coenzyme A in the mitochondria. The enzyme responsible for this reaction is pyruvate dehydrogenase (PDH), for which two subunits have been found to be encoded by the *C. elegans* gen-

ome (Castelein et al., 2008). Its activity is inhibited when PDH is phosphorylated by the pyruvate dehydrogenase kinase (PDHK), encoded by *pdhk-2* in *C. elegans*. Thus, PDHK acts as a molecular switch that regulates the flow of glycolytic products into the Krebs cycle.

Inhibiting the expression of either PDH subunits completely suppressed the lifespan extension induced by *slcf-1* RNAi (Table 1 and Fig. 3G) while it reduced the lifespan of wild-type animals by only 12% (Table 1 and Fig. 3F). These data show that the extension of lifespan observed in *slcf-1(tm2258)* worms requires PDH, which may be constitutively activated in these mutants. In agreement with this hypothesis, inhibition of *pdhk-2* expression, which is predicted to induce higher PDH activity, increased average lifespan by 20% (Table 1 and Fig. 3F) but did not further increase the lifespan of *slcf-1* RNAi-treated worms (Table 1 and Fig. 3G). These results show that pyruvate availability modulates worm lifespan and that the PDH/PDHK complex is required for the effect of SLCF-1 function on lifespan.

***slcf-1* inactivation alters mitochondria metabolism and induces ROS accumulation**

The importance of the PDH/PDHK complex for lifespan extension prompted us to investigate whether *slcf-1* function might rely on the modulation of mitochondrial function to exert its effects on lifespan. Mutations in genes encoding different components of the electron transport chain have been shown to affect *C. elegans* lifespan (Rea et al., 2007). Thus, mutation in *isp-1*, which encodes the Rieske iron sulfur protein subunit of the mitochondrial complex III, extends lifespan, while mutations of *mev-1*, which is orthologous to the human gene encoding succinate dehydrogenase of complex II, shortens it.

We found that *slcf-1* inactivation did not further extend the lifespan of *isp-1* mutants (Fig. 4A and Table 1), which is consistent with SLCF-1 regulating lifespan through an effect on mitochondria function. Conversely, mutation in *mev-1* reduced the lifespan of both *slcf-1* and wild-type by 35% and 24% respectively (Fig. 4B and Table 1), thus suggesting that *mev-1* mediates at least part of the effects of *slcf-1* on longevity.

We next examined the consequence of *slcf-1* inactivation on mitochondrial metabolism. Previous reports in yeast and *C. elegans* associated the beneficial effects of dietary restriction with higher respiration rate (Lin et al., 2002; Bishop & Guarente, 2007; Schulz et al., 2007). Surprisingly, oxygen consumption of *slcf-1* mutants was not higher than wild-type animals; however, it increased from day 1 to day 5 of adulthood while ATP content

Fig. 2 *slcf-1* inactivation mimics dietary restriction in worms fed *ad libitum*. (A) Survival curves of wild-type animals fed with RNAi clones for *daf-2* or *slcf-1* (50% diluted with control vector RNAi bacteria) or with a mix of both. Control worms are wild-type animals fed with bacteria containing an empty vector. Survival curves represent a pool of data from three independent experiments (two RNAi experiments and one using *daf-2(e1370)* mutants that gave similar results). (B) Mean lifespan of wild-type and *slcf-1(tm2258)* animals across a range of serial dilution of bacteria on NGM plates (5×10^{10} to 5×10^6 UV-killed bacteria mL^{-1}). (C) Mean lifespan of wild-type and *slcf-1(tm2258)* animals across a range of serial dilution of bacterial liquid cultures (OD = 1.5 to OD = 0). (D) Survival curves of wild-type and *slcf-1(tm2258)* animals at the *ad libitum* (AL) food concentrations (5×10^{10} of UV-killed bacteria) and dietary deprivation condition (DD). (E, F) Expression of SLCF-1::GFP in L4 larvae in presence of food or after 12 hours of food deprivation (E) and corresponding fluorescence quantification (F). Scale bar: 400 μm . AA: *ad libitum*; DD: dietary deprivation. (G) *slcf-1(tm2258)* mutants show a mild fecundity phenotype compared to *eat-2(ad465)* mutants. See Table 1 for additional data.

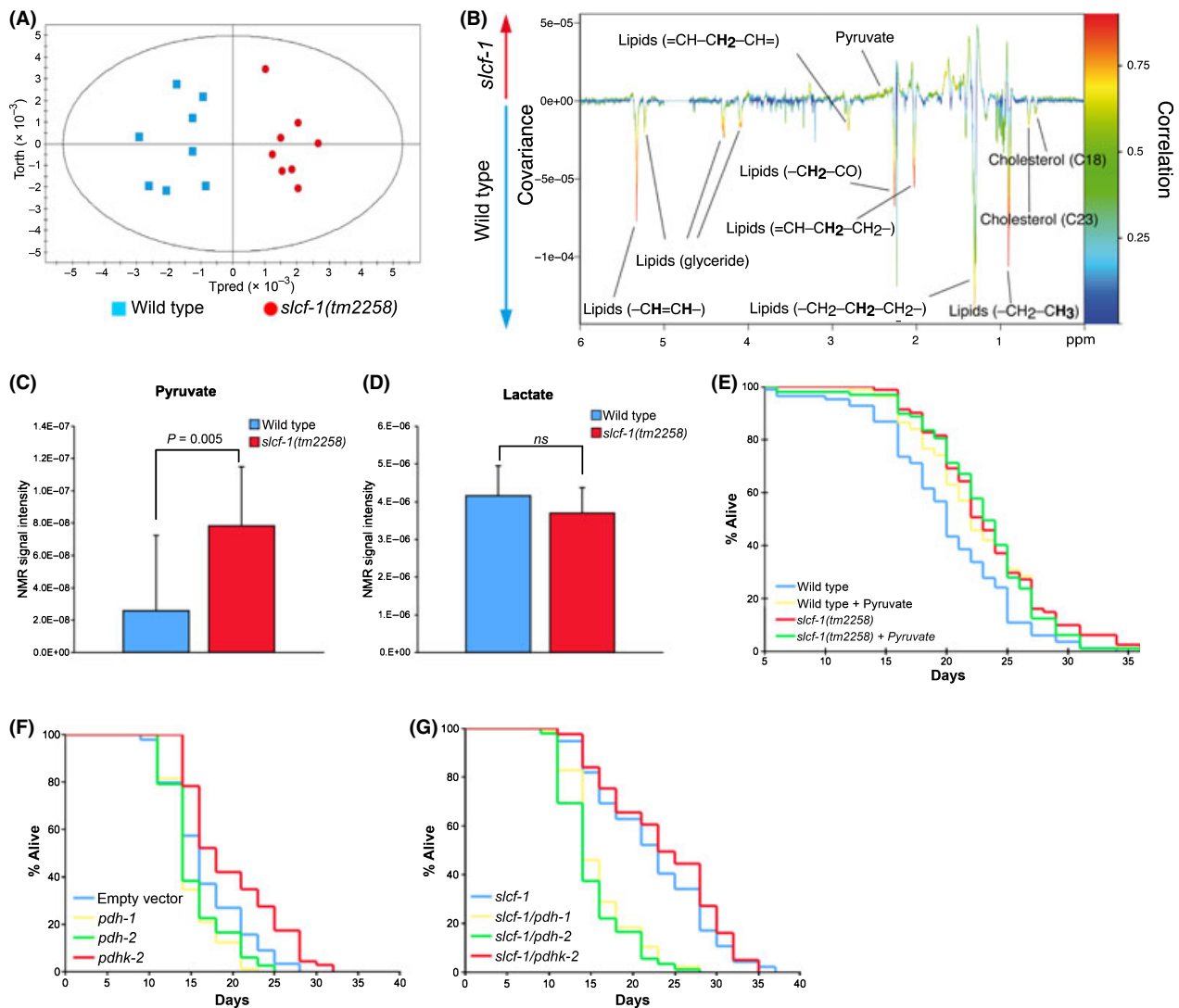
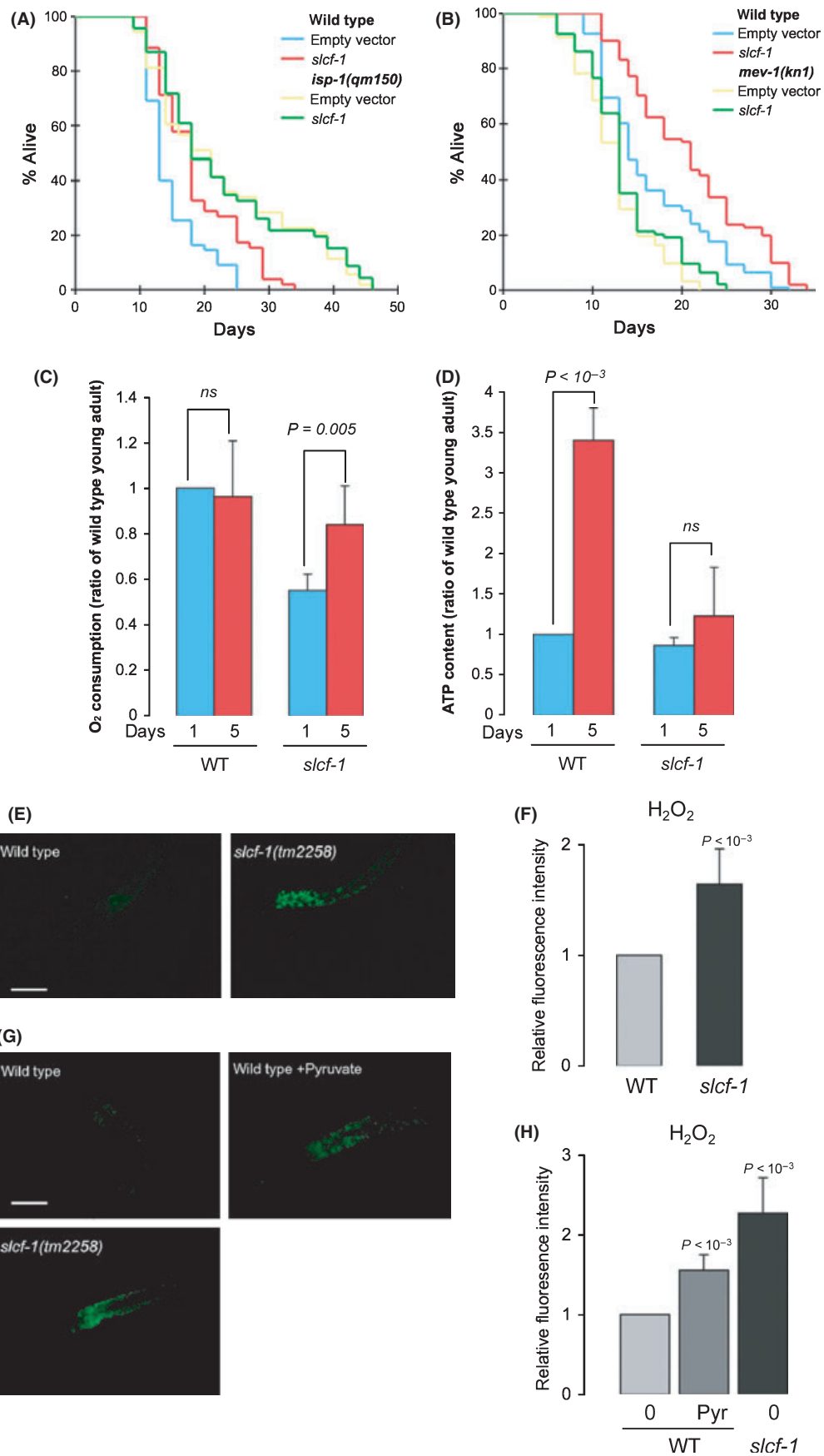


Fig. 3 Lifespan regulation by SLCF-1 relies on pyruvate homeostasis. (A–D) ^1H HRMAS NMR-based metabolomic characterization of *slcf-1* mutants. (A) Score plots of orthogonal partial least-squares discriminant model analysis (OPLS-DA) between N2 and *slcf-1(tm2258)* strains ($R^2 = 0.88$, $Q^2 = 0.80$). (B) Annotated loadings (OPLS-DA model coefficients) for wild-type and *slcf-1(tm2258)* worms. Upward peaks indicate increased concentration in *slcf-1(tm2258)* and downward peaks increased concentration in N2. The color code associated with signals indicates the correlation derived from the unit-variance model (scale on the right) between metabolites and strains. For this analysis, the correlation was considered to be significant when higher than 0.60 (yellow and red color code), which corresponds to a significant level of 1% (Pearson's correlation test, $P = 0.01$). (C, D) NMR intensity for signals assigned respectively to pyruvate (1.33 ppm) (C) and lactate (2.38 ppm) (D) and (E) Survival curves of wild-type and *slcf-1(tm2258)* animals treated or not with pyruvate. (F, G) Survival curves of wild-type animals fed with *pdh-1*, *pdh-2*, and *pdhk-2* RNAi clones 50% diluted with control vector (F) or in combination with *slcf-1* RNAi clone (G). Data were confirmed using *pdhk-2(tm3086)* and *pdhk-2(tm3075)* mutants (Table S1). Similar results were obtained with *slcf-1(tm2258)* mutants. See Table 1 for additional data.

remained stable (Fig. 4C,D). Higher oxygen consumption can be associated with a rise in reactive oxygen species (ROS) production, and we were able to demonstrate that *slcf-1(tm2258)* mutants did show higher levels of hydrogen peroxide than wild-type animals (Fig. 4E,F).

We then hypothesized that ROS production might be a consequence of higher pyruvate level and confirmed that pyruvate treatment of young adult wild-type worms did induce hydrogen peroxide accumulation (Fig. 4G,H). These data support a functional link between pyruvate homeostasis and ROS production.

Fig. 4 *slcf-1* inactivation alters mitochondria metabolism and levels of reactive oxygen species. (A, B) Survival curves of *isp-1(qm150)* mutants (A) and *mev-1(kn1)* mutants (B) fed either control bacteria not expressing any dsRNA (HT115) or bacteria expressing dsRNA that targets *slcf-1* gene. See Table 1 for additional data. (C) Mitochondria oxygen consumption and (D) ATP content in wild-type and *slcf-1(tm2258)* animals at young adult stage or at day 5 of adulthood. Statistical analyses were performed by a Student's *t*-test. (E, F) Pictures of *slcf-1(tm2258)* and wild-type young adults stained with DCFDA, which reveals hydrogen peroxide levels (scale bar: 100 μm) (E) and fluorescence quantification (F). (G, H) Pictures of wild-type young adults stained with DCFDA after pyruvate treatment (scale bar: 100 μm) (G) and fluorescence quantification (H). WT: wild-type.



Overall, our data support a model in which the increased pyruvate level in *slcf-1* mutants would be responsible for higher ROS generation via an alteration of mitochondria function.

The long lifespan of *slcf-1* mutants results from a hormetic stress response

Consistent with the burst of ROS observed, young adult stage *slcf-1* mutants were more sensitive than wild-type when challenged with an additional oxidative stressor such as the ROS generator paraquat (Fig. 5A). However, the resistance to paraquat observed in *slcf-1* mutants increased progressively with age to reach a level equal to or greater than wild-type animals at day 5 and 7 of adulthood, respectively (Fig. 5B and C). Our observations thus indicate that DR is a stressful condition that may result in a hormetic stress response that contributes to increased longevity.

To test this model, we first assessed the impact of ROS accumulation on the lifespan of *slcf-1* mutants by treating worms with the antioxidant N-acetyl cysteine (NAC). Although NAC only slightly reduced the lifespan of wild-type animals, it fully suppressed *slcf-1* mutants lifespan phenotype (Fig. 5D). Furthermore, when *slcf-1* mutants were treated with the antioxidant NAC during the first 5 days of adulthood, they then

remained sensitive to paraquat in comparison with the wild-type (Fig. 5E). These results show that the increase in ROS at young adult stage is required for both setting up adaptation to oxidative stress and for lifespan extension.

Specific DR effectors are essential for adaptation to hormetic stress

Previous studies in *C. elegans* have revealed that several molecular factors are required for the lifespan increase according to DR regimen (Greer & Brunet, 2009; Mair *et al.*, 2009). Several DR effectors have been proposed to affect lifespan by reducing oxidative damages via the transcriptional regulation of ROS-detoxifying enzymes. We thus investigated the role of DR effectors in the adaptation of *slcf-1* mutant to oxidative stress. For this, DR effectors were inactivated during the first 5 days of adulthood of *slcf-1* mutants, which were then tested for their resistance to paraquat.

When *PTEN/daf-18*, *AMPK/aak-2*, *FOXA/pha-4*, *SIRT/SIR-2.1* or *HSF1/hsf-1* were inactivated, the stress resistance of *slcf-1(tm2258)* mutants at day 5 of adulthood was significantly reduced compared to *slcf-1(tm2258)* worms treated with control RNAi bacteria, thus supporting their role in metabolic adaptation of *slcf-1* mutants (Fig. 6A). Conversely, when

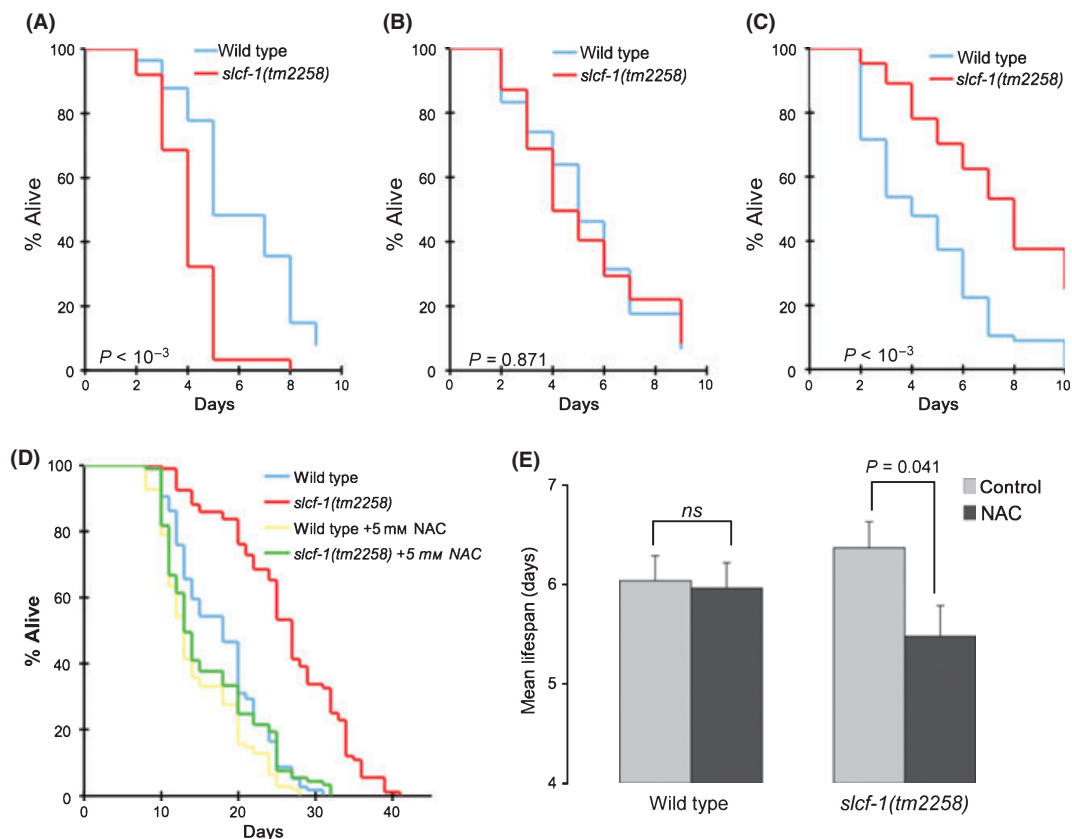


Fig. 5 Lifespan phenotype of *slcf-1(tm2258)* mutants relies on a hormetic stress response. (A–C) Survival curves of wild-type and *slcf-1(tm2258)* animals treated with paraquat from the young adult stage (A), day 5 (B) or day 7 of adulthood (C). (D) Survival curves of wild-type and *slcf-1(tm2258)* animals treated or not with 5 mM of N-acetyl cysteine (NAC). (E) Mean lifespan of wild-type and *slcf-1(tm2258)* animals on paraquat at day 5 of adulthood with or without NAC treatment from the young adult stage.

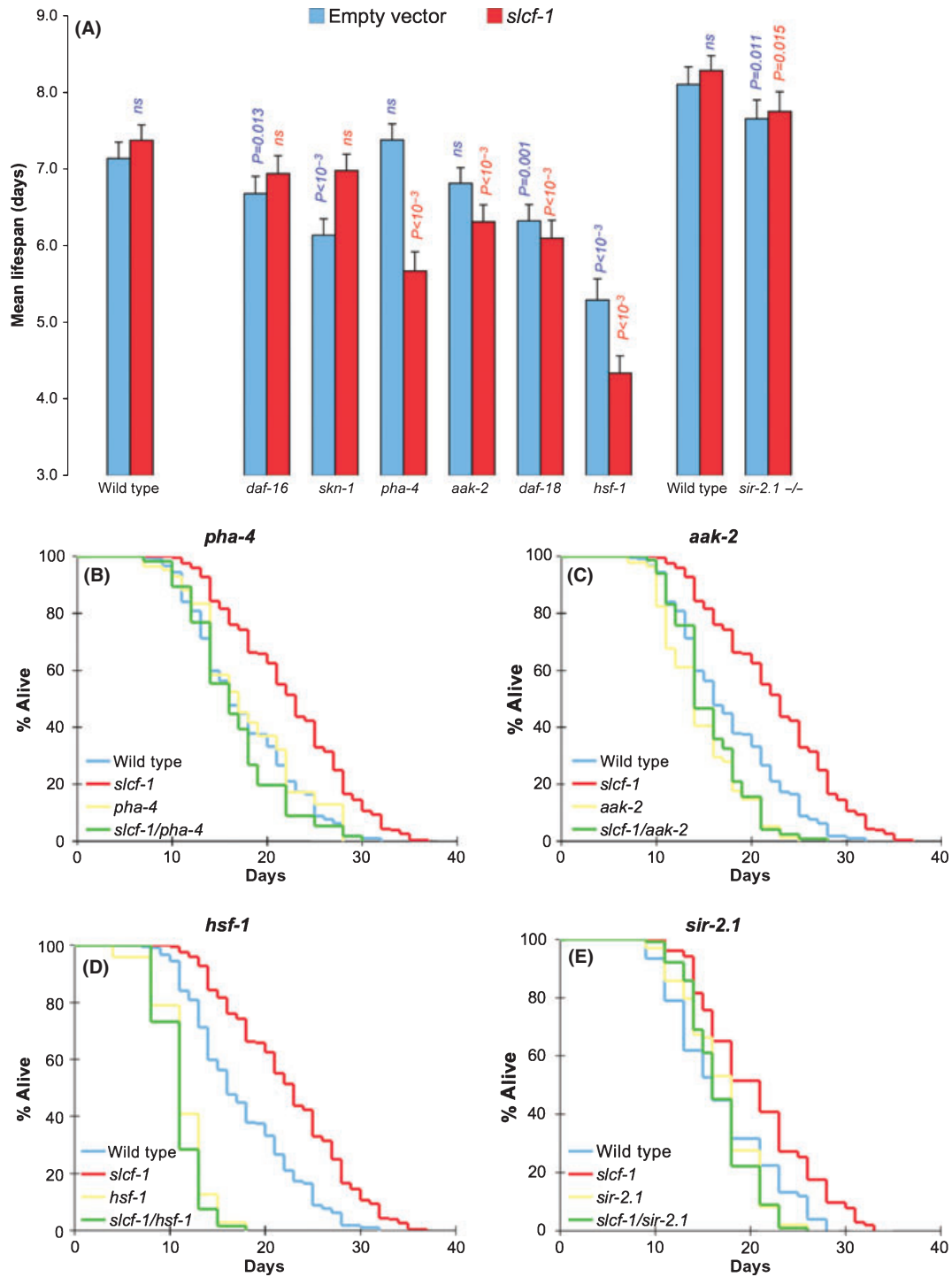


Fig. 6 Specific DR effectors are essential for adaptation to hormetic stress. (A) Histogram showing average lifespans of *slcf-1(tm2258)* worms fed with bacteria expressing *daf-16*, *skn-1*, *pha-4*, *aak-2*, *daf-18* or *hsf-1* dsRNA during the first 5 days of adulthood and then challenged with the ROS generator paraquat. The involvement of *sir-2.1* was tested in double mutants *slcf-1(tm2258);sir-2.1(ok434)* thus in condition in which *sir-2.1* is constitutively inactivated. Results have been confirmed with double mutants *slcf-1(tm2258);daf-16(mu86)* and *slcf-1(tm2258);daf-18(e1375)*. P-values in blue comparing RNAi treatment population to the vector control, and P-values in red compare double *slcf-1* with specific DR effectors RNAi treatment to *slcf-1* RNAi alone. (B–D) Survival curves of wild-type worms fed with bacteria expressing *pha-4* (B), *aak-2* (C), *hsf-1* (D) dsRNA mixed with either control bacteria or bacteria expressing *slcf-1* dsRNA. (E) Survival curves of *sir-2.1(ok434)* mutants and wild-type animals fed either control bacteria not expressing any dsRNA (HT115) or bacteria expressing dsRNA that targets *slcf-1* gene. See Table 1 for additional data.

NRF2/skn-1 or *FOXO/daf-16* were inactivated, *slcf-1(tm2258)* mutants showed an adaptative response to oxidative stress similar to control (Fig. 6A).

Two classes of genes could be distinguished among those that suppress adaptation: *FOXA/pha-4* and *AMPK/aak-2* affect the stress resistance of *slcf-1* mutants only, while others modulate both *slcf-1* and wild-type stress response.

In agreement with their role in the adaptation of *slcf-1* mutants to oxidative stress, lifespan analysis revealed that *FOXA/PHA-4*, *AMPK/AAK2* and *HSF1/HSF-1* and *SIRT/SIR2.1* were required for the lifespan phenotype of *slcf-1* mutants (Table 1 and Fig. 6B–D).

Overall, our results showed that the hormetic stress response required for lifespan extension in *slcf-1(tm2258)* mutants relies on the coordinated function of several effectors as illustrated in our model Fig. S8 (Supporting information). Those effectors interact in a complex manner, because genetic interaction between these genes and *PDHK/pdhk-2* for lifespan regulation indicated that *AMPK/AAK-2* and *SIRT/SIR-2.1* would lie upstream, while other factors such as *FOXA/PHA-4* would act downstream and/or in parallel in a manner similar to *HSF1/HSF-1* and *PTEN/DAF-18* (Figs S7 and S8, Table S1). Finally, *slcf-1* lifespan phenotype also requires *FOXO/DAF-16* and *NRF2/SKN-1* (Fig. S9), which appeared not to be involved in the early adaptive process (Fig. 6A). Further molecular analysis should shed light on the precise molecular interactions between these different factors.

A question that still remains unanswered regarding the role of DR on lifespan is whether specific metabolites rather than overall nutrients levels may be critical for DR beneficial effects. A previous study (Schulz *et al.*, 2007) demonstrated that treating worms with 2-deoxy-D-glucose (2DG), a nonmetabolizable analog of glucose that inhibits glycolysis, extended average lifespan by 15% in an *AMPK/AAK-2*-dependent and *SIRT/SIR-2.1*-independent manner. Furthermore, the authors provided evidence for a mitohormetic control of lifespan in *C. elegans*. Consistent with this work, the mode of action of *SLCF-1* supports the concept of mitohormesis. However, the magnitude of lifespan extension achieved is significantly higher with *slcf-1* mutants than with glucose restriction. Moreover, our study shows that lifespan can be prolonged by increasing pyruvate concentration rather than by lowering it, as one could expect from several articles (Schulz *et al.*, 2007) (Lee *et al.*, 2009) (Schlotterer *et al.*, 2009) that have reported glucose addition to shorten *C. elegans* lifespan. One reason for this discrepancy is that glucose or 2DG treatments do not only affect pyruvate level, but also other pathways (Ralser *et al.*, 2008) that may have deleterious effects on longevity.

In *Drosophila*, Grandison *et al.* (2009) have shown that adding essential amino acids to the dietary restriction regimen increased fertility and decreased lifespan, similar to the effects of full feeding. It would thus be interesting to investigate the consequence of adding essential amino acids on various metabolite levels and particularly on pyruvate homeostasis.

Finally, considering that *SLCF-1* belongs to an evolutionary conserved gene family, the next challenge will be to identify the

precise functional mammalian orthologue. Interestingly, some mammalian *SLC16s* transporters also show a polarized expression at the basolateral membrane of intestinal cells (Iwanaga *et al.*, 2006). Specific drugs targeting *SLC16* family members are already in development (Wilson *et al.*, 2005) (Ovens *et al.*, 2010) and one can tentatively suggest that treating mammals with those drugs may mimic the benefits associated with DR on health span.

Experimental procedures

Strains and RNAi experiments

Caenorhabditis elegans strains were cultured at 20°C on nematode growth media agar plates seeded with *E. Coli* strain OP50 unless stated otherwise. Strains used were wild-type Bristol N2, *daf-16(mu86)* I, *rrf-3(pk1426)* II, *eat-2(ad465)* II, *daf-2(e1370)* III, *daf-18(e1375)* IV, *isp-1(qm150)* IV, *mev-1(kn1)* III, *rrf-3(pk1426);daf-18(e1375)*, *sid-1(qt2)* V, *sir-2.1(ok434)* IV, and RW1596 [*myo-3(st386)* V; *stEx30*, (Herndon *et al.*, 2002)]. Strains were provided by the *Caenorhabditis* Genetics Center (University of Minnesota). *slcf-1(tm2258)* X, *pdhk-2(tm3075)* III, and *pdhk-2(tm3086)* III mutants were obtained from the *C. elegans* knockout consortium directed by Pr Mitani and outcrossed five times for *slcf-1(tm2258)* mutants and two times for *pdhk-2(tm3075)* and *pdhk-2(tm3086)* mutants in our wild-type strain. *slcf-1(tm2258);daf-18(e1375)*, *slcf-1(tm2258);sir-2.1(ok434)* and *slcf-1(tm2258);daf-16(mu86)* double mutants were obtained by genetic crosses.

Bacterial feeding RNAi experiments were carried out essentially as described previously (Kamath *et al.*, 2001). Clones used were *slcf-1* (F59F5.1), *daf-16* (R13H8.1), *daf-18* (T07A9.6), *aak-2* (T01C8.1), *pha-4* (F38A6.1), *skn-1* (T19E7.2), *pdh-1* (T05H10.6), *pdh-2* (C04C3.3), *pdhk-2* (ZK370.5), *hsf-1* (Y53C10A.12), *dcr-1* (K12H4.8), and for *slc16* family members homologs: C10E2.6, C49F8.2, M03B6.2, K05B2.5, C01B4.8, Y59A8B.21, T02G5.12, F10G7.5. Clones were purchased from GeneService Ltd (GeneService Ltd, Cambridge, UK) except for *daf-2* that was kindly provided by C. Kenyon. Y59A8B.21 was constructed by inserting a PCR product containing exons 2–4 of Y59A8B.21 (EcoRI tagged primers ccggaattccggcggtat-aaatagcccaagc and ccggaattccggtcccaatgcgtttaagacc) into the EcoRI-digested RNAi feeding vector pL4440. Each clone was sequenced to confirm its identity. Double RNAi experiments were carried out by mixing the bacterial cultures directly before seeding the NGM plates. Controls were RNAi clone 50% diluted with control vector RNAi bacteria.

Cloning of *slcf-1* cDNA and protein sequences comparison

Total RNAs were isolated from mixed-stage wild-type worms using Trizol. cDNA was generated by reverse transcriptase–polymerase chain reaction (RT–PCR) (SIGMA Omniscript). *slcf-1* cDNA was amplified by using *slcf-1*-specific primers, *slcf-1F1*

(forward: CGGAATTCATGACCATTGAGAGAGCTACTCG, start codon underlined) and *slcf-1R1* (reverse: CACTTCTGTGGAAAA-TTCATAAGTAGAATTCCG stop codon underlined). This PCR product was cloned into pCR2.1-TOPO vector (Invitrogen, Carlsbad, CA, USA). Complete cDNA was sequenced and is identical to Wormbase-predicted cDNA.

The proteins most homologous to the SLCF-1 protein (NP-509762) were identified by Blast. TMS predictions and multiple sequence alignment were carried out using HMMTOP.

Construction of *slcf-1::gfp* transgene

slcf-1-predicted gene F59F5.1 is present in the fosmid clone WRM0639bB08. This clone was digested with EcoRI to release a 6.1-kb fragment that included 2410 bp upstream from start codon and 840 bp downstream from stop codon. This fragment was ligated into pBluescript digested with EcoRI. The AelI site located in the multiple cloning site of pBluescript was then eliminated by digestion with SacI. This SacI digestion removed 320 bp from 5' end of the gene and 60 bp from the vector, giving pFS23. GFP sequence was recovered from pPD119.16 (kind gift from Andy Fire laboratory) by digesting with Ecl136II and ligated in frame into pFS23 linearized with AelI. The resulting construct pFS25 contains the *slcf-1* gene with GFP inserted just before the stop codon. To test the lifespan rescue, *slcf-1(tm2258)* mutant was co-injected with 20 ng μL^{-1} of pFS25 together with the injection marker pRF4(*rol-6*) at 100 ng μL^{-1} . Three independent lines were generated.

Lifespan and stress resistance assays

Lifespan tests were performed as described by Masse *et al.* (2008). Worms lifespan assays were performed at 20°C unless noticed otherwise. Animals that crawled off the plate or had an 'exploded vulva' phenotype were censored. *slcf-1* RNAi and *slcf-1(tm2258)* lifespan assays were also performed in the absence of 5-FU and gave similar results.

NAC (N-acetyl cysteine) was used at a final concentration of 5 mM from a 0.5 M aqueous stock. Animals were exposed to NAC from the young adult stage.

Sodium pyruvate was added at a final concentration of 2.5 mM to NGM plates containing carbenicillin (100 $\mu\text{g mL}^{-1}$) and seeded with UV-killed OP50.

To lower DR effectors (*PTEN/DAF-18*, *AMPK/AAK-2*, *HSF1/HSF-1*, *NRF2/SKN-1*, *FOXO/DAF-16*, *SIRT/SIR-2.1*, *FOXA/PHA-4*) expression only during the first 5 days of adulthood, *slcf-1(tm2258)* mutants were grown on RNAi bacteria containing the empty vector from hatching to the young adult stage and then transferred to DR effectors RNAi bacteria. After 5 days of RNAi treatment, worms were transferred to plates containing paraquat and seeded with *dcr-1* RNAi bacteria. Control animals were grown during the first 5 days of adulthood on RNAi bacteria containing the empty vector and then transferred to plates containing paraquat and seeded with *dcr-1* RNAi bacteria.

Survival analyses were performed using the Kaplan–Meier method, and the significance of differences between survival curves was calculated using the log rank test. The statistical software used was XLSTAT 2007 (XLSTAT, Brooklyn, NY, USA), and all *P*-values < 0.05 were considered significant.

Paraquat assays were performed according to Masse *et al.* (2008).

Dietary restriction protocols

All experiments were carried out in 20°C incubators. Solid dietary restriction (SDR) was performed as previously described by Greer *et al.* (2007) with UV-killed bacteria. OP50 bacteria were serially diluted from 5×10^{10} to 5×10^6 bacteria mL^{-1} . Bacteria were resuspended in LB and then UV-irradiated for 5 min in a UV Stratalinker 2400 (9999 Joules m^{-2}). Adult worms were placed on these various concentrations of bacteria starting at day 7 of life (day 4 of adulthood). Dietary restriction condition was utilized 5×10^8 bacteria mL^{-1} and *ad libitum* was 5×10^{10} bacteria mL^{-1} .

Dietary deprivation (DD) was performed as previously described by Smith *et al.* (2008) with slight modifications. Briefly, OP50 bacteria were grown in 100 mL of LB in a 1-L flask O/N at 37°C, spun down at $\sim 2500g$ for 10 min, then concentrated fivefold before seeding. Control plates (NGM agar in 50-mm diameter Petri dishes) were seeded with 200 μL of concentrated OP50. The bacterial food source for all experiments was UV-killed by a 5-min exposure of plates to a UV source in a Stratalinker (9999 J m^{-2}). Lifespan assays were initiated by allowing adult hermaphrodites to lay eggs overnight on NGM containing UV-killed OP50 and carbenicillin (100 $\mu\text{g mL}^{-1}$). Three days later, L4 were transferred to fresh NGM + UV-killed OP50 supplemented with 5-FU (50 μM) to prevent eggs from hatching. At the second day of adulthood, animals were transferred to experimental media: either NGM + UV-killed OP50 + carbenicillin/5-FU or NGM + carbenicillin/5-FU. Fed animals were transferred to fresh plates every 3 days for the first 2 weeks of each lifespan experiment and then as necessary to prevent depletion of the food source. The viability of each animal was determined every 2–3 days by assaying for movement in response to agitation of the plate or gentle prodding.

Bacterial dilution was performed as previously described by Mair *et al.* (2009). Briefly, worms were grown until day 1 of adulthood on NGM plates previously seeded with OP50 bacteria. At day 1 of adulthood, 50 μL of 5-FU (100 mg mL^{-1}) were added to each plate to arrest progeny development. Twenty-four hours after the addition of 5-FU, approximately 15 worms were transferred to a six-well cell culture plate containing 3 mL of S-basal supplemented with cholesterol (5 mg mL^{-1}), carbenicillin (50 $\mu\text{g mL}^{-1}$), kanamycin (10 $\mu\text{g mL}^{-1}$), tetracycline (1 $\mu\text{g mL}^{-1}$), and 5-FU (100 mg mL^{-1}) containing OP50 bacteria at various concentrations (from optical density OD = 1.5 to OD = 0). Approximately 60 total worms were used for each condition (4 wells containing 15 worms per well). Plates were gently shaken at 20°C, and worms were scored as alive as

described before. Live worms were switched to fresh liquid medium containing the appropriate dilution of bacteria every third day.

Assessment of hydrogen peroxide level

Measurements of H₂O₂ levels were carried using a protocol slightly modified from that previously described (Harding *et al.*, 2003). Fifty synchronized young adults were washed off the plate and collected by centrifugation at 800g in M9 buffer and then washed twice in 1 mL of M9 buffer. Worms were incubated 1 h in 1 mL of M9 buffer to eliminate gut-unspecific staining because of living bacteria. Worms were then resuspended in 500 µL of DCHF-DA (dichlorohydrofluorescein diacetate; Molecular Probes Inc., Eugene, OR, USA.) and incubated on a rotator for 1 h wrapped in foil. Worms were centrifuged at 3000 rpm, washed in 1 mL M9, rapidly resuspended in 1 mL ice cold 30% sucrose solution that was then covered with 250 µL ice cold 0.1 N NaCl and centrifuged at 2200g in a 4°C table-top centrifuge for 5 min. Worms on the interphase were collected by transferring the upper phase to a new tube containing 1 mL of M9 buffer and pelleted at 3000 rpm before washing three times in 1 mL M9, and transferring onto NGM plates seeded with OP50 bacteria for 2 h. ROS levels were monitored by fluorescence microscopy.

Animals were mounted on 2% agarose pads in a droplet of 10 mM tetramisole (Sigma-Aldrich, Saint Louis, MO, USA) and examined using a Leica DMRB microscope equipped for both DIC and epifluorescence. Images were obtained using a Nikon DXM1200 Digital Camera (Nikon, Paris, France). Quantification of fluorescence intensity was performed with the software LUCIA-GF (Lucia GF, Nikon, Paris, France).

Respiration assays

Oxygen consumption rates were measured using a Clark-type oxygen electrode as previously described by Lee *et al.* (2003) with slight modifications. A thousand synchronized young adults or 5-day-old adults were washed four times by successive centrifugation at 3000 rpm for 5 min and resuspended in 500 µL of M9 buffer. Thereafter, worms were transferred to the chamber of a Clark-type oxygen electrode (Rank Brothers Ltd., Cambridge, UK) maintained at 20°C using a recirculating water bath and connected to a BD12E recorder model (Kipp and Zonen, Delft, The Netherlands). Basal endogenous respiration of animal was determined by measuring the linear rate of oxygen consumption for 10 min, then 400 µM of cyanide (potassium salt) was added to allow the measurement of the rate of nonmitochondrial oxygen consumption rate in animals. After recording the respiration rates, animal suspensions were collected from the chamber for total protein determination using the Biorad (Bio-Rad Laboratories Inc., Hercules, California, USA) method according to manufacturer's instructions. The four populations (wild-type and *slcf-1(tm2258)*, young adults and 5-day-old adults) were always assayed in

the same experiment, and the experiment was repeated three times.

Quantification of ATP

ATP levels were measured as described previously (Kimura *et al.*, 2007). Three hundred young adults or 5-day-old adults were washed four times with 1 mL M9 buffer, resuspended in cell lysis buffer and immersed in liquid nitrogen immediately. The frozen samples were boiled for 15 min to release ATP, and dilution buffer was added. Samples were centrifuged at 15000g for 5 min. The supernatant was diluted fivefold with dilution buffer. ATP levels were measured by using the ATP bioluminescent HSII kit (Roche Applied Science, Mannheim, Germany) with the Luminoskan Ascent (Termo Electron SA, Courtaboeuf, France). ATP levels were normalized to protein concentration. The four populations (wild-type and *slcf-1(tm2258)*, young adults and 5-day-old adults) were always assayed in the same experiment and the experiment was repeated three times.

¹H HR-MAS-NMR and multivariate statistics analysis

Worm samples were prepared as described by Blaise *et al.* (2007). NMR experiments were carried out on a Bruker Avance (Bruker Avance, Vienna, Austria) spectrometer operating at 700 MHz and 293 K as described elsewhere (Blaise *et al.*, 2007). ¹H chemical shifts were internally referenced to the alanine CH₃ doublet at δ = 1.48 ppm. NMR spectra were scaled to total intensity and reduced to 9000 10⁻³ ppm wide regions (buckets) from 0 to 9 ppm using AMIX (Bruker Avance, Vienna, Austria).

An O-PLS-DA model was built to discriminate between spectra obtained from the N2 and *slcf-1(tm2258)* strains. The goodness-of-fit of the model is indicated by the R² and cross-validation Q² statistics. Individual spectra were visualized on the scores plot, whereas NMR variables were visualized in the model coefficient plot or loadings plot.

Discriminant signals were attributed in reference to the spectral attribution previously performed on worms (Blaise *et al.*, 2007) and from the literature (Tukiainen *et al.*, 2008).

Acknowledgments

We are grateful to M. Kaeberlein and E.L Greer and W. Mair for their help in setting up dietary restriction protocols. We thank the *C. elegans* knockout consortium directed by Pr Mitani for *slcf-1(tm2258)* mutants and the *Caenorhabditis* Genetics Center for providing all other strains used in this work. We thank Patricia Guillaumot and Ivan Mikaelian, for their help in *slcf-1* expression in mammalian cells, and Monique Buisson, for her technical advices. We are grateful to Renaud Legouis and Gilles Mithieux for fruitful discussions.

This study was supported by grants from Association pour la Recherche sur le Cancer (ARC, F. Solari #4957) and CNRS (AO 'Longévité et Vieillessement', 2009). L. Mouchiroud and P. Kas-

turi were recipients of fellowships from the French Ministry of Research and ARC and from the Centre National de la Recherche Scientifique, respectively. Marc Dumas is funded by a Young Investigator Award from Agence Nationale de la Recherche (#ANR-07-JC-0042-01). The *Caenorhabditis* Genetics Center is funded by the NIH National Center for Research Resources (NCRR).

Author contributions

L. Mouchiroud performed all lifespan experiments, expression pattern characterization, ATP, ROS and paraquat resistance stress assays, preparation of worms for metabolomic studies and wrote the experimental procedures and legends. L. Molin and P. Kasturi isolated *slcf-1* cDNA, constructed expression vectors and transgenic lines. L. Molin performed dietary deprivation lifespan tests. M. Triba performed metabolomics analysis under the supervision of M.E.Dumas and L. Ségalat. D. Rousselle did respiration measurements. M.C. Wilson performed transport tests in *Xenopus* oocytes guided by A.P. Halestrap who also checked the manuscript for correct English style. I. Masse isolated *slcf-1* RNAi clone from the initial lifespan screen. N. Dalli  re performed liquid culture DR and pathogen resistance tests. M. Billaud decided with F. Solari of the research project and commented both on results and on the manuscript. F. Solari designed experiments, analyzed data, and wrote the paper. All authors edited the manuscript.

References

- Apfeld J, O'Connor G, McDonagh T, DiStefano PS, Curtis R (2004) The AMP-activated protein kinase AAK-2 links energy levels and insulin-like signals to lifespan in *C. elegans*. *Genes Dev.* **18**, 3004–3009.
- Bishop NA, Guarente L (2007) Two neurons mediate diet-restriction-induced longevity in *C. elegans*. *Nature* **447**, 545–549.
- Blaise BJ, Giacomotto J, Elena B, Dumas ME, Toulhoat P, Segalat L, Emsley L (2007) Metabotyping of *Caenorhabditis elegans* reveals latent phenotypes. *Proc. Natl. Acad. Sci. USA* **104**, 19808–19812.
- Blaise BJ, Giacomotto J, Triba MN, Toulhoat P, Piotto M, Emsley L, Segalat L, Dumas ME, Elena B (2009) Metabolic profiling strategy of *Caenorhabditis elegans* by whole-organism nuclear magnetic resonance. *J. Proteome. Res.* **8**, 2542–2550.
- Bluhner M, Kahn BB, Kahn CR (2003) Extended longevity in mice lacking the insulin receptor in adipose tissue. *Science* **299**, 572–574.
- Castelein N, Hoogewijs D, De Vreese A, Braeckman BP, Vanfleteren JR (2008) Dietary restriction by growth in axenic medium induces discrete changes in the transcriptional output of genes involved in energy metabolism in *Caenorhabditis elegans*. *Biotechnol. J.* **3**, 803–812.
- Colman RJ, Anderson RM, Johnson SC, Kastman EK, Kosmatka KJ, Beasley TM, Allison DB, Cruzen C, Simmons HA, Kemnitz JW, Weindruch R (2009) Caloric restriction delays disease onset and mortality in rhesus monkeys. *Science* **325**, 201–204.
- Grandison RC, Piper MD, Partridge L (2009) Amino-acid imbalance explains extension of lifespan by dietary restriction in *Drosophila*. *Nature* **462**, 1061–1064.
- Greer EL, Brunet A (2009) Different dietary restriction regimens extend lifespan by both independent and overlapping genetic pathways in *C. elegans*. *Aging Cell* **8**, 113–127.
- Greer EL, Dowlatshahi D, Banko MR, Villen J, Hoang K, Blanchard D, Gygi SP, Brunet A (2007) An AMPK-FOXO pathway mediates longevity induced by a novel method of dietary restriction in *C. elegans*. *Curr. Biol.* **17**, 1646–1656.
- Halestrap AP, Meredith D (2004) The SLC16 gene family—from monocarboxylate transporters (MCTs) to aromatic amino acid transporters and beyond. *Pflugers Arch.* **447**, 619–628.
- Harding HP, Zhang Y, Zeng H, Novoa I, Lu PD, Calfon M, Sadri N, Yun C, Popko B, Paules R, Stojdl DF, Bell JC, Hettmann T, Leiden JM, Ron D (2003) An integrated stress response regulates amino acid metabolism and resistance to oxidative stress. *Mol. Cell* **11**, 619–633.
- Herndon LA, Schmeissner PJ, Dudaronek JM, Brown PA, Listner KM, Sakano Y, Paupard MC, Hall DH, Driscoll M (2002) Stochastic and genetic factors influence tissue-specific decline in ageing *C. elegans*. *Nature* **419**, 808–814.
- Holzenberger M, Dupont J, Ducos B, Leneuve P, Geloen A, Even PC, Cervera P, Le Bouc Y (2003) IGF-1 receptor regulates lifespan and resistance to oxidative stress in mice. *Nature* **421**, 182–187.
- Hsu AL, Murphy CT, Kenyon C (2003) Regulation of aging and age-related disease by DAF-16 and heat-shock factor. *Science* **300**, 1142–1145.
- Iwanaga T, Takebe K, Kato I, Karaki S, Kuwahara A (2006) Cellular expression of monocarboxylate transporters (MCT) in the digestive tract of the mouse, rat, and humans, with special reference to slc5a8. *Biomed Res.* **27**, 243–254.
- Kalaany NY, Sabatini DM (2009) Tumours with PI3K activation are resistant to dietary restriction. *Nature* **458**, 725–731.
- Kamath RS, Martinez-Campos M, Zipperlen P, Fraser AG, Ahringer J (2001) Effectiveness of specific RNA-mediated interference through ingested double-stranded RNA in *Caenorhabditis elegans*. *J. Genome. Biol.* **2**: (1), research0002.1-research0002.10.
- Kappeler L, De Magalhaes Filho CM, Dupont J, Leneuve P, Cervera P, Perin L, Loudes C, Blaise A, Klein R, Epelbaum J, Le Bouc Y, Holzenberger M (2008) Brain IGF-1 receptors control mammalian growth and lifespan through a neuroendocrine mechanism. *PLoS Biol.* **6**, e254.
- Kemp BJ, Church DL, Hatzold J, Conradt B, Lambie EJ (2009) Gem-1 encodes an SLC16 monocarboxylate transporter-related protein that functions in parallel to the gon-2 TRPM channel during gonad development in *Caenorhabditis elegans*. *Genetics* **181**, 581–591.
- Kenyon C, Chang J, Gensch E, Rudner A, Tabtiang R (1993) A *C. elegans* mutant that lives twice as long as wild type. *Nature* **366**, 461–464.
- Kimura K, Tanaka N, Nakamura N, Takano S, Ohkuma S (2007) Knockdown of mitochondrial heat shock protein 70 promotes progeria-like phenotypes in *Caenorhabditis elegans*. *J. Biol. Chem.* **282**, 5910–5918.
- Lakowski B, Hekimi S (1998) The genetics of caloric restriction in *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. USA* **95**, 13091–13096.
- Lane MA, Ingram DK, Roth GS (1999) Calorie restriction in nonhuman primates: effects on diabetes and cardiovascular disease risk. *Toxicol. Sci.* **52**, 41–48.
- Lee SS, Lee RY, Fraser AG, Kamath RS, Ahringer J, Ruvkun G (2003) A systematic RNAi screen identifies a critical role for mitochondria in *C. elegans* longevity. *Nat. Genet.* **33**, 40–48.
- Lee SJ, Murphy CT, Kenyon C (2009) Glucose shortens the life span of *C. elegans* by downregulating DAF-16/FOXO activity and aquaporin gene expression. *Cell Metab.* **10**, 379–391.
- Lin SJ, Kaerberlein M, Andalis AA, Sturtz LA, Defossez PA, Culotta VC, Fink GR, Guarente L (2002) Calorie restriction extends *Saccharomyces cerevisiae* lifespan by increasing respiration. *Nature* **418**, 344–348.

- Maeda H, Gleiser CA, Masoro EJ, Murata I, McMahan CA, Yu BP (1985) Nutritional influences on aging of Fischer 344 rats: II. Pathology. *J. Gerontol.* **40**, 671–688.
- Mair W, Panowski SH, Shaw RJ, Dillin A (2009) Optimizing dietary restriction for genetic epistasis analysis and gene discovery in *C. elegans*. *PLoS ONE* **4**, e4535.
- Masoro EJ (2005) Overview of caloric restriction and ageing. *Mech. Ageing Dev.* **126**, 913–922.
- Masse I, Molin L, Mouchiroud L, Vanhems P, Palladino F, Billaud M, Solari F (2008) A novel role for the SMG-1 kinase in lifespan and oxidative stress resistance in *Caenorhabditis elegans*. *PLoS ONE* **3**, e3354.
- Mattson MP, Wan R (2005) Beneficial effects of intermittent fasting and caloric restriction on the cardiovascular and cerebrovascular systems. *J. Nutr. Biochem.* **16**, 129–137.
- Ovens MJ, Davies AJ, Wilson MC, Murray CM, Halestrap AP (2010) AR-C155858 is a potent inhibitor of monocarboxylate transporters MCT1 and MCT2 that binds to an intracellular site involving transmembrane helices 7–10. *Biochem. J.* **425**, 523–530.
- Panowski SH, Wolff S, Aguilaniu H, Durieux J, Dillin A (2007) PHA-4/Foxa mediates diet-restriction-induced longevity of *C. elegans*. *Nature* **447**, 550–555.
- Partridge L, Gems D, Withers DJ (2005) Sex and death: what is the connection? *Cell* **120**, 461–472.
- Ralser M, Wamelink MM, Struys EA, Joppich C, Krobisch S, Jakobs C, Lehrach H (2008) A catabolic block does not sufficiently explain how 2-deoxy-D-glucose inhibits cell growth. *Proc. Natl. Acad. Sci. USA* **105**, 17807–17811.
- Rea SL, Ventura N, Johnson TE (2007) Relationship between mitochondrial electron transport chain dysfunction, development, and life extension in *Caenorhabditis elegans*. *PLoS Biol.* **5**, e259.
- Schlottterer A, Kukudov G, Bozorgmehr F, Hutter H, Du X, Oikonomou D, Ibrahim Y, Pfisterer F, Rabbani N, Thornalley P, Sayed A, Fleming T, Humpert P, Schwenger V, Zeier M, Hamann A, Stern D, Brownlee M, Bierhaus A, Nawroth P, Morcos M (2009) *C. elegans* as model for the study of high glucose-mediated life span reduction. *Diabetes* **58**, 2450–2456.
- Schulz TJ, Zarse K, Voigt A, Urban N, Birringer M, Ristow M (2007) Glucose restriction extends *Caenorhabditis elegans* life span by inducing mitochondrial respiration and increasing oxidative stress. *Cell Metab.* **6**, 280–293.
- Selman C, Kerrison ND, Cooray A, Piper MD, Lingard SJ, Barton RH, Schuster EF, Blanc E, Gems D, Nicholson JK, Thornton JM, Partridge L, Withers DJ (2006) Coordinated multitissue transcriptional and plasma metabolomic profiles following acute caloric restriction in mice. *Physiol. Genomics* **27**, 187–200.
- Smith ED, Kaerberlein TL, Lydum BT, Sager J, Welton KL, Kennedy BK, Kaerberlein M (2008) Age- and calorie-independent life span extension from dietary restriction by bacterial deprivation in *Caenorhabditis elegans*. *BMC Dev. Biol.* **8**, 49.
- Steinkraus KA, Smith ED, Davis C, Carr D, Pendergrass WR, Sutphin GL, Kennedy BK, Kaerberlein M (2008) Dietary restriction suppresses proteotoxicity and enhances longevity by an hsf-1-dependent mechanism in *Caenorhabditis elegans*. *Aging Cell* **7**, 394–404.
- Tukiaainen T, Tynkkynen T, Makinen VP, Jylanki P, Kangas A, Hokkanen J, Vehtari A, Grohn O, Hallikainen M, Soininen H, Kivipelto M, Groop PH, Kaski K, Laatikainen R, Soininen P, Pirttila T, Ala-Korpela M (2008) A multi-metabolite analysis of serum by 1H NMR spectroscopy: early systemic signs of Alzheimer's disease. *Biochem. Biophys. Res. Commun.* **375**, 356–361.
- Walford RL, Mock D, Verdery R, MacCallum T (2002) Caloric restriction in biosphere 2: alterations in physiologic, hematologic, hormonal, and biochemical parameters in humans restricted for a 2-year period. *J. Gerontol. A Biol. Sci. Med. Sci.* **57**, B211–B224.
- Wang Y, Tissenbaum HA (2006) Overlapping and distinct functions for a *Caenorhabditis elegans* SIR2 and DAF-16/FOXO. *Mech. Ageing Dev.* **127**, 48–56.
- Wang Z, Masternak MM, Al-Regaiey KA, Bartke A (2007) Adipocytokines and the regulation of lipid metabolism in growth hormone transgenic and calorie-restricted mice. *Endocrinology* **148**, 2845–2853.
- Wilson MC, Meredith D, Fox JE, Manoharan C, Davies AJ, Halestrap AP (2005) Basigin (CD147) is the target for organomercurial inhibition of monocarboxylate transporter isoforms 1 and 4: the ancillary protein for the insensitive MCT2 is EMBIGIN (gp70). *J. Biol. Chem.* **280**, 27213–27221.
- Winston WM, Sutherland M, Wright AJ, Feinberg EH, Hunter CP (2007) *Caenorhabditis elegans* SID-2 is required for environmental RNA interference. *Proc. Natl. Acad. Sci. USA* **104**, 10565–10570.
- Wolkow CA, Munoz MJ, Riddle DL, Ruvkun G (2002) Insulin receptor substrate and p53 orthologous adaptor proteins function in the *Caenorhabditis elegans* daf-2/insulin-like signaling pathway. *J. Biol. Chem.* **277**, 49591–49597.

Supporting Information

Additional supporting information may be found in the online version of this article:

Fig. S1 Expression of SLCF-1 and transport assays for lactate and pyruvate in *Xenopus oocytes*.

Fig. S2 Survival curves of wild-type worms fed with RNAi clones corresponding to each *C. elegans* SLC16 family members.

Fig. S3 *slcf-1* mutation delays the appearance of physiological aging markers.

Fig. S4 *slcf-1::GFP* expression rescued *slcf-1* mutants lifespan phenotype and did not reduce wild-type lifespan.

Fig. S5 *slcf-1(tm2258)* mutants showed the same rhythm of pharyngeal contraction compared to wild-type animals.

Fig. S6 *SLC-1::GFP* expression is down-regulated by food intake.

Fig. S7 Genetic interaction between *pdhk* and DR effector genes for lifespan control.

Fig. S8 Model for lifespan regulation by SLC-1 in condition of dietary restriction.

Fig. S9 *daf-16* and *skn-1* are required for *slcf-1* lifespan phenotype.

Table S1 Summary of supplemental lifespan experiments.

As a service to our authors and readers, this journal provides supporting information supplied by the authors. Such materials are peer-reviewed and may be re-organized for online delivery, but are not copy-edited or typeset. Technical support issues arising from supporting information (other than missing files) should be addressed to the authors.