

Supplemental Table 1. Characteristics of the three groups of mice used in the present adult-onset calorie-restriction study recorded at 24-months of age.

	Young mice	Aged+AL mice	Aged+CR mice
Number of animals (at study entry)	8	10	10
Number of deaths (during 12 months of observation)	-	3	1
Causes of death	-	Neoplasia (n=2) Cirrhosis (n=1)	Neoplasia (n=1)
Body weight (g)	25.8 ± 1.2	35.4 ± 6.4 ^{a,c}	20.0 ± 0.6 ^b
Blood glucose (mg/dl)	120.4 ± 35.4	120.0 ± 26.6 ^c	42.6 ± 12.6 ^a
Insulin (ng/ml)	0.50 ± 0.14	1.29 ± 0.27 ^{a,c}	0.08 ± 0.01
Free fatty acid (mEq/l)	1.30 ± 0.18	0.93 ± 0.20	0.63 ± 0.03 ^b
Triglyceride (mg/dl)	59.2 ± 5.2	57.3 ± 13.4 ^d	24.6 ± 2.2 ^a
Cholesterol (mg/dl)	113.9 ± 5.1	183.4 ± 19.3 ^{a,c}	90.8 ± 8.6
Adiponectin (µg/ml)	4.08 ± 0.15	4.09 ± 0.41 ^d	5.22 ± 0.23 ^b
TNFα (pg/ml)	46.5 ± 2.1	92.2 ± 8.4 ^{a,c}	47.6 ± 2.4
Hematocrit (%)	45.4 ± 0.5	40.1 ± 1.3 ^b	41.8 ± 0.6 ^a
Left kidney weight (mg)	133.3 ± 3.8	186.6 ± 9.8 ^{a,c}	97.2 ± 10.9 ^a
Systolic blood pressure (mmHg)	112.3 ± 3.3	126.4 ± 1.7 ^{b,c}	101.4 ± 2.2 ^b

Data are mean ± SEM.

^a*p* < 0.01 vs Young mice, ^b*p* < 0.05 vs Young mice,

^c*p* < 0.01 vs Aged+CR mice, ^d*p* < 0.05 vs Aged+CR mice

Young group (n=8), Aged+AL group (n=7), Aged+CR group (n=9).
AL, ad libitum; CR, calorie restriction.

Supplemental Table 2. Characteristics of three groups of mice used in the present study of calorie-restriction/*SIRT1*^{+/-} mice at 12-months of age.

	WT+AL mice	<i>SIRT1</i> ^{+/-} +AL mice	<i>SIRT1</i> ^{+/-} +CR mice
Number of animals (at study entry)	6	10	10
Number of death (during 6 months of observation)	1	3	3
Cause of death	Neoplasia (n=1)	Neoplasia (n=3)	Neoplasia (n=3)
Body weight (g)	28.9 ± 1.8	26.9 ± 2.7	23.7 ± 0.8 ^a
Blood glucose (mg/dl)	123.4 ± 34.1	128.0 ± 16.3	62.6 ± 8.6 ^a
Insulin (ng/ml)	0.54 ± 0.34	0.76 ± 0.27	0.11 ± 0.07 ^a

Data are mean ± SEM.

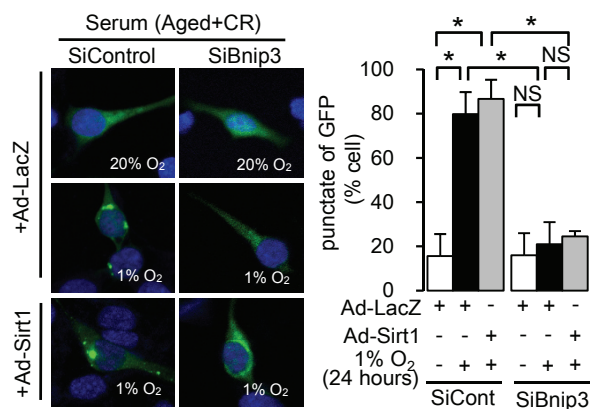
^a*p* < 0.05 vs the other groups of mice

WT group (n=5), *SIRT1*^{+/-}+AL group (n=7), *SIRT1*^{+/-}+CR group (n=7).
AL, ad libitum; CR, calorie restriction.

Supplemental Table 3. Sequences of primers used in real-time PCR.

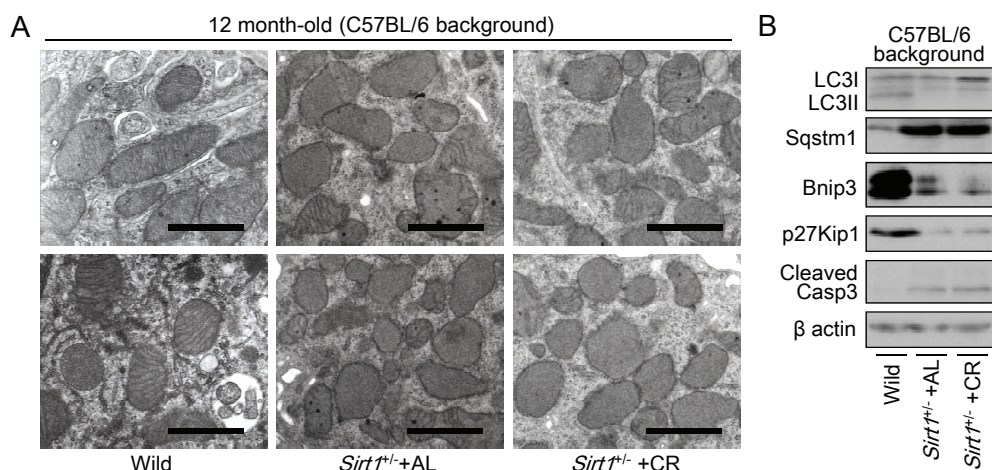
	Accession	Forward	Reverse
Primers for real-time PCR analysis			
18S	NR_003278	5'-Ttccgataacgaacgagactct-3'	5'-tggctgaacgccacttgtc-3'
Sirt1	NM_019812	5'-agaaccaccaaagcggaaaa-3'	5'-aatcccacaggagacagaaacc-3'
LC3	NM_025735	5'-aggccttctctctgctggtc-3'	5'-ccgtcttcctctctctctgtt-3'
beclin1	NM_019584	5'-tgcaggtgagcttcgtgtg-3'	5'-ctgggctgtggtaagtaaggag-3'
Atg12	NM_026217	5'-aacaagaatgggctgtggag-3'	5'-tctggggaaggggcaaa-3'
Atg5	NM_053069	5'-gagcggccttccatcca-3'	5'-cctggctcctcttctcca-3'
Bnip3	NM_009760	5'-cagcatgaatctggacgaagtag-3'	5'-tgttggtatcttgggtgtctgg-3'
Lamp2	NM_001017959	5'-aagacaactcctgtgccata-3'	5'-gaccaataaaataagccagcaac-3'
Cln3	NM_009907	5'-gggtttctggatcttgggtct-3'	5'-gttggtgggtgtgggtgtt-3'
Rab7	NM_009005	5'-ggacacagccggtcaagaa-3'	5'-catcaaacaccagaacacagca-3'
Primers for detection of point mutations in cytochrome b gene			
Cytb	YP_001686710	5'-catcgttgaattcaactgcag-3'	5'-caagaccagagtaattgtttatac-3'
Primers for detection of D-17 mutations in mitochondrial DNA with real-time PCR			
D-17	YP_001686710	5'-tcatgaccaatgaactctg-3'	5'-aggctcgcggactagtatat-3'
Cytb	YP_001686710	5'-tcgcttccacttcatcttac-3'	5'-atcctgttctgaggaggaag-3'
Primers for ChIP analysis			
HRE (Bnip3 promoter)		5'-cctagctagccggctccacttctgcatt agacc-3'	5'-cggaggatcttgcgccctcagttctg aggccagg-3'
FHRE (Bnip3 promoter)		5'-acttgcatgtgcaaggccct-3'	5'-agaagctatcttagtagcag-3'
FHRE (p27kip1 promoter)		5'-acacacacatcctggcaaaag-3'	5'-agtgtcccaaagaagcatgg-3'
FHRE (Bim promoter)		5'-gggcgggtacattctgagt-3'	5'-caggctgcgacaggtagt-3'

Supplemental Figure 1



Supplemental Figure 1. Role of Sirt1-Bnip3 interaction in hypoxia-induced autophagy under CR serum. Determination of hypoxia (1% O₂, 24 h)-induced autophagy in control and Bnip3-knockdown cells infected with either adenoviral SIRT1 or LacZ under CR serum. Data are mean ± SEM. *p<0.05. NS: no significant difference. CR: calorie restriction.

Supplemental Figure 2



Supplemental Figure 2. Effect of calorie restriction (CR) on cell adaptation in the kidney of aged *Sirt1*^{+/-} mice of C57BL/6 background. (A) Electron micrographs of renal proximal tubular cells. Scale bars: 1 μ m. (B) LC3II formation and expression levels of Sqstm1, Bnip3, p27Kip1 and cleaved caspase 3 in the kidney. Wild, 12-month-old wild type mice; *Sirt1*^{+/-}+AL: 12-month-old *Sirt1*^{+/-} mice fed ad libitum; *Sirt1*^{+/-}+CR, 12-month-old *Sirt1*^{+/-} mice on CR for 6 months. To generate *Sirt1*^{+/-} mice on C57BL/6 background, *Sirt1*^{+/-} mice were backcrossed with wild type C57BL/6 mice for more than seven generations. Male *Sirt1*^{+/-} mice and their WT littermates on C57BL/6 background were provided food and water ad libitum for first 6 months. At 6 months of age, they were divided into three groups, WT mice with AL, *Sirt1*^{+/-} mice with AL and *Sirt1*^{+/-} mice with CR, and were followed for next 6 months. At the 12 months of age, these mice were finally sacrificed and their kidney phenotypes were analyzed.