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TRPV1 Pain Receptors Regulate Longevity and Metabolism by Neuropeptide Signaling

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SUMMARY

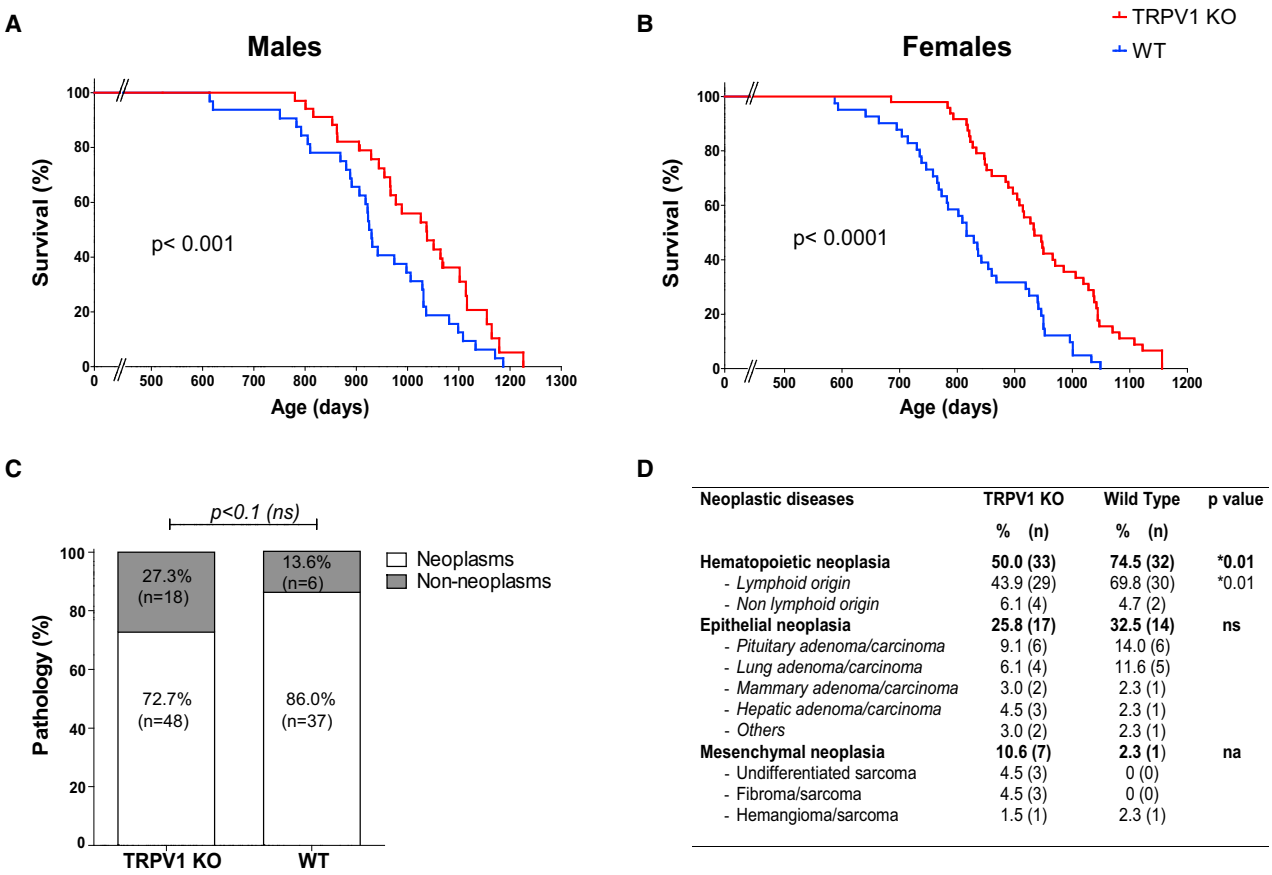
The sensation of pain is associated with increased mortality, but it is unknown whether pain perception can directly affect aging. We find that mice lacking TRPV1 pain receptors are long-lived, displaying a youthful metabolic profile at old age. Loss of TRPV1 inactivates a calcium-signaling cascade that ends in the nuclear exclusion of the CREB-regulated transcriptional coactivator CRTC1 within pain sensory neurons originating from the spinal cord. In long-lived TRPV1 knockout mice, CRTC1 nuclear exclusion decreases production of the neuropeptide CGRP from sensory endings innervating the pancreatic islets, subsequently promoting insulin secretion and metabolic health. In contrast, CGRP homeostasis is disrupted with age in wild-type mice, resulting in metabolic decline. We show that pharmacologic inactivation of CGRP receptors in old wild-type animals can restore metabolic health. These data suggest that ablation of select pain sensory receptors or the inhibition of CGRP are associated with increased metabolic health and control longevity.

INTRODUCTION

The necessity for an organism to sense potentially harmful stimuli in its environment has resulted in the evolution of receptors and their associated signaling cascades designed to recognize and appropriately respond to deleterious environments. Neural receptors that detect noxious stimuli often trigger pain sensations in order to promote avoidance and protect the organism from further harm. The transient receptor potential cation channel subfamily V member 1 (TRPV1) is expressed in afferent sensory neurons that detect extremely high temperatures and painful stimuli in target tissues such as the dermal and epidermal layers of the skin, the oral and nasal mucosa, and joints (Caterina et al., 1997, 2000). The cell bodies of these neurons relay information regarding environmental stimuli to the CNS via projection to the dorsal horn of the spinal cord. In addition to their diminished pain sensitivity, another striking phenotype of TRPV1 null mutant mice is their protection against diet-induced obesity, suggesting a prominent role for TRPV1 in metabolism (Motter and Ahern, 2008). Unmyelinated C-fibers expressing TRPV1 form a dense meshwork innervating the pancreas, and their stimulation causes the release of neuropeptides, substance P, and calcitonin gene-related peptide (CGRP), which promote either neurogenic inflammation (Noble et al., 2006) or antagonize insulin release in in vivo assays, respectively (Pettersson et al., 1986). The relationship between the release of CGRP, aging, and the overall health and metabolism of the organism, however, remains unknown.

The experience of pain necessarily causes a stress to the organism, however, what remains unknown is the extent to which the perception, rather than the experience of pain elicits long-term consequences for the organism. Clinical studies have observed a correlative decrease in lifespan and overall decrease in health in patients that experience chronic levels of pain (McBeth et al., 2003; Nitter and Forseth, 2013; Torrance et al., 2010). Likewise, inversely, in invertebrates in which the aging process is strongly affected by environmental factors, alterations in canonical sensory perception can acutely influence normal aging. For example, ablation of sensory neurons in the worm extends lifespan and mutations that impair chemosensory signal transduction increase longevity in both worms and flies (Alcedo and Kenyon, 2004; Apfeld and Kenyon, 1999; Libert et al., 2007). It is not known whether sensory perception, and especially the activation of pain receptors upon noxious stimulus, affects aging in mammals. Moreover, the underlying molecular mechanisms behind the organism-wide dissemination and reaction to this sensation, and its consequence on organismal physiology, remain relatively unknown.

In this study, we asked if reduced pain perception could positively regulate mammalian aging by targeting evolutionary conserved TRPVs. We provide evidence that mice lacking



Neoplastic diseases	TRPV1 KO % (n)	Wild Type % (n)	p value
Hematopoietic neoplasia	**50.0 (33)**	**74.5 (32)**	***0.01**
- *Lymphoid origin*	43.9 (29)	69.8 (30)	*0.01
- *Non lymphoid origin*	6.1 (4)	4.7 (2)	
Epithelial neoplasia	**25.8 (17)**	**32.5 (14)**	**ns**
- *Pituitary adenoma/carcinoma*	9.1 (6)	14.0 (6)	
- *Lung adenoma/carcinoma*	6.1 (4)	11.6 (5)	
- *Mammary adenoma/carcinoma*	3.0 (2)	2.3 (1)	
- *Hepatic adenoma/carcinoma*	4.5 (3)	2.3 (1)	
- *Others*	3.0 (2)	2.3 (1)	
Mesenchymal neoplasia	**10.6 (7)**	**2.3 (1)**	**na**
- Undifferentiated sarcoma	4.5 (3)	0 (0)	
- Fibroma/sarcoma	4.5 (3)	0 (0)	
- Hemangioma/sarcoma	1.5 (1)	2.3 (1)	

Figure 1. TRPV1 Disruption Extends Mouse Lifespan

(A and B) Kaplan-Meyer survival curves for wild-type (WT) and TRPV1 knockout (KO) mice are shown. (A) Male data. (B) Female data. Survival data are reported in Table 1.

(C) Necropsy analysis. Incidence of neoplastic and nonneoplastic diseases. Results reported as percentage of mice showing occurrence of either neoplastic or strictly nonneoplastic diseases ($p < 0.1$, ns, Fisher's exact test).

(D) Nature of neoplastic diseases. Results reported as percentage (total number) of mice with the disease (*$p < 0.05$, Fisher's exact test). ns, not significant, na, not applicable.

See also Figure S1 and Tables S1 and S2.

TRPV1 are long-lived and display a youthful metabolic profile at old age. To understand the mechanism of this increased longevity, we examined whether the chemosensory TRPVs of *Caenorhabditis elegans* also played a role in aging. We find that worm TRPVs function upstream of calcineurin to regulate the transcriptional output of the nutrient sensing cAMP-responsive element-binding protein, CREB, and its coactivator CRTC1. Similarly, the lifespan extension of mice lacking TRPV1 appears to be regulated by inactivation of the CRTC1/CREB pathway in sensory neurons, conserved with results in the worm. Mechanistically, we find that transcriptional activation of CRTC1/CREB in TRPV1 neurons induces the release of the neuropeptide CGRP that antagonizes insulin release from pancreatic β cells. Lastly, our data indicate that genetic and pharmacological homeostasis of CGRP restores metabolic health in old mice. We thus report the discovery of a novel neuroendocrine circuitry that represses longevity by adjusting the metabolic activity through the CRTC1/CREB circuit.

RESULTS

Mice Lacking TRPV1 Are Youthful and Long-Lived

We asked if loss of TRPV1, the capsaicin receptor, altered longevity in mice. To our surprise, animals lacking TRPV1 receptors were exceptionally long-lived as indicated by their Kaplan-Meier survival curves, median, and maximal lifespan (Figures 1A and 1B; Table 1). Under normal fed ad libitum conditions, the TRPV1 mutation was not sex-specific in its effects: longevity in both genders was extended to a similar extent, with 11.9% increase in male TRPV1 mutants and 15.9% increase in median female lifespan compared to wild-type, isogenic C57BL/6 controls (WT). On average, TRPV1 mutant males lived almost 100 days and TRPV1 mutant females lived 130 days longer than control animals (mean lifespan, Table 1). Maximum lifespan was strongly extended in female mutant mice.

We assessed cancer incidence by conducting postmortem necropsy and histopathology (Figures 1C and 1D). The

Table 1. Comparative Survival Characteristics of TRPV1 Mutant and WT Mice

	n	Median	Min–Max	Mean	Log Rank Test	Max Lifespan
Females						
TRPV1 KO	48	946	685–1,156	957.48 ± 16.66	$\chi^2 = 18.35$, $p < 0.0001$	$p < 0.001$
WT	42	816	375–1,049	827.98 ± 18.47		
Males						
TRPV1 KO	38	1,038	780–1,226	1,036.31 ± 23.32	$\chi^2 = 9.92$, $p < 0.001$	ns
WT	32	927.5	614–1,187	937 ± 24.79		

prevalence of cancer tended to be reduced in TRPV1 mutant versus control mice (Figure 1C). Neoplasms, which are the primary cause of death observed in our mouse cohort, comprised a wide panel of cancer types (Figure 1D). Notably, the incidence of hematopoietic neoplasia of lymphoid origin, the most prevalent cancer observed in both genotypes, was reduced in TRPV1 mutant animals. Other cancer types including sarcomas, carcinomas, pituitary, and lung adenomas were also present in both genotypes, however, the small number of observations did not allow for any conclusion regarding the incidence of these pathologies among genotypes. Furthermore, we did not observe significant differences in the incidence of nonneoplastic diseases between genotypes (Figure 1C; Table S1 available online).

Consistent with improved longevity and reduced aging, 30-month-old TRPV1 mutant mice displayed improved spatial memory compared to their age-matched control animals when challenged with a training, retention, and reversal paradigm in a Barnes maze test (Figures S1A–S1C). Decline in motor coordination, measured by the latency to fall from a rotating rod, was also delayed in the long-lived TRPV1 mutant mice (Figure S1D).

Because the presence of TRPV1 has been detected in the hippocampus (Mezey et al., 2000; Sasamura et al., 1998), we examined the neuronal density in the CA1, CA3, and dentate gyrus (DG) areas of the hippocampus by Nissl staining (Figure S1E). The distribution of Nissl-positive neurons in these areas was similar in WT and TRPV1 mutant mice, suggesting that improved cognitive function in the TRPV1 mutants is not due to increased neuronal numbers in the TRPV1 mutant animals, but rather to a global improved healthspan in these mice.

Long-Lived TRPV1 Mutant Mice Do Not Have Reduced Growth Rates or Altered IGF-1 Levels

Reduced growth hormone (GH) and/or insulin growth factor (IGF-1) signaling have been tightly linked to longevity, often resulting in delayed growth and small adult animals (Blüher et al., 2003; Ortega-Molina et al., 2012; Selman et al., 2008). We initially hypothesized that the increased longevity and health of the TRPV1 mutant strain was caused by inactivation of the GH/IGF-1 axis. Unlike GH/IGF-1 mice, TRPV1 mutant mice appeared similar in size to WT mice (Figure 2A). Their growth curves were identical to WT controls in both genders throughout aging (Figure 2B), in contrast to mouse models with altered activity in either growth hormone (GH) or IGF-1 signaling (Blüher et al., 2003; Brown-Borg et al., 1996; Coschigano et al., 2000, 2003; Flurkey et al., 2001, 2002; Holzenberger et al., 2003; Ortega-Molina et al., 2012; Selman et al., 2008, 2009; Zhang et al., 2012). Analysis of body composition of age- and gender-matched controls confirmed that organs including lung, liver, kidney, spleen, pancreas, brain, heart, and different fat pads (inguinal, gonadal, and retroperitoneal) were the same size in both genotypes (Figure 2C). Basic histological analysis by hematoxylin and eosin (H&E) staining of key metabolic tissues (liver, white fat, heart) did not reveal morphological differences between the wild-type and TRPV1 mutant mice (Figure S2). Consistent with a normal body weight, GH content in the pituitary was normal in the TRPV1 mutant adult mice (Figure 2D). In addition, plasma levels of IGF-1, which is regulated by GH, were identical in both genotypes (Figure 2E). Therefore, loss of TRPV1 appears to modulate the aging process in a mechanism independent of the GH/IGF-1 axis.

Long-Lived TRPV1 Mutant Mice Have a Youthful Metabolic Profile

Altered metabolic programs have been associated with many longevity pathways ranging from worms to mice. We investigated whether the TRPV1 mutant mice had a distinct metabolic profile in comparison to wild-type animals. Analysis of body temperature, indicative of metabolic activity and reported to be low in dietary restricted and long-lived Ames dwarf mice (Brown-Borg et al., 1996), was unaffected in age-matched TRPV1 mutant mice compared to control animals (Figure S3A). Similarly, food intake, which can influence lifespan through dietary restriction, was unaffected (Figure S3A). A general assessment of blood factors indicative of metabolic activity revealed no difference in fasting insulin levels, leptin, adiponectin, or circulating triglycerides and cholesterol across genotypes (Figure S3B). There were no alterations in the expression of brown fat-specific genes characteristic of thermogenesis and brown fat development in the interscapular brown adipose tissue (Figure S3C). However, we found that TRPV1 mutant animals retained a youthful metabolic program compared to age-matched control mice (Figure 3A). In particular, the respiratory exchange ratio (RER), which measures the daily transition from glucose to lipid metabolism, retained a youthful circadian shift from night to day in old TRPV1 mutant animals. At 3 months, both WT and TRPV1 mutant mice displayed identical circadian oscillations from night to day. However, at late age, the TRPV1 mutants retained circadian transitions of similar order of magnitude compared to that of young mice, whereas WT animals lost their circadian shift. In fact, decline in energy expenditure involving a lower RER is commonly observed in old mice indicating that aging mice have a proportional substrate preference toward fat (Houtkooper et al., 2011). We clearly observe that the TRPV1 mutant mice do not have this preference in late life.

Concurrent with improved metabolism, oxygen consumption was enhanced in TRPV1 mutants at both young and old time points compared to age-matched WT controls at the peak of nocturnal activity (Figure S3D). TRPV1 mutants maintained their VO_2 maximum consumption late in life, in contrast to WT animals that display a decline in maximum VO_2 consumed with age. Interestingly, we also found that voluntary activity was slightly

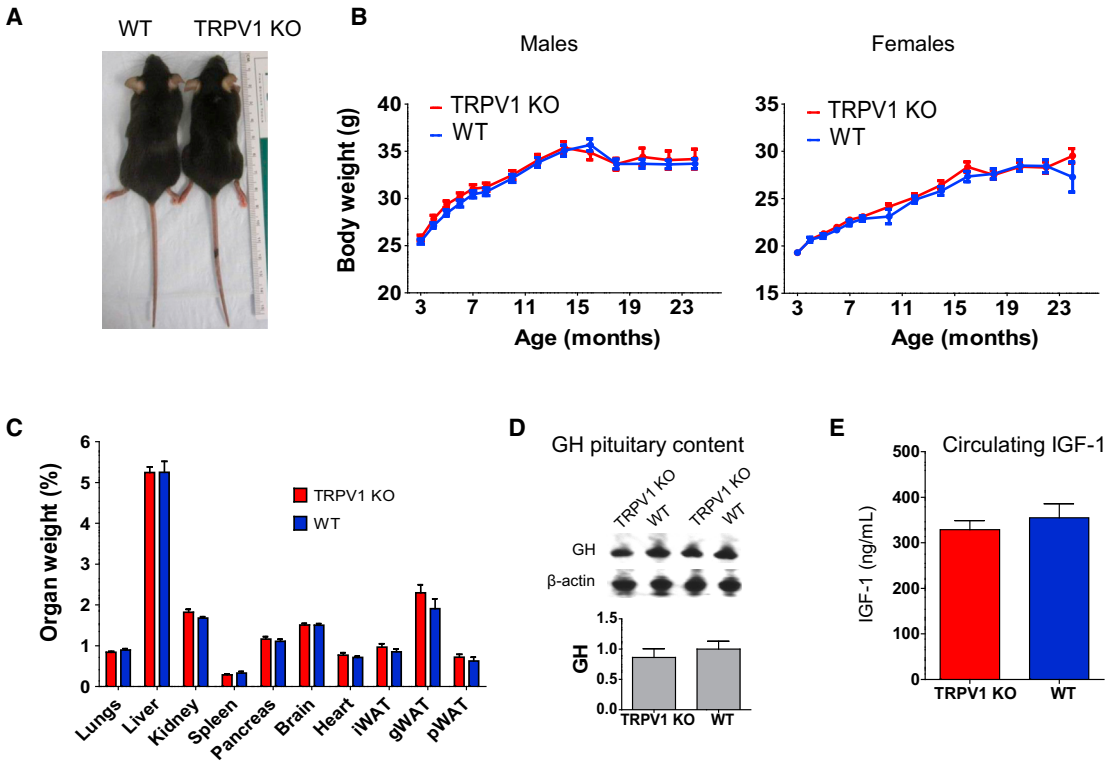


Figure 2. Evaluation of the Requirement of the GH/IGF-1 Axis in TRPV1 Mutant Mice

(A) Representative pictures of adult TRPV1 KO (6 months old, left: WT, right: TRPV1 KO).

(B) Whole-body growth of TRPV1 KO and WT mice from 3 to 24 months old, (left) male data (TRPV1 KO, n = 38; WT, n = 32), (right) female data (TRPV1 KO, n = 48; WT, n = 42). Means ± SEM, ns, two-way ANOVA.

(C) Body composition of 24-month-old TRPV1 KO and WT males. Organ weight of lungs, liver, kidney, spleen, pancreas, brain, heart, inguinal white adipose tissue (iWAT), gonadal white adipose tissue (gWAT), and peritoneal white adipose tissue (pWAT) are expressed as a percentage of total body weight. Means ± SEM, ns, Student's t test.

(D) Western blotting of growth hormone (GH) from pituitary glands (n = 5 mice per lane, 6 months old). Loading control: β-actin.

(E) ELISA analysis of plasma IGF-1 levels in fed male mice (n = 15, 6 months old). Means ± SEM, ns, Student's t test.

See also Figure S2.

higher in young TRPV1 mutant animals but diminished to the same extent as WT when old (Figure S3E). Taken together, these data suggest than the youthful energy expenditure observed in old TRPV1 mutant animals is not due to increased voluntary exercise, but appears to be due to more efficient maintenance of metabolism with age.

Long-Lived TRPV1 Mutant Mice Have Altered Insulin Metabolism

To uncover the mechanism underlying the youthful metabolic phenotype of the TRPV1 mutant mice, we characterized the glucose metabolic profile of these mice. We hypothesized that TRPV1 mutant animals should be glucose tolerant as suggested previously by studies focusing on chemo-denervation or pharmacological inhibition of TRPV1 channels (Gram et al., 2007; Tanaka et al., 2011). We found that TRPV1 mutant mice were markedly more glucose tolerant than WT at 3 months and 22 months of age, which correspond respectively to "young" and "old" time points in a glucose tolerance test (GTT) (Figure 3B). Fasted glucose levels were unchanged between mutant and wild-type mice. As improved glucose tolerance is often associated with increased insulin sensitivity, we analyzed how efficiently TRPV1 mutant mice were clearing blood glucose after insulin injection through an insulin tolerance test (ITT) (Figure 3C). Despite improved glucose tolerance, we found that blood glucose levels from young TRPV1 mutant mice decreased by ~40% following acute insulin challenge, contrary to a 67% drop in WT controls. Both old WT and TRPV1 mutant mice displayed age-onset insulin resistance, expressed by a diminished glucose response to insulin challenge relatively to the young time point.

As insulin resistance is a hallmark of type 2 diabetes, leading to β cell failure, we analyzed pancreatic islet morphology in adult mice. We found no difference in islet architecture when staining for insulin and glucagon in islets from mutant or wild-type mice, suggesting that TRPV1 mutant islets are normal (Figure 3D). Further investigation revealed that β cell mass was increased in the TRPV1 mutant mice, suggesting that β cell survival was improved in these mice independently of their proliferation or neogenesis rate (Figures 3E and S3F). Gene expression of isolated islet mRNA through quantitative real-time PCR (qRT-PCR) did not reveal transcriptional differences between TRPV1

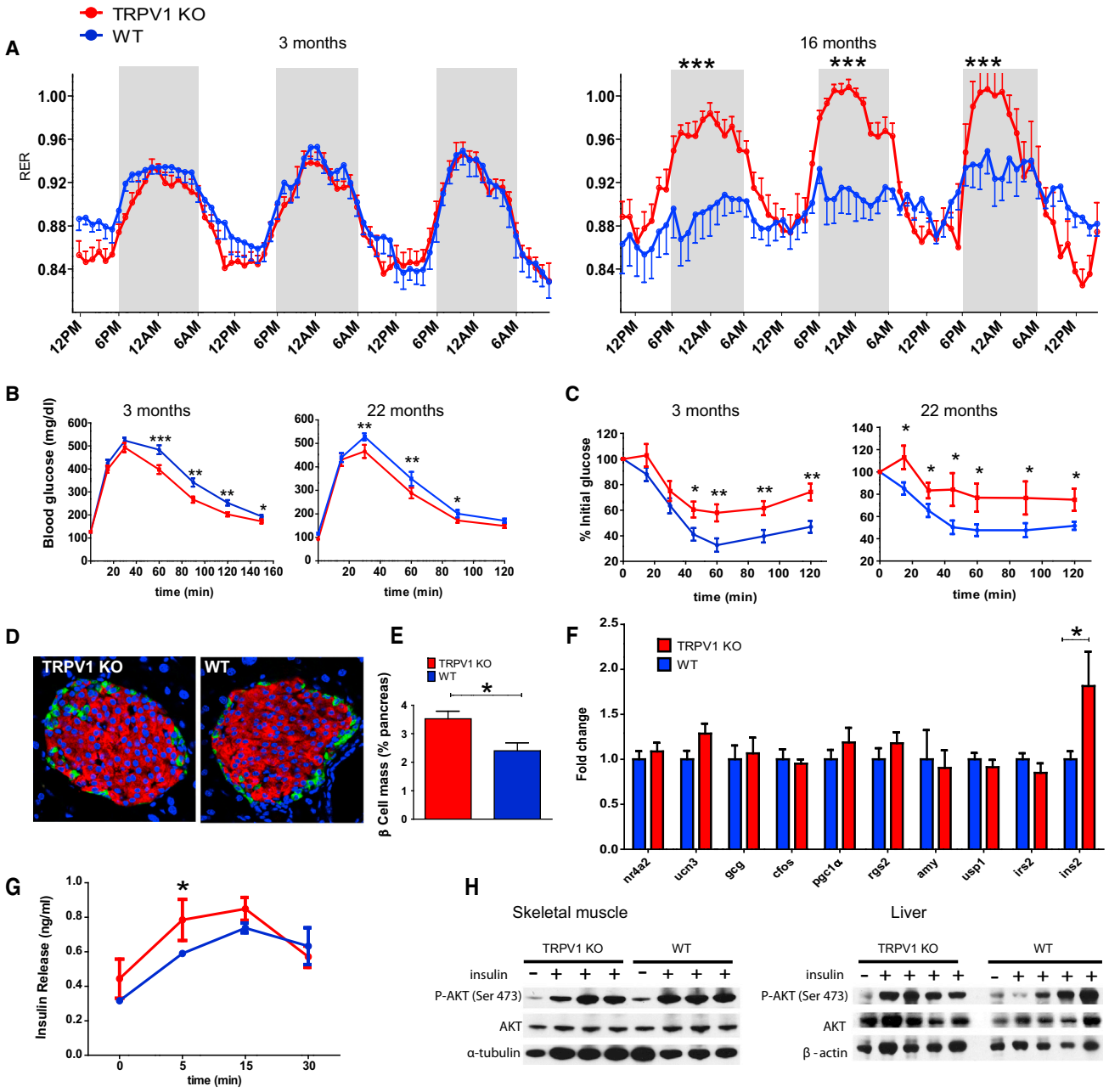


Figure 3. Analysis of Whole-Body Energy Expenditure and Insulin Resistance

(A) Respiratory exchange ratio (RER) analysis of males TRPV1 KO and WT controls. n = 8, means ± SEM, ***p < 0.0001, one-way ANOVA.

(B) Glucose tolerance tests at 3 months and 22 months, n = 16, means ± SEM, ***p < 0.0001, **p < 0.001, *p < 0.01, Student's t test.

(C) Insulin tolerance test at 3 months and 22 months expressed as a percentage of the initial glucose levels, n = 11–16, means ± SEM, **p < 0.001, *p < 0.01, Student's t test.

(D) Pancreata were fixed and immunolabeled against insulin (red) and glucagon (green), DAPI nuclear dye (blue).

(E) β cell mass of 24-month-old TRPV1 KO and WT mice, n = 4, means ± SEM, *p < 0.05, Student's t test.

(F) Quantitative PCR analysis of isolated pancreatic islets from TRPV1 KO and WT mice, n = 8, means ± SEM, *p < 0.01, Student's t test.

(G) Glucose stimulated insulin secretion assay, n = 5, means ± SEM, *p < 0.01, Student's t test.

(H) Western blot analysis of phospho-AKT (Ser 473) and total AKT in TRPV1 KO and WT muscle (left) and liver (right) in sham control (–) and insulin-stimulated (+) mice, n = 5. α-tubulin and β-actin were respectively used as loading controls for muscle and liver tissues.

See also Figure S3.

mutants and WT islet endocrine markers (Figure 3F). Levels of glucagon (gcg), urocortin-3 (ucn3), amylin (amy), insulin-receptor substrate 2 (irs2), the nuclear hormone receptor nr4a2, the fos gene cFos, the ubiquitin carboxyl-terminal hydrolase 1 (usp1), the regulator of G protein signaling 2 (rgs2), and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (pgc1α) were unchanged across genotypes.

Because of the increased β cell mass observed in the TRPV1 mutant mice, we assumed that the improved glucose tolerance was achieved through insulin hypersecretion in response to glucose in order to compensate for their mild insulin resistance. Consistent with this hypothesis, either chemo-denervation of TRPV1 neurons or chemical inhibition of TRPV1 in rodents increases glucose-dependent insulin secretion (Gram et al., 2007; Tanaka et al., 2011). Indeed, qRT-PCR analysis of pancreatic islet mRNA revealed that the insulin gene (ins2) was ~2-fold upregulated in the TRPV1 mutant cells. To directly assess the link between TRPV1 and insulin secretion, we performed a glucose stimulated insulin secretion experiment (GSIS) where insulin levels are monitored upon glucose injection (Figure 3G). We found that insulin release was significantly more pronounced in TRPV1 mutant mice than WT, thus explaining the improved glucose tolerance of TRPV1 mutants. Consistent with TRPV1 mutant mice being healthy and not harboring symptoms of type 2 diabetes, fasted insulin levels were similarly low as compared to wild-type mice (Figures 3G and S3B). Therefore, the TRPV1 mutant mice are not hyperinsulinemic, but rather display an elevation in prandial insulin to respond more robustly to glucose challenge.

To further investigate the nature of the insulin resistance of the TRPV1 mutant mice, we evaluated insulin-stimulated signaling in liver and skeletal muscle by analyzing AKT phosphorylation, which constitutes a downstream effector of the insulin receptor pathway. Upon intravenous insulin injection, phosphorylation of serine 473 of the serine/threonine kinase AKT protein was unaltered in both tissues of the TRPV1 mutant animals compared to control animals (Figure 3H), indicating that insulin signaling is functional in TRPV1 mutants in response to insulin.

Taken together, loss of TRPV1 results in long-lived mice that are not growth-retarded and do not have altered IGF-1 levels or responsiveness, yet have a youthful, metabolic profile with increased glucose tolerance mediated by increased insulin secretion upon glucose challenge. These data suggest that impaired TRPV1 sensory neuron function has beneficial effects on glucose homeostasis and longevity. Intrigued by the ability of these mice to live long by a mechanism not canonical to established longevity paradigms (GH/IGF-1 and dietary restriction), we sought to uncover the mechanism by which TRPV1 neurons could modulate this prolongevity signal.

C. elegans TRPV Regulates Lifespan through CRTC1/CREB

Confronted with the complexity of the mammalian neuroendocrine system and its impact upon metabolism, we turned to the nematode *C. elegans* to reveal the possible mechanism by which TRPV1 could modulate metabolism and the aging process. Despite a higher level of integration and compensation, the functional organization of the mammalian chemosensory organs displays many striking similarities to worms at both anatomical and signaling levels. Conserved signaling molecules include members of the TRPV channel family. In worms, *osm-9* and *ocr-2* function as a channel complex that transduces signals from olfactory, nociceptive, and serotonergic neurons (Colbert et al., 1997; Tobin et al., 2002; Zhang et al., 2004). Much like the mouse, loss of TRPV in the worm resulted in increased longevity (Figure 4A; Table S3). Using null mutants of both *osm-9(ky4)* and *ocr-2(ak47)*, we observed that loss of either *trpv* resulted in a modest increase in longevity, consistent with the functional redundancy of this receptor pair (Colbert et al., 1997; Tobin et al., 2002; Zhang et al., 2004). However, loss of both *osm-9* and *ocr-2* resulted in a robust extension of lifespan up to 32% compared to control animals.

In response to ligands, TRPV receptors transduce signals to the cytoplasm of affected cells through intracellular calcium increase (Venkatachalam and Montell, 2007). One of the major transponders of calcium flux in the cell is calcineurin (Mellström et al., 2008). The worm calcineurin ortholog, the calcium-activated calcineurin catalytic A subunit, *tax-6*, plays an intricate role in the aging process (Dong et al., 2007; Mair et al., 2011). Loss of *tax-6* results in long-lived animals and hyperactivation results in short lifespan. One essential target of *tax-6* to regulate the aging process in worms is the highly conserved *crtc-1* (CREB-regulated transcriptional coactivator 1). Dephosphorylation of CRTC1 on serines 76 and 179 by *tax-6* results in nuclear localization, modulation of CREB transcriptional targets and reduced longevity. Opposing calcineurin, AMPK monitors energy sources and phosphorylates CRTC1, retaining CRTC1 in the cytoplasm. Consistent with loss of *tax-6* resulting in increased longevity, increased activity of AMPK results in increased longevity through phosphorylation of CRTC1 at serines 76 and 179, sites counteracted by *tax-6* (Mair et al., 2011). While the circuitry downstream of calcineurin to regulate longevity is well established, upstream mediators of this pathway are unknown.

Because of the link between longevity and calcium regulation, we asked if *tax-6* and *crtc-1* could function downstream of *trpv*-mediated longevity. Upon tricaine treatment, a drug that increases intracellular calcium in cells, CRTC1 shuttles to the nucleus in wild-type animals, but remains strictly cytoplasmic in *tax-6(ok2065)* mutants (Mair et al., 2011). Similarly to *tax-6* mutant worms, *trpv* mutants (*osm-9; ocr-2* double-mutant animals) retained cytoplasmic localization of CRTC1 upon tricaine treatment (Figure 4B), indicating that TRPV functions upstream within the *tax-6/crtc* pathway.

Consistent with TRPV functioning upstream in the calcineurin pathway, the increased longevity caused by loss *of trpv* in the worm was completely dependent upon the CRTC1 longevity pathway. Inactivating *tax-6*, which extends lifespan in wild-type animals, did not further increase the lifespan of the *trpv* mutants (Figure 4C). Concordant with *tax-6* modulating longevity through posttranslational modifications of CTRC1, we found that the increased longevity of the *trpv* mutants was abrogated when *crtc-1* was mutated at the calcineurin dephosphorylation sites S76A, S179A, making it constitutively nuclear (Figure 4D). Therefore the lifespan extension caused by loss of *trpv* signaling was completely dependent on nuclear exclusion of CRTC1 at the same phosphorylation sites used for regulation by AMPK and

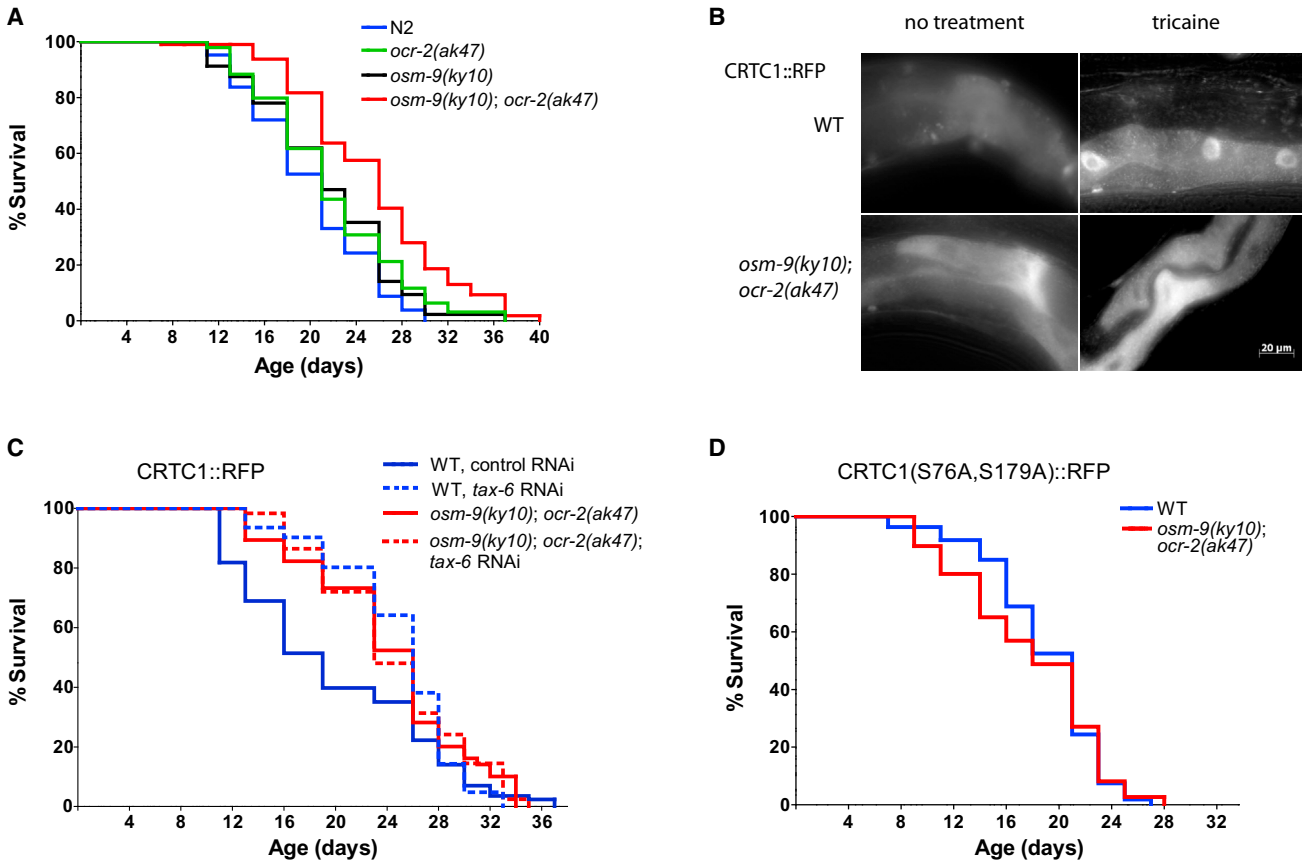


Figure 4. *trpv* Mutations in *C. elegans* Require *crtc-1* to Regulate Lifespan

(A) Survival curves of *trpv* double-null mutant worms *osm-9* (*ky10*); *ocr-2* (*ak47*) compared to single mutants.

(B) Representatives images of CRTC1::RFP cytoplasmic to nuclear shuttling in intestinal cells upon tricaine treatment in *trpv* mutant (*osm-9 (ky10); ocr-2 (ak47)*) worms and WT control.

(C) Survival curves of transgenic worms expressing CRTC1::RFP on calcineurin (*tax-6*) RNAi (dashed lines) compared to control worms fed GFP RNAi (plain lines).

(D) Survival curves of the *trpv* mutants in a constitutively nuclear *crtc-1* strain mutated at the TAX-6 dephosphorylation sites (S76A, S179A) compared to WT controls. All statistical analysis can be found in Table S3.

calcineurin. Taken together, these results indicate that a subset of chemosensory neurons utilizes a TRPV calcium signaling cascade to adjust the worm metabolism to environmental conditions by modulating CRTC1/CREB activity that ultimately dictates longevity of the animal.

TRPV1 Regulates CRTC1/CREB in Mice

Intrigued by the finding that TRPV acts upstream of the calcineurin signaling cascade that regulates CRTC1/CREB to profoundly affect aging in the worm, we asked if this same system might be engaged in the long-lived TRPV1 mutant mice. We examined the cellular distribution of the most abundant CREB-regulated transcriptional coactivator in neuronal tissues, CRTC1, in dissociated dorsal root ganglion (DRG) neuronal cultures from TRPV1 mutant and wild-type mice (Figure 5).

Previous findings indicate that in hippocampal cultures, CRTC1 shuttles to the nucleus of excitatory cells under glutamatergic synaptic activity using a calcium/calcineurin cascade (Ch'ng et al., 2012; Kovács et al., 2007). The persistence of the nuclear localization of CRTC1 is regulated by cAMP levels. To evaluate whether calcium and cAMP cascades regulate dynamic shuttling of CRTC1 in DRG neurons, we analyzed the nuclear to cytoplasmic trafficking of CRTC1 in the cell bodies of DRG neurons isolated from TRPV1 mutants by pharmacologically manipulating these pathways (Figure 5A). Immunoreactivity either directly against TRPV1 or the TRPV1 comarker CGRP allowed for recognition of small to medium-size nociceptor neurons expressing TRPV1 (Bernardini et al., 2004; Caterina et al., 1997; Price and Flores, 2007; Szallasi et al., 1995). We observed CRTC1 immunoreactivity in peripheral DRG neurons, with a cellular distribution similar to hippocampal cultures (Ch'ng et al., 2012; Kovács et al., 2007). Indeed, under basal conditions, CRTC1 localizes to axons, dendrites and the soma of the primary neurons (Figure 5B). We then used the sodium channel blocker Tetrodotoxin (TTX) to reduce endogenous synaptic activity and prevent calcium entry through NMDA receptors. Under TTX pretreatment, the distribution of CRTC1 remained mostly cytoplasmic as observed in basal conditions (Figure 5C).

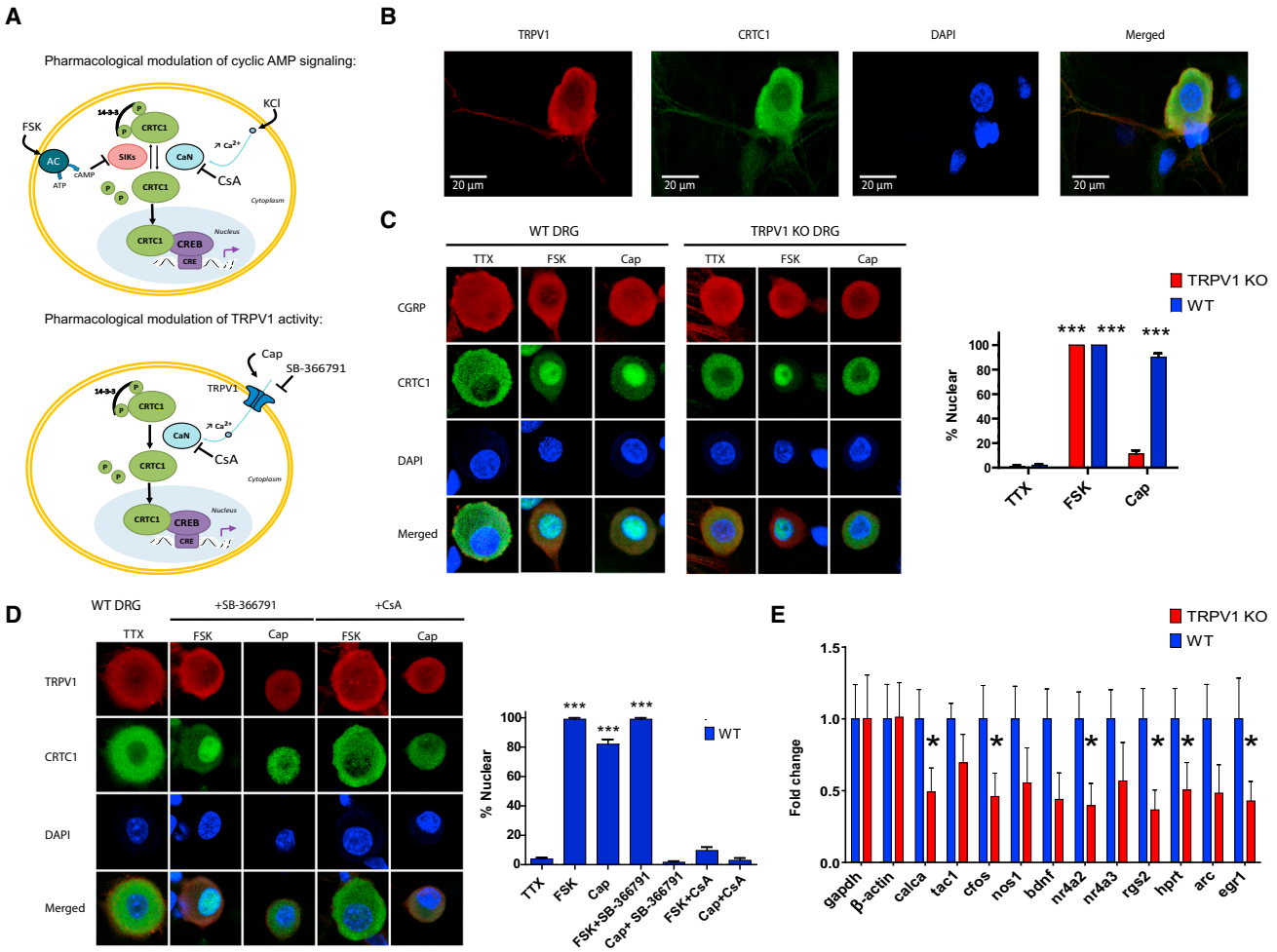


Figure 5. Nuclear Translocation of CRTC1 Requires Activation of TRPV1

(A) Schematic representing the modulation of cAMP signaling and TRPV1 activity on CRTC1 shuttling in DRG neurons upon the pharmacological manipulations used in (B–D). Maximal projections of z stack sections throughout the nucleus of the neuronal bodies.

(B) Wild-type mouse DRGs primary cultures were immunostained with antibodies against TRPV1 (red), CRTC1 (green), DAPI (blue).

(C) Left: DRG neuronal cultures were subjected to FSK (25 µM), agonist of L-type calcium channels inducing cAMP release, and Cap (1 µM), a potent TRPV1 agonist. Immunostaining was performed after fixation with antibodies against CGRP (red), CRTC1 (green), DAPI (blue). Right: quantification of the mean nuclear to cytoplasmic ratio, relative to vehicle treatment. n = 90–120 cells analyzed, means ± SEM, ***$p < 0.0001$, Student's t test.

(D) Left: WT DRGs cultures were pretreated with vehicle, the calcineurin inhibitor CsA (10 µM) and the TRPV1 antagonist SB-366791 (10 µM) prior to 1 hr stimulation with FSK and Cap. Neurons were fixed and immunostained with antibodies against TRPV1 (red), CRTC1 (green), DAPI (blue). Right: quantification of the mean nuclear to cytoplasmic ratio, relative to vehicle treatment. n = 90–120 cells analyzed, means ± SEM, ***$p < 0.0001$, Student's t test.

(E) Quantitative PCR analysis was carried out to examine CREB-dependent transcript induction in untreated DRG neurons from TRPV1 KO and WT, n = 7, means ± SEM, *$p < 0.05$, Student's t test.

To establish conditions to monitor CRTC1 shuttling, stimulation of the neurons with a combination of Forskolin (FSK), which raises cAMP levels therefore decreasing CRTC phosphorylation (Kovács et al., 2007; Screaton et al., 2004), and KCl, which triggers calcium entry and activation of calcineurin resulting in CRTC dephosphorylation, induced CRTC1 nuclear entry in WT and TRPV1 mutant DRG cells and served as our positive control (Figure 5C).

Activation of L-type voltage-gated calcium channels (LVGCCs) provokes robust calcium currents in neurons and results in hippocampal CRTC1 shuttling (Ch'ng et al., 2012). To test whether activation of TRPV1 and subsequent calcium entry were modulating CRTC1 directly, we applied a natural TRPV1 agonist, Capsaicin (Cap) to DRG neurons and observed accumulation of CRTC1 in the nuclei of CGRP-positive cells of WT DRGs cultures (Figure 5C). The nuclear entry was similar to treatment with FSK and KCl (Figure 5C). More importantly, Cap-induced CRTC1 shuttling was abolished in TRPV1 mutant DRG neurons (Figure 5C). As an additional test of TRPV1's role in CRTC1 nuclear shuttling, blockade of TRPV1 by a selective chemical antagonist of TRPV1 (SB-366791) resulted in robust nuclear exclusion of

CRTC1 in WT DRG neurons (Figure 5D), much like that found in TRPV1 mutant DRG neurons.

These findings demonstrate that calcium entry through TRPV1 is sufficient to promote nuclear redistribution of CRTC1 and is likely to cause calcineurin activation. To examine the requirement of the calcineurin pathway downstream of TRPV1, we incubated the DRG neurons with the calcineurin inhibitor Cyclosporin A (CsA). As observed previously in hippocampal cells (Kovács et al., 2007), CsA prevented FSK+KCl-mediated CRTC1 nuclear accumulation in TRPV1 positive DRG neurons (Figure 5D). CsA pretreatment also inhibited Cap's ability to force CRTC1 into the nucleus in WT DRG neurons (Figure 5D). Taken together, our data clearly indicate that TRPV1 is a potent regulator of CRTC1 shuttling via calcineurin activity in both invertebrates and vertebrates.

TRPV1 Regulates CREB Target Genes in DRGs

The ability of CRTC1 to shuttle to the nucleus under TRPV1 activity demonstrates the existence of a plastic transcriptional mechanism adapting rapidly to external outputs. The nuclear exclusion of CRTC1 in TRPV1 mutant DRG neurons suggests that CREB transcriptional activity is likely to be altered in the DRG neurons of TRPV1 mutant mice. Under inflammatory conditions, TRPV1 expressing DRG neurons utilize a CREB signaling cascade to induce neurogenic inflammation through the release of CGRP, by the binding of CREB onto the CGRP promoter (Nakanishi et al., 2010). We hypothesized that in the TRPV1 mutant mice, CREB transcriptional activity is downregulated due to the nuclear exclusion of CRTC1. Indeed, mRNA expression analysis from DRG neurons revealed that previously characterized CREB target genes were reduced in long-lived TRPV1 mutants compared to wild-type animals (Figure 5E).

CGRP, but Not Substance P, Regulates Insulin Secretion

The long-lived TRPV1 mutant mice enjoy a youthful metabolic program characterized by improved glucose homeostasis due to increased insulin secretion upon glucose challenge. Because the DRG neurons form a dense meshwork innervating pancreatic β cells (Akiba et al., 2004; Razavi et al., 2006), we hypothesized that the TRPV1 neurons within the DRG might secrete a factor that inhibits insulin release from pancreatic β cells, thereby establishing accurate insulin homeostasis. Furthermore, because CRTC1 localization is altered in the TRPV1 neurons, we speculated that the secreted factor(s) that stem from TRPV1 neurons in the DRG might be under transcriptional control by CRTC1/CREB. In our analysis of CREB regulated genes within DRG neurons, the induction of calcitonin-related polypeptide α (calca) and tachykinin 1 (tac1) transcripts, precursors of two TRPV1 secreted neuropeptides, CGRP and substance P, respectively, were reduced in the TRPV1 mutant mice (Figure 5E). Therefore, we asked whether either substance P or CGRP could affect insulin secretion from pancreatic β cells.

To mimic pancreatic β cell glucose-mediated insulin release, we used mouse insulinoma MIN6 cells (Huising et al., 2010). When stimulated with 16.8 mM of glucose, MIN6 cells release up to 150 ng/ml of insulin. However, when the same cells are treated with doses ranging between 100 nM to 500 nM of either recombinant rat α-CGRP or β-CGRP (Figures 6A and 6B), which are nearly identical at the amino acid level (Emeson et al., 1989), we found that insulin release was greatly blunted. In particular, 100 nM α-CGRP treatment significantly reduced insulin release by 46% and 500 nM β-CGRP by 58% of the maximum insulin response. Human α-CGRP also exhibited significant blockade of the insulin response, albeit to a lesser extent (Figure S4A). Furthermore, treatment with 100 nM to 500 nM substance P did not alter insulin secretion of MIN6 cells, suggesting that CGRP's effect on insulin secretion is specifically mediated by a CGRP receptor (Figure 6C). Interestingly, CGRP did not inhibit the potentiation of glucose-stimulated insulin secretion under high glucose by incretins as observed upon challenge with Exendin 4 (Figure S4B). These data confirm previous observations that CGRP inhibits glucose-mediated insulin secretion from pancreatic β cells (Ahrén et al., 1987; Kogire et al., 1991).

CGRP Homeostasis Modulates Metabolic Health

The pathogenesis of type 2 diabetes involves a low-grade inflammation, which might continuously stimulate TRPV1 neurons and therefore putatively sustain CGRP release and exacerbation of the inflammatory response (Suri and Szallasi, 2008). Consistent with these results, we observed a trend toward lower levels of inflammatory markers including chemokines and pro-inflammatory genes in brain and muscle tissue from TRPV1 mutant mice (Figure S4C). Similarly, aging results in chronic low-grade inflammation (Woods et al., 2012) and increased levels of circulating CGRP have been detected in aged rats (Melnyk and Himms-Hagen, 1995). Taken together, decreased levels of circulating CGRP might be beneficial for improved metabolic function and longevity, while high levels might be detrimental. To test this hypothesis, we measured circulating CGRP levels in serum from control and TRPV1 mutant mice (Figure 6D). As observed in rats, we found that serum levels of CGRP increase by 42% with age in WT mice (6 versus 24 months). However, and rather strikingly, this increase was blocked in TRPV1 mutant mice. In fact, CGRP levels remain unchanged between young and old TRPV1 mutant mice.

To ask whether increased CGRP might be detrimental to health of older animals, we challenged old wild-type mice with the CGRP receptor antagonist, $CGRP_{8-37}$ (Poyner et al., 1998), in an effort to restore CGRP homeostasis (Figures 6E and 6F). After 6 weeks of treatment, we found that the old mice displayed a reappearance of a more youthful metabolic circadian shift from night to day indicative of metabolic rejuvenation as measured by their RER, contrary to vehicle-treated control mice. As observed in the TRPV1 mutant mice, oxygen consumption was also increased in $CGRP_{8-37}$-treated mice compared to controls. These data indicate that pharmacological inhibition of the CGRP pathway is sufficient to restore metabolic youth in old animals and counteract the natural CGRP accumulation with age that is detrimental to wild-type animals.

DISCUSSION

Although it has been shown that genetic manipulation of the CRTC1/CREB pathway modulates aging of *C. elegans* (Dong et al., 2007; Mair et al., 2011) and that disruption of

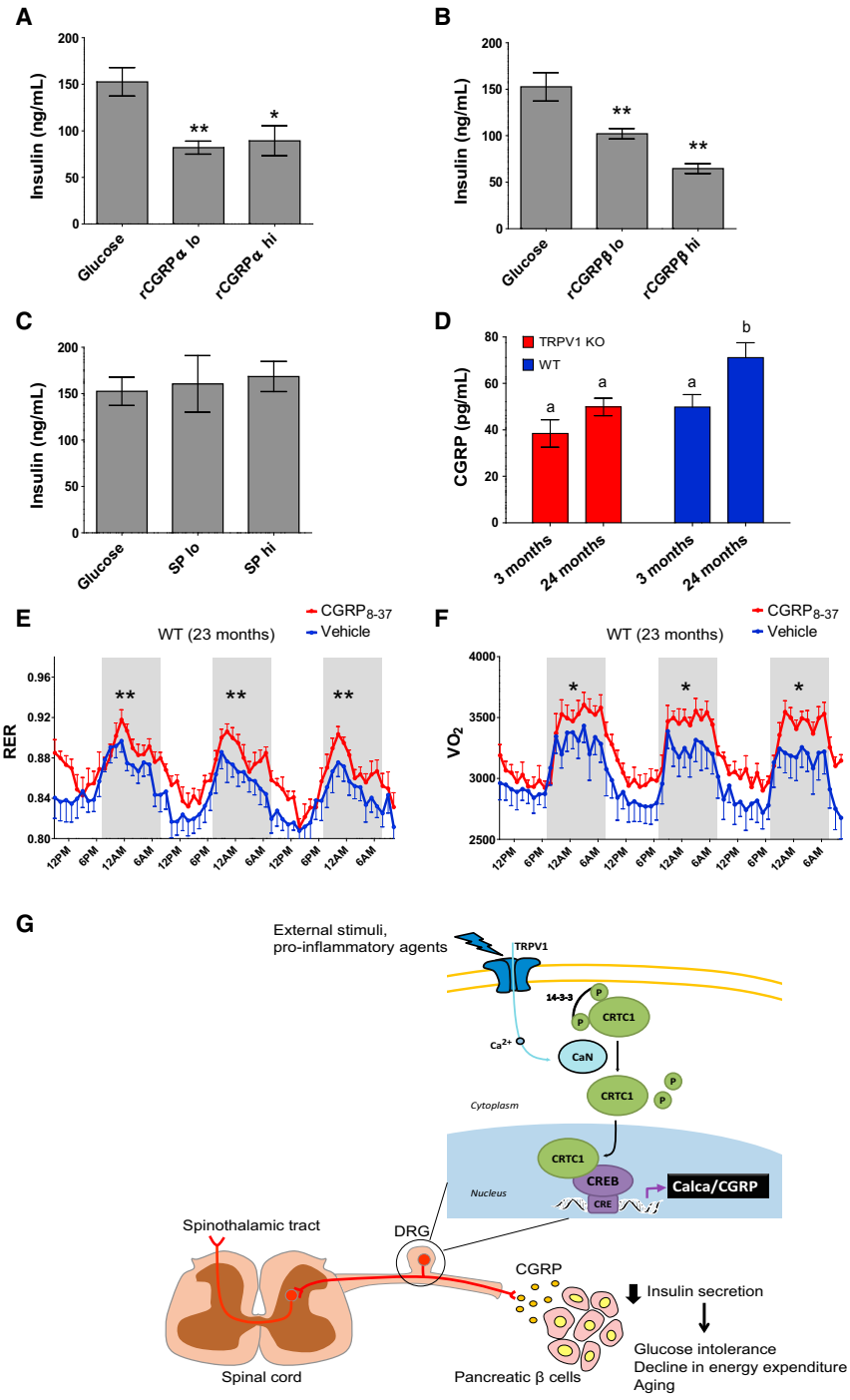


Figure 6. CGRP Is a Neuroendocrine Regulator of Metabolism

(A–C) Insulin secretion assay of mouse insulinoma cells (MIN6) upon 16.8 mM glucose stimulation. Cells were incubated with 0.1–0.5 μM synthetic peptides for (A) rat CGRPα, (B) rat CGRPβ, and (C) rat. Substance P, n = 4, means ± SEM, **p < 0.01, *p < 0.05, Student's t test.

(D) Serum levels of CGRP measured by ELISA in blood samples from WT and TRPV1 mutants at 3 and 24 months, n = 7, means ± SEM a, ns; b, p < 0.05.

(E and F) Respiratory exchange ratio (RER) analysis (E) and oxygen consumption (F) of 23-month-old male WT animals treated with $CGRP_{8–37}$ or vehicle control, n = 10, means ± SEM,**p < 0.01, *p < 0.05, Student's t test.

(G) Model for the neuroendocrine regulation of metabolism by TRPV1 expressing neurons. Stimulation of TRPV1 by external stimuli promotes CGRP secretion from DRG neurons onto the pancreatic β cells and inhibition of insulin release. TRPV1 activation results in calcium influx and activation of calcineurin, allowing dephosphorylation of CRTC1 and release from 14-3-3 proteins, resulting in nuclear internalization of CRTC1 and transcription of its targets, such as CGRP. CGRP accumulation has detrimental effects on energy expenditure, glucose tolerance, and aging. In contrast, loss of TRPV1 promotes lifespan extension through increased insulin secretion, metabolic health by inactivating of the CRTC1/CREB signaling cascade.

See also Figure S4.

chemosensory perception extends lifespan in worms and flies (Alcedo and Kenyon, 2004; Apfeld and Kenyon, 1999; Libert et al., 2007), whether similar mechanisms regulate mammalian longevity were unknown. The results presented here provide evidence that genetic deletion of TRPV1, an ion channel critical for nociception, extends mouse and *C. elegans* lifespan by regulating the activity of CRTC1 in peripheral sensory neurons.

Given the existing precedents linking TRPV1 to metabolism, we characterized the insulin metabolism of these mice in depth. Using a combination of in vivo and in vitro approaches, we found that TRPV1 mutant animals have improved glucose tolerance and increased energy expenditure throughout aging, despite a mild insulin resistance. Except for the latter, these phenotypes could underlie the exceptional longevity of these mice. It should be noted that insulin resistance has been observed in a variety of long-lived mice associated with improved glucose tolerance (Kurosu et al., 2005; Selman et al., 2008; Taguchi et al., 2007) and therefore is not necessarily an indicator of poor health or lifespan shortening. Energy expenditure, on the opposite, has emerged as a putative longevity biomarker, as associated with lifespan extension (Ortega-Molina et al., 2012; Speakman et al., 2004). Improved energy expenditure throughout aging prevents systemic damage associated from fat storage and fat metabolism by ensuring a healthier transition between carbohydrate and fat metabolism. Consistent with improved energy expenditure, TRPV1 mutant mice present beneficial effects on glucose tolerance

and resistance to obesity induced by high-fat diet (Motter and Ahern, 2008). Interestingly, a decrease in cancer incidence of neoplastic lymphoid origin has been previously associated with increased energy expenditure and increased lifespan as observed in the TRPV1 mutant mice (Ortega-Molina et al., 2012), reinforcing the connection between improved energy expenditure and youthful aging.

Because CGRP is secreted locally from TRPV1 fibers in the pancreas, our data argue in favor of a role of TRPV1 on lifespan by modulating peripheral nervous system function, rather than for a role in the brain where TRPV1 is also present and affects hippocampal synaptic plasticity (Chávez et al., 2010; Marsch et al., 2007). Additionally, the weak presence of TRPV1 in neuroglia (Mezey et al., 2000; Sasamura et al., 1998) could underlie a contribution of glial cells to assist DRG neurons in regulating insulin secretion from pancreatic islets. Consistent with peripheral neuronal control, previous research supports a direct role of TRPV1 neurons in antagonizing insulin secretion in the β cells through release of CGRP (Gram et al., 2005; 2007; Pettersson et al., 1986), suggesting that the improved cognitive function observed in TRPV1 mutant mice is more likely due to improved healthspan in these mice rather than the loss of TRPV1 in the hippocampus.

How does TRPV1 regulate the aging process in sensory neurons? Interestingly, disruption of calcium homeostasis in neurons is observed with aging and documented in the context of age-related hippocampal dysfunction, a highly age-sensitive structure (Moreno et al., 2012; West, 1993; West et al., 1994). TRPV1 activation induces calcium influx in neurons, leading to calcium-mediated signal transduction, including activation of CaMK and PKC isoenzymes. Therefore, preserving calcium balance by disrupting TRPV1 signaling is likely to be beneficial. Because calcium- and cAMP-dependent pathways that control gene expression share many common players and points of crosstalk ultimately leading in the phosphorylation of CREB at serine 133 and transcriptional activation, the CREB pathway regulated by its coactivator CRTC1, constitutes a highly likely candidate to integrate TRPV1 signals. Findings in both *C. elegans* and mouse primary DRG neurons strongly support a role for CRTC in shuttling in and out the nucleus in a calcineurin-dependent manner to mediate calcium- and cAMP-dependent, phosphorylation-independent activation of CREB. Our results further demonstrate that the silencing of CREB transcriptional regulation affects the synthesis of the CREB target CGRP.

Remarkably, we and others have observed that CGRP levels increase drastically with age (Melnyk and Himms-Hagen, 1995), and could contribute to the development of age-associated type 2 diabetes by blocking insulin secretion from β cells. Consistent with this hypothesis, low-grade inflammation is associated with age-onset type 2 diabetes and proinflammatory agents might stimulate TRPV1 neurons causing sustained CGRP release and a higher state of inflammation leading to aggravated diabetes. Therefore, sustained high CGRP levels are likely to negatively impact on β cell function and metabolic health. In contrast, the maintenance of low CGRP levels achieved by the TRPV1 mutant mice or drug treatment with the CGRP receptor antagonist $CGRP_{8–37}$, is associated with a youthful metabolism in old mice, a delay in age-associated disease and increased longevity.

Taken together, these findings illustrate that TRPV channels function in sensory neurons as an evolutionary conserved system integrating multiple sensory inputs and transducing them into neuroendocrine signals that promote longevity by adjusting the metabolic activity through the CRTC1/CREB circuit. In particular, these data highlight the role of the neuropeptide CGRP as a critical neuroendocrine regulator of longevity in mammals and possible biomarker of predictive lifespan and healthspan. Interestingly, the extremely long-lived naked mole rat, which lives over 30 years, is naturally lacking CGRP in DRGs (Park et al., 2003). The pharmacological manipulation of TRPV1 and/or CGRP might not only be useful for pain but also to improve glucose homeostasis and aging. Consistent with this idea, diets rich in capsaicin, which can over stimulate TRPV1 neurons and cause their death, have long been linked to lower incidents of diabetes and metabolic dysregulation in humans (Westerterp-Plantenga et al., 2005).

EXPERIMENTAL PROCEDURES

Mice and Diet

The longevity study was generated from homozygous TRPV1 mutants (Caterina et al., 2000) backcrossed 11 times to C57BL/6J and wild-type (WT) C57BL/6J breeder pairs both obtained from the Jackson Laboratory. Mice were housed in groups of two to five same-sex littermates under pathogen-free conditions, with ad libitum access to water and chow (PicoLab Rodent 20 5053*, LabDiet). Individuals were monitored daily and weighed monthly, but were otherwise left undisturbed until they died. Survival was assessed using male and female mice, and all animals were dead by the time of this report. Kaplan-Meier survival curves were constructed using known birth and death dates, and differences between groups were evaluated using the log-rank test. All procedures were approved by the Institutional Animal Care and Use Committees (IACUC) of The Salk Institute for Biological Studies and UC Berkeley.

Metabolic Studies

Indirect calorimetric studies were conducted in a Comprehensive Lab Animal Monitoring System (Columbus Instruments). For GTT and GSIS, glucose (2 g/kg weight) was intraperitoneally administered and blood glucose was measured from tail bleeds at indicated times after injection using a One Touch Ultra glucometer (LifeScan). For the ITT, 5 hr fasted mice were injected with 1 u/kg of human insulin (Humulin; Eli Lily) and glucose was measured as in the GTT. Insulin-stimulated signaling in liver and muscle tissue was performed as previously described (Jordan et al., 2011). Osmotic pumps (Alzet) diffusing $CGRP_{8–37}$ (Tocris Bioscience) at 5.5 mg/ml were implanted in 22-month-old C57BL/6J males (National Institute of Aging).

Quantitative PCR Analysis and Protein Analysis

Pancreatic islets were isolated as previously described (Huising et al., 2010). DRGs (25–40) were excised from each mouse and homogenized in TriZol. RNA was isolated using TriZol/chloroform extraction and RNEasy QIAGEN columns. RNA was converted into cDNA using QuantiTect reverse-transcription kit. Gene expression was assessed by qRT-PCR using SYBR green. Primers sequences are listed in Table S4.

Cell Culture Studies

MIN6 insulinoma cells were cultured as previously described (Huising et al., 2010). DRG neurons were dissociated by trituration in papain after collagenase treatment and seeded on a monolayer of cortical astrocytes. Neurons were pretreated 4 hr with TTX (1 μM) prior either vehicle control or antagonist blockade (2 hr) followed by 1 hr drug stimulation supplemented with

Leptomycin B to inhibit nuclear export. Cap, FSK, and Leptomycin B were obtained from Sigma-Aldrich, SB366791 and TTX from Tocris Biosciences, and CsA from Calbiochem. Neurons were then fixed in 4% paraformaldehyde in PBS and prepared for immunohistochemistry. All antibodies are detailed in the Extended Experimental Procedures.

C. elegans Experiments

osm-9(ky4), ocr-2(ak47), and N2 strains were obtained from the *Caenorhabditis* Genetics Center. AGD418 (N2; uthIs205 [Pcrtc-1::crtc-1::RFP::unc-54 3′UTR, rol-6]) and AGD466 (N2, uthEX222 [crtc-1p::crtc-1 cDNA (S76A, S179A)::tdTOMATO::unc-54 3′UTR; rol-6(su1006)]) were generated elsewhere (Mair et al., 2011).

Full methods and associated references are available in the online version of the paper.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Extended Experimental Procedures, four figures, and four tables and can be found with this article online at http://dx.doi.org/10.1016/j.cell.2014.03.051.

AUTHOR CONTRIBUTIONS

C.E.R. and A.D. planned the majority of the experiments and wrote the paper. C.E.R. executed most of the experiments. M.O.H. performed pancreatic islets isolations and MIN6 assays. P.F. and J.H. performed animal husbandry maintenance and assisted C.E.R. with mouse procedures. M.L. performed mouse necropsy analysis and was assisted by R.V.A., C.D.M.F., and C.M.

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REFERENCES

Ahrén, B., Mårtensson, H., and Nobin, A. (1987). Effects of calcitonin gene-related peptide (CGRP) on islet hormone secretion in the pig. Diabetologia *30*, 354–359.

Akiba, Y., Kato, S., Katsube, K., Nakamura, M., Takeuchi, K., Ishii, H., and Hibi, T. (2004). Transient receptor potential vanilloid subfamily 1 expressed in pancreatic islet β cells modulates insulin secretion in rats. Biochem. Biophys. Res. Commun. *321*, 219–225.

Alcedo, J., and Kenyon, C. (2004). Regulation of C. elegans longevity by specific gustatory and olfactory neurons. Neuron *41*, 45–55.

Apfeld, J., and Kenyon, C. (1999). Regulation of lifespan by sensory perception in Caenorhabditis elegans. Nature *402*, 804–809.

Bernardini, N., Neuhuber, W., Reeh, P.W., and Sauer, S.K. (2004). Morphological evidence for functional capsaicin receptor expression and calcitonin gene-related peptide exocytosis in isolated peripheral nerve axons of the mouse. Neuroscience *126*, 585–590.

Blüher, M., Kahn, B.B., and Kahn, C.R. (2003). Extended longevity in mice lacking the insulin receptor in adipose tissue. Science *299*, 572–574.

Brown-Borg, H.M., Borg, K.E., Meliska, C.J., and Bartke, A. (1996). Dwarf mice and the ageing process. Nature *384*, 33.

Caterina, M.J., Schumacher, M.A., Tominaga, M., Rosen, T.A., Levine, J.D., and Julius, D. (1997). The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature *389*, 816–824.

Caterina, M.J., Leffler, A., Malmberg, A.B., Martin, W.J., Trafton, J., Petersen-Zeitz, K.R., Koltzenburg, M., Basbaum, A.I., and Julius, D. (2000). Impaired nociception and pain sensation in mice lacking the capsaicin receptor. Science *288*, 306–313.

Ch'ng, T.H., Uzgil, B., Lin, P., Avliyakulov, N.K., O'Dell, T.J., and Martin, K.C. (2012). Activity-dependent transport of the transcriptional coactivator CRTC1 from synapse to nucleus. Cell *150*, 207–221.

Chávez, A.E., Chiu, C.Q., and Castillo, P.E. (2010). TRPV1 activation by endogenous anandamide triggers postsynaptic long-term depression in dentate gyrus. Nat. Neurosci. *13*, 1511–1518.

Colbert, H.A., Smith, T.L., and Bargmann, C.I. (1997). OSM-9, a novel protein with structural similarity to channels, is required for olfaction, mechanosensation, and olfactory adaptation in Caenorhabditis elegans. J. Neurosci. *17*, 8259–8269.

Coschigano, K.T., Clemmons, D., Bellush, L.L., and Kopchick, J.J. (2000). Assessment of growth parameters and life span of GHR/BP gene-disrupted mice. Endocrinology *141*, 2608–2613.

Coschigano, K.T., Holland, A.N., Riders, M.E., List, E.O., Flyvbjerg, A., and Kopchick, J.J. (2003). Deletion, but not antagonism, of the mouse growth hormone receptor results in severely decreased body weights, insulin, and insulin-like growth factor I levels and increased life span. Endocrinology *144*, 3799–3810.

Dong, M.-Q., Venable, J.D., Au, N., Xu, T., Park, S.K., Cociorva, D., Johnson, J.R., Dillin, A., and Yates, J.R., 3rd. (2007). Quantitative mass spectrometry identifies insulin signaling targets in C. elegans. Science *317*, 660–663.

Emeson, R.B., Hedjran, F., Yeakley, J.M., Guise, J.W., and Rosenfeld, M.G. (1989). Alternative production of calcitonin and CGRP mRNA is regulated at the calcitonin-specific splice acceptor. Nature *341*, 76–80.

Flurkey, K., Papaconstantinou, J., Miller, R.A., and Harrison, D.E. (2001). Lifespan extension and delayed immune and collagen aging in mutant mice with defects in growth hormone production. Proc. Natl. Acad. Sci. USA *98*, 6736–6741.

Flurkey, K., Papaconstantinou, J., and Harrison, D.E. (2002). The Snell dwarf mutation Pit1(dw) can increase life span in mice. Mech. Ageing Dev. *123*, 121–130.

Gram, D.X., Hansen, A.J., Wilken, M., Elm, T., Svendsen, O., Carr, R.D., Ahrén, B., and Brand, C.L. (2005). Plasma calcitonin gene-related peptide is increased prior to obesity, and sensory nerve desensitization by capsaicin improves oral glucose tolerance in obese Zucker rats. Eur. J. Endocrinol. *153*, 963–969.

Gram, D.X., Ahrén, B., Nagy, I., Olsen, U.B., Brand, C.L., Sundler, F., Tabanera, R., Svendsen, O., Carr, R.D., Santha, P., et al. (2007). Capsaicin-sensitive sensory fibers in the islets of Langerhans contribute to defective insulin secretion in Zucker diabetic rat, an animal model for some aspects of human type 2 diabetes. Eur. J. Neurosci. *25*, 213–223.

Holzenberger, M., Dupont, J., Ducos, B., Leneuve, P., Géloën, A., Even, P.C., Cervera, P., and Le Bouc, Y. (2003). IGF-1 receptor regulates lifespan and resistance to oxidative stress in mice. Nature *421*, 182–187.

Houtkooper, R.H., Argmann, C., Houten, S.M., Cantó, C., Jeninga, E.H., Andreux, P.A., Thomas, C., Doenlen, R., Schoonjans, K., and Auwerx, J. (2011). The metabolic footprint of aging in mice. Sci. Rep. *1*, 134.

Huising, M.O., van der Meulen, T., Vaughan, J.M., Matsumoto, M., Donaldson, C.J., Park, H., Billestrup, N., and Vale, W.W. (2010). CRFR1 is expressed on pancreatic β cells, promotes β cell proliferation, and potentiates insulin secretion in a glucose-dependent manner. Proc. Natl. Acad. Sci. USA *107*, 912–917.

Jordan, S.D., Krüger, M., Willmes, D.M., Redemann, N., Wunderlich, F.T., Brönneke, H.S., Merkwirth, C., Kashkar, H., Olkkonen, V.M., Böttger, T., et al. (2011). Obesity-induced overexpression of miRNA-143 inhibits insulin-stimulated AKT activation and impairs glucose metabolism. Nat. Cell Biol. *13*, 434–446.

Kogire, M., Ishizuka, J., Thompson, J.C., and Greeley, G.H., Jr. (1991). Inhibitory action of islet amyloid polypeptide and calcitonin gene-related peptide on release of insulin from the isolated perfused rat pancreas. Pancreas *6*, 459–463.

Kovács, K.A., Steullet, P., Steinmann, M., Do, K.Q., Magistretti, P.J., Halfon, O., and Cardinaux, J.-R. (2007). TORC1 is a calcium- and cAMP-sensitive coincidence detector involved in hippocampal long-term synaptic plasticity. Proc. Natl. Acad. Sci. USA *104*, 4700–4705.

Kurosu, H., Yamamoto, M., Clark, J.D., Pastor, J.V., Nandi, A., Gurnani, P., McGuinness, O.P., Chikuda, H., Yamaguchi, M., Kawaguchi, H., et al. (2005). Suppression of aging in mice by the hormone klotho. Science *309*, 1829–1833.

Libert, S., Zwiener, J., Chu, X., Vanvoorhies, W., Roman, G., and Pletcher, S.D. (2007). Regulation of Drosophila life span by olfaction and food-derived odors. Science *315*, 1133–1137.

Mair, W., Morantte, I., Rodrigues, A.P.C., Manning, G., Montminy, M., Shaw, R.J., and Dillin, A. (2011). Lifespan extension induced by AMPK and calcineurin is mediated by CRTC-1 and CREB. Nature *470*, 404–408.

Marsch, R., Foeller, E., Rammes, G., Bunck, M., Kössl, M., Holsboer, F., Zieglgänsberger, W., Landgraf, R., Lutz, B., and Wotjak, C.T. (2007). Reduced anxiety, conditioned fear, and hippocampal long-term potentiation in transient receptor potential vanilloid type 1 receptor-deficient mice. J. Neurosci. *27*, 832–839.

McBeth, J., Silman, A.J., and Macfarlane, G.J. (2003). Association of widespread body pain with an increased risk of cancer and reduced cancer survival: a prospective, population-based study. Arthritis Rheum. *48*, 1686–1692.

Mellström, B., Savignac, M., Gomez-Villafuertes, R., and Naranjo, J.R. (2008). Ca2+-operated transcriptional networks: molecular mechanisms and in vivo models. Physiol. Rev. *88*, 421–449.

Melnyk, A., and Himms-Hagen, J. (1995). Resistance to aging-associated obesity in capsaicin-desensitized rats one year after treatment. Obes. Res. *3*, 337–344.

Mezey, É., Tóth, Z.E., Cortright, D.N., Arzubi, M.K., Krause, J.E., Elde, R., Guo, A., Blumberg, P.M., and Szallasi, A. (2000). Distribution of mRNA for vanilloid receptor subtype 1 (VR1), and VR1-like immunoreactivity, in the central nervous system of the rat and human. Proc. Natl. Acad. Sci. USA *97*, 3655–3660.

Moreno, H., Burghardt, N.S., Vela-Duarte, D., Masciotti, J., Hua, F., Fenton, A.A., Schwaller, B., and Small, S.A. (2012). The absence of the calcium-buffering protein calbindin is associated with faster age-related decline in hippocampal metabolism. Hippocampus *22*, 1107–1120.

Motter, A.L., and Ahern, G.P. (2008). TRPV1-null mice are protected from diet-induced obesity. FEBS Lett. *582*, 2257–2262.

Nakanishi, M., Hata, K., Nagayama, T., Sakurai, T., Nishisho, T., Wakabayashi, H., Hiraga, T., Ebisu, S., and Yoneda, T. (2010). Acid activation of Trpv1 leads to an up-regulation of calcitonin gene-related peptide expression in dorsal root ganglion neurons via the CaMK-CREB cascade: a potential mechanism of inflammatory pain. Mol. Biol. Cell *21*, 2568–2577.

Nitter, A.K., and Forseth, K.Ø. (2013). Mortality rate and causes of death in women with self-reported musculoskeletal pain: Results from a 17-year follow-up study. Scand. J. Pain *4*, 86–92.

Noble, M.D., Romac, J., Wang, Y., Hsu, J., Humphrey, J.E., and Liddle, R.A. (2006). Local disruption of the celiac ganglion inhibits substance P release and ameliorates caerulein-induced pancreatitis in rats. Am. J. Physiol. Gastrointest. Liver Physiol. *291*, G128–G134.

Ortega-Molina, A., Efeyan, A., Lopez-Guadamillas, E., Muñoz-Martin, M., Gómez-López, G., Cañamero, M., Mulero, F., Pastor, J., Martinez, S., Romanos, E., et al. (2012). Pten positively regulates brown adipose function, energy expenditure, and longevity. Cell Metab. *15*, 382–394.

Park, T.J., Comer, C., Carol, A., Lu, Y., Hong, H.-S., and Rice, F.L. (2003). Somatosensory organization and behavior in naked mole-rats: II. Peripheral structures, innervation, and selective lack of neuropeptides associated with thermoregulation and pain. J. Comp. Neurol. *465*, 104–120.

Pettersson, M., Ahrén, B., Böttcher, G., and Sundler, F. (1986). Calcitonin gene-related peptide: occurrence in pancreatic islets in the mouse and the rat and inhibition of insulin secretion in the mouse. Endocrinology *119*, 865–869.

Poyner, D.R., Soomets, U., Howitt, S.G., and Langel, U. (1998). Structural determinants for binding to CGRP receptors expressed by human SK-N-MC and Col 29 cells: studies with chimeric and other peptides. Br. J. Pharmacol. *124*, 1659–1666.

Price, T.J., and Flores, C.M. (2007). Critical evaluation of the colocalization between calcitonin gene-related peptide, substance P, transient receptor potential vanilloid subfamily type 1 immunoreactivities, and isolectin B4 binding in primary afferent neurons of the rat and mouse. J. Pain *8*, 263–272.

Razavi, R., Chan, Y., Afifiyan, F.N., Liu, X.J., Wan, X., Yantha, J., Tsui, H., Tang, L., Tsai, S., Santamaria, P., et al. (2006). TRPV1+ sensory neurons control β cell stress and islet inflammation in autoimmune diabetes. Cell *127*, 1123–1135.

Sasamura, T., Sasaki, M., Tohda, C., and Kuraishi, Y. (1998). Existence of capsaicin-sensitive glutamatergic terminals in rat hypothalamus. Neuroreport *9*, 2045–2048.

Screaton, R.A., Conkright, M.D., Katoh, Y., Best, J.L., Canettieri, G., Jeffries, S., Guzman, E., Niessen, S., Yates, J.R., 3rd, Takemori, H., et al. (2004). The CREB coactivator TORC2 functions as a calcium- and cAMP-sensitive coincidence detector. Cell *119*, 61–74.

Selman, C., Lingard, S., Choudhury, A.I., Batterham, R.L., Claret, M., Clements, M., Ramadani, F., Okkenhaug, K., Schuster, E., Blanc, E., et al. (2008). Evidence for lifespan extension and delayed age-related biomarkers in insulin receptor substrate 1 null mice. FASEB J. *22*, 807–818.

Selman, C., Tullet, J.M.A., Wieser, D., Irvine, E., Lingard, S.J., Choudhury, A.I., Claret, M., Al-Qassab, H., Carmignac, D., Ramadani, F., et al. (2009). Ribosomal protein S6 kinase 1 signaling regulates mammalian life span. Science *326*, 140–144.

Speakman, J.R., Krol, E., and Johnson, M.S. (2004). The functional significance of individual variation in basal metabolic rate. Physiol. Biochem. Zool. *77*, 900–915.

Suri, A., and Szallasi, A. (2008). The emerging role of TRPV1 in diabetes and obesity. Trends Pharmacol. Sci. *29*, 29–36.

Szallasi, A., Nilsson, S., Farkas-Szallasi, T., Blumberg, P.M., Hökfelt, T., and Lundberg, J.M. (1995). Vanilloid (capsaicin) receptors in the rat: distribution in the brain, regional differences in the spinal cord, axonal transport to the periphery, and depletion by systemic vanilloid treatment. Brain Res. *703*, 175–183.

Taguchi, A., Wartschow, L.M., and White, M.F. (2007). Brain IRS2 signaling coordinates life span and nutrient homeostasis. Science *317*, 369–372.

Tanaka, H., Shimaya, A., Kiso, T., Kuramochi, T., Shimokawa, T., and Shibasaki, M. (2011). Enhanced insulin secretion and sensitization in diabetic mice on chronic treatment with a transient receptor potential vanilloid 1 antagonist. Life Sci. *88*, 559–563.

Tobin, D.M., Madsen, D.M., Kahn-Kirby, A., Peckol, E.L., Moulder, G., Barstead, R., Maricq, A.V., and Bargmann, C.I. (2002). Combinatorial expression of TRPV channel proteins defines their sensory functions and subcellular localization in C. elegans neurons. Neuron *35*, 307–318.

Torrance, N., Elliott, A.M., Lee, A.J., and Smith, B.H. (2010). Severe chronic pain is associated with increased 10 year mortality. A cohort record linkage study. Eur. J. Pain *14*, 380–386.

Venkatachalam, K., and Montell, C. (2007). TRP channels. Annu. Rev. Biochem. *76*, 387–417.

West, M.J. (1993). Regionally specific loss of neurons in the aging human hippocampus. Neurobiol. Aging *14*, 287–293.

West, M.J., Coleman, P.D., Flood, D.G., and Troncoso, J.C. (1994). Differences in the pattern of hippocampal neuronal loss in normal ageing and Alzheimer's disease. Lancet *344*, 769–772.

Westerterp-Plantenga, M.S., Smeets, A., and Lejeune, M.P.G. (2005). Sensory and gastrointestinal satiety effects of capsaicin on food intake. Int J Obes (Lond) *29*, 682–688.

Woods, J.A., Wilund, K.R., Martin, S.A., and Kistler, B.M. (2012). Exercise, inflammation and aging. Aging Dis. *3*, 130–140.

Zhang, S., Sokolchik, I., Blanco, G., and Sze, J.Y. (2004). Caenorhabditis elegans TRPV ion channel regulates 5HT biosynthesis in chemosensory neurons. Development *131*, 1629–1638.

Zhang, Y., Xie, Y., Berglund, E.D., Coate, K.C., He, T.T., Katafuchi, T., Xiao, G., Potthoff, M.J., Wei, W., Wan, Y., et al. (2012). The starvation hormone, fibroblast growth factor-21, extends lifespan in mice. eLife *1*, e00065.