Lipid-mediated regulation of SKN-1/Nrf in response to germ cell absence

Michael J. Steinbaugh$^{1,2}$, Sri Devi Narasimhan$^{1,2}$, Stacey Robida-Stubbs$^{1,2}$, Lorenza E. Moronetti Mazzeo$^{1,2}$, Jonathan M. Dreyfuss$^{1,3}$, John M. Hourihan$^{1,2}$, Prashant Raghavan$^{1,2}$, Theresa N. Operaña$^{1,2}$, Reza Esmailie$^{1,2}$, and T. Keith Blackwell$^{1,2}$

$^{1}$ Research Division, Joslin Diabetes Center, Boston, MA 02215, USA
$^{2}$ Department of Genetics and Harvard Stem Cell Institute, Harvard Medical School, Boston, MA 02215, USA
$^{3}$ Department of Biomedical Engineering, Boston University, Boston, MA 02215, USA

Contact: keith.blackwell@joslin.harvard.edu

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Abstract

In *C. elegans*, ablation of germline stem cells (GSCs) extends lifespan, but also increases fat accumulation and alters lipid metabolism, raising the intriguing question of how these effects might be related. Here we show that a lack of GSCs results in a broad transcriptional reprogramming, in which the conserved detoxification regulator SKN-1/Nrf increases stress resistance, proteasome activity, and longevity. SKN-1 also activates diverse lipid metabolism genes and reduces fat storage, thereby alleviating the increased fat accumulation caused by GSC absence. Surprisingly, SKN-1 is activated by signals from this fat, which appears to derive from unconsumed yolk that was produced for reproduction. We conclude that SKN-1 plays a direct role in maintaining lipid homeostasis, in which it is activated by lipids. This SKN-1 function may explain the importance of mammalian Nrf proteins in fatty liver disease, and suggests that particular endogenous or dietary lipids might promote health through SKN-1/Nrf.
Introduction

The nematode C. elegans has been invaluable for identifying mechanisms that slow aging and may prevent chronic disease (Kenyon, 2010). An intriguing finding that was first made in this organism is that when germline stem cells (GSCs) are ablated, mechanisms are activated in somatic tissues that protect against stress and increase lifespan (Hsin and Kenyon, 1999; Kenyon, 2010; Antebi, 2013; Hansen et al., 2013). GSC loss also increases lifespan in D. melanogaster (Flatt et al., 2008), and castration has been associated with longevity in men (Min et al., 2012), suggesting that this relationship might be conserved. These beneficial effects of GSC removal may have evolved to maximize reproductive fitness under adversity (Partridge et al., 2005; Kenyon, 2010). This relationship provides paradigms for how tissue non-autonomous signals influence aging (Kenyon, 2010), and how a stem cell population communicates with the “niche” that sustains it (Jones and Wagers, 2008).

In C. elegans, the effects of GSC absence have been studied by laser ablation of GSC precursors, which results in a complete loss of GSCs, or by analysis of genetic mutants in which GSC proliferation is inhibited so that the GSC number is very low, and mature germ cells are not formed (Hsin and Kenyon, 1999; Arantes-Oliveira et al., 2002; Kenyon, 2010). For simplicity, we will refer to each of these types of animals as GSC(-) animals. The lifespan extension seen in GSC(-) animals (GSC(-) longevity) requires the action of several conserved transcription factors in the intestine, the counterpart of the mammalian liver, digestive system, and adipose tissue (Kenyon, 2010; Antebi, 2013; Hansen et al., 2013). DAF-16/FOXO is needed for longevity from GSC ablation or reduced insulin/IGF-1 signaling (IIS), but is regulated differently by each pathway (Lin et
al., 2001; Libina et al., 2003; Kenyon, 2010). GSC(-) longevity also requires HLH-30/TFEB, PHA-4/FOXA, and the nuclear receptors DAF-12/FXR, NHR-80/HNF4, and NHR-49/PPARα (Hsin and Kenyon, 1999; Goudeau et al., 2011; Lapierre et al., 2011; O'Rourke and Ruvkun, 2013; Ratnappan et al., 2014). Under most conditions, GSC(-) longevity also depends upon a hormonal signal from the somatic gonad that activates DAF-12 (Kenyon, 2010; Antebi, 2013). Aside from the identification of mechanisms required for DAF-16 function, we understand little about how GSCs influence these transcription factors (Kenyon, 2010; Antebi, 2013).

One hallmark of GSC(-) animals is enhancement of both proteostasis and stress resistance. During aging, GSC(-) animals maintain more robust responses to thermal and proteotoxic stress (Ben-Zvi et al., 2009). They also exhibit a striking daf-16-dependent increase in activity of the proteasome (Vilchez et al., 2012), a multisubunit complex which degrades proteins that the ubiquitylation system has tagged for decay (Glickman and Ciechanover, 2002; Goldberg, 2003). In addition, GSC removal enhances immunity (Alper et al., 2010), and boosts oxidative stress resistance through an undetermined DAF-16-independent mechanism (Libina et al., 2003).

Another notable characteristic of GSC(-) animals is that many aspects of lipid metabolism are altered. Expression of particular fatty acid (FA) oxidation, FA desaturation, and triglyceride lipase genes is increased, as is total lipase activity (Wang et al., 2008; Goudeau et al., 2011; Lapierre et al., 2011; McCormick et al., 2012; Ratnappan et al., 2014). Given that lipid catabolism activities are elevated, it seems paradoxical that GSC(-) animals also exhibit dramatically increased fat accumulation (O'Rourke et al., 2009). Interestingly, GSC(-) longevity seems to depend upon
particular lipid metabolism processes. Production of the unsaturated free FA (FFA) oleic acid (OA) is required (Goudeau et al., 2011), as are the triglyceride lipases LIPL-4/LIPA and FARD-1/FAR2 (Wang et al., 2008; McCormick et al., 2012). It is of intense interest to determine whether the fat accumulation seen with GSC ablation might derive from production and storage of particular beneficial fats, or a salutary overall balance of lipid metabolism that is consistent with longevity (Ackerman and Gems, 2012; Hansen et al., 2013).

The C. elegans transcription factor SKN-1 controls a broad detoxification response to oxidative and xenobiotic stress, and is orthologous to the mammalian Nrf1/2/3 (NF-E2-related factor) proteins (An and Blackwell, 2003; Oliveira et al., 2009; Park et al., 2009). SKN-1/Nrf proteins have been implicated in longevity from C. elegans to rodents (An and Blackwell, 2003; Bishop and Guarente, 2007; Leiser and Miller, 2010; Sykiotis and Bohmann, 2010; Steinbaugh et al., 2012; Ewald et al., 2015). Recent findings raise the question of whether these transcription regulators might also have important functions in lipid homeostasis. SKN-1/Nrf proteins influence expression of lipid metabolism genes (Oliveira et al., 2009; Paek et al., 2012; Hayes and Dinkova-Kostova, 2014; Tsujita et al., 2014), and SKN-1 has been linked to fat mobilization under particular starvation or dietary conditions (Paek et al., 2012; Pang et al., 2014). Mice that lack Nrf1 in the liver develop non-alcoholic fatty liver disease (NAFLD) that progresses to non-alcoholic steatohepatitis (NASH), and Nrf2−/− mice develop NASH on a high-fat diet (Xu et al., 2005; Okada et al., 2013; Tsujita et al., 2014). However, reduced Nrf protein function is thought to predispose to NASH by impairing hepatic stress resistance (Xu et al., 2005; Lee et al., 2013). An understanding of NAFLD is a
high priority, because its incidence is increasing as a sequella of metabolic syndrome (Cohen et al., 2011).

Here we examined the role of SKN-1 in the effects of GSC absence on lifespan, stress resistance, and lipid metabolism. Genetic inhibition of GSCs activates SKN-1, thereby increasing lifespan and stress resistance. Expression profiling revealed that GSC(-) animals upregulate stress defense, extracellular matrix, and lipid metabolism genes, in many cases dependent upon skn-1. SKN-1 is also required for GSC inhibition to increase proteasome activity. SKN-1 is needed for GSC(-) longevity but reduces lipid storage, arguing against the idea that GSC(-) animals simply accumulate beneficial fat. Instead, these high fat levels appear to derive from unconsumed yolk that was produced for reproduction. Unexpectedly, in GSC(-) animals SKN-1 appears to be activated by specific FA signals, defining a new mechanism of SKN-1/Nrf protein regulation and GSC-to-soma communication. This homeostatic function of SKN-1 in lipid metabolism suggests that Nrf proteins have a similar role in preventing NASH.

**Results**

**SKN-1 promotes longevity and stress resistance in the absence of GSCs**

To investigate the importance of skn-1 in GSC(-) animals, we analyzed temperature sensitive (ts) mutations in glp-1/Notch, which is required for GSC proliferation (Kimble and Crittenden, 2005). glp-1(ts) mutants that undergo larval development at the non-permissive temperature of 25°C (GSC(-) animals) are sterile, exhibit a markedly reduced number of GSCs, and live considerably longer than WT controls (Arantes-Oliveira et al., 2002) (Figures 1A and 1B). By contrast, this lifespan extension was blocked in a skn-1 mutant background (Figures 1A and 1B). Lack of
skin-1 also impaired lifespan extension when glp-1(ts) animals were downshifted to 20°C after development was complete (Table 1). Similar results were obtained with or without 5-fluoro-2'-deoxyuridine (FUdR), which inhibits offspring formation in the control (Table 1). Consistent with these findings, in an earlier experiment in which glp-1(ts) extended lifespan by less than 7%, skin-1 knockdown by RNA interference (RNAi) prevented this increase (Vilchez et al., 2012). In contrast to daf-16, skin-1 was also required for GSC inhibition to increase oxidative stress resistance (Figures 1C-1F; Table 2).

**GSCs regulate intestinal DAF-16 and SKN-1 through different mechanisms**

We investigated whether the benefits of GSC absence simply require that SKN-1 be present, or involve activation of SKN-1. SKN-1 accumulates in intestinal nuclei in response to certain stresses, or inhibition of mechanisms that include IIS, mTORC2, glycogen synthase kinase-3 (GSK-3), translation elongation, and the ubiquitin ligase WDR-23 (An and Blackwell, 2003; Tullet et al., 2008; Choe et al., 2009; Park et al., 2009; Li et al., 2011; Robida-Stubbs et al., 2012). The levels of a SKN-1::GFP (green fluorescent protein) fusion in intestinal nuclei were also notably elevated in GSC(-) animals (Figures 2A-2B). This was associated with increased expression of direct SKN-1 target genes, apparently through activation of their intestinal expression (Figures 2C-2E). The KRI-1/KRIT1 ankyrin-repeat protein and the TCER-1/TCERG1 transcription factor are required for GSC absence to induce DAF-16 nuclear accumulation and extend lifespan (Berman and Kenyon, 2006; Ghazi et al., 2009). In contrast, in GSC(-) animals SKN-1 nuclear accumulation was only partially or minimally affected by loss of kri-1 or tcer-1, respectively, but was abolished by knockdown of the
pmk-1/p38 kinase (Figures 2F and 2G), which phosphorylates SKN-1 and under most circumstances is required for SKN-1 nuclear accumulation (Inoue et al., 2005). In GSC(-) animals, DAF-12 is needed for DAF-16 nuclear accumulation and activity (Berman and Kenyon, 2006; Kenyon, 2010; Antebi, 2013), and induces expression of the microRNAs mir-84 and mir-241, which target inhibitors of DAF-16 (Shen et al., 2012). In GSC(-) animals, daf-12 knockdown only mildly affected SKN-1::GFP accumulation (Figure 2G), and SKN-1 target gene induction was generally not impaired by daf-12 or mir-241;mir-84 mutations (Figure 2H). The absence of GSCs therefore activates SKN-1 in the intestine, but through a different mechanism from DAF-16.

**SKN-1 reprograms stress resistance and metabolism in GSC(-) animals**

To investigate how SKN-1 promotes longevity and stress resistance upon GSC loss, we used RNA sequencing (RNA-seq) to identify genes that are (1) expressed at higher levels in adult somatic tissues when germ cells are largely absent, and (2) dependent upon SKN-1 (Figure 3A). We compared intact glp-1(ts) (GSC(-)) animals to wild-type GSC(+) controls at the non-permissive temperature of 25°, analyzing day-one adults in which development was complete, and performing differential expression analyses on the normalized RNA-seq data for 12,595 expressed genes (Figure 3B). We detected similar expression levels of SKN-1 upregulated targets in qRT-PCR analyses of the samples used for sequencing, giving us confidence in our RNA-seq results (Figure 3–figure supplement 1A). Moreover, in the GSC(-) gene set, the canonical DAF-16 targets mtl-1 and sod-3 (Murphy et al., 2003; Kenyon, 2010) were upregulated (Supplementary file 1a), and functional groups that are characteristic of germline-specific genes were under-represented (Figure 3–figure supplement 1B).
No previous studies have globally profiled genes that are upregulated in the soma in response to germ cell loss. mRNAs that are present at higher relative levels in GSC(-) samples compared to WT would include not only those genes, but also genes that are expressed only in somatic cells, because the germline accounts for about two-thirds of all adult nuclei (Kimble and Crittenden, 2005). To gauge the maximal extent of this background, we examined mRNAs that are expressed specifically in somatic tissues. These somatic-specific mRNAs were enriched 3-4 fold in the GSC(-) samples, approximately the level predicted from the 2:1 proportion of germline to somatic nuclei (Figure 3–figure supplement 1C). Accordingly, if an mRNA that is not somatic-specific was present at a four-fold elevated level in GSC(-) samples, we considered this mRNA to be upregulated in the soma in response to GSC absence, although we expect that this stringent cutoff would miss many upregulated genes.

In GSC(-) animals, 1,306 and 615 genes were upregulated more than 4- and 5-fold, respectively, indicating a broad remodeling of transcription (Figure 3–figure supplements 1D and 1E; Supplementary file 1a). In the latter set, which is more amenable to functional annotation analysis because of its smaller size, the most prominently overrepresented category was collagen (Figure 3C; Supplementary file 1a). Although collagens may be expressed primarily in the soma, this overrepresentation is likely to be meaningful because of the extent to which these genes were upregulated (Supplementary file 1a), and because collagens are generally overrepresented in other longevity-associated gene sets, with certain collagens being critical for lifespan extension (Ewald et al., 2015). Also notably upregulated in GSC(-) animals were genes involved in detoxification, immunity (C-type lectin and galectin), and
metabolism, particularly FA oxidation and other lipid metabolism processes (Figure 3C; Supplementary file 1a).

We used RNAi to investigate the contribution of skn-1 to gene expression in GSC(-) animals (Figure 3A). A previous microarray analysis of skn-1 RNAi-treated L4 larvae at 20°C found that in the absence of acute stress, SKN-1 upregulates genes involved in processes that include detoxification, lipid metabolism, immunity, and proteostasis (Oliveira et al., 2009; Li et al., 2011). Similar processes were prominent in the sets of genes for which skn-1 RNAi reduced expression at 25°C in day-one WT adults (“SKN-1-upregulated in WT genes”; ≥ 33% reduction, \( P < 0.05 \) (Supplementary file 1b), or day-one GSC(-) animals (“SKN-1-upregulated in GSC(-) genes”; ≥ 33% reduction, \( P < 0.05 \) (Supplementary file 1c). Our finding that SKN-1 nuclear occupancy is increased in GSC(-) animals (Figure 2) predicts that GSC inhibition would induce SKN-1 to activate genes. Accordingly, skn-1 RNAi reduced expression of 87 genes that were upregulated at least four-fold in glp-1(ts) compared to WT (Figures 3D and 3E; Supplementary file 1d). This number probably underestimates the full contribution of SKN-1, because RNAi only partially reduces its activity. In addition to detoxification and lipid metabolism genes, these 87 genes included many involved in extracellular matrices (ECMs), as expected from the skn-1-dependence of many ECM genes that are upregulated in other long-lived C. elegans (Ewald et al., 2015). Many of these skn-1-dependent GSC(-) genes appear to be direct SKN-1 targets, as predicted by presence of SKN-1 binding sites in their upstream regions, and direct binding of SKN-1 in genome-wide chromatin immunoprecipitation (ChIP) surveys (Supplementary file 1d). In summary, SKN-1 upregulates numerous genes that are associated with
phenotypes seen in GSC-ablated animals, including increased stress resistance, immunity, and longevity, as well as alterations in lipid metabolism.

**SKN-1 increases proteasome activity in response to GSC loss**

A previous RNAi experiment suggested that SKN-1 is dispensable for the elevated proteasome activity seen in GSC(-) animals (Vilchez et al., 2012). We reexamined this question because SKN-1 maintains proteasome gene expression and intestinal proteasome activity in WT *C. elegans* (Li et al., 2011), and because proteasome genes were prominent in the SKN-1-upregulated GSC(-) gene set (Supplementary file 1c). The proteasome holocomplex consists of a 20S barrel-like structure in which proteins are degraded, and a 19S regulatory cap that directs ubiquitylated proteins into this structure (Glickman and Ciechanover, 2002; Goldberg, 2003). In general, and consistent with previous findings (Vilchez et al., 2012), the relative levels of proteasome subunit mRNAs were lower in GSC(-) animals (Figure 4–figure supplement 1A), possibly because of the lack of germ cells. In both WT and GSC(-) animals, *skn-1* knockdown comparably decreased expression of 19S and 20S proteasome subunit genes (Figure 4A and Figure 4–figure supplement 1A), the majority of which appear to be direct transcriptional targets of SKN-1 (Figure 4B). As these findings would predict, in GSC(-) animals lack of *skn-1* dramatically reduced proteasome activity at days one and five of adulthood (Figures 4C, 4D, and Figure 4–figure supplements 1B-1G). It is possible that in the earlier analysis (Vilchez et al., 2012), RNAi might not have inhibited *skn-1* expression sufficiently to detect its importance for proteasome activity in GSC(-) animals.
The increased proteasome activity of GSC(-) animals is thought to derive from DAF-16-dependent transcriptional upregulation of the RPN-6.1/PSMD11 subunit, which connects the 19S and 20S proteasome particles (Vilchez et al., 2012). *rpn-6.1* appears to be unique among proteasome subunit genes, in that its mRNA levels are proportionally higher in GSC(-) animals (Vilchez et al., 2012) (**Figure 4E**). *skn-1* was required for this increased *rpn-6.1* expression (**Figure 4E**), and binding site and ChIP studies suggested that *rpn-6.1* is upregulated directly by both SKN-1 and DAF-16 (**Figure 4F**). We conclude that by promoting expression of multiple proteasome subunit genes, including *rpn-6.1*, SKN-1 plays a central role in the increased proteasome activity that results from GSC loss.

**SKN-1 regulates lipid metabolism**

Genetic GSC inhibition increased expression of lipid metabolism genes that represent a wide range of processes, including FFA formation from triglyceride lipolysis, as well as FA oxidation, desaturation, and elongation (**Figure 5A**; **Supplementary file 1a**). Many of these genes were also upregulated by SKN-1 (**Figure 5A**; **Supplementary files 1a-1c**). Of particular note, the high-confidence GSC(-) and SKN-1-upregulated gene set included the conserved lysosomal triglyceride lipase *lipl-3*, which increases *C. elegans* lifespan when overexpressed, and is normally induced by fasting (O'Rourke and Ruvkun, 2013). This gene set also included the FA oxidation genes *acs-10* (acyl-CoA synthetase), *cpt-3* (carnitine palmitoyltransferase), and *ech-9* (enoyl-CoA hydratase) (**Figure 5A**; **Supplementary file 1d**). This suggested that SKN-1 might have a major role in lipid metabolism, and how it is influenced by GSC absence.
Given that SKN-1 increases both lifespan and stress resistance in GSC(-) animals, its effects on lipid metabolism should also be beneficial. If the elevated fat storage in GSC(-) animals reflects simply elevated production and storage of “good” lipids, we might expect skn-1 to support this fat production. We investigated whether SKN-1 affects fat storage in WT and GSC(-) animals by oil red O (ORO) staining of fixed animals, a method that reliably indicates fat accumulation (O'Rourke et al., 2009). Remarkably, ablation of skn-1 by either mutation or RNAi significantly increased lipid levels in either WT or GSC(-) day-one adults, so that glp-1(ts);skn-1(-) animals exhibited markedly high levels of ORO staining (Figures 5B, 5C, and Figure 5–figure supplements 1A, 1B). As an independent method of assessing fat accumulation in the intestine, we examined levels of the predicted short-chain FA dehydrogenase DHS-3 (Zhang et al., 2012). Proteomic and microscopy analyses have shown that DHS-3 localizes almost exclusively to intestinal lipid droplets (Figure 5–figure supplement 2A), and marks the vast majority of these lipid droplets in vivo (Zhang et al., 2012; Na et al., 2015). Consistent with ORO staining, lack of skn-1 increased accumulation of a DHS-3::GFP (green fluorescent protein) fusion in the intestine in WT and GSC(-) animals, without affecting expression of the dhs-3 mRNA (Figure 5–figure supplements 2B, 2C, and 3). An analysis of total triglyceride levels also indicated that SKN-1 reduces the overall level of fat accumulation (Figure 5–figure supplement 2D). DAF-16 increases lipid accumulation in response to reduced IIS, and influences expression of some lipid metabolism genes in response to GSC removal (Wang et al., 2008; McCormick et al., 2012). However, consistent with a previous study (O'Rourke et al., 2009), we found that loss of daf-16 did not substantially affect overall fat storage in
GSC(-) animals (Figures 5D, 5E, and Figure 5–figure supplement 1C). Together, our data indicate that SKN-1 is required to prevent excess fat accumulation under normal feeding conditions, and that SKN-1 but not DAF-16 reduces the lipid load that accumulates in response to GSC loss.

**GSC loss activates SKN-1 through lipid signaling**

Given that SKN-1 inhibits fat storage, we considered whether the SKN-1 activation seen in GSC(-) animals might be triggered by lipid accumulation. It is possible that GSC loss simply increases production of certain fats. However, GSC ablation or inhibition prevents formation of oocytes, which endocytose lipid-rich yolk that is synthesized in the intestine (Grant and Hirsh, 1999). Fat storage might therefore be increased indirectly by GSC loss, through accumulation of unused yolk lipids. We tested a key prediction of this model by examining yolk accumulation and distribution, which can be visualized with GFP-tagged vitellogenin (YP170/VIT-2::GFP), a major yolk lipoprotein (Grant and Hirsh, 1999). VIT-2::GFP was visible primarily in oocytes and embryos in WT day-one adults, but accumulated to extremely high levels throughout the body cavity in the absence of GSCs (Figures 6A, 6B, and Figure 6–figure supplement 1). Apparently, yolk production was not slowed sufficiently to compensate for the lack of gametogenesis. The failure to consume yolk-associated lipid could account for the increase in overall fat storage seen in GSC(-) animals.

We investigated whether accumulation of yolk-associated lipids might induce SKN-1 to mount a protective response. Supporting this idea, when the oocyte-specific yolk receptor *rme-2* is knocked down, yolk accumulates to high levels (Grant and Hirsh, 1999), and in the intestine SKN-1 accumulates in nuclei and its target gene *gst-4* is
activated (Figures 6C-6F). Additionally, \textit{rme-2} RNAi increased stress resistance in a \textit{skn-1}-dependent manner (Figure 6G and Table 2). When \textit{de novo} lipogenesis was prevented by knockdown of the SREBP1 ortholog \textit{sbp-1} (Yang et al., 2006), SKN-1::GFP failed to accumulate in intestinal nuclei in response to GSC inhibition (Figures 6H, 6I), but not oxidative stress (Figure 6–figure supplement 2A) or reduced IIS (\textit{daf-2} mutants, Figure 6–figure supplement 2B). The \textit{sbp-1} lipogenesis defect can be rescued by supplementation with 600 μM OA (Yang et al., 2006), which is the most abundant FA in olive oil, chicken egg yolk, and human adipose tissue (National Research Council, 1976; Kokatnur et al., 1979). In \textit{C. elegans}, the abundance of OA is increased in GSC(-) animals, and its synthesis by the FA desaturases \textit{FAT-6} and \textit{FAT-7} (SCD orthologs) is required for GSC(-) lifespan extension (Goudeau et al., 2011). \textit{fat-6/7} were also required for SKN-1 to accumulate in nuclei after GSC inhibition (Figure 6J). Moreover, in GSC(-) animals subjected to \textit{sbp-1} RNAi, SKN-1 nuclear accumulation was fully restored by OA supplementation (Figures 6H and 6I).

Consistent with their importance for SKN-1 function, \textit{sbp-1} and \textit{fat-6/7} were required for GSC absence to increase stress resistance (Figure 6–figure supplement 3).

Together, our data suggest that certain unsaturated lipids are required for SKN-1 to be activated in response to GSC loss, but not necessarily by other stimuli, and therefore that lipid accumulation \textit{per se} might activate SKN-1. Accordingly, feeding of either OA or coconut oil (CO) activated \textit{gst-4} in WT animals in a \textit{skn-1}-dependent manner without impairing development or reproduction (Figure 6–figure supplements 2C-2E). Under these conditions, CO feeding provided OA (300 μM with 0.1% CO) along with multiple saturated fatty acids. CO feeding strongly induced nuclear
accumulation of SKN-1 but not DAF-16 (Figure 6–figure supplements 2F and 2G),
and sbp-1 RNAi did not impair DAF-16 nuclear accumulation in GSC(-) animals (Figure
6–figure supplements 2H and 2I), supporting the notion that GSCs regulate SKN-1
and DAF-16 differently.

The lipid overload that results from reproductive failure might induce stress that
activates SKN-1. Oxidative stress induced by sodium arsenite (AS) robustly activates
the p38/PMK-1 kinase through phosphorylation, leading in turn to SKN-1 activation
(Inoue et al., 2005). GSC loss induced SKN-1 nuclear accumulation at least as
dramatically as AS treatment (Figure 2B and Figure 6–figure supplement 2A) but did
not detectably increase PMK-1/p38 activity (Figure 6–figure supplement 2J),
suggesting that any stress arising from the lack of GSCs might not be sufficient on its
own to explain SKN-1 activation.

By breaking down triglycerides, the lysosomal lipases LIPL-1/3 and LIPL-4
enable production of specific unsaturated FFAs that promote autophagy and longevity
(Lapierre et al., 2011; O'Rourke et al., 2013; O'Rourke and Ruvkun, 2013; Folick et al.,
2015). Some of these FAs are escorted from the lysosome to the nucleus by the
conserved lipid-binding protein LBP-8/FABP1 (Folick et al., 2015; Han and Brunet,
2015). In GSC(-) animals, SKN-1 nuclear accumulation was inhibited modestly by lipl-4
RNAi but more strongly by lipl-1/3 double knockdown (Figure 6J). Furthermore, lipl-3
RNAi reduced stress resistance in GSC(-) but not WT animals (Figure 6–figure
supplements 3A and 3B). Given that fat storage is increased in lipl-1/3 mutants
(O'Rourke and Ruvkun, 2013), our data suggest that in GSC(-) animals SKN-1 activity
may depend upon particular lipl-1/3-dependent products, not lipid levels per se.
Knockdown of *lbp-8* or other LBPs also interfered with SKN-1::GFP nuclear accumulation in GSC(-) animals, and *lbp-8* RNAi impaired SKN-1-dependent *gst-4* activation by OA, indicating involvement of FA transport (Figures 6J and 6K). Together, our data suggest that in GSC(-) animals, excessive lipid levels lead to production of OA- and LIPL-1/3-dependent FAs that activate SKN-1, possibly through FA-based signaling (Figure 7).

**Discussion**

The question of how events in one tissue can influence aging in others is of fundamental importance. The effects of GSC loss in *C. elegans* provide a paradigm for investigating this problem, as well as interactions between a stem cell population and its environment. Here we determined that GSC inhibition leads to a broad transcriptional reprogramming in somatic tissues that involves activation of SKN-1, and that SKN-1 is required for many beneficial effects of GSC absence, including lifespan extension. Previous work showed that SKN-1 is required for lifespan to be extended by reduced insulin/IGF-1, mTORC1, or mTORC2 signaling, by dietary restriction, and by low-level mitochondrial ROS production (Bishop and Guarente, 2007; Tullet et al., 2008; Robida-Stubbs et al., 2012; Zarse et al., 2012; Schmeisser et al., 2013; Mizunuma et al., 2014; Moroz et al., 2014; Ewald et al., 2015). Our new data further support the idea that SKN-1/Nrf proteins are broadly important for longevity assurance.

One major role of SKN-1 in GSC(-) animals is to increase proteasome activity (Figures 4C, 4D, and Figure 4–figure supplement 1). In WT animals, SKN-1 activates most proteasome subunit genes when the proteasome is inhibited (Li et al., 2011). This compensatory function is conserved in its mammalian ortholog Nrf1, which
is cleaved and activated when proteasome activity is low (Radhakrishnan et al., 2010; Steffen et al., 2010; Radhakrishnan et al., 2014; Sha and Goldberg, 2014). By contrast, in GSC(-) animals proteasome activity is elevated (Figures 4C, 4D) (Vilchez et al., 2012), suggesting that additional mechanisms influence SKN-1/Nrf regulation of proteasome genes. In GSC(-) animals most if not all proteasome subunit genes are dependent upon SKN-1 for their expression (Figure 4A), and SKN-1 and DAF-16 together activate the proteasome subunit gene rpn-6.1 (Figures 4E and 4F), the levels of which are rate-limiting for proteasome activity (Vilchez et al., 2012). Overexpression of either rpn-6.1 or the 20S proteasome subunit pbs-5 increases C. elegans lifespan, and in the latter case lifespan extension was shown to be skn-1-dependent (Vilchez et al., 2012; Chondrogianni et al., 2015), suggesting that enhancement of proteasome activity may be an important mechanism through which SKN-1/Nrf promotes longevity.

The evidence that GSC(-) longevity is associated with fat accumulation and altered lipid metabolism has raised an intriguing possibility, that GSC absence induces production of lipids that promote health and longevity (see Introduction). Our results are consistent with aspects of this model, but suggest an important modification. GSC(-) animals accumulate dramatically high levels of yolk lipoproteins by the first day of adulthood (Figures 6A and 6B), providing a likely reason that they accumulate so much lipid. Moreover, SKN-1 acts to reduce fat accumulation but is critical for the benefits of GSC loss (Figures 5B, 5C, and Figure 5–figure supplements 1A, 1B), suggesting that GSC(-) animals do not simply overproduce healthful lipids. Finally, excess yolk accumulation induced by another method (rme-2 RNAi) leads to increased SKN-1 nuclear accumulation and target gene activation, and skn-1-dependent stress.
resistance (Figures 6D-6G). Taken together, our data suggest that GSC(-) animals accumulate excess fat because they cannot stop production of fat that would otherwise support reproduction (Figure 7). Importantly, however, in responding to and metabolizing this fat they produce specific lipids that activate SKN-1 and other regulators, which in turn increase lifespan and may promote a more healthy balance of lipids (Figure 7).

Several lines of evidence support this model. GSC inhibition induces SKN-1 and NHR-49 to upregulate largely distinct sets of FA oxidation genes (Figure 5A) (Ratnappan et al., 2014). This effect of SKN-1 could account for its inhibitory role in fat accumulation (Figures 5B and 5C). A need to metabolize excess fat could also explain the importance of lipophagy in GSC(-) longevity (Lapierre et al., 2011; Hansen et al., 2013). With respect to signaling lipids, GSC(-) longevity requires the triglyceride lipase LIPL-4 (Wang et al., 2008), which generates unsaturated FFAs that promote longevity (O'Rourke et al., 2013; Folick et al., 2015). While LIPL-4-dependent FAs act through NHR-49 and NHR-80 (Lapierre et al., 2011; Folick et al., 2015), and possibly not SKN-1 (Figure 6J), in GSC(-) animals SKN-1 activation involves the LIPL-1/3 lipases (Figure 6J), which also promote longevity (O'Rourke and Ruvkun, 2013). This elevated SKN-1 activity also depends upon lipid transfer proteins, as well as OA (Figures 6H-6J and Figure 6–figure supplement 1C). OA is required for GSC(-) longevity (Goudeau et al., 2011) and is a precursor to unsaturated FAs that have signaling functions (O'Rourke et al., 2013; Folick et al., 2015). Finally, SKN-1 upregulates lipl-3 and lbp-8 in WT and GSC(-) animals (Figure 5A and Figure 5–figure supplement 3; Supplementary files 1a and 1c), suggesting that it may function both downstream and upstream of lipid
signals. The idea that SKN-1 can be activated by lipids that arise from prevention of reproduction and yolk consumption should be considered in evaluation of genetic or pharmacological interventions that increase SKN-1 activity.

SKN-1 is activated by lipids and regulates lipid metabolism gene expression not only in the high-fat GSC(-) model, but also in WT animals under normal feeding conditions (Figures 5 and 6; Supplementary files 1b-1d). Moreover, SKN-1 profoundly reduced fat storage by the beginning of adult life in healthy, reproductively-active animals that have not begun to age. Taken together, our findings suggest that SKN-1 plays an integral and direct role in maintaining lipid homeostasis. Our results predict that insufficient mammalian Nrf function does not lead to NAFLD/NASH simply by increasing chronic hepatic stress (Xu et al., 2005; Lee et al., 2013), and that a protective function of Nrf proteins in fat metabolism is likely to be involved. Nrf proteins therefore may provide an important line of defense against metabolic disease.

Development of NAFLD is a growing obesity-related public health issue (Cohen et al., 2011). Our data suggest that analysis of Nrf proteins could be a promising underexplored direction for investigating causes and prevention of NAFLD, and that SKN-1 and C. elegans provide a genetically tractable model that will be valuable in this effort.

Our evidence that GSCs activate SKN-1 through lipid-based signaling suggests a new mechanism through which GSCs influence the soma. The response to GSC loss therefore involves metabolic signals that reflect the altered nutritional balance within the organism. Similar interactions could be important in other stem cell contexts. For example, in the mammalian bone marrow microenvironment adipose tissue profoundly
influences the function of hematopoietic and mesenchymal stem cells (Adler et al., 2014; Muruganandan and Sinal, 2014). In addition to the mechanisms we have described, GSC(-) longevity involves endocrine signals from the somatic gonad and depends upon absence of GSCs per se, not simply reproductive cessation (Kenyon, 2010; Antebi, 2013). It will now be of interest to determine how these mechanisms, as well as other transcription factors that are required for GSC(-) longevity (see Introduction), interface with SKN-1 and its regulation.

Signaling lipids from endogenous, dietary or microbiota sources constitute an area of considerable excitement, because these signals can induce beneficial effects such as anti-inflammatory protection, enhanced insulin sensitivity, protection against metabolic disease, and increased lifespan in *C. elegans* (Wang et al., 2008; Kniazeva and Han, 2013; Lim et al., 2013; O’Rourke and Ruvkun, 2013; Folick et al., 2015; Han and Brunet, 2015). Signals derived from OA are of particular interest in this regard, because of its dietary availability in olive oil. Mechanisms through which lipid signals are known to act on transcription networks include binding to nuclear receptors, and OA-induced protein kinase A activation that ultimately leads to FA oxidation (Fu et al., 2003; Lim et al., 2013; Folick et al., 2015). By revealing SKN-1 as a new regulator of metabolism and stress defenses that is activated in response to lipids, our data suggest the exciting possibility that this might also be the case for mammalian Nrf proteins. They also suggest that a “lipohormesis” pathway in which signaling lipids confer health benefits by activating SKN-1/Nrf may not only be a characteristic of GSC-ablated animals, but also might be more broadly applicable for enhancing health- and possibly life-span.
Materials and methods

Strains

Worms were maintained on NGM plates seeded with *E. coli* (OP50) at 15°C, using standard techniques (Brenner, 1974). In all experiments, *glp-1*(ts) mutants were matched with the wild-type N2 strain used for outcrossing. The *C. elegans* strains used in this study are detailed in Table 3.

RNAi

Feeding RNAi was performed using tetracycline-resistant HT115 bacteria carrying the pL4440 plasmid with ampicillin/carbenicillin resistance (Kamath et al., 2001). RNAi cultures were grown overnight in 50 mL conical tubes at 37°C with shaking at 220 RPM in 5 mL LB medium containing 50 µg/mL carbenicillin and 12.5 µg/mL tetracycline. Cultures were diluted 1:5 the following day in LB containing carbenicillin and tetracycline to allow for re-entry into the logarithmic growth phase, grown to an OD<sub>600</sub> of 1.5 (~6 hours). Cultures were centrifuged at 4,500 RPM for 10 minutes, concentrated to a volume of 5 mL, and then induced with 1 mM IPTG prior to plating. Bacterial cultures were seeded onto standard nematode growth medium (NGM) plates containing 50 µg/mL carbenicillin, 12.5 µg/mL tetracycline, and 0.4 mM IPTG.

Lifespans

Worms were synchronized by timed egg lay, upshifted to 25°C at the L2 stage, then scored for lifespan at 25°C or 20°C, as previously described (Arantes-Oliveira et al., 2002; Robida-Stubbs et al., 2012). For analyses of *glp-1*(ts) at 20°C, worms were downshifted from 25°C upon reaching adulthood. Animals were transferred at the first day of adulthood to fresh plates containing FUdR; ACROS Organics/Thermo Fisher
Scientific, Geel, Belgium) at a concentration of 100 µg/mL to inhibit progeny development (Mitchell et al., 1979), unless otherwise indicated. RNAi-treated worms were placed on RNAi feeding plates starting at the first day of adulthood. Worms were maintained at a density of 30 worms per 6 cm plate on live bacteria, and scored every other day. Animals that crawled off the plate, ruptured, or died from internal hatching were censored. Lifespans were graphed as Kaplan-Meier survival curves with JMP Pro 12 (SAS Institute, Middleton, MA). P values for survival curve analysis were generated using log-rank test. Additional statistical analysis was performed with GraphPad Prism 6 (GraphPad Software, La Jolla, CA). P values for mean lifespan analysis were calculated by two-way ANOVA with post-hoc Holm-Šidák correction.

**Stress assays**

Synchronized animals were incubated at 25°C during development then scored for survival hourly beginning at either days 1 or 3 of adulthood. Feeding RNAi was started at the L1 stage for day 1 stress assays or post-developmentally at day 1 adulthood for stress assays performed at day 3 adulthood. For the arsenite assay, worms were incubated in M9 buffer containing 5 mM sodium arsenite (Riedel-de Haën, Seelze, Germany). For the tert-butyl hydroperoxide (TBHP) stress assay, worms were placed on solid NGM plates containing 15.4 mM TBHP (Sigma-Aldrich, St. Louis, MO). TBHP plates were freshly prepared on the day of the experiment. Survival assays were graphed as Kaplan-Meier survival curves with JMP Pro 12. P values for survival curve analysis were generated using log-rank test. Additional statistical analysis was performed with GraphPad Prism 6. P values for mean survival analysis were calculated by two-way ANOVA with post-hoc Holm-Šidák correction.
Microscopy

Animals were anaesthetized for 5 minutes in 0.06% tetramisole/M9 buffer, mounted on 2% agarose pads on glass slides under coverslips, and imaged with ZEN 2012 software on an Axio Imager.M2 microscope (Zeiss, Jena, Germany).

GFP reporter scoring

Intestinal SKN-1::GFP nuclear localization and gst-4p::GFP::NLS expression were scored as “high”, “medium”, or “low” as previously described (An and Blackwell, 2003; Ewald et al., 2015). “High” denotes strong intensity in all intestinal nuclei; “medium” indicates relatively lower intensity or distribution in approximately half of intestinal nuclei; “low” denotes weak or no visible GFP intensity in intestinal nuclei. P values were calculated by two-sided chi$^2$ test.

Intestinal DAF-16::GFP nuclear localization was scored as “high”, “medium”, or “low” as previously described (Henderson and Johnson, 2001; Berman and Kenyon, 2006; Curran and Ruvkun, 2007). “High” denotes more DAF-16::GFP observed in the nucleus compared to the cytoplasm; “medium” indicates animals with noticeable DAF-16::GFP in the nucleus but higher levels in the cytoplasm; “low” denotes entirely cytoplasmic DAF-16::GFP.

SKN-1::GFP color isolation was performed to reduce gut granule autofluorescence using selective color matching against rgb(99,159,94) with a fuzziness setting of 125 and auto contrast in Adobe Photoshop CC 2014 (Adobe, San Jose, CA). DAF-16::GFP color isolation was similarly performed using selective color matching against rgb(0,255,111) with a fuzziness setting of 100.
Samples were prepared from ~200 day 3 adult worms synchronized by timed egg lay. RNA was extracted using TRlzol (Thermo Fisher, Waltham, MA)-based phenol-chloroform extraction and purified with RNA Clean and Concentrator-5 spin columns (Zymo Research, Irvine, CA). RNA concentration and quality was assessed with a NanoDrop 1000 spectrophotometer (Thermo Fisher). cDNAs were prepared using SuperScript III First-Strand Synthesis SuperMix for qRT-PCR (Thermo Fisher). mRNA levels were quantified from biological triplicates and technical duplicates using SYBR Green (Thermo Fisher) fluorescence on a 384-well format Real Time PCR 7900 (Applied Biosystems, Foster City, CA). After an initial denaturation step (95°C for 10 min), amplification was performed using 40 cycles of denaturation (95°C for 15 s) and annealing (60°C for 1 min). Samples were analyzed by the standard curve method, with normalization to the reference genes *cdc-42* and Y45F10D.4 (Hoogewijs et al., 2008). *P* values were calculated by two-sided Student’s t-test with post-hoc Holm-Šídák correction in GraphPad Prism 6. The primers were used in this study are provided in Table 4.

**RNA sequencing (RNA-seq)**

Samples were prepared from ~5,000 synchronized, L1 arrested day-one adult animals cultured at 25°C. Worms were synchronized by sodium hypochlorite (bleach) treatment, as previously described (Porta-de-la-Riva et al., 2012). Bleach solution (9 mL ddH2O; 1 mL 1 N NaOH; 4 mL Clorox bleach) was freshly prepared before each experiment. Worms were bleached for 5 minutes, washed 5x in M9, and arrested at the L1 stage at 25°C in M9 containing 10 µg/mL cholesterol. Feeding RNAi was started at
the L1 stage. This approach only partially reduces \textit{skn-1} function, but allows analysis of larger samples than would be feasible with \textit{skn-1} mutants, which are sterile (Bowerman et al., 1992). Because these animals were not treated with FUdR, the WT adults contained an intact germline and eggs. As is explained in the \textbf{Results} section, we therefore confined our analysis to genes that were overrepresented in \textit{glp-1(ts)} animals, which lack eggs and most of the germline, and established a high-confidence cutoff for genes that were upregulated by GSC absence as opposed to simply being expressed specifically in somatic tissues. RNA was extracted using the same protocol for qRT-PCR samples. Purified RNA samples were DNase treated and assigned a RIN quality score using a Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA). Only matched samples with high RIN scores were sent for sequencing. Single read 50 bp RNA sequencing with poly(A) enrichment was performed at the Dana-Farber Cancer Institute Center for Computational Biology using a HiSeq 2000 (Illumina, San Diego, CA). FASTQ output files were aligned to the WBcel235 (Feb 2014) \textit{C. elegans} reference genome using STAR (Dobin et al., 2013). These files have been deposited at the Gene Expression Omnibus (GEO) with the accession number GSE63075. Samples averaged 75\% mapping of sequence reads to the reference genome. Differential expression analysis was performed using a custom R and Bioconductor RNA-seq pipeline (http://bioinf.wehi.edu.au/RNAseqCaseStudy/) (Gentleman et al., 2004; Anders et al., 2013; R Core Team, 2014). Quantification of mapped reads in the aligned SAM output files was performed using featureCounts, part of the Subread package (Liao et al., 2013, 2014). We filtered out transcripts that didn’t have at least one count per million reads in at least two samples. Quantile normalization and estimation of the
mean-variance relationship of the log-counts was performed by voom (Law et al., 2014).

Linear model fitting, empirical Bayes analysis and differential expression analysis was then conducted using limma (Smyth, 2005). To identify genes that are upregulated in a SKN-1-dependent manner by GSC loss, we sought genes for which \textit{glp-1(ts)} expression was higher than WT, and for which \textit{glp-1(ts);skn-1(-)} expression was reduced relative to \textit{glp-1(ts)}. To test for this pattern, if a gene’s expression change was higher in the comparison of \textit{glp-1(ts)} vs. WT and lower in the comparison of \textit{glp-1(ts);skn-1(-)} vs. \textit{glp-1(ts)}, then we calculated the minimum (in absolute value) of the \(t\)-statistics from these two comparisons, and assessed the significance of this statistic by comparing to a null distribution derived by applying this procedure to randomly generated \(t\)-statistics. We corrected for multiple testing in this and the differential expression analysis using the false discovery rate (FDR) (Benjamini and Hochberg, 1995). Heatmaps were generated using heatmap.2 in the gplots package (Warnes et al., 2014).

Functional annotations and phenotypes were obtained from Wormbase build WS246. SKN-1 transcription factor binding site analysis of hits was conducted with biomaRt, GenomicFeatures, JASPAR, MotifDb, motifStack, MotIV, and Rsamtools (Sandelin et al., 2004; Durinck et al., 2005; Durinck et al., 2009; Lawrence et al., 2013; Ou et al., 2013; Mercier and Gottardo, 2014; Shannon, 2014). JASPAR analysis was performed with the SKN-1 matrix MA0547.1 using 2 kb upstream sequences obtained from Ensembl WBcel235 (Staab et al., 2013). modENCODE SKN-1::GFP ChIP-seq analysis of hits was performed using biomaRt, ChIPpeakAnno, IRanges, and multtest (Durinck et al., 2005; Durinck et al., 2009; Gerstein et al., 2010; Zhu et al., 2010; Niu et al., 2011; Lawrence et al., 2013). SKN-1::GFP ChIP-seq peaks were generated by
Michael Snyder’s lab. We used the peak data generated from the first 3 larval stages: L1 (modENCODE_2622; GSE25810), L2 (modENCODE_3369), and L3 (modENCODE_3838; GSE48710). Human ortholog matching was performed using Wormbase, Ensembl, and OrthoList (Shaye and Greenwald, 2011). Gene lists were evaluated for functional classification and statistical overrepresentation with Database for Annotation, Visualization and Integrated Discovery (DAVID) version 6.7 (Dennis et al., 2003).

**rpn-6.1 binding site analysis**

SKN-1 and DAF-16 binding peaks within the first intron and the promoter of rpn-6.1 were previously identified by the modENCODE project (Furuyama et al., 2000; Gerstein et al., 2010; Niu et al., 2011). We identified multiple hits with the consensus binding sequence ATCAT in the TRANSFAC matrices N$SKN1_01 and N$SKN1_02 using MATCH (BioBase) that overlap with the SKN-1::GFP ChIP-seq binding peaks within the first intron and the promoter of rpn-6.1 (Kel et al., 2003; Matys et al., 2006).

Our analysis also confirmed a previously identified hit (Vilchez et al., 2012) in the N$DAF16_01 matrix with the consensus binding sequence TGT TT that overlaps with the DAF-16::GFP ChIP-seq peak within the first intron. No putative DAF-16 binding sites were identified in the rpn-6.1 promoter in the TRANSFAC MATCH analysis.

**Proteasome activity**

In vitro chymotrypsin-like proteasome activity assays were performed as previously described (Kisselev and Goldberg, 2005; Vilchez et al., 2012). Worms were bleach synchronized and maintained at 25°C from egg stage, then lysed at day 1 of adulthood, unless otherwise noted, in freshly prepared proteasome activity assay buffer.
(50 mM Tris-HCl, pH 7, 250 mM sucrose, 5 mM MgCl₂, 0.5 mM EDTA, 2 mM ATP and 1 mM dithiothreitol) using a Branson digital sonifier at 4°C. Lysates were centrifuged at 10,000 x g for 15 min at 4°C. 25 µg of protein, calculated using the BCA protein assay (#23225; Pierce Biotechnology/Thermo Fisher, Rockford, IL), was transferred to a flat 96-well microtiter plate (Nunc, Roskilde, Denmark). Samples were incubated at 25°C and fluorogenic chymotrypsin substrate (#230914; Calbiochem/EMD Millipore, San Diego, CA) was added to the plate immediately before analysis. Fluorescence (380 nm excitation; 460 nm emission) was measured every 3 min for 1 h at 25°C using a Synergy MX microplate reader with Gen5 software (Bio-Tek, Winooski, VT). Lysates were assayed in triplicate. P values were calculated by two-sided Student’s t-test in GraphPad Prism 6.

**Fixed oil red O (ORO) staining**

ORO staining was performed on fixed animals, essentially as described (O’Rourke et al., 2009; Yen et al., 2010), with some modifications. 200-300 day-one adult worms synchronized by timed egg lay were washed three times with PBS then snap frozen in a dry ice/ethanol bath. Upon thawing, worms were treated with PBS containing 2% paraformaldehyde (PFA), using 3 freeze thaw cycles with dry ice/ethanol to permeabilize the cuticle. Worms were then washed with PBS to remove the PFA. Filtered ORO solution (0.5 g of ORO powder [#O0625; Sigma-Aldrich] in 100 mL of 60% isopropanol) was prepared freshly before each experiment. Worms were stained for 3 hours in a round bottom 96 well plate in ORO solution at room temperature with gentle shaking. Longer staining periods, such as overnight incubation (O’Rourke et al., 2009),
saturated ORO staining in \textit{glp-1}(ts) animals to a level that rendered \textit{glp-1}(ts) and \textit{glp-1(ts);skn-1} strains indistinguishable.

Animals were imaged at 40X using differential interference contrast (DIC) microscopy. Quantification of ORO staining was performed on the upper intestine, directly below the pharynx. Since ORO absorbs light at 510 nm (green channel), we performed background subtraction of the red channel from the green channel in Adobe Photoshop CC (Adobe, San Jose, CA) to specifically isolate the ORO staining, as previously described (Yen et al., 2010). Quantification of mean intensity over background for each animal was performed using Fiji (http://fiji.sc). Statistical analysis was performed with GraphPad Prism 6. \( P \) values were calculated by one-way ANOVA with post-hoc Holm-Šídák correction.

**DHS-3::GFP scoring**

Using a COPAS Biosort (Union Biometrica, Holliston, MA) (Pulak, 2006), bleach-synchronized day-one adult worms were scored for GFP fluorescence. RNAi was initiated after L1 arrest. The COPAS was used to record three attributes for each individual nematode: time of flight (TOF), which corresponds to nematode length; extinction (EXT), which corresponds to the optical density; and GFP fluorescence intensity. TOF and EXT measurements are related to the size and age of the nematode; both increase with development. These parameters were used to specifically gate adult worms. GFP fluorescence was normalized to worm size as a ratio of GFP/TOF values. Representative GFP images of each strain were captured at 4X using an Olympus IX51 inverted microscope (Olympus, New Orleans, LA). \( P \) values
were calculated by one-way ANOVA with post-hoc Holm-Šídák correction in GraphPad Prism 6.

**Triglyceride quantification**

Triglyceride (TAG) levels were measured with the Triglyceride Colorimetric Assay Kit (#10010303; Cayman Chemical, Ann Arbor, MI). Samples were run according to the manufacturer’s protocol in triplicate. TAG concentrations were normalized relative to protein concentration using the BCA protein assay (Pierce Biotechnology).

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**Competing interests**

The authors declare that no competing interests exist. The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.
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Figure 1. SKN-1 promotes longevity and stress resistance in GSC(-) animals.

(A,B) Wild-type, skn-1(zu135), glp-1(bn18ts), and glp-1(bn18ts);skn-1(zu135) double mutants were assayed for lifespan at 25°C. skn-1(zu135) is a presumed null that is used throughout the study. Unless otherwise specified, glp-1(ts) refers to glp-1(bn18ts).

(A) Composite survival curve. (B) Graph of mean lifespans. (C-F) glp-1(ts) mutants require skn-1 for oxidative stress resistance. Day 3 adult glp-1(ts) and control worms treated with skn-1 RNAi or empty vector were exposed to (C,D) 5 mM sodium arsenite (AS) or (E,F) 15.4 mM tert-butyl hydroperoxide (TBHP). Data are represented as mean ± SEM. P < 0.001***. The interaction between glp-1 and skn-1 was significant for both lifespan and stress resistance (P < 0.001). Statistical analysis and replicates are in Tables 1 and 2.
Figure 2. GSCs inhibit SKN-1 activity in the intestine. (A) Representative images of SKN-1::GFP in intestinal nuclei; GFP channel (top), bright field (BF; bottom). (B) Accumulation of SKN-1::GFP in intestinal nuclei in GSC(-) animals. (C) skn-1-dependent activation of direct SKN-1 target genes (Robida-Stubbs et al., 2012) in response to GSC absence, detected by qRT-PCR. (D,E) Increased expression of gst-4p::GFP in the intestine of glp-1(ts) animals. Hypodermal gst-4p::GFP expression appeared to be unaffected. (D) Representative 10X images. (E) Intestinal gst-4p::GFP quantification. (F-H) GSCs regulate SKN-1 parallel to DAF-16 and DAF-12. In (H), SKN-1 target genes are assayed by qRT-PCR. glp-1(ts) refers to glp-1(e2141ts), and horizontal black lines indicate strains lacking GSCs. (C,H) Data are represented as mean ± SEM. n = 3 for qRT-PCR samples. (B,E,F,G) GFP quantification with high, medium, low scoring. Numbers above bars denote sample size. P < 0.05*; P < 0.01**; P < 0.001***.
Figure 3. Effects of GSC absence and skn-1 on gene expression. (A) RNA-seq experiment setup. For each condition, three biological replicates were obtained from synchronized intact day-one adults at 25°C. Arrows indicate comparisons that were made, and GSC(-) refers to glp-1(ts). (B) Heatmap of all genes evaluated, showing biological replicates. (C) DAVID functional annotation analysis of GSC(-)-upregulated genes. (D) Heatmap of genes upregulated in glp-1(ts) in a skn-1-dependent manner (87 genes; FC > 4 in GSC(-); FC < 0.67 with skn-1 RNAi in GSC(-)). (E) Functional annotation of the genes shown in (D). Genes and additional statistics are provided in Supplementary files 1a-1d.
Figure 3–figure supplement 1. Gene expression changes following GSC inhibition. (A) qRT-PCR validation of RNA samples used for RNA-seq. *gst-4 and *nit-1 are direct SKN-1 targets (Robida-Stubbs et al., 2012). Data are represented as mean ± SEM. n = 3; P < 0.05*. Analysis of a different RNA sample set is shown in Figure 2D. (B) DAVID analysis of genes that were downregulated in *glp-1(ts). Processes related to GSC maintenance and reproduction were highly represented, as expected. (C) Altered abundance of tissue-specific genes in GSC(-) animals. Adult hermaphrodite worms have 959 somatic cells and ~2,000 GSCs (Kimble and Crittenden, 2005). A representative somatic-specific gene would therefore be predicted to be present at higher relative abundance in GSC(-) samples after normalization to either total RNA, or reference housekeeping genes. Accordingly, representative somatic tissue-specific genes (Richmond, 2005; Moerman and Williams, 2006; Chikina et al., 2009) were present at 3-4-fold higher relative levels in the GSC(-) samples. By contrast, a germline-specific gene (*efl-1) was underrepresented (~4x) in GSC(-) samples. Reference genes that are ubiquitously expressed in all tissues and commonly used for qRT-PCR normalization (Hoogewijs et al., 2008) do not have altered relative abundance in the GSC(-) samples. (D,E) Frequency distribution plots of mRNA levels in *glp-1(ts) worms relative to wild-type. A cutoff of FC > 4 denotes 1,306 out of 12,595 genes sequenced (10.4%).
**Figure 4.** SKN-1 increases proteasome activity in response to GSC absence. (A) Reduction in relative proteasome gene subunit mRNA levels by skn-1 RNAi, detected by RNA-seq. (B) Venn diagram indicating the number proteasome subunit genes (pas, pbs, rpn, rpt families) that have SKN-1::GFP ChIP-seq peak hits near the transcription start site at the indicated larval stage (Niu et al., 2011). (C, D) SKN-1-dependence of increased chymotrypsin-related proteasome activity in GSC-ablated worms. The slopes from (C) are graphed in (D). Additional experiments are in Figure 4—figure supplement 1. (E) SKN-1-dependence of rpn-6.1 upregulation. Data are represented as mean ± SEM. n = 3 for all experiments. $P < 0.05^*$; $P < 0.01^{**}$; $P < 0.001^{***}$. (F) Direct binding of SKN-1 and DAF-16 to the rpn-6.1 gene, indicated by TRANSFAC transcription factor binding prediction and modENCODE GFP ChIP-seq analyses. Both the predicted promoter and first intron of rpn-6.1 are highly enriched for SKN-1 and DAF-16 binding.
Figure 4—figure supplement 1. SKN-1-dependence of the increased proteasome activity in GSC(-) animals. (A) GSC absence reduces relative proteasome subunit gene mRNA abundance, detected by RNA-seq. (B,C) The skn-1(zu135) mutation suppresses the increase in 26S proteasome activity seen in day-one adult glp-1(ts) animals. (D,E) skn-1 RNAi administered from the egg stage suppresses proteasome activity in day-one adult glp-1(ts) animals. (F,G) skn-1 RNAi administered post-developmentally, starting at day 1 adulthood, significantly reduces proteasome activity in day 5 adult glp-1(ts) animals. (B,D,F) Kinetic curves of chymotrypsin-like proteasome activity. (C,E,G) Graphs of proteasome activity slopes. Data are represented as mean ± SEM. n = 3; P < 0.001***.
Figure 5. SKN-1 regulates lipid metabolism in GSC(-) animals. (A) Functional map of lipid metabolism gene expression. Left columns show the effects of GSC absence (GSC(-) vs WT), and right columns the effects of skn-1 RNAi in GSC(-) animals. SKN-1 regulates genes involved in FA oxidation, breakdown of triacylglycerols (triglycerides, TAG) to free FAs, production of mono- and poly-unsaturated FAs (MUFA, PUFA), and FA transport. Color coding reflects relative representation in RNA-seq data, with blue and yellow indicating increased and decreased expression, respectively. (B-E) Increased fat levels in glp-1(ts) and skn-1 mutants but not daf-16 mutants. (D,E) glp-1(ts) refers to glp-1(e2141ts). Representative 40X DIC images of fixed ORO-stained worms are shown in (B,D), with quantification provided in (C,E). Additional images and quantification are provided in Figure 5–figure supplement 1. Data are represented as mean ± SEM. Numbers above bars denote sample size. P < 0.001***.
**Figure 5—figure supplement 1. Representative ORO staining images with quantification.** Representative images divided into approximate quintiles, ordered by mean pixel intensity, are shown for each strain. BF images are presented on the left and background subtracted images with quantification on the right. (A) *glp-1(bn18ts)* and *skn-1(zu135)* mutants. (B) *glp-1(ts)* mutants treated with *skn-1* and *sbp-1* RNAi. Increased staining was observed both with *skn-1* mutants and *skn-1* RNAi. RNAi against *sbp-1*, which is required for lipogenesis (Yang et al., 2006), decreases ORO staining in both WT and *glp-1(ts)* genetic backgrounds. (C) *glp-1(e2141ts)* and *daf-16(mu86)* mutants. Numbers indicate mean pixel intensity above background (see Methods for additional details).
**Figure 5—figure supplement 2. Analysis of the intestinal lipid droplet marker DHS-3::GFP, and triglyceride levels.** *skn-1* RNAi increases DHS-3::GFP intensity in both wild-type and GSC(-) animals, whereas *sbp-1* RNAi decreases DHS-3::GFP intensity. (A) 10X slide mounted DHS-3::GFP images. White arrows indicate intestine-specific expression. (B,C) COPAS Biosorter quantification of DHS-3::GFP in live day-one adult worms. (B) Representative 10X inverted scope images of worms suspended in M9 buffer used for COPAS scoring. (C) Graph of mean DHS-3::GFP fluorescence, assayed by COPAS. Numbers above bars denote sample size. Asterisks directly above bars indicate *P* values relative to WT or RNAi control. Asterisks above black lines denote effect of RNAi in *glp-1(ts)* background. RNAi was started from egg stage and animals were raised at 25°C. (D) Triglyceride (TAG) levels are significantly elevated in *skn-1* mutants. Data are represented as mean ± SEM. *P* < 0.01**; *P* < 0.001***.
Figure 5–figure supplement 3. RNA-seq counts of select lipid metabolism and yolk transporter genes. Expression of the known SKN-1 target genes *gst-4* and *nit-1* are elevated in GSC(-) animals in a *skn-1*-dependent manner. The TAG lipase *lipl-3* and FABP *lbp-8* are similarly elevated in GSC(-) animals in a *skn-1*-dependent manner, but expression of *sbp-1*, *dhs-3*, and yolk protein vitellogenins (VIT genes) are not affected by *skn-1* RNAi. *rme-2* expression is germline enriched but regulated independently of *skn-1*. Open circles denote replicates. Vertical lines indicate mean counts per million (CPM).
Figure 6. GSC absence activates SKN-1 through FA signaling. (A) Accumulation of yolk transporter vitellogenin (VIT-2::GFP) in the soma of GSC(-) animals. Detailed higher magnification images are provided in Figure 6–figure supplement 1. (B) COPAS quantification of VIT-2::GFP. Data are represented as mean ± SEM. (C) Knockdown of the oocyte-specific yolk receptor rme-2 promotes somatic VIT-2 accumulation. (D-F) rme-2 RNAi activates SKN-1 in the intestine. (D,E) SKN-1::GFP accumulates in intestinal nuclei in rme-2 RNAi-treated worms. (F) In rme-2 RNAi-treated worms, gst-4p::GFP levels in the intestine are increased at the L4 stage, and increased further by day 1 adulthood. (G) rme-2 RNAi enhances resistance to AS, in a skn-1-dependent manner (see replicates in Table 2). (H,I) An OA-dependent signal is required for SKN-1 to be activated by GSC inhibition but not oxidative stress. In GSC(-) animals, SKN-1 nuclear accumulation is abolished by sbp-1 RNAi, and rescued by OA supplementation. SKN-1 remains capable of responding to oxidative stress (30 min AS exposure) after sbp-1 RNAi in GSC(-) (H,I) or WT (Figure 6–figure supplement 2A) worms. (J) Dependence of SKN-1::GFP accumulation in GSC(-) animals on FAT-6/7-mediated FA desaturation, and proteins that generate free unsaturated FAs (LIP1/-3 lipases), or transport them to the nucleus (LBP-6/7/8). (K) OA and CO increase skn-1-dependent gst-4p::GFP expression in the intestine. lbp-8 RNAi reduces induction by OA. (A,C) Representative 10X GFP images. (D,H) Representative 40X GFP images of day-one adults. (E,F,I-K) GFP quantification with high, medium, low scoring. Numbers above bars denote sample size. P < 0.001***.
Figure 6–figure supplement 1. Enlarged VIT-2::GFP images. 40X GFP and BF images of (A) GSC(+) and (B) GSC(-) animals are shown. Note that glp-1(ts);VIT-2::GFP are presented with 5x lower exposure times due to increased VIT-2::GFP intensity in GSC(-) animals. All worms shown are day-one adults raised at 25°C. Arrowheads indicate the normal distribution of VIT-2::GFP in the intestine and oocytes, and arrows indicate the ectopic accumulation of VIT-2::GFP seen in GSC(-) animals. Numbers superimposed on images denote GFP exposure times.
Figure 6–figure supplement 2. SKN-1 is activated in response to FA signaling.

Accumulation of SKN-1 in intestinal nuclei in (A) response to arsenite (AS) exposure for 30 min or (B) daf-2 mutants is not impaired by sbp-1 RNAi. (C) Effects of oleic acid (OA) and coconut oil (CO) doses on intestinal gšt-4p::GFP expression, compared to AS treatment. (D) Larval development and (E) egg laying rate are not affected by OA or CO treatment. CO supplementation induces intestinal (F) SKN-1 nuclear accumulation but not (G) DAF-16 accumulation. (H,I) DAF-16 accumulation in glp-1(e2141ts) is unaffected by sbp-1 RNAi. (H) Representative 40X DIC images of day-one adults. (J) PMK-1 (p38 kinase) phosphorylation is not affected by GSC removal, consistent with a previous report (Alper et al., 2010). PMK-1 phosphorylation is increased dramatically by AS oxidative stress, and reflects activation of its kinase activity (Inoue et al., 2005). (A-C,F,G,I) GFP quantification with high, medium, low scoring. Numbers above bars denote sample size. *P < 0.05; **P < 0.01; ***P < 0.001.
Figure 6–figure supplement 3. Fatty acid desaturation is required for GSC(-) stress resistance. *glp-1(ts)* and control day-one adult worms treated with *lipl-3*, *sbp-1*, *fat-6/7* mix, *skn-1*, or empty vector RNAi were exposed to 5 mM AS. Knockdown of *lipl-3* (A,B), and either *sbp-1* (A,B) or *fat-6/7* (C,D) abolished the increase in AS resistance seen in *glp-1(ts)* animals. (B,D) Data are represented as mean ± SEM. \( P < 0.05^*; P < 0.01^{**}; P < 0.001^{***}. \) The interaction between *glp-1(ts)* and *fat-6/7, lipl-3, sbp-1*, and *skn-1* were significant (\( P < 0.001 \)). Additional information and statistics are provided in Table 2.
Figure 7. SKN-1 regulation in the GSC longevity pathway. GSC absence results in activation of transcription factors in the intestine, with SKN-1 being regulated in parallel to DAF-12 and DAF-16. Yolk transport to oocytes is disrupted by GSC loss, resulting in lipid accumulation in the intestine and body cavity. The resulting SKN-1 activation requires OA, the FAT-6/7 FA desaturases, and the lysosomal lipases LIPL-1/3. This lipid-based signaling to SKN-1 depends partially upon LBP-8, which transports FAs from the lysosome to the nucleus. SKN-1 induces transcription of genes involved in stress resistance, detoxification, proteasome maintenance, extracellular matrix, and lipid metabolism, thereby reducing fat storage, and increasing stress resistance and lifespan. Magenta denotes processes that are active in the presence of GSCs.
### Table 1. Lifespans.

Percent lifespan extension refers to *glp-1*(ts) versus wild-type or *skn-1* control. *P* values were calculated by log-rank test. The interaction effect of *glp-1*(ts) and *skn-1* were calculated by two-way ANOVA using mean lifespan. The last *P* value reflects the specific requirement of *skn-1* for *glp-1*(ts) lifespan, as opposed to its

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<th>Median Lifespan (days)</th>
<th>75th % (days)</th>
<th>N</th>
<th>% Mean Lifespan Ext. vs. N2</th>
<th><em>P</em> value (log-rank) vs. <em>glp-1</em></th>
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<td>two-way ANOVA <em>glp-1</em>(ts) and <em>skn-1</em> interaction</td>
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<td><strong>Replicate lifespans at 25°C with FUdR</strong></td>
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effect on lifespan in general. Homozygous \textit{skn-1} mutants produce eggs that do not hatch because of a catastrophic defect in developmental patterning, but do not exhibit known defects in the germline itself (Bowerman et al., 1992).
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<th>% Mean Survival Ext.</th>
<th>P value vs. N2 * vs. glp-1 † vs. rme-2 ‡</th>
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<td>22.5</td>
<td>22.5</td>
<td>124/124</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>glp-1(bn18ts) + vector RNAi</td>
<td>38.49 ± 1.5</td>
<td>47.5</td>
<td>47.5</td>
<td>98/98</td>
<td>58.03</td>
<td>&lt; 0.0001*</td>
</tr>
<tr>
<td></td>
<td>glp-1(bn18ts) + fat-6/7 mix RNAi</td>
<td>12.04 ± 0.7</td>
<td>9.0</td>
<td>22.5</td>
<td>153/153</td>
<td>-22.66</td>
<td>&lt; 0.0001†</td>
</tr>
<tr>
<td></td>
<td>glp-1(bn18ts) + skn-1 RNAi</td>
<td>23.69 ± 0.9</td>
<td>28.0</td>
<td>32.5</td>
<td>116/116</td>
<td>13.13</td>
<td>&lt; 0.0001†</td>
</tr>
</tbody>
</table>

| #7  | N2 + vector RNAi | 6.55 ± 0.3 | 7.0 | 8.0 | 104/104 | - | - |
|     | N2 + skn-1 RNAi | 5.08 ± 0.2 | 5.0 | 7.0 | 103/103 | - | - |
|     | glp-1(bn18ts) + vector RNAi | 19.36 ± 0.1 | 19.0 | 20.0 | 97/97 | 195.57 | < 0.0001* |
|     | glp-1(bn18ts) + skn-1 RNAi | 7.86 ± 0.4 | 8.0 | 12.0 | 100/100 | 54.72 | < 0.0001† |

| #8  | N2 + vector RNAi | 8.31 ± 0.4 | 9.0 | 10.0 | 98/98 | - | - |
|     | N2 + skn-1 RNAi | 6.89 ± 0.3 | 8.0 | 9.0 | 90/90 | - | - |
|     | glp-1(bn18ts) + vector RNAi | 16.95 ± 1.0 | 20.0 | 26.0 | 81/81 | 103.97 | < 0.0001* |
|     | glp-1(bn18ts) + skn-1 RNAi | 9.90 ± 0.4 | 10.0 | 12.0 | 91/91 | 43.69 | < 0.0001† |

| #9  | N2 + vector RNAi | 9.02 ± 0.3 | 9.0 | 11.0 | 84/108 | - | - |
|     | N2 + skn-1 RNAi | 6.16 ± 0.2 | 6.0 | 7.0 | 63/63 | - | - |
|     | glp-1(bn18ts) + vector RNAi | 11.62 ± 0.3 | 11.0 | 13.0 | 61/61 | 28.82 | < 0.0001* |
|     | glp-1(bn18ts) + skn-1 RNAi | 6.48 ± 0.1 | 8.0 | 7.0 | 63/65 | 5.19 | < 0.0001† |

| #10 | N2 | 4.29 ± 0.2 | 4.0 | 4.0 | 65/94 | - | - |
|     | skn-1(zu135) | 4.51 ± 0.1 | 5.0 | 3.0 | 73/74 | - | - |
|     | glp-1(bn18ts) | 6.25 ± 0.2 | 6.0 | 4.0 | 73/74 | 45.69 | < 0.0001* |
|     | glp-1(bn18ts);skn-1(zu135) | 4.78 ± 0.1 | 5.0 | 4.0 | 73/75 | 5.99 | < 0.0001† |

---

**Sodium arsenite (day 3 adulthood)** | 25°C during development, 20°C from D1; RNAi from D1

**TBHP (day 3 adulthood)** | 25°C during development, 20°C from D1; RNAi from D1
Table 2. Stress resistance assays. Survival after sodium arsenite or TBHP treatment was assayed in adult animals. The increase in oxidative stress resistance of glp-1(ts) (GSC(-)) animals was impaired by loss of fat-6/7, lipl-3, sbp-1, and skn-1. Representative assays are shown. Percent survival extension refers to glp-1(ts) or rme-2 RNAi vs. the matching wild-type or skn-1 control. *P* values were calculated by log-rank test. The interaction effect of GSC(-) or rme-2 with skn-1, or fat-6/7, lipl-3, and sbp-1 were calculated by two-way ANOVA using mean lifespan. The last *P* value reflects the specific requirement of each gene for GSC(-) or rme-2 stress resistance as opposed to its effect on stress resistance in general.
<table>
<thead>
<tr>
<th>Code</th>
<th>Genetic Background</th>
<th>Transgene</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA003</td>
<td>daf-12(rh61rh411) X</td>
<td>–</td>
<td>(Shen et al., 2012)</td>
</tr>
<tr>
<td>AA983</td>
<td>glp-1(e2141ts) III;</td>
<td>–</td>
<td>(Shen et al., 2012)</td>
</tr>
<tr>
<td></td>
<td>daf-12(rh61rh411) X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA1049</td>
<td>mir-241(n4315) V;</td>
<td>–</td>
<td>(Shen et al., 2012)</td>
</tr>
<tr>
<td></td>
<td>mir-84(n4037) X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA1709</td>
<td>glp-1(e2141ts) III;</td>
<td>–</td>
<td>(Shen et al., 2012)</td>
</tr>
<tr>
<td></td>
<td>mir-241(n4315) V;</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>mir-84(n4037) X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA2735</td>
<td>glp-1(e2141ts) III</td>
<td>–</td>
<td>(Shen et al., 2012)</td>
</tr>
<tr>
<td>CF1903</td>
<td>glp-1(e2141ts) III</td>
<td>–</td>
<td>(Berman and Kenyon, 2006)</td>
</tr>
<tr>
<td>CF1935</td>
<td>daf-16(mu86) I;</td>
<td>muls109[daf-16p::GFP::DAF-16 + odr-1p::RFP] X</td>
<td>(Berman and Kenyon, 2006)</td>
</tr>
<tr>
<td></td>
<td>glp-1(e2141ts) III</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL2166</td>
<td>N2</td>
<td>dvIs19[pAF15(gst-4p::GFP::NLS)] III</td>
<td>(Link and Johnson, 2002)</td>
</tr>
<tr>
<td>DH1033</td>
<td>sqt-1(sc103) II</td>
<td>bls1[vit-2p::VIT-2::GFP + rol-6(su1006)] X</td>
<td>(Grant and Hirsh, 1999)</td>
</tr>
<tr>
<td>EU31</td>
<td>skn-1(zu135) IV</td>
<td>–</td>
<td>(Bowerman et al., 1992)</td>
</tr>
<tr>
<td>LD001</td>
<td>N2</td>
<td>Idls7[SKN-1b/c::GFP + rol-6(su1006)]</td>
<td>(An and Blackwell, 2003)</td>
</tr>
<tr>
<td>LD002</td>
<td>N2</td>
<td>Idls1[SKN-1b/c::GFP + rol-6(su1006)]</td>
<td>(An and Blackwell, 2003)</td>
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<td>LD1025</td>
<td>daf-2(e1370) III</td>
<td>Idls7[SKN-1b/c::GFP + rol-6(su1006)]</td>
<td>(Tullet et al., 2008)</td>
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<td>LD1425</td>
<td>glp-1(bn18ts) III</td>
<td>Idls1[SKN-1b/c::GFP + rol-6(su1006)]</td>
<td>This study</td>
</tr>
<tr>
<td>LD1434</td>
<td>glp-1(bn18ts) III;</td>
<td>–</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td>skn-1(zu135) IV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LD1473</td>
<td>kri-1(ok1251) I;</td>
<td>Idls1[SKN-1b/c::GFP + rol-6(su1006)]</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td>glp-1(bn18ts) III</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LD1474</td>
<td>fctc-1(tm1452) II;</td>
<td>Idls1[SKN-1b/c::GFP + rol-6(su1006)]</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td>glp-1(bn18ts) III</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LD1548</td>
<td>N2</td>
<td>Is[dhs-3p::DHS-3::GFP] I</td>
<td>(Zhang et al., 2012)</td>
</tr>
<tr>
<td>LD1549</td>
<td>glp-1(bn18ts) III</td>
<td>Is[dhs-3p::DHS-3::GFP] I</td>
<td>This study</td>
</tr>
<tr>
<td>LD1644</td>
<td>sqt-1(sc103) II;</td>
<td>bls1[vit-2p::VIT-2::GFP + rol-6(su1006)] X</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td>glp-1(bn18ts) III</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LD1653</td>
<td>glp-1(bn18ts) III</td>
<td>–</td>
<td>(Dorsett et al., 2009)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Outcrossed from DG2389</td>
</tr>
<tr>
<td>LD1744</td>
<td>glp-1(bn18ts) III</td>
<td>IdEx119[pAF15(gst-4p::GFP::NLS) + rol-6(su1006)]</td>
<td>This study</td>
</tr>
<tr>
<td>TJ356</td>
<td>N2</td>
<td>zls356[daf-16p::DAF-16a/b::GFP + rol-6] IV</td>
<td>(Henderson and Johnson, 2001)</td>
</tr>
</tbody>
</table>

Table 3. *C. elegans* strains used in this study.
Table 4. qRT-PCR primers used in this study. Select primer sequences were obtained from previous publications (Robida-Stubbs et al., 2012; Vilchez et al., 2012; O'Rourke and Ruvkun, 2013).

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sequence</th>
<th>Annotation</th>
<th>Primer pair</th>
</tr>
</thead>
<tbody>
<tr>
<td>gst-4</td>
<td>K08F4.7</td>
<td>Glutathione S-transferase</td>
<td>FWD: CCCATTTTACAAGTCGATGG REV: CTTCCCTCTCGCAGTTTCCA</td>
</tr>
<tr>
<td>F20D6.11</td>
<td>F20D6.11</td>
<td>Flavin-adenine dinucleotide (FAD)-binding oxidoreductase</td>
<td>FWD: GGAAATTCTCGGTAGAATCGAA REV: ACGATCAGCAACTCGAACA</td>
</tr>
<tr>
<td>nit-1</td>
<td>ZK1058.6</td>
<td>Nitrilase</td>
<td>FWD: AATCCCTCGACTATCCTTTTG REV: AGCGAATCGTTTCTTTTG</td>
</tr>
<tr>
<td>rpn-6.1</td>
<td>F57B9.10</td>
<td>19S non-ATPase subunit</td>
<td>FWD: AATTGGAAAAAGCACCCTGAAATGT REV: TTTGATGTGGAAGTGAATGGTCATTTG</td>
</tr>
<tr>
<td>lipl-3</td>
<td>R11G11.14</td>
<td>Lysosomal triglyceride lipase</td>
<td>FWD: ATGGGCAGGCAATCCCACCA REV: AGTTGCTCGGCAATTATA</td>
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<tr>
<td>*cdc-42</td>
<td>R07G3.1</td>
<td>Housekeeping gene</td>
<td>FWD: CTGCGGACAGGAAGATTACG REV: CTCGGACATTCTCGGAATGAA</td>
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<tr>
<td>*Y45F10D.4</td>
<td>Y45F10D.4</td>
<td>Housekeeping gene</td>
<td>FWD: GTGCCCTCAATCGATTCGAC REV: GTTCTTGCAAGTGCAAGTACG</td>
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</tbody>
</table>

1237 1238 1239
Supplementary files

[see Supplementary files 1-4.xlsx file]

Supplementary file 1a. List of genes activated by GSC absence. The gene list is sorted by functional grouping, then by fold change of mRNA expression in glp-1(ts) relative to wild-type. A fold change cutoff of 4 ($P < 0.05$; $n = 1,306$) was used (see Figure 3–figure supplement 1 for rationale). A more conservative fold change cutoff of 5 that captured fewer genes ($n = 615$) was used for DAVID cluster analysis. CPM denotes counts per million, FC denotes fold change, and FDR denotes false discovery rate.

Supplementary file 1b. List of genes activated by SKN-1 in wild-type animals. The gene list is sorted by functional grouping, then by fold change of mRNA expression in WT skn-1 RNAi-treated worms relative to WT control vector-treated worms. A fold change cutoff of 0.67 ($n = 295$) was used to enrich for higher confidence SKN-1 targets. CPM denotes counts per million, FC denotes fold change, and FDR denotes false discovery rate.

Supplementary file 1c. List of genes activated by SKN-1 in GSC(-) animals. The gene list is sorted by functional grouping, then by fold change of mRNA expression in glp-1(ts) skn-1 RNAi-treated worms relative to glp-1(ts) control vector-treated worms. A fold change cutoff of 0.67 ($P < 0.05$; $n = 529$) was used to enrich for higher confidence SKN-1 targets. CPM denotes counts per million, FC denotes fold change, and FDR denotes false discovery rate.

Supplementary file 1d. List of genes activated by GSC absence in a SKN-1-dependent manner. The gene list is sorted by functional grouping, then by fold change of mRNA expression in glp-1(ts) skn-1 RNAi-treated worms relative to glp-1(ts) control vector-treated worms. We employed a GSC(-) fold change cutoff of > 4 and skn-1 RNAi cutoff of < 0.67 to generate the list (see Figure 3–figure supplement 1 for GSC(-) FC cutoff rationale). Statistics were generated by min analysis. Predicted SKN-1 binding sites were determined using 1.5 kb upstream sequences from WBcel235 and the SKN-1 JASPAR matrix (Staab et al., 2013). SKN-1::GFP ChIP-seq binding analysis was performed using the L1, L2, and L3 data sets available from modENCODE (Niu et al., 2011). Additional details are available in Materials and methods. CPM denotes counts per million, FC denotes fold change, and FDR denotes false discovery rate.
Relative mRNA levels

WT
daf-12
mir-241;mir-84

*  

glp-1(ts)

**  

vector
daf-12 pmk-1

*** 

SKN-1::GFP

**  

WT

glp-1(ts)

kri-1;glp-1(ts)
tcer-1;glp-1(ts)

*** 

15°C 25°C

glp-1(ts);gst-4p::GFP

*** 

15°C 25°C

glp-1(ts);gst-4p::GFP

BF

Merge

A B C

D E F G H
GSC(-) upregulated and SKN-1 dependent (n = 87)

Sorted by skn-1 RNAi fold change in glp-1(ts)

GW + EV
skn-1 RNAi
glp-1(ts) + EV

Enrichment score

All genes (n = 12,595)

Hierarchical clustering

Enrichment score

GSC(-) upregulated and SKN-1 dependent (n = 87)

Sorted by skn-1 RNAi fold change in glp-1(ts)
A RNA-Seq

**skin-1 RNAi in GSC(-)**

**skin-1 RNAi in WT**

Relative mRNA levels

B SKN-1::GFP ChIP-Seq proteasome gene hits

C Chymotrypsin-like proteasome activity

D Proteasome activity (slope)

**skn-1 RNAi**

**glp-1(ts)**

**glp-1(ts);skn-1**

E Relative mRNA levels

F chromosome III

**rpn-6.1**

GFP ChIP-Seq

DAF-16

GFP ChIP-Seq

SKN-1

TRANSFAC

DAF-16

TRANSFAC
SKN-1 effects:
- ↑ stress resistance
- ↑ proteostasis
- ↑ collagens/ECM
- ↓ fat levels
- ↑ lifespan

FA signal?

lipogenesis

lipid metabolism
- ↑ TAG lipases
- ↑ FA desat. & elong.
- ↑ FA transporters
- ↑ FA β-oxidation

yolk transport

GSC

oocyte

RME-2

DAF-16

SKN-1

PMK-1

KRI-1

mir-84

mir-241

DAF-12

DAF-16

FXR

NHR-80

NHR-49

PPARα

PHA-4

FOXA

HNF4

FOXO

TCER-1

HLH-30

TFEB

GLP-1

intestine

gonad

oocyte

nucleus

lipids