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SHORT TAKE

Host cell factor 1 inhibits SKN-1 to modulate oxidative stress responses in *Caenorhabditis elegans*

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Summary

Host cell factor-1 (HCF-1) is a conserved regulator of the longevity and stress response functions of DAF-16/FOXO. SKN-1 transcription factor is an evolutionarily conserved xenobiotic stress regulator and a pro-longevity factor. Here, we demonstrate that SKN-1 contributes to the enhanced oxidative stress resistance incurred by *hcf-1* mutation in *C. elegans*. HCF-1 prevents the nuclear accumulation of SKN-1 and represses the transcriptional activation of SKN-1 specifically at target genes involved in cellular detoxification pathways. Our findings reveal a novel and context-specific regulatory relationship between two highly conserved longevity and stress response factors HCF-1 and SKN-1.

Key words: aging; DAF-16; HCF-1; oxidative stress; SKN-1; transcription.

Introduction, results, discussion

Animal cells are equipped with mechanisms to cope with fluctuations in reactive oxygen species (ROS) produced as by-products of oxygen metabolism. Excessive ROS leads to disruption of redox homeostasis and contributes to the development of various diseases (Molavi & Mehta, 2004; Jomova *et al.*, 2010; Reuter *et al.*, 2010; Rains & Jain, 2011). Two conserved mechanisms responsible for combating oxidative stress involve FOXO forkhead and NF-E2-related (Nrf) transcription factors (Huang & Tindall, 2007; Sykiotis & Bohmann, 2010).

Host cell factor-1 (HCF-1) is an evolutionarily conserved transcriptional regulator key to cell cycle progression, aging, and stress response (Lee *et al.*, 2007; Tyagi *et al.*, 2007; Li *et al.*, 2008; Rizki *et al.*, 2011). Inactivation of *hcf-1* prolongs lifespan and increases resistance to oxidative stress in a manner dependent on *daf-16*, the worm ortholog of FOXO transcription factors (Li *et al.*, 2008). Given that *skn*-1, the sole ortholog of Nrf factors in *C. elegans*, is a major mediator of detoxification and longevity (An & Blackwell, 2003; Tullet *et al.*, 2008), we assessed whether *skn-1* participates in the modulation of longevity and stress responses by *hcf-1*. We used RNAi to diminish *skn-1* activity in mutant *hcf-1(pk924)* and wild-type N2 worms (Oliveira *et al.*, 2009) and examined either normal lifespan or survival upon exposure to oxidative stress-inducing agents, *tert*-Butyl Hydroperoxide (*t*-BOOH), paraquat, sodium arsenite (NaAs), or heat stress at 32 °C. As expected, *hcf-1* mutants depended on *daf-16* to live long and be resistant to oxidative and thermal stress [(Li *et al.*, 2008) and Figs 1 and S1, Table S1]. Interestingly, reducing *skn-1* activity blunted the *t*-BOOH, paraquat, and NaAs-response exhibited by *hcf-1* mutants but did not significantly affect the longevity or thermotolerance of *hcf-1*-deficient worms (Figs 1 and S1; Table S1). To rule out the possibility that the lack of effect of *skn-1* RNAi in lifespan was because of inefficient inactivation of *skn-1* or that a mild suppression effect was masked by the strong longevity phenotype of *hcf-1* mutants, we treated *skn-1(zu67)* mutants with *hcf-1* RNAi and monitored their lifespan. *skn-1(zu67)* mutation, which introduces a premature stop codon in the *skn-1a* and *c* isoforms and abolishes SKN-1 protein levels (Bowerman *et al.*, 1993; Tullet *et al.*, 2008), did not suppress the mild lifespan extension exerted by *hcf-1* knockdown (Fig. S1C; Table S1E). In fact, for reasons we do not yet understand, we consistently observed that *hcf-1* RNAi extended lifespan to a greater degree in the *skn-1(zu67)* mutant compared to wild-type worms. We cannot exclude the possibility that the *skn-1b* isoform may have a role in *hcf-1*-mediated longevity. Our findings indicate that while DAF-16 strongly contributes to all phenotypes associated with *hcf-1* mutation, SKN-1 appears to be more specific for oxidative stress response and may not be involved in the thermal stress and lifespan functions of HCF-1.

Our results, which demonstrate that HCF-1 inhibits both DAF-16 and SKN-1 under oxidative stress conditions, raised the question of whether DAF-16 and SKN-1 act in concert or independently downstream of HCF-1. We next examined the genetic relationship between DAF-16 and SKN-1 downstream of HCF-1. We fed N2, *hcf-1(pk924), daf-16(mgDf47)* and *daf-16(mgDf47); hcf-1(pk924)* worms control or *skn-1* RNAi, and monitored their survival after exposure to paraquat, *t*-BOOH, and NaAs. Upon treatment with any of the three compounds, simultaneously depleting both *daf-16* and *skn-1* annulled the *hcf-1* mutant-induced resistance to a greater extent than did depleting either one alone (Figs 1 and S1D; Table S1F–H), suggesting that DAF-16 and SKN-1 act in parallel pathways to provide protection against oxidative insults in *hcf-1* mutants. As expected, *skn-1* knockdown did not lead to additional hypersensitivity to thermal stress in the absence of *daf-16,* corroborating our proposal that DAF-16 but not SKN-1 is engaged by HCF-1 at higher temperatures (Fig. S1E; Table S1I). Overall, our data are consistent with a model in which HCF-1 inhibits DAF-16 and SKN-1 through independent means when worms are challenged by elevated oxidative stress.

Given that HCF-1 is a prominent coregulator of transcription factors, we asked whether it antagonizes the transcriptional activity of SKN-1. To examine the effects of HCF-1 on SKN-1 activity, we monitored the expression of multiple direct targets of SKN-1 in the absence of *hcf-1*. Under elevated stress or reduced IIS conditions, SKN-1 directly induces detoxification genes such as γ-glutamyl cysteine synthetase (*gcs-1*) and glutathione S-transferases (*gst*) in the intestine (An & Blackwell, 2003; Tullet *et al.*, 2008). When we diminished *hcf-1* levels by RNAi knockdown, intestinal expression of SKN-1 target gene reporters, *gcs-1::gfp, gst-4::gfp* and *gst-7::gfp,* was elevated (Fig. S2A). The elevation in gene expression was because of SKN-1 since the induction was abolished in the *gcs-1::gfp* transgenic worms carrying the *skn-1(zu67)* mutation

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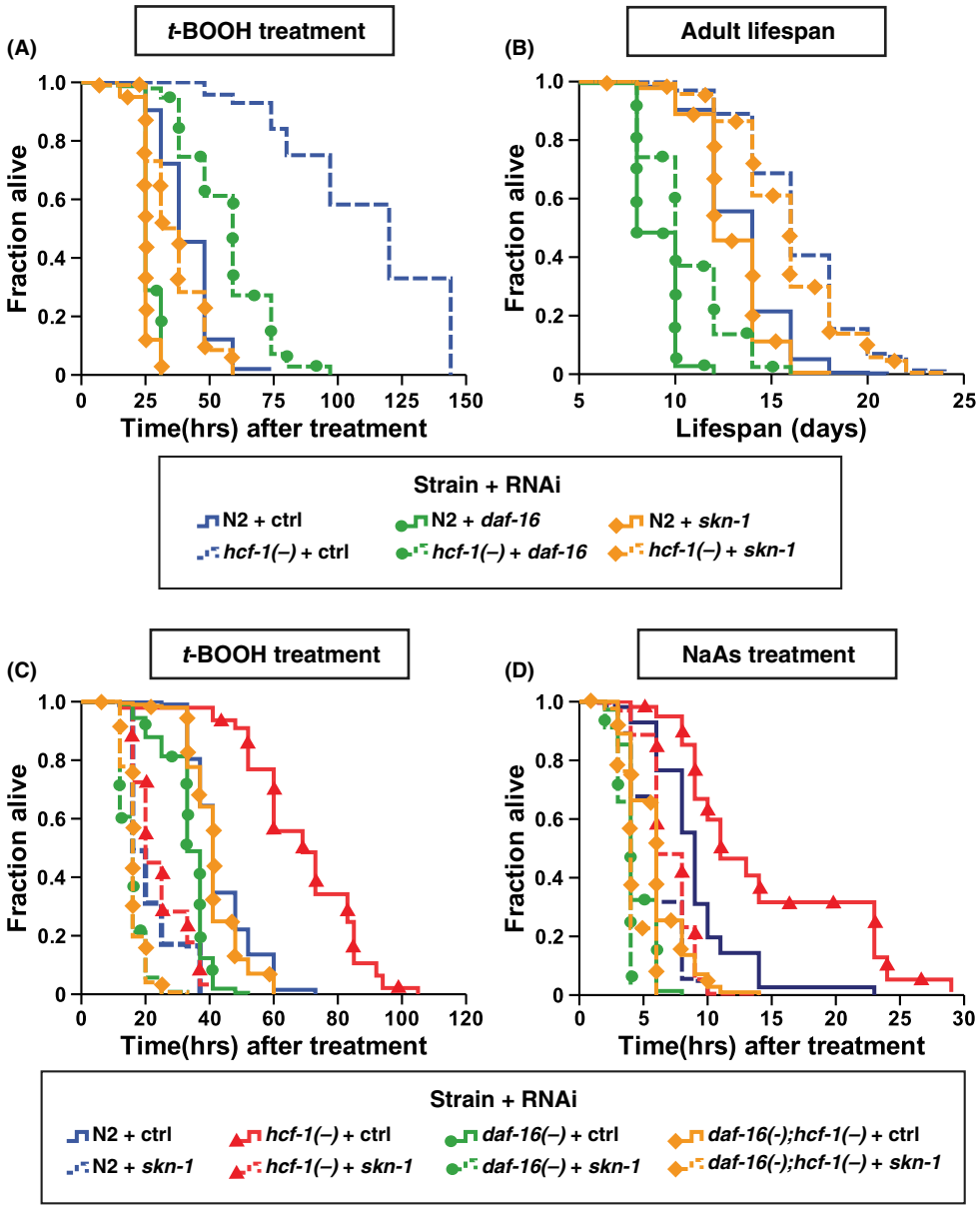

Fig. 1 *skn-1* mediates the oxidative stress resistance conferred by *hcf-1(pk924)* mutation. Survival plots of wt N2, *hcf-1(pk924)*, *daf-16(mgDf47)*, and *daf-16(mgDf47); hcf-1(pk924)* strains fed with control (L4440) or *skn-1* RNAi. (A) Worms were exposed to 4 mM *t*-BOOH. *daf-16* RNAi was used once, whereas results with *skn-1* knockdown were repeated three times. (B) Survival plots represent adult lifespan. wt N2 and *hcf-1(pk924)* were treated with indicated RNAi. Pooled data from two experiments for *daf-16* RNAi and three experiments for ctrl and *skn-1* RNAi are combined and plotted. (C–D) wt N2, *hcf-1(pk924)*, *daf-16(mgDf47)*, and *hcf-1(pk924);daf-16(mgDf47)* strains were fed the indicated RNAi bacteria. All graphs represent combined data from two independent experiments. Animals were exposed to (C) 4 mM *t*-BOOH or (D) 10 mM NaAs.

(Fig. S2B). The absence of *hcf-1* further induced *gcs-1::gfp* expression when the animals were exposed to NaAs (Fig. S2C). These data indicate that HCF-1 represses SKN-1-mediated gene expression.

To gain a global insight into the transcriptional regulation of SKN-1 by HCF-1, we compared the transcriptomes of *hcf-1* mutants to those of *skn-1*-depleted worms. We compared the genes differentially expressed in *hcf-1(pk924)* mutant worms relative to N2 wild-type worms (referred to as *hcf-1*(–) profile) (Rizki *et al.*, 2011) to those changed in wild-type worms treated with control RNAi relative to *skn-1* RNAi (referred to as *skn-1*(+) profile) (Oliveira *et al.*, 2009). Cluster analysis revealed a group of genes that were upregulated in the absence of *hcf-1* and the presence of *skn-1*, consistent with the idea that SKN-1 activation in *hcf-1* mutants may account for their altered expression (Figs 2 and S2D; Table S3). Chi-squared analysis revealed that the overlap between *hcf-1*(–) and *skn-1(+)* profiles was greater than expected by random chance ($P = 0.007$). Using reverse-transcription coupled quantitative PCR (RT-qPCR), we demonstrated that the elevated expression of representatives of this group of genes in *hcf-1* mutants was indeed dependent on SKN-1 activity, as diminished *skn-1* activity largely abolished the induced gene expression produced by *hcf-1* mutation (Fig. 2). In support of the idea that HCF-1 affects SKN-1's oxidative stress response functions, Gene Ontology analysis revealed that among this group of *hcf-1*(–)/*skn-1(+)*-upregulated genes, Phase II detoxification genes such as *gst*s were by far the most highly enriched class, and Phase I detoxification genes including short-

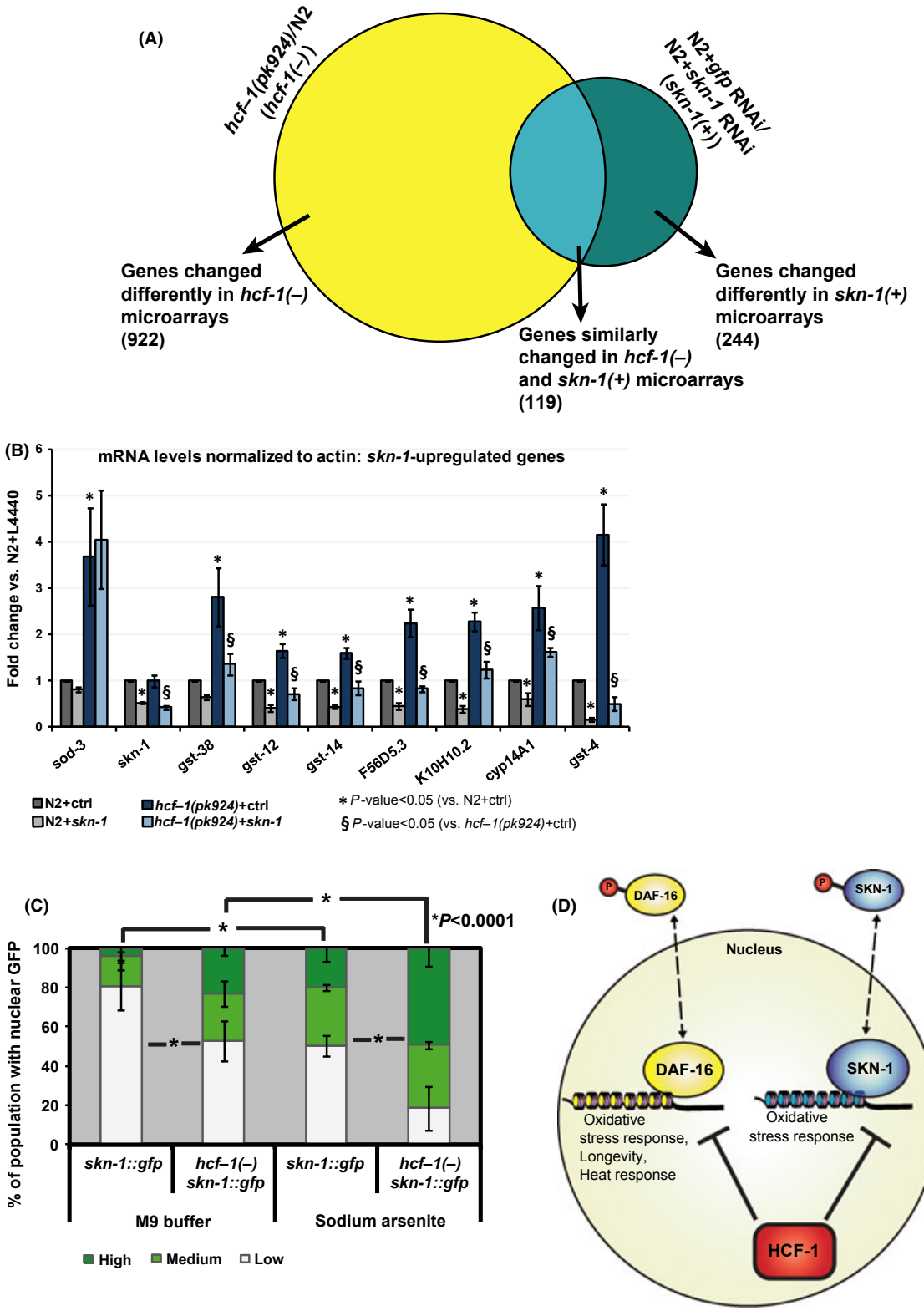

Fig. 2 HCF-1 limits SKN-1's nuclear accumulation to inhibit the expression of oxidative stress responsive SKN-1 target genes. (A) Venn diagram showing the overlap between *hcf-1(pk924)*/N2 and ctrl RNAi/*skn-1* RNAi microarrays. (B) mRNA levels of indicated genes quantified by RT-qPCR. Fold change relative to N2+ctrl RNAi averaged from three independent experiments is plotted and error bars represent ±SEM. $P < 0.05$ is considered statistically significant. (C) Wild-type or *hcf-1(pk924)* mutant worms expressing a SKN-1B/C::GFP reporter are exposed to 5 mM NaAs or M9 and nuclear SKN-1 levels examined. Mean percentages for low, medium, and high from three independent experiments are displayed. Error bars denote ±SEM. (D) HCF-1 inhibits the nuclear accumulation of SKN-1 and regulates SKN-1's transcriptional activity parallel to DAF-16 to modulate defenses against oxidative stress.

chain dehydrogenases were highly overrepresented (Table S2). A small group of co-downregulated genes did not reveal enriched functional GO-term classes (Table S2). We next examined how the genes similarly changed in *hcf-1*(–) and *skn-1(+)* arrays were affected by *daf-16*. We found that only 44% of the *hcf-1/skn-1*- affected genes overlapped with HCF-1-regulated DAF-16 targets (Fig. S2E). In support of our model that SKN-1 and DAF-16 work at least partially independently downstream of HCF-1 to respond to oxidative stress, we found that Phase I and Phase II detoxification GO terms were enriched in the gene set specifically regulated by *hcf-1/skn-1*, whereas the *daf-16*-overlapping subset was enriched in transcription function (Fig. S2F). Our findings establish HCF-1 as a specific transcriptional regulator of SKN-1, where HCF-1 governs SKN-1 activity at a group of genes whose main function is to combat oxidative insults and toxicity.

As SKN-1 nuclear localization is a key regulatory step of its activation (An & Blackwell, 2003; An *et al.*, 2005; Inoue *et al.*, 2005; Kahn *et al.*, 2008; Tullet *et al.*, 2008; Choe *et al.*, 2009), we examined whether HCF-1 affects the subcellular localization of SKN-1. We monitored the effect of *hcf-1(pk924)* mutation on SKN-1's subcellular localization using a transgenic strain carrying the SKN-1B/C::GFP reporter (An & Blackwell, 2003). Under unstressed conditions, attenuating *hcf-1* function increased the nuclear accumulation of SKN-1 protein (Figs 2 and S3). Interestingly, the amount of elevated intestinal nuclear SKN-1 was even further augmented in *hcf-1* mutants upon exposure to NaAs and *t*-BOOH (Figs 2 and S3). It is possible that the additional increase in nuclear SKN-1 localization or the enhancement of SKN-1's transcriptional activity (Fig. S2C) is because of SKN-1 being more active in the absence of *hcf-1* and being able to respond to acute stress more effectively. However, we cannot exclude the possibility that stressors may have parallel mechanisms, both through and independent of HCF-1, to affect SKN-1 activation. Overall, our results indicate that HCF-1 regulates the activity of SKN-1 by inhibiting its nuclear accumulation.

In this study, we revealed a new regulatory relationship between HCF-1 and the key oxidative stress factor SKN-1. We demonstrated that HCF-1 inhibits transcriptional activation by SKN-1 on specific Phase I & II detoxification genes, in part by preventing its nuclear accumulation. HCF-1 lacks enzymatic activity and acts largely by assembling different protein complexes. Whether HCF-1 also binds SKN-1 has yet to be determined. Interestingly, SKN-1 carries a conserved HCF-1-binding motif, DHSY, which is a major site of interaction between HCF-1- and HCF-1-interacting factors in mammalian cells (Freiman & Herr, 1997).

Our observation that SKN-1's nuclear localization is exaggerated in *hcf-1* loss of function mutants provides an important clue for the mechanism by which HCF-1 inhibits SKN-1. However, using the SKN-1::GFP reporter, we cannot discern whether the significant rise in nuclear SKN-1 is because of increased cytoplasm–nucleus shuttling, increased nuclear import or reduced nuclear export, and elevated *skn-1* expression or protein stability. Future studies to elicit the exact mechanism by which HCF-1 regulates the levels of nuclear SKN-1 will be necessary.

In summary, we uncovered a novel functional interaction between HCF-1 and SKN-1, two major contributors to aging and organismal stress response processes. Our findings further our knowledge of coordinated response mechanisms required to sense and fight against harmful toxic insults. The highly conserved nature of both HCF-1 and SKN-1 proteins raise the possibility that a similar regulatory relationship between mammalian HCF and Nrf factors may represent an important defense against oxidative stress-induced ailments such as cancer and cardiovascular disease.

Experimental procedures

All strains were cultured under standard growth conditions (Brenner, 1974).

For lifespan and stress assays, adult animals were allowed to lay eggs overnight at 16 °C and the progeny grown at 25 °C. Worms were kept at 25 °C for the remainder of the experiments (see Data S1, Supplemental Experimental Procedures for details).

GFP fluorescence scoring was essentially carried out as described in (An & Blackwell, 2003; Tullet *et al.*, 2008) and described in Data S1. For RT-qPCR, total RNA was extracted (Tri-reagent) and reverse transcribed (iScript cDNA synthesis Kit; BioRad, Hercules, CA, USA), and cDNAs were quantified by SYBR Green quantitative PCR on a Roche LightCycler 480 (Roche Applied Science, Indianapolis, IN, USA) real-time PCR machine [see Data S1)].

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Author contributions

Gizem Rizki and Siu Sylvia Lee conceived and designed the experiments. Gizem Rizki, Colette Lafontaine Picard, and Charles Pereyra performed the experiments and analyzed the data. Gizem Rizki and Siu Sylvia Lee wrote the paper.

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Supporting Information

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

Fig. S1 *skn-1* is required for the elevated oxidative stress resistance of *hcf-1(pk924)* mutants.

Fig. S2 Absence of *hcf-1* induces transcriptional activity of SKN-1 under basal and high oxidant conditions.

Fig. S3 Absence of *hcf-1* induces nuclear accumulation of SKN-1 under basal and oxidative stress conditions.

Table S1. Quantitative data for survival plots.

Table S2 Enriched GO terms of *hcf-1*(–) and *skn-1*(+) clusters.

Table S3. Genes co-regulated by HCF-1 and SKN-1.

Data S1 Supplemental Experimental Procedures.

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