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Casein kinase 1 gamma integrates oxidative stress and innate immune responses

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Abstract

Animals utilize associated pathways to elicit responses to oxidative stress and infection. The molecular mechanisms coordinating these pathways remain unclear. Here, using *C. elegans* we identified the highly conserved casein kinase 1 gamma CSNK-1 (also known as CK1γ or CSNK1G), as a key regulator of these processes. *csnk-1* interacted with the *bli-3/tsp-15/doxa-1* dual oxidase genes by nonallelic noncomplementation to negatively regulate animal survival in excess iodide, an oxidative stressor. A conserved interaction was detected between DOXA-1 and CSNK-1 and between their human homologs DUOXA2 and CSNK1G2. *csnk-1* deficiency resulted in upregulated expression of innate immunity genes and increased animal survival in the pathogenic *Pseudomonas aeruginosa* PA14. Phosphoproteomic analyses identified decreased phosphorylation of key innate immunity regulators NSY-1 MAPKKK and LIN-45 Raf in *csnk-1(lf)* mutants. Indeed, NSY-1 and LIN-45 pathways were required for the increased survival of *csnk-1*-deficient animals in PA14. Further analyses suggest that CSNK-1 and SKN-1 Nrf2 might act in parallel to regulate oxidative stress response. We propose that CSNK-1 CSNK1G plays a novel pivotal role in integrating animal’s responses to oxidative stress and pathogens.
Introduction

Reactive oxygen species (ROS) are key signaling molecules that regulate metabolism, cell growth, cell survival and cell proliferation via different pathways (Schieber and Chandel, 2014). However, excessive levels of ROS can damage proteins, lipids and nucleic acids and cause oxidative stress. Animals utilize conserved molecular mechanisms to regulate ROS levels and mitigate oxidative stress (Schieber and Chandel, 2014; Sies et al., 2017). Endogenous ROS are primarily generated by the NADPH oxidases and mitochondria (Sies et al., 2017). The nuclear factor erythroid 2-related factor 2 (Nrf2) pathway and the NF-κB pathway play major roles in mediating defense responses to oxidative stress triggered by endogenous or exogenous stressors (Blackwell et al., 2015; Lingappan, 2018; Sies et al., 2017).

Oxidative stress and immune responses share key regulators. The Nrf2 pathway is involved in innate immunity, antiviral response and inflammation (Battino et al., 2018; Herengt et al., 2021; Saha et al., 2020). Mitochondrial ROS are essential second messengers for innate and adaptive immune responses (Weinberg et al., 2015). NADPH oxidase mediates host defense by generating antimicrobial ROS (Moghadam et al., 2021) and loss of function in phagocyte NADPH oxidase complex can lead to chronic granulomatous disease, a form of excessive inflammation caused by severe bacterial and fungal infections (Segal et al., 2012). Hence, an important question arises as to how animals coordinate the response to oxidative stress and the defense against pathogens.

The nematode Caenorhabditis elegans uses conserved pathways to regulate oxidative stress and innate immune responses (Blackwell et al., 2015; Ewald, 2018; Kim and Ewbank, 2018;
McCallum and Garsin, 2016). *C. elegans* SKN-1 protein is homologous to the mammalian Nrf2 (Walker et al., 2000), can similarly mediate oxidative stress response (An and Blackwell, 2003), and has broad homeostatic functions in stress response, aging, immunity, lipid metabolism, unfolded protein response and other processes (Blackwell et al., 2015). The NSY-1-SEK-1-PMK-1 p38 MAPK pathway and the LIN-45-MEK-2-MPK-1 ERK MAPK pathway mediate key innate immune responses (Kim and Ewbank, 2018; Kim et al., 2002; Nicholas and Hodgkin, 2004). SKN-1 functions downstream of the p38 MAPK pathway to regulate oxidative stress response and innate immunity (Inoue et al., 2005; Papp et al., 2012). *C. elegans* also has a BLI-3 dual oxidase that is the only functional NADPH oxidase encoded by its genome (Edens et al., 2001). BLI-3, the tetraspanin protein TSP-15 and the dual oxidase maturation factor DOXA-1 form a complex to regulate *C. elegans* cuticle formation and response to oxidative stress (Edens et al., 2001; Ewald, 2018; Ewald et al., 2017; Moribe et al., 2004, 2012; Thein et al., 2009; Xu et al., 2015, 2018). BLI-3 regulates innate immune response via the p38 MAPK and the SKN-1 pathways (Chavez et al., 2009; Hoeven et al., 2011; McCallum and Garsin, 2016).

We recently found that the survival phenotype of *C. elegans* in excess iodide provides a new tool for dissecting the functions of genes regulating oxidative stress response (Xu et al., 2015, 2018). Screens for mutants that can survive in excess iodide isolated multiple loss-of-function (lf) mutations in *bli-3*, *tsp-15*, *doxa-1*, *wdr-23* (encoding a negative regulator of SKN-1) (Choe et al., 2009) and gain-of-function (gf) mutations in *skn-1* (Xu et al., 2015, 2018).

To further understand how *C. elegans* responds to oxidative stress, we characterized a
previously isolated mutant (*mac397*) that can grow into adults in 5 mM NaI (Xu et al., 2018).

*mac397* affected the conserved *casein kinase 1 gamma* gene *csnk-1*. We found that *csnk-1* interacted with the *bli-3/tsp-15/doxa-1* complex to negatively regulate animal survival in excess iodide. Transcriptome analyses suggest that CSNK-1 was a negative regulator of innate immune gene expression, which was validated by increased survival of *csnk-1(lf)* mutants in the pathogenic bacteria *Pseudomonas aeruginosa* PA14. Phosphoproteomic analyses identified decreased phosphorylation of NSY-1 and LIN-45 in *csnk-1(lf)* mutants, and these kinases were required for the negative effects of CSNK-1 on innate immunity. Together our results suggest that CSNK-1 CSNK1G integrates animal’s responses to oxidative stress and pathogen infections.
Results

csnk-1 negatively regulates C. elegans survival in excess iodide, an oxidative stressor

We previously performed an ethyl methanesulfonate (EMS) screen for F₁ mutants that can grow into adults in 5 mM NaI (the survival, hereafter) and isolated six unidentified mutants (Xu et al., 2018). Within the mapped region of the original mac397 isolate (Supplemental Materials and Methods), genomic sequencing detected a point mutation in the csnk-1 gene (exon4:c.G482A:p.R161H, named mac397. Fig. 1A and Table S1), a point mutation in tsp-15 (exon3:c.G331A:p.G111R, Table S1), and coding variations in nine other genes (Table S1).

Further analyses suggest that csnk-1 can specifically inhibit the survival (Tables S1 and S2, Supplemental Materials and Methods) and the tsp-15 mutation (named mac499, Table S3, Supplemental Materials and Methods) caused a loss of function.

csnk-1 encodes the C. elegans ortholog of casein kinase 1 gamma (Manning, 2005). Mammals have three casein kinase 1 gamma genes (CSNK1G1, CSNK1G2 and CSNK1G3) (Schittek and Sinnberg, 2014). CSNK1Gs, especially in the kinase domain, are highly conserved (Fig. S1). mac397 caused an R161H (arginine to histidine) change in the kinase domain of CSNK-1 (Fig. 1A, 1B and S1).

To confirm the effect of csnk-1 on the survival, we generated two frameshift mutations in csnk-1, mac494 and mac495 (Fig. 1A and Table S2), using the CRISPR/Cas9 method.

Homozygous csnk-1(mac494) and csnk-1(mac495) progeny of heterozygous hermaphrodite parents can grow into adults in 5 mM NaI, while heterozygous progeny failed to do so (Fig. 1C and Table S2). The homozygous adults can lay multiple eggs on regular NGM plates.
However, very few of the eggs would hatch (Fig. 1D). Together these results suggest that *csnk-1* is essential for *C. elegans* embryonic development and also negatively affects the survival in excess iodide at postembryonic stages.

To further understand the function of *csnk-1*, we expressed a *csnk-1* cDNA transgene driven by an endogenous *csnk-1* promoter (3.1 kb upstream of the start codon) in *csnk-1(mac494lf)* mutants (reference loss-of-function allele if not specified, hereafter). We found that the transgene significantly rescued the survival phenotype of *csnk-1(lf)* mutants (Table 1).

We next examined how *mac397* R161H affects CSNK-1 activity. Driven by the endogenous *csnk-1* promoter, a *csnk-1(R161H)* mutant cDNA transgene failed to rescue the survival phenotype of *csnk-1(lf)* mutants (Table 1). Neither did this transgene promote the survival of wildtype animals (Table 1). These results suggest that *mac397* causes a loss of function in *csnk-1*.

To understand the tissue-specific functions of *csnk-1*, we first examined the activity of the *csnk-1* promoter. A GFP transgene driven by the promoter was broadly expressed when injected into wildtype animals (Fig. 1E). The expression was obvious in the pharynx, head neurons, intestine, ventral cord, vulval muscles, body-wall muscles, dorsal cord and ventrodorsal commissures (Fig. 1F-J). Expression was also observed in the epidermis (hypodermis) (Fig. 1F and H).
We next performed tissue-specific rescue experiments. Driven by an epidermis-specific promoter (dpy-7p) (Supplemental Materials and Methods), the csnk-1 cDNA transgene strongly rescued the survival of csnk-1(lf) mutants (Table 1). However, the transgene driven by an intestine-specific promoter (nhx-2p) failed to rescue (Table 1). Therefore, csnk-1 probably functions in the epidermis to affect the survival. Similar results were previously reported for tsp-15 (Xu et al., 2018).

To examine whether the function of CSNK1G was conserved, we expressed the human CSNK1G1, CSNK1G2 or CSNK1G3 cDNA transgene driven by the dyp-7 promoter in csnk-1(lf) mutants. We found that all three transgenes significantly rescued the survival phenotype (Table 1). Therefore, the function of csnk-1 is conserved and the three human CSNK1Gs exhibit similar activities.

CSNK1G proteins contain a conserved C-terminal palmitoylation signal sequence (TKCCCFFKR) required for the membrane localization and activities of the kinases (Davidson et al., 2005; Li et al., 2016). A conserved palmitoylation sequence was also found in CSNK-1 (VKCCCCRRR, residues 386 to 394, Fig. S1). We generated two mutant csnk-1 transgenes disrupting this sequence (p.C388-K407del and p.C388-390S, Fig. S1) and found that both transgenes failed to rescue the survival phenotype of csnk-1(lf) mutants (Table 1). Therefore, the conserved C-terminal palmitoylation sequence might be essential for CSNK-1 function.

csnk-1 interacts with bli-3/tsp-15/doxa-1 to negatively regulate the survival
Considering that csnk-1 recessively inhibited the survival (Table S2), that we identified a tsp-15(mac499) loss-of-function mutation in the mac397 isolate (Table S1 and Table S3, Supplemental Materials and Methods), and that tsp-15(mac499) also recessively inhibited the survival (Table S3), we speculated that the survival of the mac397 isolate as F₁ progeny in the original screen (Xu et al., 2018) might be caused by the co-presence of csnk-1(mac397)/+ and tsp-15(mac499)/+. Such a genetic interaction is known as nonallelic noncomplementation, which can be found between partial alleles of two genes encoding proteins that physically interact with each other or function in the same pathway (Kusch and Edgar, 1986; Yook et al., 2001). Previously we observed a similar interaction between bli-3 and doxa-1 (Xu et al., 2018).

To further dissect the interaction between csnk-1 and bli-3-related genes, we generated double heterozygotes carrying csnk-1(lf)/+ and tsp-15(lf)/+, bli-3(lf)/+ or doxa-1(lf)/+ mutations.

On regular NGM plates, tsp-15(lf) +/+ csnk-1(lf) males grew and behaved like wild type (Table 2). In 5 mM NaI, a few of these males were able to develop into scrawny adults (Table 2), corroborating the survival of the mac397 hermaphrodite isolate in the original F₁ screen (Supplemental Materials and Methods). bli-3(lf) csnk-1(lf)/+ hermaphrodites exhibited similar phenotypes (Fig. 2A and 2B, Table 2).

Unlike tsp-15 or bli-3, csnk-1(lf)/+; doxa-1(mac55lf)/+ hermaphrodites exhibited obvious blistered and dumpy phenotypes on regular NGM plates (Fig. 2C and Table 2). These animals survived much better than tsp-15(lf) +/+ csnk-1(lf) or bli-3(lf) csnk-1(lf)/+ mutants in 5 mM NaI (Table 2). This special interaction between csnk-1 and doxa-1 was verified using a second
doxa-1 allele, mac67 (Table 2), which was previously characterized as a loss-of-function mutation (Xu et al., 2018).

Meanwhile, we examined the morphology of double homozygous mutants between csnk-1(lf) and bli-3(lf) or doxa-1(lf). Compared to single mutants, bli-3(lf) csnk-1(lf) double mutants were strongly scrawny, often with obvious blisters (Fig. S2A-I, compare wild type and single mutants with double mutants. Fig. S3, compare A-B with C-F). Different from bli-3(lf) csnk-1(lf) double mutants, csnk-1(lf); doxa-1(lf) double mutants were apparently larger, often with obvious blisters (Fig. S2J-L and Fig. S3G-H). Together with the noncomplementation analyses, these results suggest that csnk-1 interacts with the bli-3/tsp-15/doxa-1 complex and probably has a closer interaction with doxa-1.

We previously found that bli-3 and tsp-15 loss-of-function mutants had reduced ROS levels (Xu et al., 2015). If csnk-1 interacts with bli-3/tsp-15/doxa-1, ROS levels might be affected by csnk-1(lf). Indeed, using 2’,7’-dichlorofluorescin diacetate (DCFDA) staining, we found that the DCFDA fluorescent intensity in csnk-1(lf) mutants was obviously reduced compared to wildtype animals (Fig. 2D and S4).

DOXA-1 and CSNK-1 are detected in a same protein complex
To understand the interaction between CSNK-1 and DOXA-1, we first examined whether these proteins colocalize in subcellular structures. We expressed a csnk-1::mCherry fusion transgene and a doxa-1::GFP fusion transgene in the epidermis and detected colocalization of mCherry and GFP in epidermal subcellular structures of L3 larva (Fig. 2E-G). The
colocalization appeared specific for CSNK-1 and DOXA-1 because GFP alone did not
colocalize with CSNK-1::mCherry, nor did mCherry alone colocalize with DOXA-1::GFP (Fig.
S5).

We next examined whether CSNK-1 and DOXA-1 coexist in a same protein complex. Total
protein extracts prepared from HEK293T cells transiently transfected with plasmids expressing
FLAG-tagged CSNK-1 (FLAG::CSNK-1) and/or HA-tagged DOXA-1 (DOXA-1::HA) were used
for immunoprecipitation and immunoblotting. When both fusion proteins were co-expressed,
FLAG::CSNK-1 could be coimmunoprecipitated with DOXA-1::HA using an anti-HA antibody
(Fig. 2H, right lanes), while co-expressing FLAG::CSNK-1 and HA-tag alone did not lead to the
detection of the FLAG::CSNK-1 (Fig. 2H, left lanes).

DOXA-1 is homologous to the human DUOX maturation factor 1 and 2 (DUOXA1 and
DUOXA2) that are required for DUOX activities (Grasberger and Refetoff, 2006; Moribe et al.,
2012). We performed similar immunoprecipitation and immunoblotting experiments on human
DUOXA2 and CSNK1G2 (the homolog exhibiting a relatively stronger rescue effect on the
survival phenotype) (Table 1), and tag-independent interaction between the two proteins could
still be detected (Fig. 2I). In addition, immunofluorescence detected co-localization of
FLAG::CSNK1G2 and DUOXA2::HA on the plasma membrane of co-transfected HeLa cells
(Fig. 2J). Together these results suggest that DOXA-1 and CSNK-1 might interact directly or
co-exist in a same protein complex, and that this relationship is likely conserved.
DUOXAs have a shorter extracellular N-terminal domain, five transmembrane domains, a large ectodomain between transmembrane domains 2 and 3, and a longer intracellular C-terminal domain (Grasberger and Refetoff, 2006). We inspected DOXA-1 using Scansite 4 (www.scansite4.mit.edu) and found three predicted casein kinase 1 phosphorylation sites in the C-terminal domain (Fig. S6). We mutated each individual residue to alanine in doxa-1 transgenes and tested whether they could rescue the survival of doxa-1(lf) mutants. Interestingly, the T343A mutation ablated the rescuing ability, while the other two mutations did not (Table 3). Therefore, T343 in the C-terminal domain of DOXA-1 may be important for DOXA-1 activity.

csnk-1 is a negative regulator of innate immunity gene expression and animal survival in the pathogenic PA14

To understand how csnk-1 affects global gene expression, we analyzed the transcriptome of csnk-1(lf) mutant L4 larva (Fig. 3A, heat map). In total, 968 genes exhibited more than 1.5-fold changes in expression (up-regulated or down-regulated) in csnk-1(lf) mutants (Table S4A), among which 579 exhibited more than 2-fold changes (398 up and 181 down) (Fig. 3B, Table S4B and S4C). Gene Ontology (GO) analyses of genes with more than 2-fold changes identified “defense response to other organisms” and “innate immune response” among the most significantly affected biological processes (Fig. S7A and Table S4D). The KEGG analyses identified “drug metabolism-cytochrome P450” and “metabolism of xenobiotics by cytochrome P450” as the most significantly affected pathways (Fig. S7A and Table S4D).
Based on the transcriptome, the expression levels of genes in the “defense response to other organism” term were variable (Fig. S7B, 30 up and 4 down) and all 21 genes in the “innate immune response” term were included in this group (Fig. S7B and Table S4G, genes in blue, 20 up and 1 down). We therefore examined whether up-regulated genes and down-regulated genes might carry out different functions. The results indicated that “defense response to other organisms” and “innate immune response” terms were primarily affected by up-regulated genes (Fig. 3C, Table S4E and S4F), while the “drug metabolism-cytochrome P450” pathway was affected by both up-regulated and down-regulated genes (Table S4E and S4F).

These findings suggest that csnk-1 might affect innate immune response. To test this, we examined the survival of csnk-1(lf) mutants in the pathogenic bacteria Pseudomonas aeruginosa PA14. Indeed, csnk-1(lf) mutants were significantly more resistant to the killing by PA14 compared to wildtype animals (Fig. 3D), and this phenotype was rescued by the csnk-1 transgene driven by the endogenous csnk-1 promoter or the intestine-specific nhx-2 promoter, but not by the epidermis-specific dpy-7 promoter (Fig. 3D). Hence, intestinal csnk-1 probably negatively regulates C. elegans survival in PA14.

**NSY-1 and LIN-45 mediate the negative effects of CSNK-1 on animal survival in PA14**

As a kinase, the effects of CSNK-1 might be mediated by downstream phosphorylation signals. To identify protein phosphorylation affected by CSNK-1, we performed mass spectrometry-based phosphoproteomic analyses on csnk-1(lf) mutant L4 larva.
The analyses detected 11,644 peptides, containing 16,946 phosphorylation sites in 4,082 proteins (Fig. S8A). Among these, 1,278 phosphorylation sites in 731 proteins exhibited more than 20% phosphorylation level changes in csnk-1(lf) mutants (Fig. S8A and Table S5A). Most of the sites were phosphoserine (1027/1278, p-Ser), followed by phosphothreonine (208/1278, p-Thr) and phosphotyrosine (43/1278, p-Tyr) (Fig. S8A). We postulated that some proteins with decreased phosphorylation in csnk-1(lf) mutants (Table S5B) might be candidate phosphorylation substrates of CSNK-1, while those with increased phosphorylation (Table S5C) might be indirectly affected by CSNK-1.

We performed KEGG pathway analyses on these proteins (Tables S5D and S5E). Decreased phosphorylation primarily affected proteins in calcium signaling, MAPK signaling, ErbB signaling, and ABC transporters pathways (Fig. S8B and Table S5D), while increased phosphorylation primarily affected proteins in MAPK signaling, FoxO signaling and calcium signaling pathways (Fig. S8B and Table S5E). These proteins also affected multiple GO terms (Table S5D and S5E).

To identify potential downstream regulators of CSNK-1, we performed protein-protein association analyses on proteins assigned to the KEGG pathways (Table S5F). Here we included an innate immunity regulator DKF-2 (Ren et al., 2009), which exhibited decreased phosphorylation in csnk-1(lf) mutants (Table S5B) but was not assigned to pathways by KEGG analyses. The association analyses identified three larger protein clusters and two smaller clusters (Fig. 4A). Key regulators of innate immunity, such as NSY-1, LIN-45, MPK-1 and DAF-16 (Garsin et al., 2003; Kim and Ewbank, 2018; Kim et al., 2002; Nicholas and Hodgkin, 2004),
were central nodes of two larger clusters, and DKF-2 was in the same cluster with NSY-1 and DAF-16 (Fig. 4A).

Among these regulators, LIN-45, NSY-1 and DKF-2 exhibited decreased phosphorylation, while DAF-16 and MPK-1 exhibited increased phosphorylation in csnk-1(lf) mutants (Table S5 and Fig. S9A). Sequence alignment indicated that p-Ser486 of NSY-1 was highly conserved (Fig. S9B). p-Thr256 and p-Tyr258 of MPK-1 were also highly conserved (Fig. S9B). However, phosphorylated residues of DKF-2, DAF-16 and LIN-45 were not (Fig. S9B). Interestingly, four residues of CSNK-1, two of which were highly conserved, exhibited decreased phosphorylation in csnk-1(lf) mutants (Table S5B and Fig. S1, red asterisks).

NSY-1 and LIN-45 represent two major pathways for pathogen defense in C. elegans (Kim and Ewbank, 2018). We next examined whether they are involved in the negative regulation of innate immunity by CSNK-1.

We treated wildtype or csnk-1(lf) animals with nsy-1(RNAi) and quantified their survival in PA14. nsy-1(RNAi) decreased the survival of wildtype animals in PA14 compare to control RNAi (Fig. 4B and S10A), consistent with previous findings (Kim et al., 2002). Importantly, the increased survival of csnk-1(lf) mutants in PA14 was significantly suppressed by nsy-1(RNAi) (Fig. 4B and S10A). Furthermore, RNAi knockdown of sek-1 and pmk-1, two downstream effectors of nsy-1 (Kim and Ewbank, 2018), also significantly suppressed the increased survival of csnk-1(lf) mutants in PA14 (Fig. 4C and S10B).
We next examined the effects of lin-45(RNAi). lin-45(RNAi) itself did not obviously affect the survival of wildtype animals in PA14 (Fig. 4B and S10A). However, it significantly suppressed the increased survival of csnk-1(lf) mutants (Fig. 4B and S10A). Consistent with the notion that MPK-1 is a downstream effector of LIN-45 (Kim and Ewbank, 2018), mpk-1(RNAi) exhibited similar suppressive effects as lin-45(RNAi) (Fig. 4B and S10A). Therefore, NSY-1 and LIN-45 likely mediate the negative regulation of innate immune response by CSNK-1.

Our findings predicted that CSNK-1 might negatively regulate the expression of certain genes that were activated by NSY-1 and its downstream effectors. To examine this, we compared differentially expressed genes in csnk-1(lf) mutants (Table S4) with previously reported genes affected by nsy-1 (Cheesman et al., 2016), pmk-1 and atf-7 (Fletcher et al., 2019). Indeed, significantly more genes up-regulated by nsy-1, pmk-1 or atf-7 were down-regulated by csnk-1, while none or only a small number of genes down-regulated by nsy-1, pmk-1 or atf-7 were up-regulated by csnk-1 (Fig. S11A and S11B).

csnk-1 and skn-1 might function in parallel to affect oxidative stress response

SKN-1 Nrf2 is a major regulator of oxidative stress response and we previously found that skn-1(gf) mutants can survive in excess iodide (Xu et al., 2018). To investigate how csnk-1 interacts with skn-1, we generated csnk-1; skn-1 double mutants and compared the gene expression of csnk-1(lf) mutants with that of skn-1(gf) or skn-1-deficient mutants.
First, we found that \textit{csnk-1(lf); skn-1(lf)} double mutants can survive in 5 mM NaI (Table 4A). Since \textit{skn-1(lf)} single mutants could not survive in 5 mM NaI (Table 4A) (Xu et al., 2018), this result suggests that \textit{csnk-1} might function in parallel with or downstream of \textit{skn-1}.

We next examined the survival of \textit{csnk-1(lf); skn-1(gf)} double mutants in a much higher concentration of NaI (50 mM), a test that we previously used to detect the additive or synergistic effects between \textit{bli-3/tsp-15/doxa-1} loss-of-function mutations and \textit{skn-1(gf)} mutations (Xu et al., 2018). We found that \textit{csnk-1(lf); skn-1(gf)} double mutants now can survive in 50 mM NaI, while either single mutant failed to (Table 4B).

Finally we compared the differentially expressed genes in \textit{csnk-1(lf)} mutants (Table S4) with those affected by \textit{skn-1}-deficiency or \textit{skn-1(gf)} (Oliveira et al., 2009; Xu et al., 2018). Here only a small fraction of differentially expressed genes was shared between \textit{csnk-1(lf)} and \textit{skn-1(gf)/skn-1}-deficient animals (Fig. S11C). Together these results suggest that \textit{csnk-1} and \textit{skn-1} probably function in parallel to regulate oxidative stress response.
Discussion

In this study, we found that CSNK-1 interacts with the BLI-3/TSP-15/DOXA-1 dual oxidase complex to negatively regulate animal survival in an oxidative stressor. CSNK-1 also negatively regulates the expression of innate immunity genes and innate immune response, which appears to be mediated by NSY-1 and LIN-45. Furthermore, CSNK-1 and SKN-1 might function in parallel to regulate oxidative stress responses. Together, our results suggest that CSNK-1 is a conserved coordinator of animal’s responses to oxidative stress and pathogens.

The functions of CSNK1Gs remain to be understood

Mammalian casein kinase 1 (CSNK1) family has seven members, CSNK1A (CK1\(\alpha\)), CSNK1B (CK1\(\beta\), cow only), CSNK1G1/2/3 (CK1\(\gamma_1/2/3\)), CSNK1D (CK1\(\delta\)) and CSNK1E (CK1\(\varepsilon\)) (Schittek and Sinnberg, 2014). CSNK1G requires palmitoylation-mediated membrane localization for its functions (Davidson et al., 2005; Li et al., 2016). Most studies on CSNK1 were focused on A, D and E members, while the functions of the G members are less understood. The C. elegans genome contains three genes, \textit{kin-19}, \textit{kin-20} and \textit{csnk-1}, that encode orthologs of CSNK1A, CSNK1D and CSNK1G (Manning, 2005) (wormbase.org).

CSNK1Gs have been shown to regulate Wnt, Hh and JNK pathways (Davidson et al., 2005; Jiang, 2017; Li et al., 2016, 2020b). Mammalian CSNK1G1/3 can stimulate necroptosis by interacting with receptor-interacting kinase 3 (RIPK3) (Lee et al., 2019), a function that is similarly carried out by CSNK1A, CSNK1D and CSNK1E (Hanna-Addams et al., 2020) while attenuated by CSNK1G2 (Li et al., 2020a). CSNK1G also can phosphorylate presenilin 1 of the \(\gamma\)-secretase complex (Bustos et al., 2017) and was associated with developmental delay and
autism spectrum disorder (Gold et al., 2020), implicating this type of kinases in major neurological diseases.

In *C. elegans*, csnk-1 is essential for embryonic development by affecting the asymmetric spindle positioning in embryos (Panbianco et al., 2008; Redemann et al., 2010; Rodriguez-Garcia et al., 2018; Singh and Pohl, 2014) and oocyte meiosis (Flynn and McNally, 2017). Consistent with these studies, we found that eggs laid by csnk-1(lf) mutants failed to hatch.

**CSNK-1 interacts with BLI-3/TSP-15/DOXA-1 dual oxidase complex to regulate oxidative stress response**

Excess iodide can induce increased ROS generation in mammalian cells and *C. elegans* (Corvilain et al., 2000; Xu et al., 2015). We previously found that wildtype *C. elegans* exhibited larval arrest at 5 mM or higher concentrations of NaI, probably as a response to oxidative stress (Xu et al., 2015). We showed that loss of function in the BLI-3/TSP-15/DOXA-1 dual oxidase complex or activation of the SKN-1 pathway can lead to the survival of animals in excess iodide (Xu et al., 2015, 2018).

In this study, we made several new findings on CSNK-1. First, csnk-1(lf) mutants can survive in excess iodide, and this appears to be a specific function of csnk-1 as knocking down kin-19 (CSNK1A), kin-20 (CSNK1D), gsk-3 (GSK3B), kin-1 (PKA) or kin-2 (PKA) by RNAi failed to cause this phenotype (Table S2). Second, csnk-1(lf) mutants exhibited reduced levels of endogenous ROS. Third, the conserved palmitoylation signal was required for CSNK-1 activity, suggesting that CSNK-1 might function on plasma membrane. Fourth, csnk-1 interacted with
bli-3/tsp-15/doxa-1 in a nonallelic noncomplementation manner, suggesting that CSNK-1 might function in the same pathway and/or physically interact with BLI-3/TSP-15/DOXA-1. Fifth, CSNK-1::mCherry and DOXA-1::GFP fusion proteins were colocalized in subcellular structures of the epidermis, and human FLAG::CSNK1G2 and DUOXA2::HA fusion proteins were colocalized on the plasma membrane of cultured cells. Sixth, CSNK-1 and DOXA-1 were detected in a same protein complex, and human CSNK1G2 and DUOXA2 exhibited a similar interaction. Together, these results support the hypothesis that CSNK-1 interacts with DOXA-1 to affect the activity of BLI-3/TSP-15/DOXA-1 dual oxidase complex and ROS generation.

Interestingly, we found that a potential casein kinase 1 phosphorylation site in DOXA-1 C-terminal domain was essential for DOXA-1 activity. However, it is unclear whether this is a direct phosphorylation site of CSNK-1. In addition, we have not addressed whether the interaction between DOXA-1 and CSNK-1 is required for the dual oxidase activity. Future studies are warranted to answer these important questions.

The transgene rescue experiments suggest that CSNK-1 primarily functions in the epidermis to affect the survival phenotype, which was previously shown for tsp-15 and skn-1(gf) (Xu et al., 2018). Nevertheless, the interaction between CSNK-1 and the dual oxidase complex might extend beyond the epidermis. This possibility is supported by the broad expression of csnk-1 and the appearance of pleiotropic defects in growth and morphology of double mutants between csnk-1(lf) and bli-3(lf) or doxa-1(lf), which might involve defects in tissues beyond epidermis.
NSY-1 and LIN-45 mediate the negative effects of CSNK-1 on innate immunity

Evolutionarily conserved pathways regulate innate immune responses in *C. elegans* (Kim and Ewbank, 2018). The NSY-1-SEK-1-PMK-1 p38 MAPK pathway plays key roles in intestinal immunity (Kim and Ewbank, 2018; Kim et al., 2002), while the LIN-45-MEK-2-MPK-1 ERK pathway is critical for immune response to rectal infection by *M. nematophilum* (Kim and Ewbank, 2018; Nicholas and Hodgkin, 2004). We found that multiple innate immunity genes were up-regulated in *csnk-1(lf)* mutants, and *csnk-1(lf)* mutants exhibited increased survival in the pathogenic bacteria PA14. Therefore, CSNK-1 is likely a new regulator of innate immunity.

The phosphoproteomic analyses detected reduced phosphorylation of NSY-1 and LIN-45 in *csnk-1(lf)* mutants. However, the phosphorylation of MPK-1 was increased in *csnk-1(lf)* mutants. The phosphorylated residues in NSY-1 and MPK-1 are highly conserved, implying functional importance.

We found that RNAi targeting *nsy-1*, *sek-1* or *pmk-1* essentially abrogated the increased survival of *csnk-1(lf)* mutants in PA14, and RNAi targeting *lin-45* or *mpk-1* significantly suppressed the increased survival of *csnk-1(lf)* mutants. These results suggest that the NSY-1 and LIN-45 pathways function downstream of CSNK-1. Together with the phosphoproteomic results that LIN-45 exhibited decreased phosphorylation while MPK-1 exhibited increased phosphorylation in *csnk-1(lf)* mutants, these findings suggest that CSNK-1-dependent phosphorylation of NSY-1 and LIN-45 (direct or indirect) negatively regulates the activities of these kinases, the expression of downstream innate immunity genes and innate immune response.
Supporting this hypothesis, we found that significantly more genes depending on *nsy-1* for expression (Cheesman et al., 2016) overlapped with those genes down-regulated by *csnk-1*. We made similar observations for genes depending on PMK-1 and ATF-7 for up-regulation (Fletcher et al., 2019) and genes down-regulated by *csnk-1*. [ATF-7 is a downstream transcription activator of the NSY-1 pathway (Shivers et al., 2010)].

We identified four CSNK-1 S/T residues with decreased phosphorylation in *csnk-1(lf)* mutants (Fig. S1 and Table S5), implying autophosphorylation. It was previously shown that CSNK1D and CSNK1E autophosphorylation provides a feedback inhibitory regulation of the kinase activity (Gietzen and Virshup, 1999; Rivers et al., 1998). Therefore, it will be interesting to examine whether CSNK-1 autophosphorylation might serve a similar purpose.

Several key details of how CSNK-1 regulates innate immunity remain unanswered. It is unclear whether NSY-1 and LIN-45 are phosphorylation substrates of CSNK-1, whether the reduced phosphorylation of NSY-1 and LIN-45 in *csnk-1(lf)* mutants affects their kinase activities, and how CSNK-1 interacts with other regulators of innate immunity (Kim and Ewbank, 2018). These are important questions that need to be answered for understanding the function of CSNK-1.

**Parallel functions of CSNK-1 and SKN-1 in oxidative stress response**

SKN-1 is a key activator of stress responses in *C. elegans* (Blackwell et al., 2015). We previously found that *skn-1(gf)* and a *bli-3* missense mutation (*mac40*) that affects the oxidase...
domain together can cause animal survival in a higher concentration of iodide (50 mM NaI), while either single mutation failed to do so (Xu et al., 2018). In addition, bli-3(lf); skn-1(lf) double mutants could not survive in 5 mM NaI, while bli-3(lf) single mutants can (Xu et al., 2015, 2018).

We found that csnk-1(lf); skn-1(gf) double mutants can survive in 50 mM NaI, which is like bli-3(mac40lf); skn-1(gf) mutants. However, csnk-1(lf); skn-1(lf) double mutants also can survive in 5 mM NaI, which is different from bli-3(lf); skn-1(lf) mutants. Besides, only a small fraction of genes affected by csnk-1 was also affected by skn-1. Based on these results, we postulate that CSNK-1 and SKN-1 probably function in parallel to regulate oxidative stress response, in which CSNK-1 activity might be mediated by BLI-3 and also by the expression of SKN-1-independent stress response genes.

Transmembrane transporters with decreased phosphorylation in csnk-1(lf) mutants

GO analyses found that a prominent group of proteins with decreased phosphorylation in csnk-1(lf) mutants were transmembrane transporters, including solute carriers and ABC transporters (Fig. S8C and S8D, Table S5D and S5G). A detailed analysis of the relationship between CSNK-1 and these transporters is beyond the scope of our current study. Nevertheless, it could be interesting to explore along this line, considering that these transporters are important for cellular homeostasis and that the functions of most of them are unclear (César-Razquin et al., 2015). In addition, GO analyses on proteins with increased phosphorylation in csnk-1(lf) mutants identified terms such as “cytoskeletal protein binding”, “developmental process”, “supramolecular complex”, etc., to be significantly affected (Table S5E). These changes imply
that CSNK-1 might have broad functions beyond its effects in oxidative stress and innate immune responses.

A working model for CSNK-1 in postembryonic stages

Combining our results and the summarized findings on CSNK1G, oxidative stress and innate immune responses (Blackwell et al., 2015; Jiang, 2017; Kim and Ewbank, 2018; McCallum and Garsin, 2016; Moghadam et al., 2021), we propose a working model for the function of CSNK-1 in postembryonic *C. elegans* (Fig. 4D). In this model, pathogen infection probably triggers an unknown event that leads to CSNK-1-dependent activation of the BLI-3 dual oxidase via DOXA-1. Activated BLI-3 generates an ROS surge for killing pathogens. The increased ROS level is subsequently sensed by the p38 MAPK pathway to activate SKN-1 for the expression of antioxidant genes. Meanwhile, activated CSNK-1 may cause self-inhibition via auto-phosphorylation. This will attenuate the inhibitory phosphorylation of NSY-1 and LIN-45, which will activate innate immune response to further fight against pathogens. As aforementioned, multiple questions about this model remain unanswered and extensive future studies are warranted for understanding the detailed molecular mechanisms.

Conclusions

Oxidative stress response is intimately related to innate and adaptive immunity. It is critical that the activities of the regulators and pathways are coordinated to generate efficient and balanced responses to oxidative stress and pathogens. We suggest that CSNK1G plays a key role in integrating these processes.
Materials and Methods

See Supplemental Materials and Methods.

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Author Contributions

YH, ZX and LM designed the experiments. YH performed the experiments with assistance of ZX. YH, ZX and LM analyzed the data. YH, ZX and LM wrote the manuscript.

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Figure 1. Characterization of *csnk-1*.

(A) *csnk-1* gene structure (www.wormbase.org). The locations of the *mac397* mutation and the two deletion mutations, *mac494* and *mac495*, are indicated. The position of the *sgRNA* used in the CRISPR/Cas9-based mutagenesis experiment is shown as a red bar. (B) Partial sequence alignment of CSNK1G across species showing the R161H change caused by *mac397*. *C. e.*:
C. elegans; D. m.: Drosophila; M. m.: mouse; H. s.: human. (C) Percentage of L1 larva that can grow into adults on plates with 5 mM NaI. Results were based on two biological replicates. 100 L1 larva were analyzed in each replicate. Statistics: two-tailed unpaired t-test. **: p < 0.01. (D) Hatching rate of eggs laid by csnk-1(If) homozygous mutants. All eggs laid by a single adult on regular NGM agar plates were examined. Results were based on three individual adults. Statistics: two-tailed unpaired t-test. ****: p < 0.0001. (E-J) Expression pattern of a csnk-1p::GFP transgene in adults. (E) Picture of a whole transgenic adult. (F) Higher-resolution picture of the anterior part. (G) Higher-resolution picture of the posterior part. (H, I, J) Higher-resolution pictures of the middle part at different focal planes.
Figure 2. CSNK-1 interacts with DOXA-1.

(A, B, C) Morphology of double heterozygous mutants between csnk-1(lf) and bli-3(lf) or doxa-1(lf) mutations. bli-3(lf) csnk-1(lf)/hT2 (used as bli-3(lf) csnk-1(lf)/+ + here) mutants had wildtype-like morphology, while csnk-1(lf)/hT2; doxa-1(lf)/hT2 (used as csnk-1(lf)/+; doxa-1(lf)/+ here) mutants exhibited strong blistered and dumpy phenotype. Arrows point to blisters. (D) Fluorescent intensities of DCFDA-stained L4 larva. Results were based on 3 biological replicates. Statistics: two-tailed unpaired t-test. ***: p < 0.001. (E, F, G) Fluorescent pictures of epithelial subcellular structures of a transgenic L3 larva co-expressing dpy-7p::csnk-1::mCherry and dpy-7p::doxa-1::GFP transgenes. (H) Western blotting detecting the interaction between tagged DOXA-1 and CSNK-1 co-expressed in HEK293T Cells.
Immunoprecipitation was performed using an anti-HA antibody. (I) Western blotting detecting the interaction between tagged DUOXA2 and CSNK1G2 co-expressed in HEK293T Cells. Immunoprecipitation was performed using an anti-HA antibody. (J) Membrane colocalization of tagged DUOXA2 and CSNK1G2 expressed in HeLa Cells. DUOXA2::HA fusion protein is shown in green, FLAG::CSNK1G2 is shown in red, and DAPI staining is shown in blue.
Figure 3. csnk-1 affects innate immune response.

(A) Heat map of differentially expressed genes in csnk-1(lf) mutants compared with wildtype animals. (B) Volcano plot of differentially expressed genes in csnk-1(lf) mutants. FC: fold change. (C) List of GO terms and KEGG pathways significantly affected by up-regulated genes in csnk-1(lf) mutants. The number of genes in each category is shown in the parenthesis. (D) Survival curve of wildtype animals and csnk-1(lf) mutants in PA14. Results were based on 3 to 4 parallel plates. p values were determined using the log-rank test comparing mutants with wild type.
Figure 4. The *nsy-1* and *lin-45* pathways are required for the increased survival of *csnk-1(lf)* mutants in PA14.

(A) Protein-protein association analysis of KEGG pathway proteins with altered phosphorylation in *csnk-1(lf)* mutants. Key regulators of innate immunity are shown in bold. PPI: protein-protein interaction. (B) *nsy-1(RNAi)*, *lin-45(RNAi)* and *mpk-1(RNAi)* significantly suppressed the increased survival of *csnk-1(lf)* mutants in PA14. Similar effects were observed for *sek-1(RNAi)* and *pmk-1(RNAi)* (C). *p* values were determined using the log-rank test. (D) A working model for CSNK-1 in oxidative stress and innate immune responses.
Table 1. Effects of *csnk-1* transgenes on the survival phenotype of *csnk-1(lf)* mutants in 5 mM NaI.

| Background     | Transgene                        | Tg line | Ratio of L1 larva growing into adults (%) |
|----------------|----------------------------------|---------|------------------------------------------|
|                |                                  |         | Experiment 1 | Experiment 2 |
| WT             | No                               | 0       | 0/100 (0%) | 0/100 (0%)   |
|                | *csnk-1p::csnk-1_cDNA(p.R161H)* | 1       | 0/100 (0%) | 0/100 (0%)   |
|                |                                  | 2       | 0/100 (0%) | 0/100 (0%)   |
|                | No                               | 0       | 78/100 (78%) | 80/100 (80%) |
|                | *csnk-1p::csnk-1_cDNA*           | 1       | 6/100 (6%)  | 13/100 (13%) |
|                |                                  | 2       | 3/100 (3%)  | 5/100 (5%)   |
|                | *csnk-1p::csnk-1_cDNA(p.R161H)*  | 1       | 81/100 (81%) | 74/100 (74%) |
|                |                                  | 2       | 62/100 (62%) | 56/100 (56%) |
|                | *dpy-7p::csnk-1_cDNA*            | 1       | 0/100 (0%)  | 1/100 (1%)   |
|                | (epidermis promoter)             | 2       | 5/100 (5%)  | 1/100 (1%)   |
|                | *nhx-2p::csnk-1_cDNA*            | 1       | 66/85 (77.6%) | 65/83 (78.3%) |
|                | (intestine promoter)             | 2       | 48/76 (63.2%) | 56/77 (72.7%) |
| *csnk-1(mac494lf)* | *dpy-7p::HsCSNK1G1_cDNA*        | 1       | 20/100 (20%) | 15/80 (18.8%) |
|                |                                  | 2       | 19/113 (16.8%) | 23/100 (23%)  |
|                | *dpy-7p::HsCSNK1G2_cDNA*         | 1       | 10/100 (10%) | 5/100 (5%)    |
|                |                                  | 2       | 12/100 (12%) | 6/100 (6%)    |
|                | *dpy-7p::HsCSNK1G3_cDNA*         | 1       | 23/100 (23%) | 21/100 (21%)  |
|                |                                  | 2       | 42/100 (42%) | 37/100 (37%)  |
|                | *csnk-1p::csnk-1_cDNA(p.C388_K407del)* | 1 | 60/100 (60%) | 58/100 (58%) |
|                |                                  | 2       | 74/100 (74%) | 63/100 (63%) |
|                | *csnk-1p::csnk-1_cDNA(p.C388-390S)* | 1 | 59/105 (56.2%) | 67/100 (67%) |
|                |                                  | 2       | 72/100 (72%) | 78/100 (78%) |

For *csnk-1(lf)* background, all transgenes were maintained in the *csnk-1(mac494lf)/hT2* heterozygous background except for *csnk-1p::csnk-1_cDNA* or *dpy-7p::csnk-1::cDNA*, one line of which was maintained in the *csnk-1(mac494lf)* homozygous background.
Table 2. Nonallelic noncomplementation interaction between *csnk-1* and *tsp-15*, *bli-3* or *doxa-1*.

| Genotype                        | Phenotype (No Nal)          | Survival (5 mM Nal)          |
|---------------------------------|-----------------------------|------------------------------|
| WT                              | WT                          | No                           |
| *csnk-1(mac494lf)/+*            | WT-like                     | No                           |
| *csnk-1(mac495lf)/+*            | WT-like                     | No                           |
| *tsp-15(mac500lf)/+*            | WT-like (male)              | No (male)                    |
| *tsp-15(mac501lf)/+*            | WT-like (male)              | No (male)                    |
| *bli-3(mac40lf)/+*              | WT-like (male)              | No (male)                    |
| *bli-3(e767lf)/+*               | WT-like (male)              | No (male)                    |
| *doxa-1(mac55lf)/+*             | WT-like (male)              | No (male)                    |
| *doxa-1(mac67lf)/+*             | WT-like (male)              | No (male)                    |
| *tsp-15(mac500lf) +/+ csnk-1(mac494) | WT-like (male)        | Weak (a few, scrawny) (male) |
| *tsp-15(mac500lf) +/+ csnk-1(mac495) | WT-like (male)          | Weak (a few, scrawny) (male) |
| *tsp-15(mac501lf) +/+ csnk-1(mac494) | WT-like (male)        | Weak (a few, scrawny) (male) |
| *tsp-15(mac501lf) +/+ csnk-1(mac495) | WT-like (male)          | Weak (a few, scrawny) (male) |
| *bli-3(mac40lf) csnk-1(mac494lf)/+ +* | WT-like                  | Weak (a few, scrawny)        |
| *bli-3(mac40lf) csnk-1(mac495lf)/+ +* | WT-like                  | Weak (a few, scrawny)        |
| *bli-3(e767lf) csnk-1(mac494lf)/+ +* | WT-like                  | Weak (a few, scrawny)        |
| *bli-3(e767lf) csnk-1(mac495lf)/+ +* | WT-like                  | Weak (a few, scrawny)        |
| *csnk-1(mac494lf)/+; doxa-1(mac55lf)/+* | Bli, Dpy                | Strong (plenty, Bli, Dpy)    |
| *csnk-1(mac495lf)/+; doxa-1(mac55lf)/+* | Bli, Dpy                | Strong (plenty, Bli, Dpy)    |
| *csnk-1(mac494lf)/+; doxa-1(mac67lf)/+* | Bli (male)               | Strong (plenty, Bli) (male)  |
| *csnk-1(mac495lf)/+; doxa-1(mac67lf)/+* | Bli (male)               | Strong (plenty, Bli) (male)  |

*tsp-15(mac500lf)* and *tsp-15(mac501lf)* mutations were knockin genocopies of *tsp-15(mac499lf)* (Table S3). *bli-3(lf)* and *doxa-1(lf)* mutations were previously described (Supplemental Materials and Methods). *csnk-1(lf)/hT2* was used as *csnk-1(lf)/+. Single mutant heterozygous males were generated by crossing wildtype males with corresponding mutant hermaphrodites. We failed to place *tsp-15(lf)* and *csnk-1(lf)* on the same chromosome due to their close linkage. We therefore examined male progeny from crosses between *tsp-15(lf)* males and *csnk-1(lf)/hT2* hermaphrodites. *bli-3(lf) csnk-1(lf)/hT2* and *csnk-1(lf)/hT2; doxa-1(lf)*
1(mac55lf)/hT2 heterozygotes were used here as bli-3(lf) csnk-1(lf)/+ + and csnk-1(lf)/+; doxa-1(lf)/+ mutants. csnk-1(lf)/+; doxa-1(mac67lf)/+ double heterozygous males were generated by crossing doxa-1(mac67lf) males with csnk-1(lf)/hT2 hermaphrodites.

Table 3. Mutations on predicted casein kinase 1 phosphorylation sites affect DOXA-1 activity differentially.

| Background | doxa-1p:: doxa-1 cDNA | Total Tg lines | Survival (5 mM NaI) |
|------------|------------------------|----------------|---------------------|
| No         | No                     |                | Yes                 |
| WT         | 3                      | 0/3            |                     |
| doxa-1(mac55lf) | p.T343A | 4              | 4/4                 |
|            | p.S346A                | 3              | 0/3                 |
|            | p.S364A                | 3              | 0/3                 |

Survival of doxa-1(mac55lf) animals carrying the indicated doxa-1 transgenes in 5 mM NaI were observed.
Table 4. (A) Survival of animals carrying different combinations of \textit{csnk-1(lf)} and \textit{skn-1(zu135lf)} mutations. (B) \textit{csnk-1(lf)} and \textit{skn-1(gf)} mutations exhibit additive or synergistic effects on the survival in 50 mM NaI.

### A

| P₀ genotype | F₁ genotype | No Nal | 5 mM Nal |
|-------------|-------------|--------|---------|
| +/+; +/+    | ND          | 0      |         |
| +/+; skn-1(lf)/+ | ND    | 0      |         |
| \textit{csnk-1(mac494lf)/+; skn-1(lf)/+} | ND | 0 |         |
| \textit{csnk-1(mac494lf)/+; +/+} | ND | 0 |         |
| \textit{csnk-1(mac494lf); +/+} | 7 | 60 |         |
| \textit{csnk-1(mac494lf); skn-1(lf)/+} | 14 | 136 |         |
| \textit{csnk-1(mac494lf); skn-1(lf)} | 3 | 29 |         |
| \textit{csnk-1(mac494lf)/+; skn-1(lf)} | 15 | 0 |         |
| +/+; skn-1(lf) | 7 | 0 |         |

| P₀ genotype | F₁ genotype | No Nal | 5 mM Nal |
|-------------|-------------|--------|---------|
| +/+; +/+    | ND          | 0      |         |
| +/+; skn-1(lf)/+ | ND    | 0      |         |
| \textit{csnk-1(mac495lf)/+; skn-1(lf)/+} | ND | 0 |         |
| \textit{csnk-1(mac495lf)/+; +/+} | ND | 0 |         |
| \textit{csnk-1(mac495lf); +/+} | 4 | 6 |         |
| \textit{csnk-1(mac495lf); skn-1(lf)/+} | 10 | 27 |         |
| \textit{csnk-1(mac495lf); skn-1(lf)} | 1 | 10 |         |
| \textit{csnk-1(mac495lf)/+; skn-1(lf)} | 15 | 0 |         |
| +/+; skn-1(lf) | 6 | 0 |         |

### B

| Genotype                              | Survival (50 mM NaI) |
|---------------------------------------|----------------------|
| \textit{csnk-1(mac494lf)}             | No                   |
| \textit{csnk-1(mac495lf)}             | No                   |
| \textit{skn-1(lax120gf)}              | No                   |
| \textit{skn-1(mac53gf)}               | No                   |
| \textit{csnk-1(mac494lf); skn-1(lax120gf)} | Yes |
| \textit{csnk-1(mac495lf); skn-1(lax120gf)} | Yes |
| \textit{csnk-1(mac494lf); skn-1(mac53gf)} | Yes |
| \textit{csnk-1(mac495lf); skn-1(mac53gf)} | Yes |
For (A), F₁ progeny of csnk-1(lf)/+; skn-1(zu135lf)/+ animals were grown in plates with or without 5 mM NaI. For plates without NaI, F₁ adults were randomly picked to separate plates and examined for egg laying and egg hatching. Individuals that laid hatched eggs were not analyzed further as they were not homozygous at csnk-1 or skn-1 locus. Individuals that only laid unhatched eggs were genotyped for csnk-1(lf) and skn-1( lf) mutations. For plates with 5 mM NaI, F₁ adults were individually genotyped for csnk-1(lf) and skn-1( lf) mutations. ND: individuals of these genotypes were not determined.
Figure S1. CSNK1G protein sequence alignment.

C.e.: C. elegans CSNK-1; D.m.: Drosophila Gilgamesh; M.m.: mouse CSNK1G2; H.s.: human CSNK1G1, CSNK1G2, CSNK1G3. Green bar: location of mac494 and mac495 mutations. Red box: kinase domain. Red arrowhead: mac397 mutation. Red stars: potential autophosphorylation sites. Blue box: palmitoylation signal.
Figure S2. With csnk-1(lf), bli-3(lf) and doxa-1(lf) exhibit differential synthetic effects on the morphology.

(A) A wildtype adult. (B, C) csnk-1(lf) mutant adults. (D, G) Representative bli-3(e767lf) and bli-3(mac40lf) mutant adults. (E, F, H, I) Representative bli-3(lf) csnk-1(lf) double mutants. (J) A doxa-1(mac55lf) mutant adult. (K, L) Representative csnk-1(lf); doxa-1(mac55lf) double mutants. Arrows point to obvious blisters. All images are of the same scale as shown in (A).
Figure S3. Typical double heterozygous and double homozygous mutants carrying *csnk-1(lf)* and *bli-3(lf)* or *doxa-1(lf)* mutations.

(A, B) *csnk-1(lf)* single heterozygous and homozygous mutants. (C, D, E, F) *bli-3(lf); csnk-1(lf)* double heterozygous and homozygous mutants. (G, H) *csnk-1(lf); doxa-1(mac55lf)* double heterozygous and homozygous mutants. The mutations were balanced with *hT2[qls48]*. Arrowheads indicate single or double heterozygous mutants. Arrows indicate single or double homozygous mutants.
Figure S4. DCFDA fluorescent intensities of wildtype animals and \textit{csnk-1(lf)} mutants.

(A) A picture of DCFDA-stained WT L4 larva. (B) A picture of DCFDA-stained L4 \textit{csnk-1(mac494lf)} mutants. Arrow points to a \textit{csnk-1(mac494lf)} homozygous animal. Arrowhead points to a \textit{csnk-1(mac494lf) /hT2[qIs48]} (I; III) animal. (C) Quantification of whole-body DCFDA fluorescent intensities of individual animals. Three biological replicates, shown as red, green and blue triangles, were analyzed. Pooled results were compared. Statistics: two tailed unpaired \textit{t}-test. ****: $p < 0.0001$. 
Figure S5. CSNK-1::mCherry does not colocalize with GFP and DOXA-1::GFP does not colocalize with mCherry.

(A, B, C) A transgenic L3 larvae co-expressing GFP (A) and CSNK-1::mCherry (B) in the epidermis. The merged picture is shown in (C).

(D, E, F) A transgenic 3-fold embryo co-expressing DOXA-1::GFP (D) and mCherry (E) in the epidermis. The merged picture is shown in (F). For unknown reasons, DOXA-1::GFP was not detected in larva of these transgenic lines. We therefore used embryos to observe whether DOXA-1::GFP exhibits colocalization with mCherry.
Figure S6. Sequence alignment of DOXA-1 and its homologs.

Three predicted casein kinase 1 gamma phosphorylation sites in DOXA-1 are indicated with red arrowheads. Prediction was made with Scansite 4 (scansite4.mit.edu). C.e.: *C. elegans* DOXA-1; D.m.: *Drosophila* Moladietz; M.m.: mouse DUOXA1; H.s.: human DUOXA1 and DUOXA2.
Figure S7. Differentially expressed genes in *csnk-1(lf)* mutants are enriched in the “defense response to other organism” term.

(A) GO terms and KEGG pathways significantly affected by differentially expressed genes in *csnk-1(lf)* mutants. (B) Expression levels of defense response genes based on transcriptome analyses. Fold changes are shown on the right of each column. Genes in the “Innate immune response” term are shown in blue.
Figure S8. Phosphoproteomic analyses of csnk-1(lf) mutants.

(A) Summary of total peptides, proteins and sites (black columns) in csnk-1(lf) mutants identified by phosphoproteomic analyses. Numbers of proteins and sites with significantly altered phosphorylation (fold change >0.2) are shown in red and green columns. Numbers of different types of phosphorylation sites are indicated on right. (B) KEGG pathways significantly affected by proteins with decreased or increased phosphorylation. (C) GO terms significantly affected by proteins with decreased phosphorylation. (D) List of transmembrane transporters with decreased phosphorylation in csnk-1(lf) mutants.
Figure S9. Partial sequence alignments of innate immunity regulators with altered phosphorylation in csnk-1(lf) mutants.

(A) Phosphorylation changes of key immunity regulators in csnk-1(lf) mutants. (B) Protein sequence alignment surrounding the phosphorylation sites. Residues with significantly altered phosphorylation are shown in red on top of each alignment. Conserved residues are enclosed with thick red lines, while non-conserved residues are enclosed with thin red lines. Protein sequences were aligned at https://www.ebi.ac.uk/Tools/msa/clustalo/.
Figure S10. Effects of RNAi targeting the *nsy-1* and *lin-45* pathways on the survival of *csnk-1(\text{lf})* mutants in PA14 (biological replicate).

(A) *nsy-1(RNAi), lin-45(RNAi)* and *mpk-1(RNAi)* significantly suppressed the increased survival of *csnk-1(\text{lf})* mutants in PA14. (B) *sek-1(RNAi)* and *pmk-1(RNAi)* significantly suppressed the increased survival of *csnk-1(\text{lf})* mutants in PA14. *p* values were determined using the log-rank test.
Figure S11. Venn diagrams of shared differentially expressed genes (DEG) between csnk-1(lf) mutants and other strains.

(A) Comparison with DEGs identified in nsy-1(ag3lf) mutants. (B) Comparison with DEGs in pmk-1(km25lf) and atf-7(qd22qd130lf) mutants. (C) Comparison with DEGs in skn-1(gf) and skn-1(-) animals. Up: up-regulated. Down: down-regulated.
Table S1. Deleterious genetic variations detected by whole-genome sequencing between the mapped SNPs in the original mac397 isolate.

| Strain       | Affected genes detected by genomic seq. | Variation detail | Genetic location | Development of WT eggs 4 days after RNAi treatment |
|--------------|----------------------------------------|------------------|------------------|--------------------------------------------------|
|             |                                        |                  |                  | 0 mM Nal  | 5 mM Nal                           |
| mac397 (Chr. 1 -6 to 13) |                                        |                  |                  |                                                   |
| rpb-7 (0.78) | exon2:c.G238A:p.G80S                    | I : -4.66         |                  | A       | L2                               |
| lrp-2 (1.00) | exon18:c.A14252T:p.K4751I               | I : -1.9          |                  | A       | L2                               |
| asic-2 (1.00) | exon7:c.G877A:p.D293N                   | I : 2.1           |                  | A       | L2                               |
| col-63 (1.00) | exon2:c.C911T:p.S304F                   | I : 2.7           |                  | ND      | ND                               |
| tsp-15 (1.00) | exon3:c.G331A:p.G111R                   | I : 3.32          |                  | A       | A                                |
| rbpl-1 (1.00) | exon6:c.C860T:p.T287I                   | I : 3.37          |                  | egg     | egg                             |
| ugg-2 (0.87) | exon1:c.T2A:p.M1K                      | I : 3.74          |                  | A       | L2                               |
| K07A1.1 (0.85) | exon4:c.C545T:p.P182L                   | I : 3.8           |                  | A       | L1/L2                            |
| ate-1 (1.00) | exon6:c.T889G:p.C297G                   | I : 3.81          |                  | A       | L2                               |
| sys-1 (1.00) | exon4:c.C659T:p.S220F                   | I : 4.13          |                  | A       | L1/L2                            |
| csnk-1 (0.78) | exon4:c.G482A:p.R161H                   | I : 4.77          |                  | A       | L1/L2/L4                         |

Ratios of mutated sequences are shown in parentheses. A ratio of 1.00 suggests homozygous, and a ratio less than 1.00 suggests heterozygous. A: adults. ND: not determined.
Table S2. Phenotype of animals carrying *csnk-1* deletion mutations or treated with RNAi targeting kinase-encoding genes.

| Genotype                  | Phenotype (0 mM Nal) | Survival (5 mM Nal) |
|---------------------------|----------------------|---------------------|
| control RNAi              | WT                   | L2/L3 arrest        |
| *csnk-1*(RNAi)            | Grow to adults       | Grow to adults      |
| *csnk-1*(mac494)/+        | WT-like              | L2/L3 arrest        |
| *csnk-1*(mac494)          | Grow to adults       | Grow to adults      |
| *csnk-1*(mac495)/+        | WT-like              | L2/L3 arrest        |
| *csnk-1*(mac495)          | Grow to adults       | Grow to adults      |
| *kin-19*(RNAi) (CSNK1A)   | WT-like              | L2/L3 arrest        |
| *kin-20*(RNAi) (CSNK1D)   | WT-like              | L2/L3 arrest        |
| *gsk-3*(RNAi) (GSK3B)     | WT-like              | L2/L3 arrest        |
| *kin-1*(RNAi) (PKA)       | WT-like              | L2/L3 arrest        |
| *kin-2*(RNAi) (PKA)       | Dpy                  | L2/L3 arrest        |

Mammalian orthologs or homologs are indicated in the parentheses.
Table S3. Characterization of *tsp-15(mac499lf)* mutation.

| Strain             | Transgene            | Tg line | Survival (5 mM Nal) |
|--------------------|----------------------|---------|---------------------|
| WT                 | No                   | 0       | No                  |
| *tsp-15(mac499lf)* | No                   | 0       | Yes                 |
| *tsp-15(mac499lf)/+* | No                   | 0       | No                  |
| *tsp-15(mac499lf)* | *tsp-15p::tsp-15_gDNA* | 1     | No                  |
| *tsp-15(mac499lf)* | *tsp-15p::tsp-15_gDNA* | 2     | No                  |
| *tsp-15(mac499lf)* | *tsp-15p::tsp-15_gDNA* | 3     | No                  |
| *tsp-15(mac500lf)* | No                   | No      | Yes                 |
| *tsp-15(mac500lf)/+* | No                   | No      | No                  |
| *tsp-15(mac501lf)* | No                   | No      | Yes                 |
| *tsp-15(mac501lf)/+* | No                   | No      | No                  |

tsp-15(mac500lf) and tsp-15(mac501lf) are knockin genocopies of tsp-15(mac499lf) generated by the CRISPR/Cas9 method.

Table S4. List of differentially expressed genes in *csnk-1(lf)* mutants.

Table S5. List of proteins with altered phosphorylation in *csnk-1(lf)* mutants.
### Table S6. PCR primers for generating the indicated cDNAs.

| cDNA                          | Forward primer     | Reverse primer                                           |
|-------------------------------|--------------------|----------------------------------------------------------|
| csnk-1p                        | cgGGATCCggtgcgagatttgagcc gccgccggt | ggGTACCTgcgcgaaggtgccgccagtt gc |
| csnk-1 cDNA                   | ggGGATCCCatgacgaaacacaacgcggggg | cgGAATTCCcatattttgtgatcgtggcgc |
| csnk-1 cDNA(p.R161H)           | caaacaccaacacacatgttcg | aataacgctccgatcagaaacatggg |
| csnk-1 cDNA(p.C388_K407del)    | gaattaacctgacgacgctgt     | ttccaccttcctcactggaatttctg |
| csnk-1 cDNA(p.C388-390S)       | gaaatccttattttctcaggagac | acttcctactttcgacttttaactc |
| HsCSNK1G1 cDNA                | cgGGATCCatggacccatcttactgggagggg | cgGAATTCCctacttggggcgcggcag |
| HsCSNK1G2 cDNA                | cgGGATCCatgggacttgggaacagggg | cgGAATTCCtacttggctgcgtgcag |
| HsCSNK1G3 cDNA                | cgGGATCCatgggaaataaaagggagaaaagg | cgGAATTCCtacttgggctgtatgcag |
| doxa-1cDNA(p.T343A)            | gataagcgctgagtcaagt         | actgatatatcttggttgctgcaac |
| doxa-1cDNA(p.S346A)            | gagaacaggttggtacaccttac    | agcagttcttacatcttgtatatact |
| doxa-1cDNA(p.S364A)            | ctgcctttgcagcagccatcctc    | ctgcctttgcagcagccatcctc |
| HsDUOX2A2 cDNA                | ggGGATCCatgaccctgtggaggggcga | cgGAATTCCtacaggttagttgatatatactt |

Restriction sites are shown in uppercase.

### Table S7. PCR primers for generating RNAi plasmids targeting the indicated genes.

| RNAi bacteria | Forward primer | Reverse primer |
|---------------|----------------|----------------|
| rbp-7         | aaCTGCAGcccagaacggtctggtcttc | cccAAGCTTcctgtcagcggctttctcac |
| asi-2         | aaCTGCAGttctgtacctgacagcagtgc | cccAAGCTTtaattgtagcgggtactcttcg |
| ate-1         | aaCTGCAGggagacgagagggtgtgaggc | cccAAGCTTcattttccggaatagtgagcattgc |
| kin-1         | aaCTGCAGGctgtctctgagactttgtag | ggGTACCccacgagtgggtataacgagaag |
| kin-2         | aaCTGCAGctctgtctgtagtacgagct | ggGTACCccagagcagatcctgtacttttg |
| kin-19        | aaCTGCAGAtggcgagggtgtgacttggctg | ggGTACCccagatgtaaacacttcttgagcag |
| kin-20        | aaCTGCAGGgagatggcaattccgttgctg | ggGTACCccatctcttctgctagtactta |
| nsy-1         | aaCTGCAGGgagatggcaattccctggctg | ggGTACCccatctcttctgctagtactta |
| lin-45        | aaCTGCAGGgagatggcaattccctggctg | ggGTACCccatctcttctgctagtactta |
| pmk-1         | aaCTGCAGGgagatggcaattccctggctg | ggGTACCccatctcttctgctagtactta |
| sek-1         | aaCTGCAGGgagatggcaattccctggctg | ggGTACCccatctcttctgctagtactta |

Restriction sites are shown in uppercase.
Table S8. Genomic target sequences for generating *tsp-15* knockin and *csnk-1* knockout strains.

| Gene  | sgRNA target sequence         | Nucleotides | Exon | Number of lines examined | Knockout lines | Knockin lines |
|-------|-------------------------------|-------------|------|--------------------------|----------------|---------------|
| *tsp-15* | sgRNA1: GCTGTACAGTTATT | 301~320     | 3    | 78                       | ND             | 2             |
|       | sgRNA2: TATAATGATGTAAGATT    | 345~364     | 3    |                           |                |               |
| *csnk-1* | sgRNA1: TTTTGTTACTGGC     | 32~51       | 1    | 25                       |                |               |

The sequence of *tsp-15* knockin repair template is shown. ND: not determined.
Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- CSNK1SupMXM.pdf
- TableS5D2.xlsx
- TableS4D2.xlsx