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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 CK1 γ 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.

47 **Introduction**

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

57
58 Oxidative stress and immune responses share key regulators. The Nrf2 pathway is involved in
59 innate immunity, antiviral response and inflammation (Battino et al., 2018; Herengt et al., 2021;
60 Saha et al., 2020). Mitochondrial ROS are essential second messengers for innate and
61 adaptive immune responses (Weinberg et al., 2015). NADPH oxidase mediates host defense
62 by generating antimicrobial ROS (Moghadam et al., 2021) and loss of function in phagocyte
63 NADPH oxidase complex can lead to chronic granulomatous disease, a form of excessive
64 inflammation caused by severe bacterial and fungal infections (Segal et al., 2012). Hence, an
65 important question arises as to how animals coordinate the response to oxidative stress and
66 the defense against pathogens.

67
68 The nematode *Caenorhabditis elegans* uses conserved pathways to regulate oxidative stress
69 and innate immune responses (Blackwell et al., 2015; Ewald, 2018; Kim and Ewbank, 2018;

70 McCallum and Garsin, 2016). *C. elegans* SKN-1 protein is homologous to the mammalian Nrf2
71 (Walker et al., 2000), can similarly mediate oxidative stress response (An and Blackwell,
72 2003), and has broad homeostatic functions in stress response, aging, immunity, lipid
73 metabolism, unfolded protein response and other processes (Blackwell et al., 2015). The NSY-
74 1-SEK-1-PMK-1 p38 MAPK pathway and the LIN-45-MEK-2-MPK-1 ERK MAPK pathway
75 mediate key innate immune responses (Kim and Ewbank, 2018; Kim et al., 2002; Nicholas and
76 Hodgkin, 2004). SKN-1 functions downstream of the p38 MAPK pathway to regulate oxidative
77 stress response and innate immunity (Inoue et al., 2005; Papp et al., 2012). *C. elegans* also
78 has a BLI-3 dual oxidase that is the only functional NADPH oxidase encoded by its genome
79 (Edens et al., 2001). BLI-3, the tetraspanin protein TSP-15 and the dual oxidase maturation
80 factor DOXA-1 form a complex to regulate *C. elegans* cuticle formation and response to
81 oxidative stress (Edens et al., 2001; Ewald, 2018; Ewald et al., 2017; Moribe et al., 2004,
82 2012; Thein et al., 2009; Xu et al., 2015, 2018). BLI-3 regulates innate immune response via
83 the p38 MAPK and the SKN-1 pathways (Chavez et al., 2009; Hoeven et al., 2011; McCallum
84 and Garsin, 2016).

85

86 We recently found that the survival phenotype of *C. elegans* in excess iodide provides a new
87 tool for dissecting the functions of genes regulating oxidative stress response (Xu et al., 2015,
88 2018). Screens for mutants that can survive in excess iodide isolated multiple loss-of-function
89 (lf) mutations in *bli-3*, *tsp-15*, *doxa-1*, *wdr-23* (encoding a negative regulator of SKN-1) (Choe
90 et al., 2009) and gain-of-function (gf) mutations in *skn-1* (Xu et al., 2015, 2018).

91

92 To further understand how *C. elegans* responds to oxidative stress, we characterized a

93 previously isolated mutant (*mac397*) that can grow into adults in 5 mM NaI (Xu et al., 2018).
94 *mac397* affected the conserved *casein kinase 1 gamma* gene *csnk-1*. We found that *csnk-1*
95 interacted with the *bli-3/tsp-15/doxa-1* complex to negatively regulate animal survival in excess
96 iodide. Transcriptome analyses suggest that CSNK-1 was a negative regulator of innate
97 immune gene expression, which was validated by increased survival of *csnk-1(lf)* mutants in
98 the pathogenic bacteria *Pseudomonas aeruginosa* PA14. Phosphoproteomic analyses
99 identified decreased phosphorylation of NSY-1 and LIN-45 in *csnk-1(lf)* mutants, and these
100 kinases were required for the negative effects of CSNK-1 on innate immunity. Together our
101 results suggest that CSNK-1 CSNK1G integrates animal's responses to oxidative stress and
102 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.

139 However, very few of the eggs would hatch (Fig. 1D). Together these results suggest that
140 *csnk-1* is essential for *C. elegans* embryonic development and also negatively affects the
141 survival in excess iodide at postembryonic stages.

142

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

147

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

153

154 To understand the tissue-specific functions of *csnk-1*, we first examined the activity of the
155 *csnk-1* promoter. A *GFP* transgene driven by the promoter was broadly expressed when
156 injected into wildtype animals (Fig. 1E). The expression was obvious in the pharynx, head
157 neurons, intestine, ventral cord, vulval muscles, body-wall muscles, dorsal cord and
158 ventrodorsal commissures (Fig. 1F-J). Expression was also observed in the epidermis
159 (hypodermis) (Fig. 1F and H).

160

161 We next performed tissue-specific rescue experiments. Driven by an epidermis-specific
162 promoter (*dpy-7p*) (Supplemental Materials and Methods), the *csnk-1* cDNA transgene
163 strongly rescued the survival of *csnk-1(lf)* mutants (Table 1). However, the transgene driven by
164 an intestine-specific promoter (*nhx-2p*) failed to rescue (Table 1). Therefore, *csnk-1* probably
165 functions in the epidermis to affect the survival. Similar results were previously reported for *tsp-*
166 *15* (Xu et al., 2018).

167

168 To examine whether the function of *CSNK1G* was conserved, we expressed the human
169 *CSNK1G1*, *CSNK1G2* or *CSNK1G3* cDNA transgene driven by the *dyp-7* promoter in *csnk-*
170 *1(lf)* mutants. We found that all three transgenes significantly rescued the survival phenotype
171 (Table 1). Therefore, the function of *csnk-1* is conserved and the three human *CSNK1Gs*
172 exhibit similar activities.

173

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

181

182 ***csnk-1* interacts with *bli-3/tsp-15/doxa-1* to negatively regulate the survival**

183 Considering that *csnk-1* recessively inhibited the survival (Table S2), that we identified a *tsp-*
184 *15(mac499)* loss-of-function mutation in the *mac397* isolate (Table S1 and Table S3,
185 Supplemental Materials and Methods), and that *tsp-15(mac499)* also recessively inhibited the
186 survival (Table S3), we speculated that the survival of the *mac397* isolate as F₁ progeny in the
187 original screen (Xu et al., 2018) might be caused by the co-presence of *csnk-1(mac397)/+* and
188 *tsp-15(mac499)/+*. Such a genetic interaction is known as nonallelic noncomplementation,
189 which can be found between partial alleles of two genes encoding proteins that physically
190 interact with each other or function in the same pathway (Kusch and Edgar, 1986; Yook et al.,
191 2001). Previously we observed a similar interaction between *bli-3* and *doxa-1* (Xu et al., 2018).
192
193 To further dissect the interaction between *csnk-1* and *bli-3*-related genes, we generated double
194 heterozygotes carrying *csnk-1(lf)/+* and *tsp-15(lf)/+*, *bli-3(lf)/+* or *doxa-1(lf)/+* mutations.
195
196 On regular NGM plates, *tsp-15(lf) +/+ csnk-1(lf)* males grew and behaved like wild type (Table
197 2). In 5 mM NaI, a few of these males were able to develop into scrawny adults (Table 2),
198 corroborating the survival of the *mac397* hermaphrodite isolate in the original F₁ screen
199 (Supplemental Materials and Methods). *bli-3(lf) csnk-1(lf)/+ +* hermaphrodites exhibited similar
200 phenotypes (Fig. 2A and 2B, Table 2).
201
202 Unlike *tsp-15* or *bli-3*, *csnk-1(lf)/+; doxa-1(mac55lf)/+* hermaphrodites exhibited obvious
203 blistered and dumpy phenotypes on regular NGM plates (Fig. 2C and Table 2). These animals
204 survived much better than *tsp-15(lf) +/+ csnk-1(lf)* or *bli-3(lf) csnk-1(lf)/+ +* mutants in 5 mM NaI
205 (Table 2). This special interaction between *csnk-1* and *doxa-1* was verified using a second

206 *doxa-1* allele, *mac67* (Table 2), which was previously characterized as a loss-of-function
207 mutation (Xu et al., 2018).

208

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

217

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

223

224 **DOXA-1 and CSNK-1 are detected in a same protein complex**

225 To understand the interaction between CSNK-1 and DOXA-1, we first examined whether these
226 proteins colocalize in subcellular structures. We expressed a *csnk-1::mCherry* fusion
227 transgene and a *doxa-1::GFP* fusion transgene in the epidermis and detected colocalization of
228 mCherry and GFP in epidermal subcellular structures of L3 larva (Fig. 2E-G). The

229 colocalization appeared specific for CSNK-1 and DOXA-1 because GFP alone did not
230 colocalize with CSNK-1::mCherry, nor did mCherry alone colocalize with DOXA-1::GFP (Fig.
231 S5).

232

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

240

241 DOXA-1 is homologous to the human DUOX maturation factor 1 and 2 (DUOXA1 and
242 DUOXA2) that are required for DUOX activities (Grasberger and Refetoff, 2006; Moribe et al.,
243 2012). We performed similar immunoprecipitation and immunoblotting experiments on human
244 DUOXA2 and CSNK1G2 (the homolog exhibiting a relatively stronger rescue effect on the
245 survival phenotype) (Table 1), and tag-independent interaction between the two proteins could
246 still be detected (Fig. 2I). In addition, immunofluorescence detected co-localization of
247 FLAG::CSNK1G2 and DUOXA2::HA on the plasma membrane of co-transfected HeLa cells
248 (Fig. 2J). Together these results suggest that DOXA-1 and CSNK-1 might interact directly or
249 co-exist in a same protein complex, and that this relationship is likely conserved.

250

251 DUOXAs have a shorter extracellular N-terminal domain, five transmembrane domains, a large
252 ectodomain between transmembrane domains 2 and 3, and a longer intracellular C-terminal
253 domain (Grasberger and Refetoff, 2006). We inspected DOXA-1 using Scansite 4
254 (www.scansite4.mit.edu) and found three predicted casein kinase 1 phosphorylation sites in
255 the C-terminal domain (Fig. S6). We mutated each individual residue to alanine in *doxa-1*
256 transgenes and tested whether they could rescue the survival of *doxa-1(lf)* mutants.
257 Interestingly, the T343A mutation ablated the rescuing ability, while the other two mutations did
258 not (Table 3). Therefore, T343 in the C-terminal domain of DOXA-1 may be important for
259 DOXA-1 activity.

260

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

263 To understand how *csnk-1* affects global gene expression, we analyzed the transcriptome of
264 *csnk-1(lf)* mutant L4 larva (Fig. 3A, heat map). In total, 968 genes exhibited more than 1.5-fold
265 changes in expression (up-regulated or down-regulated) in *csnk-1(lf)* mutants (Table S4A),
266 among which 579 exhibited more than 2-fold changes (398 up and 181 down) (Fig. 3B, Table
267 S4B and S4C). Gene Ontology (GO) analyses of genes with more than 2-fold changes
268 identified “defense response to other organisms” and “innate immune response” among the
269 most significantly affected biological processes (Fig. S7A and Table S4D). The KEGG
270 analyses identified “drug metabolism-cytochrome P450” and “metabolism of xenobiotics by
271 cytochrome P450” as the most significantly affected pathways (Fig. S7A and Table S4D).

272

273 Based on the transcriptome, the expression levels of genes in the “defense response to other
274 organism” term were variable (Fig. S7B, 30 up and 4 down) and all 21 genes in the “innate
275 immune response” term were included in this group (Fig. S7B and Table S4G, genes in blue,
276 20 up and 1 down). We therefore examined whether up-regulated genes and down-regulated
277 genes might carry out different functions. The results indicated that “defense response to other
278 organisms” and “innate immune response” terms were primarily affected by up-regulated
279 genes (Fig. 3C, Table S4E and S4F), while the “drug metabolism-cytochrome P450” pathway
280 was affected by both up-regulated and down-regulated genes (Table S4E and S4F).

281
282 These findings suggest that *csnk-1* might affect innate immune response. To test this, we
283 examined the survival of *csnk-1(lf)* mutants in the pathogenic bacteria *Pseudomonas*
284 *aeruginosa* PA14. Indeed, *csnk-1(lf)* mutants were significantly more resistant to the killing by
285 PA14 compared to wildtype animals (Fig. 3D), and this phenotype was rescued by the *csnk-1*
286 transgene driven by the endogenous *csnk-1* promoter or the intestine-specific *nhx-2* promoter,
287 but not by the epidermis-specific *dpy-7* promoter (Fig. 3D). Hence, intestinal *csnk-1* probably
288 negatively regulates *C. elegans* survival in PA14.

289

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

291 As a kinase, the effects of CSNK-1 might be mediated by downstream phosphorylation signals.
292 To identify protein phosphorylation affected by CSNK-1, we performed mass spectrometry-
293 based phosphoproteomic analyses on *csnk-1(lf)* mutant L4 larva.

294

295 The analyses detected 11,644 peptides, containing 16,946 phosphorylation sites in 4,082
296 proteins (Fig. S8A). Among these, 1,278 phosphorylation sites in 731 proteins exhibited more
297 than 20% phosphorylation level changes in *csnk-1(lf)* mutants (Fig. S8A and Table S5A). Most
298 of the sites were phosphoserine (1027/1278, p-Ser), followed by phosphothreonine (208/1278,
299 p-Thr) and phosphotyrosine (43/1278, p-Tyr) (Fig. S8A). We postulated that some proteins
300 with decreased phosphorylation in *csnk-1(lf)* mutants (Table S5B) might be candidate
301 phosphorylation substrates of CSNK-1, while those with increased phosphorylation (Table
302 S5C) might be indirectly affected by CSNK-1.

303

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

310

311 To identify potential downstream regulators of CSNK-1, we performed protein-protein
312 association analyses on proteins assigned to the KEGG pathways (Table S5F). Here we
313 included an innate immunity regulator DKF-2 (Ren et al., 2009), which exhibited decreased
314 phosphorylation in *csnk-1(lf)* mutants (Table S5B) but was not assigned to pathways by KEGG
315 analyses. The association analyses identified three larger protein clusters and two smaller
316 clusters (Fig. 4A). Key regulators of innate immunity, such as NSY-1, LIN-45, MPK-1 and DAF-
317 16 (Garsin et al., 2003; Kim and Ewbank, 2018; Kim et al., 2002; Nicholas and Hodgkin, 2004),

318 were central nodes of two larger clusters, and DKF-2 was in the same cluster with NSY-1 and
319 DAF-16 (Fig. 4A).

320

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

328

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

332

333 We treated wildtype or *csnk-1(lf)* animals with *nsy-1(RNAi)* and quantified their survival in
334 PA14. *nsy-1(RNAi)* decreased the survival of wildtype animals in PA14 compare to control
335 RNAi (Fig. 4B and S10A), consistent with previous findings (Kim et al., 2002). Importantly, the
336 increased survival of *csnk-1(lf)* mutants in PA14 was significantly suppressed by *nsy-1(RNAi)*
337 (Fig. 4B and S10A). Furthermore, RNAi knockdown of *sek-1* and *pmk-1*, two downstream
338 effectors of *nsy-1* (Kim and Ewbank, 2018), also significantly suppressed the increased
339 survival of *csnk-1(lf)* mutants in PA14 (Fig. 4C and S10B).

340

341 We next examined the effects of *lin-45(RNAi)*. *lin-45(RNAi)* itself did not obviously affect the
342 survival of wildtype animals in PA14 (Fig. 4B and S10A). However, it significantly suppressed
343 the increased survival of *csnk-1(lf)* mutants (Fig. 4B and S10A). Consistent with the notion that
344 MPK-1 is a downstream effector of LIN-45 (Kim and Ewbank, 2018), *mpk-1(RNAi)* exhibited
345 similar suppressive effects as *lin-45(RNAi)* (Fig. 4B and S10A). Therefore, NSY-1 and LIN-45
346 likely mediate the negative regulation of innate immune response by CSNK-1.

347

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

355

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

357 SKN-1 Nrf2 is a major regulator of oxidative stress response and we previously found that *skn-*
358 *1(gf)* mutants can survive in excess iodide (Xu et al., 2018). To investigate how *csnk-1*
359 interacts with *skn-1*, we generated *csnk-1; skn-1* double mutants and compared the gene
360 expression of *csnk-1(lf)* mutants with that of *skn-1(gf)* or *skn-1*-deficient mutants.

361

362 First, we found that *csnk-1(lf); skn-1(lf)* double mutants can survive in 5 mM NaI (Table 4A).
363 Since *skn-1(lf)* single mutants could not survive in 5 mM NaI (Table 4A) (Xu et al., 2018), this
364 result suggests that *csnk-1* might function in parallel with or downstream of *skn-1*.
365
366 We next examined the survival of *csnk-1(lf); skn-1(gf)* double mutants in a much higher
367 concentration of NaI (50 mM), a test that we previously used to detect the additive or
368 synergistic effects between *bli-3/tsp-15/doxa-1* loss-of-function mutations and *skn-1(gf)*
369 mutations (Xu et al., 2018). We found that *csnk-1(lf); skn-1(gf)* double mutants now can survive
370 in 50 mM NaI, while either single mutant failed to (Table 4B).
371
372 Finally we compared the differentially expressed genes in *csnk-1(lf)* mutants (Table S4) with
373 those affected by *skn-1*-deficiency or *skn-1(gf)* (Oliveira et al., 2009; Xu et al., 2018). Here only
374 a small fraction of differentially expressed genes was shared between *csnk-1(lf)* and *skn-*
375 *1(gf)/skn-1*-deficient animals (Fig. S11C). Together these results suggest that *csnk-1* and *skn-*
376 *1* probably function in parallel to regulate oxidative stress response.

385 **Discussion**

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

392

393 **The functions of CSNK1Gs remain to be understood**

394 Mammalian casein kinase 1 (CSNK1) family has seven members, CSNK1A (CK1 α), CSNK1B
395 (CK1 β , cow only), CSNK1G1/2/3 (CK1 γ 1/2/3), CSNK1D (CK1 δ) and CSNK1E (CK1 ϵ) (Schitteck
396 and Sinnberg, 2014). CSNK1G requires palmitoylation-mediated membrane localization for its
397 functions (Davidson et al., 2005; Li et al., 2016). Most studies on CSNK1 were focused on A, D
398 and E members, while the functions of the G members are less understood. The *C. elegans*
399 genome contains three genes, *kin-19*, *kin-20* and *csnk-1*, that encode orthologs of CSNK1A,
400 CSNK1D and CSNK1G (Manning, 2005) (wormbase.org).

401

402 CSNK1Gs have been shown to regulate Wnt, Hh and JNK pathways (Davidson et al., 2005;
403 Jiang, 2017; Li et al., 2016, 2020b). Mammalian CSNK1G1/3 can stimulate necroptosis by
404 interacting with receptor-interacting kinase 3 (RIPK3) (Lee et al., 2019), a function that is
405 similarly carried out by CSNK1A, CSNK1D and CSNK1E (Hanna-Addams et al., 2020) while
406 attenuated by CSNK1G2 (Li et al., 2020a). CSNK1G also can phosphorylate presenilin 1 of the
407 γ -secretase complex (Bustos et al., 2017) and was associated with developmental delay and

408 autism spectrum disorder (Gold et al., 2020), implicating this type of kinases in major
409 neurological diseases.

410

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

415

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

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

424

425 In this study, we made several new findings on CSNK-1. First, *csnk-1(lf)* mutants can survive
426 in excess iodide, and this appears to be a specific function of *csnk-1* as knocking down *kin-19*
427 (*CSNK1A*), *kin-20* (*CSNK1D*), *gsk-3* (*GSK3B*), *kin-1* (*PKA*) or *kin-2* (*PKA*) by RNAi failed to
428 cause this phenotype (Table S2). Second, *csnk-1(lf)* mutants exhibited reduced levels of
429 endogenous ROS. Third, the conserved palmitoylation signal was required for CSNK-1 activity,
430 suggesting that CSNK-1 might function on plasma membrane. Fourth, *csnk-1* interacted with

431 *bli-3/tsp-15/doxa-1* in a nonallelic noncomplementation manner, suggesting that CSNK-1 might
432 function in the same pathway and/or physically interact with BLI-3/TSP-15/DOXA-1. Fifth,
433 CSNK-1::mCherry and DOXA-1::GFP fusion proteins were colocalized in subcellular structures
434 of the epidermis, and human FLAG::CSNK1G2 and DUOXA2::HA fusion proteins were
435 colocalized on the plasma membrane of cultured cells. Sixth, CSNK-1 and DOXA-1 were
436 detected in a same protein complex, and human CSNK1G2 and DUOXA2 exhibited a similar
437 interaction. Together, these results support the hypothesis that CSNK-1 interacts with DOXA-1
438 to affect the activity of BLI-3/TSP-15/DOXA-1 dual oxidase complex and ROS generation.

439
440 Interestingly, we found that a potential casein kinase 1 phosphorylation site in DOXA-1 C-
441 terminal domain was essential for DOXA-1 activity. However, it is unclear whether this is a
442 direct phosphorylation site of CSNK-1. In addition, we have not addressed whether the
443 interaction between DOXA-1 and CSNK-1 is required for the dual oxidase activity. Future
444 studies are warranted to answer these important questions.

445
446 The transgene rescue experiments suggest that CSNK-1 primarily functions in the epidermis to
447 affect the survival phenotype, which was previously shown for *tsp-15* and *skn-1(gf)* (Xu et al.,
448 2018). Nevertheless, the interaction between CSNK-1 and the dual oxidase complex might
449 extend beyond the epidermis. This possibility is supported by the broad expression of *csnk-1*
450 and the appearance of pleiotropic defects in growth and morphology of double mutants
451 between *csnk-1(lf)* and *bli-3(lf)* or *doxa-1(lf)*, which might involve defects in tissues beyond
452 epidermis.

453

454 **NSY-1 and LIN-45 mediate the negative effects of CSNK-1 on innate immunity**

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

462

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

467

468 We found that RNAi targeting *nsy-1*, *sek-1* or *pmk-1* essentially abrogated the increased
469 survival of *csnk-1(lf)* mutants in PA14, and RNAi targeting *lin-45* or *mpk-1* significantly
470 suppressed the increased survival of *csnk-1(lf)* mutants. These results suggest that the NSY-1
471 and LIN-45 pathways function downstream of CSNK-1. Together with the phosphoproteomic
472 results that LIN-45 exhibited decreased phosphorylation while MPK-1 exhibited increased
473 phosphorylation in *csnk-1(lf)* mutants, these findings suggest that CSNK-1-dependent
474 phosphorylation of NSY-1 and LIN-45 (direct or indirect) negatively regulates the activities of
475 these kinases, the expression of downstream innate immunity genes and innate immune
476 response.

477

478 Supporting this hypothesis, we found that significantly more genes depending on *nsy-1* for
479 expression (Cheesman et al., 2016) overlapped with those genes down-regulated by *csnk-1*.
480 We made similar observations for genes depending on PMK-1 and ATF-7 for up-regulation
481 (Fletcher et al., 2019) and genes down-regulated by *csnk-1*. [ATF-7 is a downstream
482 transcription activator of the NSY-1 pathway (Shivers et al., 2010)].

483

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

489

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

496

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

498 SKN-1 is a key activator of stress responses in *C. elegans* (Blackwell et al., 2015). We
499 previously found that *skn-1(gf)* and a *bli-3* missense mutation (*mac40*) that affects the oxidase

500 domain together can cause animal survival in a higher concentration of iodide (50 mM NaI),
501 while either single mutation failed to do so (Xu et al., 2018). In addition, *bli-3(lf); skn-1(lf)*
502 double mutants could not survive in 5 mM NaI, while *bli-3(lf)* single mutants can (Xu et al.,
503 2015, 2018).

504

505 We found that *csnk-1(lf); skn-1(gf)* double mutants can survive in 50 mM NaI, which is like *bli-*
506 *3(mac40lf); skn-1(gf)* mutants. However, *csnk-1(lf); skn-1(lf)* double mutants also can survive
507 in 5 mM NaI, which is different from *bli-3(lf); skn-1(lf)* mutants. Besides, only a small fraction of
508 genes affected by *csnk-1* was also affected by *skn-1*. Based on these results, we postulate
509 that CSNK-1 and SKN-1 probably function in parallel to regulate oxidative stress response, in
510 which CSNK-1 activity might be mediated by BLI-3 and also by the expression of SKN-1-
511 independent stress response genes.

512

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

514 GO analyses found that a prominent group of proteins with decreased phosphorylation in *csnk-*
515 *1(lf)* mutants were transmembrane transporters, including solute carriers and ABC transporters
516 (Fig. S8C and S8D, Table S5D and S5G). A detailed analysis of the relationship between
517 CSNK-1 and these transporters is beyond the scope of our current study. Nevertheless, it
518 could be interesting to explore along this line, considering that these transporters are important
519 for cellular homeostasis and that the functions of most of them are unclear (César-Razquin et
520 al., 2015). In addition, GO analyses on proteins with increased phosphorylation in *csnk-1(lf)*
521 mutants identified terms such as “cytoskeletal protein binding”, “developmental process”,
522 “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.

546 **Materials and Methods**

547 See Supplemental Materials and Methods.

548

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552

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556

557 **Author Contributions**

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

560

561 **Competing interests:** The authors have declared that no competing interests exist.

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739 **Figure 1. Characterization of *csnk-1*.**

740 (A) *csnk-1* gene structure (www.wormbase.org). The locations of the *mac397* mutation and the
741 two deletion mutations, *mac494* and *mac495*, are indicated. The position of the *sgRNA* used in
742 the CRISPR/Cas9-based mutagenesis experiment is shown as a red bar. (B) Partial sequence
743 alignment of CSNK1G across species showing the R161H change caused by *mac397*. *C. e.*:

744 *C. elegans*; *D. m.*: *Drosophila*; *M. m.*: mouse; *H. s.*: human. (C) Percentage of L1 larva that
745 can grow into adults on plates with 5 mM NaI. Results were based on two biological replicates.
746 100 L1 larva were analyzed in each replicate. Statistics: two-tailed unpaired *t*-test. **: $p < 0.01$.
747 (D) Hatching rate of eggs laid by *csnk-1(lf)* homozygous mutants. All eggs laid by a single adult
748 on regular NGM agar plates were examined. Results were based on three individual adults.
749 Statistics: two-tailed unpaired *t*-test. ****: $p < 0.0001$. (E-J) Expression pattern of a *csnk-*
750 *1p::GFP* transgene in adults. (E) Picture of a whole transgenic adult. (F) Higher-resolution
751 picture of the anterior part. (G) Higher-resolution picture of the posterior part. (H, I, J) Higher-
752 resolution pictures of the middle part at different focal planes.

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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.

766 Immunoprecipitation was performed using an anti-HA antibody. (I) Western blotting detecting
767 the interaction between tagged DUOXA2 and CSNK1G2 co-expressed in HEK293T Cells.
768 Immunoprecipitation was performed using an anti-HA antibody. (J) Membrane colocalization of
769 tagged DUOXA2 and CSNK1G2 expressed in HeLa Cells. DUOXA2::HA fusion protein is
770 shown in green, FLAG::CSNK1G2 is shown in red, and DAPI staining is shown in blue.

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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.

794 **Table 1. Effects of *csnk-1* transgenes on the survival phenotype of *csnk-1(lf)* mutants in**
795 **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%)
	<i>csnk-1p::csnk-1_cDNA(p.R161H)</i>	1	0/100 (0%)	0/100 (0%)
		2	0/100 (0%)	0/100 (0%)
<i>csnk-1(mac494lf)</i>	No	0	78/100 (78%)	80/100 (80%)
	<i>csnk-1p::csnk-1_cDNA</i>	1	6/100 (6%)	13/100 (13%)
		2	3/100 (3%)	5/100 (5%)
	<i>csnk-1p::csnk-1_cDNA(p.R161H)</i>	1	81/100 (81%)	74/100 (74%)
		2	62/100 (62%)	56/100 (56%)
	<i>dpy-7p::csnk-1_cDNA</i> (epidermis promoter)	1	0/100 (0%)	1/100 (1%)
		2	5/100 (5%)	1/100 (1%)
	<i>nhx-2p::csnk-1_cDNA</i> (intestine promoter)	1	66/85 (77.6%)	65/83 (78.3%)
		2	48/76 (63.2%)	56/77 (72.7%)
	<i>dpy-7p::HsCSNK1G1_cDNA</i>	1	20/100 (20%)	15/80 (18.8%)
		2	19/113 (16.8%)	23/100 (23%)
	<i>dpy-7p::HsCSNK1G2_cDNA</i>	1	10/100 (10%)	5/100 (5%)
		2	12/100 (12%)	6/100 (6%)
	<i>dpy-7p::HsCSNK1G3_cDNA</i>	1	23/100 (23%)	21/100 (21%)
		2	42/100 (42%)	37/100 (37%)
	<i>csnk-1p::csnk-1_cDNA(p.C388_K407del)</i>	1	60/100 (60%)	58/100 (58%)
		2	74/100 (74%)	63/100 (63%)
	<i>csnk-1p::csnk-1_cDNA(p.C388-390S)</i>	1	59/105 (56.2%)	67/100 (67%)
		2	72/100 (72%)	78/100 (78%)

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797 For *csnk-1(lf)* background, all transgenes were maintained in the *csnk-1(mac494lf)/hT2*
798 heterozygous background except for *csnk-1p::csnk-1_cDNA* or *dpy-7p::csnk-1::cDNA*, one line
799 of which was maintained in the *csnk-1(mac494lf)* homozygous background.

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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
<i>csnk-1(mac494lf)/+</i>	WT-like	No
<i>csnk-1(mac495lf)/+</i>	WT-like	No
<i>tsp-15(mac500lf)/+</i>	WT-like (male)	No (male)
<i>tsp-15(mac501lf)/+</i>	WT-like (male)	No (male)
<i>bli-3(mac40lf)/+</i>	WT-like (male)	No (male)
<i>bli-3(e767lf)/+</i>	WT-like (male)	No (male)
<i>doxa-1(mac55lf)/+</i>	WT-like (male)	No (male)
<i>doxa-1(mac67lf)/+</i>	WT-like (male)	No (male)
<i>tsp-15(mac500lf) +/+ csnk-1(mac494)</i>	WT-like (male)	Weak (a few, scrawny) (male)
<i>tsp-15(mac500lf) +/+ csnk-1(mac495)</i>	WT-like (male)	Weak (a few, scrawny) (male)
<i>tsp-15(mac501lf) +/+ csnk-1(mac494)</i>	WT-like (male)	Weak (a few, scrawny) (male)
<i>tsp-15(mac501lf) +/+ csnk-1(mac495)</i>	WT-like (male)	Weak (a few, scrawny) (male)
<i>bli-3(mac40lf) csnk-1(mac494lf)/+ +</i>	WT-like	Weak (a few, scrawny)
<i>bli-3(mac40lf) csnk-1(mac495lf)/+ +</i>	WT-like	Weak (a few, scrawny)
<i>bli-3(e767lf) csnk-1(mac494lf)/+ +</i>	WT-like	Weak (a few, scrawny)
<i>bli-3(e767lf) csnk-1(mac495lf)/+ +</i>	WT-like	Weak (a few, scrawny)
<i>csnk-1(mac494lf)/+; doxa-1(mac55lf)/+</i>	Bli, Dpy	Strong (plenty, Bli, Dpy)
<i>csnk-1(mac495lf)/+; doxa-1(mac55lf)/+</i>	Bli, Dpy	Strong (plenty, Bli, Dpy)
<i>csnk-1(mac494lf)/+; doxa-1(mac67lf)/+</i>	Bli (male)	Strong (plenty, Bli) (male)
<i>csnk-1(mac495lf)/+; doxa-1(mac67lf)/+</i>	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-*

811 *1(mac55lf)/hT2* heterozygotes were used here as *bli-3(lf) csnk-1(lf)/+ +* and *csnk-1(lf)/+; doxa-*
812 *1(lf)/+* mutants. *csnk-1(lf)/+; doxa-1(mac67lf)/+* double heterozygous males were generated by
813 crossing *doxa-1(mac67lf)* males with *csnk-1(lf)/hT2* hermaphrodites.

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

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

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819 Survival of *doxa-1(mac55lf)* animals carrying the indicated *doxa-1* transgenes in 5 mM Nal
820 were observed.

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829 **Table 4. (A) Survival of animals carrying different combinations of *csnk-1(lf)* and *skn-***
830 ***1(zu135lf)* mutations. (B) *csnk-1(lf)* and *skn-1(gf)* mutations exhibit additive or**
831 **synergistic effects on the survival in 50 mM NaI.**

A		Number of F ₁ adults	
P ₀ genotype	F ₁ genotype	No NaI	5 mM NaI
<i>csnk-1(mac494lf)/+;</i> <i>skn-1(zu135lf)/+</i>	<i>+/+; +/+</i>	ND	0
	<i>+/+; skn-1(lf)/+</i>	ND	0
	<i>csnk-1(mac494lf)/+; skn-1(lf)/+</i>	ND	0
	<i>csnk-1(mac494lf)/+; +/+</i>	ND	0
	<i>csnk-1(mac494lf); +/+</i>	7	60
	<i>csnk-1(mac494lf); skn-1(lf)/+</i>	14	136
	<i>csnk-1(mac494lf); skn-1(lf)</i>	3	29
	<i>csnk-1(mac494lf)/+; skn-1(lf)</i>	15	0
<i>csnk-1(mac495lf)/+;</i> <i>skn-1(zu135lf)/+</i>	<i>+/+; +/+</i>	7	0
	<i>+/+; skn-1(lf)/+</i>	ND	0
	<i>csnk-1(mac495lf)/+; skn-1(lf)/+</i>	ND	0
	<i>csnk-1(mac495lf)/+; +/+</i>	ND	0
	<i>csnk-1(mac495lf); +/+</i>	4	6
	<i>csnk-1(mac495lf); skn-1(lf)/+</i>	10	27
	<i>csnk-1(mac495lf); skn-1(lf)</i>	1	10
	<i>csnk-1(mac495lf)/+; skn-1(lf)</i>	15	0
B		Survival (50 mM NaI)	
Genotype			
<i>csnk-1(mac494lf)</i>		No	
<i>csnk-1(mac495lf)</i>		No	
<i>skn-1(lax120gf)</i>		No	
<i>skn-1(mac53gf)</i>		No	
<i>csnk-1(mac494lf); skn-1(lax120gf)</i>		Yes	
<i>csnk-1(mac495lf); skn-1(lax120gf)</i>		Yes	
<i>csnk-1(mac494lf); skn-1(mac53gf)</i>		Yes	
<i>csnk-1(mac495lf); skn-1(mac53gf)</i>		Yes	

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833 For (A), F₁ progeny of *csnk-1(lf)/+; skn-1(zu135lf)/+* animals were grown in plates with or
834 without 5 mM NaI. For plates without NaI, F₁ adults were randomly picked to separate plates
835 and examined for egg laying and egg hatching. Individuals that laid hatched eggs were not
836 analyzed further as they were not homozygous at *csnk-1* or *skn-1* locus. Individuals that only
837 laid unhatched eggs were genotyped for *csnk-1(lf)* and *skn-1(lf)* mutations. For plates with 5
838 mM NaI, F₁ adults were individually genotyped for *csnk-1(lf)* and *skn-1(lf)* mutations. ND:
839 individuals of these genotypes were not determined.

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858 **Figure S1. CSNK1G protein sequence alignment.**

859 *C.e.*: *C. elegans* CSNK-1; *D.m.*: *Drosophila* Gilgamesh; *M.m.*: mouse CSNK1G2; *H.s.*: human

860 CSNK1G1, CSNK1G2, CSNK1G3. Green bar: location of *mac494* and *mac495* mutations. Red

861 box: kinase domain. Red arrowhead: *mac397* mutation. Red stars: potential

862 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[qIs48]*. Arrowheads indicate single or double heterozygous mutants. Arrows indicate single or double homozygous mutants.

Figure S4. DCFDA fluorescent intensities of wildtype animals and *csnk-1(lf)* mutants.

(A) A picture of DCFDA-stained WT L4 larva. (B) A picture of DCFDA-stained L4 *csnk-1(mac494lf)* mutants. Arrow points to a *csnk-1(mac494lf)* homozygous animal. Arrowhead points to a *csnk-1(mac494lf) I/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 *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). (D, E, 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.

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C. e. NP_498886.2 -----MWWFGGNSSDYPNAAIPNFMHAFVTFSVFTIPLIAYITLLPGVR-RKVVVTVTYVLTAVGSAIIASLIYPCMASSSCMIYTCFRGHSNE 93
D. m. NP_001036362.1 MKGWFDAPRDDGGPTLYSFSNRTPFVTGTVSIVAVSVLEAFYVAFVIFPGVR-FQKFTTFSTVTLSTFGLVITLIRLGSAMHVAHATIIAPYKAFSPRE 99
M. m. NP_001292191.1 -----MAALCHTLPFYTGKPTTFPMDTTLAVITITFIDALVTFTIILPGIRGKTRIFWLLRVVTSLSFIGAAILAVNFSSEMSVGVUNNTTYKAFSPK 93
H. s. NP_001263195.1 -----MATLCHTLPFYAGPKPTTFPMDTTLASITIMIFLIALATFTIILPGIRGKTRIFWLLRVVTSLSFIGAAILAVNFSSEMSVGVUNNTTYKAFSPSE 93
H. s. NP_997464.2 -----MTLWNGVLPFYQPQRHAAGFSVELLLIVLVFALAASFLIILPGIRGHSRWFVLVRVLLSLFIGAETVAVHFSAEWPFVGVNNTNTSYKAFSAA 93

RIIARIGVEIGLQKRVNVTTKFERLLSSNDVLPGSDMTELYYNEGFDISGISMAEALHHCLENGLEMMFTSVLEBYFSLNQDSFDWGRHYRVAGHYTHAAI 193
KLPAKIGTHIGIMHVNTLTALFIGNWTP-----PDIDYNBRFTWEGANDMSANVRHALQRLGEPFETTVABYFSLGRBEGFSWGGCYRAAGYFASIML 192
WVSVDVGLGIGIGGVNITLTGTPVQQLN-----ETINYNBAFAMRLGRSYAEEYAKALEKGLDPDVLYLAEKFTF-RSPCGLYNCYRLAGHYASAML 184
WISADIGLCVGLGGVNITLTGTPVQQLN-----ETINYNBEFTWRIDGENYAEYAKALEKGLDPDVLYLAEKFTF-RSPCGLYRCYRLAGHYTSAML 184
RVTARVRRLVGLGEGNITLTGTPVHQLN-----ETIDYNBQCTWRIRKENYAAEYANALEKGLDPDVLYLAEKFTF-RSPCGLYHCYRLAGHYASATL 184

MFAPACMCIS-UVIMMFLFHNAYKSLATGTSCHLACLVLLLSPCBLRIAPTGENFERVDLTATFSFCFYLIFAIGLLCVLCGIGLGICEHWRIYTLST 292
WASLASMLIMNLLL-IAVERYGAYMKAITGALLVCTTVGYHCLLPKR-FLSIH---TEGGRLEHFFGCMYMLVIVAGLLCFIASVLISIIDLVMPHTFST 287
WVAFICWLLIANVMLSNPVLVYGGHMLLATGFLCHLAFPFSSMTTSLISPCPIR---LGTAVLHTHHGEAFWITLATGLLCHLLGLVMAVAHRMCPHRLKA 281
WVAFICWLLIANVMLSNPVLVYGGHMLLATGFLCHLAFPFSSMTTSLISPCPIH---LGASVLHTHHGEAFWITLTGCLLCVLLGLAMAVAHMCPHRLKA 281
WVAFICWLLIANVMLSNPVLVYGGHMLLATGFLCHLAFPFSSMTTSLISPCPIH---LGSSALTTCQCAAFWVTLATGVLCFLGSAVVSLOYVRESALRT 280
343 346 364
FTDASLDEHVGPWKKKLPTGGPALQVCIGAYGTNTTNSRDKNDISSDKTAGSSGFCSTSTCQSSASSASLRSCSSSIEIVHDEAELEERTHVHFLQEPFC 392
VLEVYVGYTFY---DRHVILEESDVYRKPRNRSRLEDEEGLGSRIRRRSSKARDICPGTAPRRDSFAGVSSSGGVENKGFQSDAPKSPWRYPFRRSCQ 384
FFNCSS-----EDPVLEWGSSEGGGLSPHYRSTAESEETQDIPMSVASS-ETCFKEEHPKESDCSL----- 341
FFNCSD-----EDPMLEWSPBEGGLSPRYRSMADSEKSDIDLEASSTKAYCKEAHPKDPDCAL----- 343
LLDCSA-----KDCQCRGG--SF--LILGILHQAAPDLKCTITNI----- 320

SSSST----- 397
MACCQHSHPHQHPLCQHQQHQQHHCCQLQFVGGEVVCHEPMHMCRTMSQDSGSSIASAAVCISPLHKKHALARMLPNPVERIRDMDHW 474
----- 341
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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.

A

Protein	Phospho-down	Phospho-up	Function in immunity
NSY-1	Yes	Not detected	Positive
LIN-45	Yes	Not detected	Positive
MPK-1	Not detected	Yes	Positive
DKF-2	Yes	Not detected	Positive
DAF-16	Not detected	Yes	Negative

B

(phosphorylation down) **ST**MENIK

C. elegans **NSY-1a**: 434 SLLGRKGALQNLMEYWDVATYFEVSVLAENYQKACEAALMMVKLKPPVWYLK**ST**MENIKL 493

Drosophila NP_477089.3: 360 NLIGKKGSLSSLSLEYWDVATFFEISVLANDYAKAIQAAECMFKLKPPNWWYLK**ST**IGNISL 419

Mouse NP_057902.5: 425 CLLARKGCVEKMQYYWDVGFLGAQILANDPIQVVLAAEQLYKLNAPIWYLV**ST**METFL 484

Human NP_005914.1: 461 SLLGKKGNLEKLQSYWEVGFFLGASVLANDHMRVIQASEKLFKLKTPAWYLV**ST**IVETILI 520

HSD**SLHSL**SGHHSAGGAGTSDKEPPK (phosphorylation down)

C. elegans **LIN-45b**: 90 PSTPLYPKHSD**SLHSL**SGHHSAGGAGTSDKEPPKFKYKMIMVHLFPDQHSRVEVRPGETA 150

Drosophila NP_525047.1: 108 QRQLARVHHGNDLTDLSGSQPGSQCGTLT----RQPKILLRAHLPNQRTSVEVISGVRL 164

Mouse NP_033833.1: 1 ----MEPPRG----PPVSGAEPSP-----AVGTVKVYLPNKQRTVVTVREGMSV 42

Human AAH07514.1: 1 ----MEPPRG----PPANGAEPSP-----AVGTVKVYLPNKQRTVVTVRDGMSV 42

VTDPQTDHTGFL**TEY**VATR (phosphorylation up)

C. elegans **MPK-1b**: 241 LARVTDPTDHTGFL**TEY**VATRWRAPAEIMLNSKGYTKSIDVWSVGCILAEMLSNRPLFP 300

Drosophila NP_001015122.1: 183 LARIADPEHDHTGFL**TEY**VATRWRAPAEIMLNSKGYTKSIDIWSVGCILAEMLSNRPIFP 242

Mouse NP_001033752.1: 168 LARVADPDHDHTGFL**TEY**VATRWRAPAEIMLNSKGYTKSIDIWSVGCILAEMLSNRPIFP 227

Human NP_002736.3: 170 LARVADPDHDHTGFL**TEY**VATRWRAPAEIMLNSKGYTKSIDIWSVGCILAEMLSNRPIFP 229

SCSWSGR (phosphorylation down)

C. elegans **DKF-2f**: 412 ARPSSTLGGAAATPNIFITSDDCGDAVGGNYLQMPRKDR**SCS**WSGRPLWMEIAEATRVKLQ 471

Drosophila NP_001262705.1: 182 -----LVSTEDSTGRNDSSSSSLNIPGRHQRT**SCS**SG-----SRNGLMVLR 220

Mouse NP_001164475.1: 239 -----ESHTHQEPSKRIP**SCS**WSGRPIWMEKMVMC--RVK 269

Human NP_005804.1: 239 -----ESHVHQEPSKRIP**SCS**WSGRPIWMEKMVMC--RVK 269

SAS-FGGV-----TQYSQQFLR (phosphorylation up)

C. elegans **DAF-16d**: 1 MSFSTDFDDDFNLDLHQQERS**SAS**-FGGV-----TQYSQQFLREKCSFS-----PYFHTSLE 51

Drosophila NP_001262557.1: 1 --MMDGYAQEWPRLTHTDNGLAM**SAS**QGGDLPLDVGFEPQTRARSNTWPCRPENFVEPTD 58

Mouse NP_062714.1: 1 --MAE-----APASPVPLSPLEVELDPEFEPQSRPRSCTWPLQRPQLQASPAK 46

Human NP_001446.1: 1 --MAE-----APASPAPLSLEVELDPEFEPQSRPRSCTWPLQRPQLQASPAK 46

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(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(lf)* mutants in PA14. (B) *sek-1(RNAi)* and *pmk-1(RNAi)* significantly suppressed the increased survival of *csnk-1(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.

943 **Table S1. Deleterious genetic variations detected by whole-genome sequencing**
944 **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 Nai	5 mM Nai
<i>mac397</i> (Chr. I -6 to 13)	<i>rpb-7</i> (0.78)	exon2:c.G238A;p.G80S	I : -4.66	A	L2
	<i>lrp-2</i> (1.00)	exon18:c.A14252T;p.K4751I	I : -1.9	A	L2
	<i>asic-2</i> (1.00)	exon7:c.G877A;p.D293N	I : 2.1	A	L2
	<i>col-63</i> (1.00)	exon2:c.C911T;p.S304F	I : 2.7	ND	ND
	<i>tsp-15</i> (1.00)	exon3:c.G331A;p.G111R	I : 3.32	A	A
	<i>rbpl-1</i> (1.00)	exon6:c.C860T;p.T287I	I : 3.37	egg	egg
	<i>uggt-2</i> (0.87)	exon1:c.T2A;p.M1K	I : 3.74	A	L2
	<i>K07A1.1</i> (0.85)	exon4:c.C545T;p.P182L	I : 3.8	A	L1/L2
	<i>ate-1</i> (1.00)	exon6:c.T889G;p.C297G	I : 3.81	A	L2
	<i>sys-1</i> (1.00)	exon4:c.C659T;p.S220F	I : 4.13	A	L1/L2
	<i>csnk-1</i> (0.78)	exon4:c.G482A;p.R161H	I : 4.77	A	L1/L2/L4

945
946 Ratios of mutated sequences are shown in parentheses. A ratio of 1.00 suggests homozygous,
947 and a ration less than 1.00 suggests heterozygous. A: adults. ND: not determined.

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949

950 **Table S2. Phenotype of animals carrying *csnk-1* deletion mutations or treated with RNAi**
 951 **targeting kinase-encoding genes.**

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

952
 953 Mammalian orthologs or homologs are indicated in the parentheses.

954

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956

957 **Table S3. Characterization of *tsp-15(mac499lf)* mutation.**

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

958

959 *tsp-15(mac500lf)* and *tsp-15(mac501lf)* are knockin genocopies of *tsp-15(mac499lf)* generated

960 by the CRISPR/Cas9 method.

961

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

963

964 **Table S5. List of proteins with altered phosphorylation in *csnk-1(lf)* mutants.**

965

966 **Table S6. PCR primers for generating the indicated cDNAs.**

	Forward primer	Reverse primer
<i>csnk-1p</i>	cgGGATCCgcgtccgagattggatgcctg	ggGGTACCtgcggaaggtgccagtg
<i>csnk-1 cDNA</i>	ggGGTACCatgacgaacacacgcggg	cgGAATTCctattttgttagctggggtc
<i>csnk-1 cDNA(p.R161H)</i>	caacacacaaacagcatgttc	aataacgtccgatcagaaagtttc
<i>csnk-1 cDNA(p.C388_K407del)</i>	gaattccaactgagcgccggtc	ttcacttctccactgaattgtctc
<i>csnk-1 cDNA(p.C388-390S)</i>	gaaatcctcttcctcaggagac	acttctccactgaattgtctc
<i>HsCSNK1G1 cDNA</i>	cgGGATCCatggaccatcctagtagggaaaagg	cgGAATTCtcacttggcgctgagcag
<i>HsCSNK1G2 cDNA</i>	cgGGATCCatggatttgacaagaaaggagg	cgGAATTCtcacttgtgcgtgcag
<i>HsCSNK1G3 cDNA</i>	cgGGATCCatggaaaataaaaagaagacaagg	cgGAATTCtcacttggcgctgtatgg
<i>doxa-1cDNA(p.T343A)</i>	gataaggctgctggatcaagtg	actgatatactgtttgtcgcgac
<i>doxa-1cDNA(p.S346A)</i>	ggagcaagtggaattccaatcac	agcagcttatcacttgatatac
<i>doxa-1cDNA(p.S364A)</i>	ctgacattgaggagccaatcac	ctgagcttgactcgattgac
<i>HsDUOXA2 cDNA</i>	ggGGTACCatgacctgtggaacgcgta	cgGAATTCtcacaggttagtggtgatacatt

968 Restriction sites are shown in uppercase.

969

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

RNAi bacteria	Forward primer	Reverse primer
<i>rpb-7</i>	aaCTGCAGcccagaaccgttacgatttc	cccAAGCTTtctgtagcacggtttctcac
<i>asic-2</i>	aaCTGCAGtttcgtatcctgccactgtc	cccAAGCTTtaattgagtgacgcatctcg
<i>ate-1</i>	aaCTGCAGgagcaaagaggagggtgagc	cccAAGCTTcattgagcaagtgagccatc
<i>kin-1</i>	aaCTGCAGcgtgtcttgacgactttgatag	ggGGTACCccagtggaatcgataagcaag
<i>kin-2</i>	aaCTGCAGctgctgatgatgacgac	ggGGTACCcgacaacgtcgaatggtg
<i>kin-19</i>	aaCTGCAGatggcaggtgtgatgtcagt	ggGGTACCcatgtaaatcttctggagcaga
<i>kin-20</i>	aaCTGCAGggagatggaactcgtgtcg	ggGGTACCcataccaattcctgatgagta
<i>nsy-1</i>	aaCTGCAGtgccgaaacagaaactctgg	ggGGTACCcaccagcaagacaaacaacg
<i>lin-45</i>	aaCTGCAGcgtatggcaagtcctcaact	ggGGTACCcaaatacgcaataaagtggtgca
<i>pmk-1</i>	aaCTGCAGgctgttaatgaggattgtgaa	ggGGTACCccattcatcaattgtatctgc
<i>sek-1</i>	aaCTGCAGacagcgaatcaaatcatcaatc	ggGGTACCagcagcaaatcttacctcattg

972 Restriction sites are shown in uppercase.

973

974 **Table S8. Genomic target sequences for generating *tsp-15* knockin and *csnk-1* knockout**
 975 **strains.**

Gene	sgRNA target sequence	Nucleotides	Exon	Number of lines examined	Knockout lines	Knockin lines
<i>tsp-15</i>	sgRNA1: GCTGTCATGCTTATTATTGG	301~320	3	78	ND	2
	sgRNA2: TATAATGAGTCAATTGATCA	345~364	3			
<i>csnk-1</i>	sgRNA1: TTGGTGGTACTCGCCGAAG	32~51	1	25	2	-

Gene	Repair template
<i>tsp-15</i>	TCAATGAAGTAAGCATTTTCAGGTTGAGTGGAGTATAATGAGTCAATTGATCTCGAAAGTTGAGTC TAAATACAGCCGCCGATGATGAGCATAACTGCAATAACAATGGACGCAACTGCGCACTGAAAATT

976
 977 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](#)