The neuropeptide receptor NMUR-1 regulates the specificity of *C. elegans* innate immunity against pathogen infection

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SUMMARY

A key question in current immunology is how the innate immune system generates high levels of specificity. Like most invertebrates, *Caenorhabditis elegans* does not have an adaptive immune system and relies solely on innate immunity to defend itself against pathogen attacks, yet it can still differentiate different pathogens and launch distinct innate immune responses. Here, we have found that functional loss of NMUR-1, a neuronal GPCR homologous to mammalian receptors for the neuropeptide neuromedin U, has diverse effects on *C. elegans* survival against various bacterial pathogens. Transcriptomic analyses and functional assays revealed that NMUR-1 modulates *C. elegans* transcription activity by regulating the expression of transcription factors, which, in turn, controls the expression of distinct immune genes in response to different pathogens. Our study has uncovered a molecular basis for the specificity

of *C. elegans* innate immunity that could provide mechanistic insights into understanding the specificity of vertebrate innate immunity.

Key words: *Caenorhabditis elegans*, specificity of innate immunity, neuromedin U, G protein-coupled receptor, the nervous system

INTRODUCTION

The pathogen-triggered host immune response is a multilayered process programed to fight invading microorganisms and maintain healthy homeostasis. There are two types of immune systems: the innate immune system and the adaptive immune system. The innate immune system, comprised of physical barriers as well as inducible physiological and behavioral responses, serves as the first line of defense for the host and is evolutionarily conserved across multicellular organisms. Innate immune responses are initiated by recognizing pathogen-associated molecular patterns (PAMPs) or damage caused by pathogen-encoded virulence factors (1,2). Such recognition triggers multiple internal defense responses, including the release of cytokines and chemokines, to fight invading microbes directly or indirectly. In vertebrates, when the innate immune response is insufficient to control an infection, the highly specific adaptive immune response is activated as the second line of defense (3). In this system, antigen-presenting cells (APCs) engulf and break down pathogens and present the foreign antigens on their cell surface via MHC class II molecules. Subsequently, T cells and B cells with antigen-specific receptors are activated to destroy infected cells or produce antibodies to inhibit infection, respectively.

While a large repertoire of T- and B-cell receptors and antibodies in the adaptive immune system confer remarkable specificity against different pathogens, the innate immune response

50 is traditionally believed to be non-specific. However, increasing evidence indicates that the
51 innate immune system can also generate high levels of specificity. In vertebrates, a diverse
52 array of pattern recognition receptors (PRRs), such as the Toll-like receptors (TLRs), NOD-like
53 receptors (NLRs), retinoic acid-inducible gene-I-like receptors (RLRs), and C-type lectins
54 (CLRs), recognize various PAMPs and mediate a broad pattern of specificity (4). The diversity
55 of ligand binding can be further expanded by the formation of receptor homodimers or
56 heterodimers through interactions with other PRR co-receptors (5). In addition to controlling
57 immune activation through specific receptor-ligand binding, innate immunity is also tightly
58 regulated by the nervous system to ensure that appropriate responses are mounted against
59 specific pathogens. Such regulation is critical because insufficient immune responses can
60 exacerbate infection and excessive immune responses can lead to prolonged inflammation,
61 tissue damage, or even death (6,7). The mechanisms underlying neuro-immune regulation,
62 however, are not well understood and are difficult to dissect due to the high degree of
63 complexity of the nervous and immune systems in most model organisms. Overall, the
64 molecular basis responsible for the specificity of innate immunity in vertebrates remains unclear
65 and is largely understudied, possibly because such specificity is overshadowed by the
66 concomitant adaptive immune response.
67
68 Unlike vertebrates, most invertebrates do not have an adaptive immune system and rely solely
69 on innate immunity to defend themselves against pathogen infection, yet they can still
70 differentiate between different pathogens (4). For example, *Drosophila melanogaster* possesses
71 a repertoire of receptors that mediate the distinction of different types of pathogens and fungi
72 (8). Alternative splicing of exons of the Dscam protein in insects generates thousands of
73 isoforms that are potentially involved in pathogen recognition and resistance (9). Moreover, in
74 both *D. melanogaster* and *Caenorhabditis elegans*, brief pre-exposure of animals to a pathogen

75 (termed priming or conditioning) provides strong protection against a subsequent challenge with
76 an otherwise lethal dose of the same pathogen (10). These studies demonstrate that
77 invertebrate innate immune systems can generate high levels of specificity, although the
78 underlying mechanisms remain poorly understood. Nonetheless, this allows for studies of the
79 specificity of innate immunity without the confounding variability from adaptive immunity. In
80 invertebrate organisms, the limited genetic diversity of PRRs and/or immune effectors is likely
81 insufficient to explain the high levels of specificity (9). This is exemplified by the case of *C.*
82 *elegans*. The nematode genome does not encode the majority of known PRRs, and its sole TLR
83 protein, TOL-1, does not seem to play key roles in immunity (11). However, *C. elegans* can still
84 discern different pathogens and launch distinct immune responses against them (12,13).
85 Different infections induce both pathogen-specific and shared immune genes in the worm
86 (13,14). While how the *C. elegans* immune system identifies specific pathogens remains
87 elusive, several key studies have revealed that neural regulation of immunity has homologous
88 occurrence in the nematode (15-21), which could control appropriate immune responses to
89 different pathogens. The characteristics of *C. elegans*, such as the simplicity of its nervous and
90 immune systems, genetic tractability, invariant lineage, effectiveness of RNA interference
91 (RNAi), and a transparent body that allows for the monitoring of gene expression, make this
92 organism uniquely suited for studying neural regulation of immunity. Indeed, such research in *C.*
93 *elegans* is at the forefront of the field and has revealed unprecedented details regarding the
94 molecules, cells, and signaling pathways involved in neural regulation of immunity (reviewed in
95 (22)). For example, we have described an octopaminergic neuro-immune regulatory circuit in
96 great detail (19,23-25) as well as a neural-cuticle defense regulatory circuit mediated by the
97 neuropeptide receptor NPR-8 (26). Others have also revealed serotonergic, dopaminergic,
98 cholinergic, and neuropeptidergic immunoregulatory pathways that control innate immune

99 responses to various pathogens (reviewed in (22)). These studies have greatly facilitated our
100 understanding of complex neuro-immune signaling mechanisms.
101
102 While the above-mentioned *C. elegans* studies showed how the nervous system regulates
103 immunity against certain pathogens, they did not address whether or how such regulation
104 generates specificity against different pathogens. During our work on neuro-immune regulation
105 in *C. elegans*, we observed that functional loss of NMUR-1, a neuronal GPCR homologous to
106 mammalian receptors for the neuropeptide neuromedin U (NMU), had diverse effects on the
107 survival of *C. elegans* exposed to various bacterial pathogens. Furthermore, an earlier study by
108 Alcedo and colleagues showed that an *nmur-1* null mutation affected the lifespan of *C. elegans*
109 fed different *E. coli* food sources, and that such effects were dependent upon the type of *E. coli*
110 lipopolysaccharide (LPS) structures (27). These findings indicate that NMU/NMUR-1 signaling
111 might mediate the specificity of immune responses to different pathogens. NMU is known to
112 have roles in regulating immune responses in addition to its functions regulating smooth muscle
113 contractions in the uterus, reducing food intake and weight, and modifying ion transport (28,29).
114 Indeed, three independent studies in mice demonstrated that NMU from enteric neurons directly
115 activates type 2 innate lymphoid cells through NMUR1 to drive anti-parasitic immunity (30-32).
116 In the current study, we have discovered that NMUR-1 modulates *C. elegans* transcriptional
117 activity by regulating the expression of transcription factors, which, in turn, controls the
118 expression of distinct immune genes in response to different pathogens. Our study has
119 uncovered a molecular basis for the specificity of *C. elegans* innate immunity that could
120 potentially provide mechanistic insights into understanding the specificity of innate immune
121 responses in vertebrates.
122
123 **RESULTS**

124 **Functional loss of NMUR-1 differentially affects *C. elegans* survival against various**
125 **bacterial pathogens**
126 Functional loss of NMUR-1 has distinct effects on the lifespan of *C. elegans* fed different *E. coli*
127 food sources, and such effects are dependent upon the type of *E. coli* LPS structures (27).
128 Given the toxic and immunogenic nature of LPS (33), these LPS-dependent lifespan
129 phenotypes suggest that NMUR-1 might mediate distinct immune responses to different
130 pathogens. To test this notion, we examined the susceptibility of wild-type *N2* and *nmur-1* null
131 animals (*nmur-1(ok1387)* strain) to six paradigmatic pathogens that included three Gram-
132 negative bacteria (*Salmonella enterica* strain SL1344, *Pseudomonas aeruginosa* strain PA14,
133 and *Yersinia pestis* strain kim5) and three Gram-positive bacteria (*Enterococcus faecalis* strain
134 OG1RF, *Microbacterium nematophilum* strain CBX102, and *Staphylococcus aureus* strain
135 NCTC8325). Compared to wild-type animals, *nmur-1(ok1387)* animals exhibited enhanced
136 survival against *S. enterica* and *Y. pestis*, reduced survival against *E. faecalis*, and showed no
137 differences in survival against *P. aeruginosa*, *M. nematophilum*, and *S. aureus* (Fig. 1 and Fig.
138 S1). In addition, with two commonly used worm food sources, namely, *E. coli* strains OP50 and
139 HT115, we found that the *nmur-1* mutation improved worm survival against OP50 but had no
140 effect against HT115 (Fig. 1 and Fig. S1), an observation in agreement with a previous study by
141 Alcedo and colleagues (27). These distinct effects of the *nmur-1* mutation on survival, which
142 appeared to be unrelated to the Gram-negative or Gram-positive groups, indicate that NMUR-1
143 plays diverse roles in *C. elegans* defense against various pathogens.
144
145 There are many factors affecting the pathogenesis of the above-mentioned bacteria besides
146 being Gram-negative or Gram-positive. For example, each pathogen produces a unique set of
147 virulence factors that promote pathogenicity through different mechanisms (34). In turn, *C.*
148 *elegans* can launch distinct defense responses to specific pathogens (12-14). The diverse

149 effects that NMUR-1 has on *C. elegans* survival suggest that it could have multiple physiological
150 functions in the nematode's defense against these bacteria. Given NMUR-1's potential
151 versatility in function, we sought to gain insights into NMUR-1's functionality by focusing on its
152 roles in defense against *E. faecalis* and *S. enterica*, two pathogens that have opposite effects
153 on the survival of *nmur-1* mutant animals (reduced survival against *E. faecalis* but improved
154 survival against *S. enterica* (Fig. 1)). Below, we describe our study using these two pathogens
155 as infecting agents.
156

157 **Pathogen avoidance behavior does not contribute to the altered survival of *nmur-***
158 ***1(ok1387)* animals exposed to *E. faecalis* or *S. enterica***

159 Compared to wild-type animals, *nmur-1(ok1387)* animals exhibited altered survival following
160 exposure to *E. faecalis* or *S. enterica* (Fig. 1). However, no differences in survival were
161 observed between mutant and wild-type animals when they were fed worm food *E. coli* strain
162 HT115 (Fig. 1), heat-killed *E. faecalis* (Fig. S2A), or heat-killed *S. enterica* (Fig. S2B). These
163 results indicate that the *nmur-1* mutation affects *C. elegans* defense against living pathogens
164 without impacting the nematode's basic lifespan. The altered survival of mutant animals could
165 be due to pathogen avoidance behavior, a known mechanism of *C. elegans* defense (35). To
166 test this possibility, we measured animal survival using full-lawn assays in which the agar plates
167 were completely covered by pathogenic bacteria to eliminate pathogen avoidance. Consistent
168 with our original (partial-lawn) survival assays, we observed that *nmur-1(ok1387)* animals
169 exposed to *E. faecalis* died at a faster rate than wild-type controls, whereas mutants exposed to
170 *S. enterica* died at a slower rate than wild-type controls (Fig. S3). These results suggest that
171 lawn avoidance is not the reason for the altered survival observed in *nmur-1* mutants. We next
172 conducted lawn occupancy behavioral assays to compare the magnitude of pathogen
173 avoidance between wild-type and *nmur-1(ok1387)* animals. In these assays, a small lawn of

174 pathogenic bacteria was seeded in the center of an agar plate, and a set number of
175 synchronized young adult animals were placed on the lawn. The numbers of animals that
176 stayed on and off the lawn were then counted at five time points over a period of 36 hours.
177 Compared to wild-type animals, *nmur-1(ok1387)* animals overall did not show significant
178 differences in the magnitudes of pathogen avoidance (Fig. 2). Taken together, these data
179 suggest that pathogen avoidance behavior does not play a role in the altered survival of *nmur-*
180 *1(ok1387)* animals exposed to *E. faecalis* or *S. enterica*.
181
182 **Functional loss of NMUR-1 changes intestinal accumulation of *E. faecalis* and *S. enterica***
183 Certain mutations in *C. elegans* can change pathogen accumulation in the intestine, leading to
184 altered resistance to infection (36). To examine whether the *nmur-1* mutation affects bacterial
185 accumulation, we used GFP-tagged *E. faecalis* to visualize accumulated bacteria in the
186 nematode's intestine. Our results showed that the GFP fluorescence intensity in *nmur-*
187 *1(ok1387)* animals was stronger than that seen in wild-type animals (Fig. 3A). Intestinal
188 bacterial loads were quantified by counting the Colony Forming Units (CFU) of live bacterial
189 cells recovered from the intestine. The mutant animals had more *E. faecalis* CFUs than wild-
190 type animals (Fig. 3B). These results indicated that the *nmur*-1 mutation caused increased
191 intestinal accumulation of *E. faecalis*. The higher bacterial accumulation could be due to altered
192 pathogen intake, expulsion, or clearance by immunity. These possible causes were further
193 investigated. *C. elegans* feeds on bacterial food via rhythmic contractions (pumping) of its
194 pharynx, so to determine whether there were any differences in pathogen intake between wild-
195 type and *nmur-1(ok1387)* animals, we measured their pharyngeal pumping rates on bacterial
196 lawns. When feeding on *E. faecalis*, both wild-type and mutant animals showed similar pumping
197 rates, reflecting similar levels of pathogen intake (Fig. 3C). Bacterial evacuation was assessed
198 by measuring the defecation rate, defined as the time interval between expulsions of gut

199 contents. We found that the defecation rate of *nmur-1(ok1387)* animals on *E. faecalis* was
200 comparable to the rate observed in wild-type animals (Fig. 3D). Overall, these results indicate
201 that the reduced survival of *nmur-1(ok1387)* animals exposed to *E. faecalis* is likely due to
202 increased pathogen accumulation in the intestine. Because the *nmur-1* mutation did not alter *E.*
203 *faecalis* intake or expulsion, the increased pathogen accumulation likely resulted from the
204 mutants' weakened innate immunity that was unable to clear the infection from the intestine.
205
206 We next performed similar experiments to investigate intestinal accumulation, intake, and
207 expulsion of *S. enterica*. Our results showed that, compared to wild-type animals, *nmur-*
208 *1(ok1387)* animals exhibited slightly weaker GFP-tagged *S. enterica* fluorescence intensities
209 (Fig. 3E) and had significantly lower CFU counts (Fig. 3F), indicating that the *nmur*-1 mutation
210 decreased intestinal accumulation of *S. enterica*. Mutant animals also had slightly higher
211 pharyngeal pumping rates than wild-type animals (Fig. 3G), suggesting that pathogen intake
212 was increased in the mutants despite their reduced bacterial accumulation. The defecation rate
213 of *nmur-1(ok1387)* animals on *S. enterica* was comparable to that of wild-type animals (Fig. 3H).
214 These results indicate that the enhanced survival of *nmur-1(ok1387)* animals exposed to *S.*
215 *enterica* is likely due to decreased pathogen accumulation in the intestine. This decrease did not
216 result from reduced pathogen intake (pathogen intake actually improved) or increased expulsion
217 but likely resulted from enhanced innate immunity in the mutants that cleared the infection from
218 the intestine. Taken together, both the *E. faecalis* and *S. enterica* studies suggest that NMUR-1
219 could regulate innate immunity in *C. elegans* defense against pathogen infection but plays
220 opposite roles in such potential regulation against the two pathogens.
221
222 **NMUR-1 regulates *C. elegans* transcriptional activity**

223 To gain insights into how NMUR-1 regulates *C. elegans* defense at the molecular level, we
224 employed RNA sequencing to profile gene expression in wild-type and *nmur-1(ok1387)* animals
225 with or without pathogen infection. For these studies, we collected RNA samples from wild-type
226 and *nmur-1(ok1387)* animals with or without exposure to *E. faecalis* or *S. enterica* (five
227 replicates per group). The samples were then submitted to the Washington State University
228 Genomics Core for RNA-seq analysis. The resulting sequence data (FASTQ files) were
229 deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive
230 (SRA) database through the Gene Expression Omnibus (GEO). The processed gene
231 quantification files and differential expression files were deposited in the GEO. All of these data
232 can be accessed through the GEO with the accession number GSE154324.
233
234 Using the resulting sequencing data, we first compared the gene expression profiles of *nmur-*
235 *1(ok1387)* animals with those of wild-type animals under normal conditions (i.e., without
236 infection). In total, 14,206 genes were detected and quantified with a false discovery rate (FDR)
237 of 5%. Among these genes, 1,599 were upregulated and 275 were downregulated at least two-
238 fold in *nmur-1(ok1387)* animals relative to wild-type animals. The change in expression of such
239 a large number of genes in the mutant worms indicates that the lack of NMUR-1 profoundly
240 affects gene expression in *C. elegans*. Gene ontology (GO) analysis of the 1,599 upregulated
241 genes identified 26 significantly enriched molecular functions, including protein
242 kinase/phosphatase activity, ribonucleotide binding, and cuticle structure activity (Table S1).
243 Interestingly, GO analysis of the 276 downregulated genes showed 11 significantly enriched
244 molecular functions, all of which involve transcription-related DNA binding activities (Table 1A).
245 Twenty-three downregulated genes possess these activities and contribute to the functional
246 enrichment, most of which encode transcription factors involved in binding to RNA polymerase II

247 regulatory regions (Table 1B). These results indicate that NMUR-1 could affect transcription by
248 regulating the expression of these transcription factors.
249
250 Because of the high importance of transcription in the fundamental functions of life and the
251 potential regulatory role of NMUR-1 in this process, we next examined how NMUR-1, or the lack
252 thereof, impacts transcription. To this end, we developed a novel *in vitro* transcription system to
253 measure *C. elegans* transcriptional activity (37). One of the biggest obstacles for biochemical
254 studies of *C. elegans* is the high difficulty of preparing functionally active nuclear extract due to
255 its thick surrounding cuticle. In our transcription system, Balch homogenization was employed to
256 effectively disrupt worms, and functionally active nuclear extracts were obtained through
257 subcellular fractionation. Subsequently, the nuclear extracts were used to re-constitute *in vitro*
258 transcription reactions with a linear DNA template containing the worm *Δpes-10* promoter,
259 followed by PCR-based, non-radioactive detection of transcripts (37). Using this system, we
260 were able to compare the general transcriptional activity of *nmur-1(ok1387)* animals with that of
261 wild-type animals at the whole-organism level. PCR and gel analysis of the transcript products
262 showed substantial increases in transcriptional activity in reactions with mutant nuclear extract
263 compared to those with wild-type nuclear extract (Fig. 4A). To quantitatively compare the
264 transcriptional activity of wild-type and mutant nuclear extracts, we next titrated the DNA
265 template with varying amounts of each extract and used qRT-PCR to quantify transcripts at
266 each titration point. The resulting data were then fitted with the Michaelis-Menten model and the
267 non-linear least-squares method, as we described previously (37). The titration curves and two
268 titration parameters, namely, the maximum yield and the amount of nuclear extract needed to
269 reach 50% of the maximum yield, are shown in Fig. 4B. Compared to the titrations with wild-type
270 nuclear extract, those with mutant extract yielded 2.33-fold maximum number of transcripts and
271 only needed 0.67-fold extract to reach 50% of the maximum yield (Fig. 4B), indicating that the

272 reactions performed with mutant extract were more robust and had higher transcriptional
273 activity. These results were surprising given the fact that the expression of many transcription
274 factors was downregulated in the mutant animals. Taken together, our data suggest that NMUR-
275 1 suppresses transcriptional activity in wild-type animals and that functional loss of NMUR-1
276 abrogates this suppression, which explains the large number of genes (1,599 genes) that were
277 upregulated in *nmur-1(ok1387)* animals relative to wild-type animals.
278
279 We also employed *in vivo* transcription assays to evaluate how the lack of NMUR-1 affects
280 transcriptional activity in *C. elegans*. For these assays, transgenic strain CL2070, which
281 contains the *hsp-16.2p::GFP* reporter transgene, was used. This strain does not express
282 detectable GFP under standard conditions but broadly expresses GFP in all tissues after heat
283 shock, allowing for the assessment of overall transcriptional activity in living animals (38). The
284 CL2070 animals were outcrossed with our wild-type animals and then crossed with *nmur-*
285 *1(ok1387)* animals. The resulting animals (*CL2070(dvls70)* and *CL2070(dvls70);nmur-*
286 *1(ok1387))* were grown to the L4 larval stage, heat shocked at 35°C for 2 hours, and then
287 returned to 20°C for GFP mRNA quantification by qRT-PCR. Our results showed that GFP
288 expression was significantly increased in *CL2070(dvls70);nmur-1(ok1387)* animals compared to
289 *CL2070(dvls70)* animals (Fig. 4C), indicating that the *nmur-1* mutation causes higher overall
290 transcriptional activity, which is consistent with the results of our *in vitro* transcription assays.
291
292 **NMUR-1 regulates the innate immune response to *E. faecalis* by controlling the**
293 **expression of C-type lectins**
294 Next, we asked how NMUR-1 regulates gene expression in *C. elegans* in response to *E.*
295 *faecalis* infection. By examining the gene expression profiles of *nmur-1(ok1387)* animals relative
296 to wild-type animals exposed to *E. faecalis*, we found that 229 genes were upregulated and 106

297 genes were downregulated at least two-fold. GO analysis of the upregulated genes identified 11
298 significantly enriched biological processes, all of which involve defense responses to bacteria or
299 other external stimuli (Table S2). This is consistent with the fact that *E. faecalis* is a pathogenic
300 agent for *C. elegans* (39). GO analysis of the downregulated genes yielded one significantly
301 enriched molecular function involving carbohydrate binding activity (Table 2A). Fifteen of the
302 downregulated genes were related to this activity, 14 of which encode C-type lectins (Table 2B).
303 C-type lectins are a group of proteins that contain one or more C-type lectin-like domains
304 (CTLDs) and recognize complex carbohydrates on cells and tissues (40,41). C-type lectins may
305 act in pathogen recognition or function as immune effectors to fight infection (4,11). Therefore,
306 downregulation of C-type lectins in *nmur-1(ok1387)* animals might explain the reduced survival
307 observed in mutant animals following exposure to *E. faecalis*.
308
309 To determine whether NMUR-1-regulated C-type lectin genes contribute to the reduced survival
310 of *nmur-1(ok1387)* animals, we inactivated these genes using RNAi-mediated knockdown in
311 wild-type and *nmur-1(ok1387)* animals and assayed for their survival against *E. faecalis*-
312 mediated killing. Five of the most downregulated C-type lectin genes (*clec-94, clec-137, clec-*
313 *157, clec-208,* and *clec-263*) were individually targeted in these studies. While RNAi of these
314 genes did not further reduce the survival of *nmur-1(ok1387)* animals (Fig. 5B), knockdown of
315 four of the five genes (all except *clec-157*) significantly suppressed the survival of wild-type
316 animals (Fig. 5A). These results indicate that some of the NMUR-1-regulated C-type lectin
317 genes are indeed required for *C. elegans* defense against *E. faecalis* infection.
318
319 Since knockdown of C-type lectins in wild-type animals recaptured the reduced survival
320 phenotype of *nmur-1(ok1387)* animals, we next asked whether complementing the expression
321 of these C-type lectins in mutant animals could rescue their survival phenotype. To this end, we

322 employed transgenes to express *clec-94*, *clec-208*, and *clec-263*, respectively, in *nmur-*
323 *1(ok1387)* and wild-type animals. Survival assays against *E. faecalis* showed that while
324 overexpression of these C-type lectins had no effect on wild-type animal survival, the
325 expression of *clec-94* or *clec-208* partially rescued the reduced survival of the mutant animals
326 (Fig. 5C), confirming that the downregulation of C-type lectins contributes to the reduced
327 survival of *nmur-1* mutants challenged with *E. faecalis*.
328

329 **NMUR-1 suppresses the innate immune response to *S. enterica* by inhibiting unfolded**
330 **protein response (UPR) genes**

331 We also compared the gene expression profiles of *nmur-1(ok1387)* animals with those of wild-
332 type animals exposed to *S. enterica* and found that 28 genes were upregulated and 28 genes
333 were downregulated at least two-fold. While GO analysis of the downregulated genes did not
334 yield any enriched GO terms, a similar analysis of the upregulated genes identified four
335 significantly enriched biological processes and two significantly enriched molecular functions
336 (Table 3A). Among these enrichments, the biological process "protein quality control for
337 misfolded or incompletely synthesized proteins" was the most significantly enriched (FDR =
338 7.89E-5) and has a known role in immune responses to pathogen infection (42). Under an
339 environmental stress, such as pathogen infection, increased demand for protein folding causes
340 ER stress, which activates the UPR pathways to alleviate the stress and maintain protein
341 homeostasis. This process is known to be controlled by the nervous system (43). Four of the
342 upregulated genes (*T20D4.3*, *T20D4.5*, *C17B7.5*, and *npl-4.2*) are related to UPR activity and
343 contributed to the biological process enrichment (Table 3B). To determine whether these
344 NMUR-1-regulated UPR genes play any roles in the enhanced survival of *nmur-1(ok1387)*
345 animals, we inactivated these genes individually using RNAi and then assayed the animals for
346 survival against *S. enterica*. Because these genes are mainly expressed in neurons

347 (Wormbase), we employed strain *TU3401*, which is capable of RNAi-mediated gene knockdown
348 in neurons (44), in our experiments. While RNAi silencing of *T20D4.3* specifically suppressed
349 the enhanced survival of *nmur-1(ok1387);TU3401* animals following exposure to *S. enterica*,
350 RNAi of *npl-4.2* suppressed the survival of both TU3401 and *nmur-1(ok1387);TU3401* animals
351 (Fig. 6). In comparison, RNAi of *T20D4.5* or *C17B7.5* had no effect on animal survival (Fig. 6).
352 The gene *npl-4.2* has been previously implicated in ER-associated protein degradation.
353 Specifically, NPL-4 forms a complex with CDC-48 ATPase and UFD-1, which can extract
354 polyubiquitin-tagged misfolded proteins from the ER membrane and facilitate their degradation
355 by the proteasome (45,46). Less is known about *T20D4.3*, except that it reportedly encodes a
356 peptide N glycanase and functions in longevity, dauer formation, and pathogen responses (47).
357 Nonetheless, our results indicate that these UPR genes are important for *C. elegans* defense
358 against *S. enterica*, and that NMUR-1 can suppress the innate immune response to *S. enterica*
359 by inhibiting UPR genes.
360

361 **The NMUR-1 ligand CAPA-1 is required for *C. elegans* defense against *E. faecalis* but not**
362 ***S. enterica***

363 CAPA-1, a homolog of vertebrate NMU and insect *capability* peptides, is an endogenous ligand
364 of NMUR-1 (48,49). To determine whether CAPA-1 plays any roles in *C. elegans* defense, we
365 examined the survival of a loss-of-function mutant of CAPA-1, *capa-1(ok3065)*, after *E. faecalis*
366 exposure. Compared to wild-type animals, *capa-1(ok3065)* animals showed reduced survival, a
367 phenotype also displayed by *nmur-1(ok1387)* animals (Fig. 7A). We then crossed *capa-*
368 *1(ok3065)* with *nmur-1(ok1387)* animals to generate *capa-1(ok3065);nmur-1(ok1387)* double
369 mutants. These double mutants exhibited reduced survival against *E. faecalis*, similar to *nmur-*
370 *1(ok1387)* animals (Fig. 7A). These results indicate that CAPA-1 and NMUR-1 likely function in
371 the same pathway to mediate *C. elegans* defense against *E. faecalis*. To investigate whether

372 CAPA-1 is a ligand of NMUR-1 in the defense response, we tested whether exogenous
373 administration of a synthetic CAPA-1 peptide could rescue the reduced survival phenotype of
374 the mutant animals. To this end, we exposed wild-type and mutant animals to *E. faecalis* on BHI
375 media containing 1 μg/mL synthetic CAPA-1 peptide and scored their survival over time. Our
376 results showed that exogenous CAPA-1 peptide rescued the reduced survival phenotype of
377 *capa-1(ok3065)* animals but failed to rescue *nmur-1(ok1387)* animals or *capa-1(ok3065);nmur-*
378 *1(ok1387)* double mutants (Fig. 7B). These results demonstrate that the function of NMUR-1 in
379 defense depends on the presence of CAPA-1, and *vice versa*, indicating that CAPA-1/NMUR-1
380 function as a ligand/receptor pair in mediating *C. elegans* defense against *E. faecalis*.
381
382 To determine whether CAPA-1 also functions in the *C. elegans* defense response to *S. enterica*,
383 we subjected wild-type and mutant animals to survival assays against *S. enterica*. Results
384 showed that while *capa-1(ok3065)* animals displayed survival at the wild-type level, *capa-*
385 *1(ok3065);nmur-1(ok1387)* double mutants exhibited survival comparable to that of *nmur-*
386 *1(ok1387)* animals (Fig. 7C). This indicates that lacking CAPA-1 has no influence on *C. elegans*
387 defense against *S. enterica*, and that CAPA-1 might not be involved in the NMUR-1-mediated
388 innate immune response to this particular pathogen. Although CAPA-1 is the only NMUR-1
389 ligand identified so far (48,49), these results suggest that other ligands may exist, and that
390 NMUR-1 could bind to different ligands to regulate immune responses to different pathogens.
391
392 **DISCUSSION**
393 In the current study, we have shown that *C. elegans* lacking the neuronal GPCR NMUR-1
394 exhibited altered survival against various bacterial pathogens. By focusing on two pathogens, *E.*
395 *faecalis* and *S. enterica*, as infecting agents, we have demonstrated that the altered survival
396 was not caused by pathogen avoidance behavior but by differences in intestinal accumulation of

397 pathogens, likely due to changes in innate immunity. Our RNA-seq analyses showed that
398 functional loss of NMUR-1 negatively affects the expression of transcription factors involved in
399 binding to RNA polymerase II regulatory regions, which, in turn, impacts transcriptional activity
400 in *C. elegans*, as demonstrated by both *in vitro* and *in vivo* transcription assays. We also found
401 that NMUR-1 regulates the expression of different sets of genes in *C. elegans* in response to *E.*
402 *faecalis* and *S. enterica* exposure. In particular, C-type lectin genes were downregulated in
403 *nmur-1(ok1387)* animals relative to wild-type animals exposed to *E. faecalis*, whereas UPR
404 genes were upregulated following exposure to *S. enterica*. These genes have been previously
405 implicated in conserved immune signaling pathways in response to pathogen infection (11).
406 Functional assays with selected genes revealed that at least three C-type lectin genes (*clec-94*,
407 *clec-208*, and *clec-263*) and two UPR genes (*T20D4.3* and *npl-4.2*) are important for *C. elegans*
408 defense against *E. faecalis* and *S. enterica*, respectively. Taken together, we have
409 demonstrated that NMUR-1 modulates *C. elegans* transcriptional activity by regulating the
410 expression of transcription factors, which, in turn, controls the expression of distinct immune
411 genes in response to different pathogens. Overall, our study has uncovered a molecular basis
412 for the specificity of *C. elegans* innate immunity. Given the evolutionary conservation of
413 NMU/NMUR-1 signaling in immune regulation across multicellular organisms (29), our work
414 could provide mechanistic insights into understanding the specificity of innate immunity in other
415 animals, including vertebrates.
416
417 In vertebrates, a diverse array of PRRs that recognize various PAMPs have been implicated in
418 the specificity of the innate immune system (5). Although *C. elegans* does not possess the
419 majority of known PRRs, it can differentiate between different pathogens and launch distinct
420 innate immune responses to specific pathogens (12-14). Recent behavior research in *C.*
421 *elegans* indicates that its nervous system might play a major role in pathogen detection (50).

422 The nematode nervous system contains 32 chemosensory neurons (out of a total of 302
423 neurons in hermaphrodites), many of which have cilia exposed to the external environment that
424 can directly sense pathogen-derived molecules in the environment (51). Moreover, *C. elegans*
425 expresses approximately 1,300 GPCRs that may function as chemoreceptors in sensory
426 neurons to detect pathogen cues, thus contributing to the specificity of innate immunity (22). In
427 this context, a relevant question would be whether neuronal NMUR-1 mediates the specificity of
428 innate immunity by directly interacting with bacterial molecules. We have shown that NMUR-1
429 has diverse effects on *C. elegans* defense against various bacterial pathogens (Fig. 1 and Fig.
430 S1). If the hypothesis that NMUR-1 directly interacts with pathogens is correct, then this GPCR
431 should be able to bind to various pathogen-derived molecules. Such scenario seems unlikely
432 given that PRR-PAMP interactions follow a simple key-lock system, i.e., each receptor matches
433 one pathogen-associated molecule or molecular pattern (9). Therefore, it is more likely that
434 NMUR-1 mediates the ability to differentiate between pathogens by controlling neural signaling
435 than by recognizing bacterial molecules directly. Indeed, we have found that NMUR-1 regulates
436 C-type lectin genes in response to *E. faecalis* infection and UPR genes in response to *S.*
437 *enterica* infection; both of these genes are known to function in innate immune signaling
438 pathways that fight infection (11).
439
440 How does NMUR-1, a single type of neuronal GPCR, distinguish different pathogens so that it
441 can respond to some attacks with suppression of the innate immune response and amplify the
442 response to others? A basic principle in neuroscience is that the interaction of multiple neural
443 circuits mediates the functional output of a neural network and determines the final state of
444 organ function (52). Accordingly, the net effect of neural control on the immune response
445 depends on the total sum of the interactions resulting from ligand-receptor signal transduction in
446 a specific path. NMUR-1 is normally expressed in the spermathecae of the somatic gonad and

447 several different types of sensory neurons as well as in interneurons (27). By using a
448 fluorescent reporter transgene, we observed that distinct subsets of NMUR-1-expressing
449 neurons are activated upon exposure to *E. faecalis* and *S. enterica* (data not shown), indicating
450 that different neural circuits are involved in regulating the immune responses to these two
451 pathogens. This notion is further supported by the fact that CAPA-1, the only NMUR-1 ligand
452 identified so far, is required for the nematode immune response to *E. faecalis* but is dispensable
453 for its response to *S. enterica* (Fig. 7). Future studies to decipher these different neuro-immune
454 regulatory circuits would help us understand how signals pass through different components of
455 the circuits to exert a net effect on immunity. An associative learning circuit mediated by CAPA-
456 1/NMUR-1 signaling was recently uncovered in *C. elegans* in which sensory ASG neurons
457 release CAPA-1, which signals via NMUR-1 in AFD sensory neurons to specifically mediate the
458 retrieval of learned salt avoidance (49). In mice, cholinergic neurons in the intestine can sense
459 and respond to infection by expressing NMU, which directly activates type 2 innate lymphoid
460 cells in the gastrointestinal tract through NMUR1 to drive anti-parasitic immunity (30-32). These
461 studies provide useful information for deciphering NMUR-1-mediated neuro-immune regulatory
462 circuits in *C. elegans*, which will be critical for further understanding how the nervous system
463 regulates innate immunity to generate specificity by either suppressing or amplifying immune
464 responses to different pathogens.
465

466 **MATERIALS AND METHODS**

467 **Nematode strains**

468 The following *C. elegans* strains were cultured under standard conditions and fed *E. coli* HT115.
469 Wild-type N2 and *nmur-1(ok1387)* were gifts from Dr. Joy Alcedo (Wayne State University,
470 Detroit, MI). The *capa-1(ok3065)*, *CL2070(dvIs70)*, and *TU3401* strains were obtained from the
471 *Caenorhabditis elegans* Genetics Center (University of Minnesota, Minneapolis, MN). The *capa-*

472 *1(ok3065);nmur-1(ok1387)*, *nmur-1(ok1387);CL2070(dvls70)*, and *nmur-1(ok1387);TU3401*
473 mutant strains were constructed using standard genetic techniques.
474
475 **Bacterial strains**
476 The following bacterial strains were grown using standard conditions (53): *Escherichia coli*
477 HT115, *Escherichia coli* OP50, *Salmonella enterica* strain SL1344, *S. enterica SL1344::gfp*,
478 *Pseudomonas aeruginosa* strain PA14, *Yersinia pestis* strain kim5, *Enterococcus faecalis* strain
479 OG1RF, *E. faecalis OG1RF::gfp*, *Microbacterium nematophilum* strain CBX102, and
480 *Staphylococcus aureus* strain NCTC 8325.
481
482 **Survival assay**
483 *C. elegans* wild-type *N2* animals and mutant animals were maintained as hermaphrodites at
484 20°C, grown on modified NGM with 30 units/mL nystatin and 15μg/mL tetracycline, and fed *E.*
485 *coli* HT115. Bacterial lawns for the survival assays were prepared by placing a 30μL drop of an
486 overnight culture of pathogenic bacteria on each 3.5cm plate. Full-lawn survival assays were
487 prepared by spreading 30μL of overnight bacterial culture over the complete surface of each
488 3.5cm plate. Plates were incubated at 37°C for 15~16 hours, cooled down to room temperature,
489 and then seeded with synchronized 65-hour-old young adult animals. The survival assays were
490 performed at 25°C, and live animals were transferred daily to fresh plates until egg laying
491 ceased. Animals were scored at the times indicated and were considered dead when they failed
492 to respond to touch.
493
494 **Lawn occupancy assay**
495 Wild-type and mutant animals were maintained as hermaphrodites at 20°C, grown on modified
496 NGM with 30 units/mL nystatin and 15μg/mL tetracycline and fed *E. coli* HT115. The bacterial

497 lawns for the lawn occupancy assays were prepared by placing a 30μL drop of an overnight
498 culture of pathogenic bacteria on each 3.5cm agar plate. Plates were incubated at 37°C for
499 15~16 hours, cooled down to room temperature, and then seeded with synchronized 65-hour-
500 old young adult animals. The assays were performed at 25°C and animals were scored at the
501 described time points for lawn occupancy. Dead animals were censored from the results.
502
503 **Profiling bacterial accumulation in the nematode intestine**
504 Wild-type and *nmur-1(ok1387)* animals were synchronized by egg-laying. The animals were
505 grown on *E. coli* HT115 at 20°C for 65 hours or until the young adult stage. The animals were
506 then transferred to plates seeded with GFP-tagged bacteria and incubated for 24 hours at 25°C.
507 Following incubation, the animals were transferred to an empty NGM plate for 15 minutes to
508 eliminate any fluorescent bacteria stuck to the body. The animals were then transferred to a
509 new, empty NGM plate and into a droplet of M9 buffer to further wash away any bacteria
510 remaining on the body. Animals were anesthetized using 25mM Sodium Azide and visualized
511 using a Zeiss Axio Imager M2 stereoscopic microscope.
512
513 **Quantification of intestinal colonization**
514 Wild-type and *nmur-1(ok1387)* animals were synchronized by egg-laying. Sixty-five-hour-old
515 animals were transferred to plates seeded with GFP-tagged bacteria and incubated for 24 hours
516 at 25°C. Ten animals were then transferred to a 1.5mL tube containing a solution of 25mM
517 Sodium Azide with 1mg/mL ampicillin and kanamycin. The animals were soaked for 45 minutes
518 to remove any external bacteria. After 45 minutes, the animals were washed three times with
519 PBS containing 0.1% Triton to remove the remaining antibiotics, and ground in 100μL of PBS
520 with 0.1% Triton. Serial dilutions of the lysates (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}) were plated with
521 rifampicin to select for GFP-tagged bacteria and the plates were incubated at 37°C for 24 hours.

522

523 **Profiling pumping and defecation rates**

524 Wild-type and *nmur-1(ok1387)* animals were synchronized by egg-laying. Sixty-five-hour-old
525 animals were transferred to plates seeded with pathogenic bacteria and incubated for 24 hours
526 at 25°C. Pumping rates were counted by observing the contraction/relaxation cycles of the
527 terminal pharyngeal bulb using a stereoscopic microscope. Cycles were counted for 30 second
528 durations, three times for ten individual animals per condition. Defecation rates were measured
529 by timing the span between the contraction of the rectal muscles and subsequent excretion
530 using a stereoscopic microscope. Cycles were counted five times for ten individual animals per
531 condition.

532

533 **RNA interference**

534 RNAi was conducted by feeding synchronized L3 larval *C. elegans E. coli* strain HT115(DE3)
535 expressing double-stranded RNA (dsRNA) that was homologous to a target gene (54,55). *E.*
536 *coli* with the appropriate dsRNA vector were grown in LB broth containing ampicillin (100µg/mL)
537 at 37°C for 16 hours and plated on modified NGM plates containing 100µg/mL ampicillin and
538 3mM isopropyl β-D-thiogalactoside (IPTG). The bacteria were allowed to grow for 16 hours at
539 37°C. The plates were cooled away from direct light before the synchronized L3 larval animals
540 were placed on the bacteria. The animals were incubated at 20°C for 24 hours or until the
541 animals were 65 hours old. *unc-22* RNAi was included as a positive control in all experiments to
542 account for RNAi efficiency.

543

544 **Synthetic NMUR-1 ligand administration**

545 Wild-type and mutant animals were maintained as hermaphrodites at 20°C, grown on modified
546 NGM with 30 units/mL nystatin and 15µg/mL tetracycline, and fed *E. coli* HT115. The animals

547 were allowed to lay eggs for 45 minutes at 25°C on modified NGM plates seeded with *E.coli*
548 HT115. The synchronized 65-hour young adult animals were transferred to BHI medium
549 containing 0, 1, or 5μg/mL oligopeptide AFFYTPRI-NH2 and seeded with 30μL of fresh
550 pathogen broth that had been incubated at 37°C for 16 hours. The survival assays were
551 performed at 25°C and live animals were transferred daily to fresh plates until egg laying
552 ceased. The animals were scored at the times indicated and were considered dead when they
553 failed to respond to touch.
554
555 **RNA isolation**
556 Gravid adult wild-type and *nmur-1(ok1387)* animals were lysed using a solution of sodium
557 hydroxide and bleach (ratio 5:2), washed, and eggs were synchronized for 22 hours in S-basal
558 liquid medium at room temperature. Synchronized L1 larval animals were transferred onto NGM
559 plates seeded with *E. coli* HT115 and grown at 20°C for 48 hours until the animals had reached
560 the L4 larval stage. The animals were collected and washed with M9 buffer before being
561 transferred to plates containing *E. coli* HT115 or pathogenic bacteria for 24 hours at 25°C. After
562 24 hours, animals were collected and washed with M9 buffer, and RNA was extracted using
563 QIAzol (Qiagen). Total RNA was isolated using the RNeasy mini kit (Qiagen) following the
564 protocol provided by the manufacturer.
565
566 **Quantitative real-time PCR (qRT-PCR)**
567 Total RNA was obtained as described above. Two μg of RNA were used to generate cDNA
568 using the Applied Biosystems High-Capacity cDNA Reverse Transcription Kit. qRT-PCR was
569 conducted by following the prescribed protocol for PowerUp SYBR Green (Applied Biosystems)
570 on an Applied Biosystems StepOnePlus real-time PCR machine. Ten-μL reactions were set up
571 following the manufacturer's recommendations, and 20ng of cDNA were used per reaction.

572 Relative fold-changes for transcripts were calculated using the comparative CT(2-ΔΔCT)
573 method and were normalized to pan-actin (act-1, -3, -4). Amplification cycle thresholds were
574 determined by the StepOnePlus software. All samples were run in triplicate.
575
576 **RNA sequencing**
577 Total RNA samples were obtained as described above and was submitted to the WSU
578 Genomics Core for RNA-seq analyses. RNA-seq and related bioinformatic analyses were done
579 following our published protocol (26). The raw sequence data (FASTQ files) were deposited in
580 NCBI's SRA database through the GEO. The processed gene quantification files and differential
581 expression files were deposited in GEO. All of these data can be accessed through GEO with
582 the accession number GSE154324.
583
584 **In vitro transcription assay**
585 In vitro transcription assays were performed following our published protocol (37). Briefly, worm
586 nuclear extracts were prepared from synchronized young adult animals using Balch
587 homogenization. The nuclear extracts were then incubated with PESDNA (a linear DNA
588 template containing the worm *Δpes-10* promoter) and other transcription components at 30°C
589 for 30 minutes, followed by RNA purification and reverse transcription. The resulting cDNA was
590 either amplified by PCR and detected by gel electrophoresis or quantified by qRT-PCR using
591 the PowerUp SYBR green qPCR kit (Applied Biosystems, catalog # A25918).
592
593 **Plasmid construction and transgenic animal generation**
594 A *clec-94* genomic DNA fragment (4,247 bp) was amplified by PCR from mixed stage wild-type
595 *C. elegans* using the following primers: 5'- ATTGTCGACGTACAGTCCCACTACTTGTTGC -3'
596 and 5'- CTAGGATCCCTAACATTGGCCGGGAAAGAG -3'. PCR products were then cloned into

597 the *pPD95.77* vector (Fire Lab *C. elegans* vector kit; Addgene, Cambridge, MA) via the *SalI* and
598 *BamHI* sites. The resulting plasmid pPW01 (*clec-94p::clec-94::SL2::gfp*) was microinjected into
599 wild-type animals at 10ng/μL to generate strain JRS60 (Table S3). The *clec-94* genomic rescue
600 strain JRS63 (Table S3) was generated by crossing JRS60 with *nmur-1(ok1387)* animals.
601
602 A *clec-208* genomic DNA fragment (3,556 bp) was amplified by PCR from mixed stage wild-type
603 *C. elegans* using the following primers: 5'- TTTGTCGACTTTCTGTTCTTGCTACTCTCTACC -3'
604 and 5'- TTTTCTAGACTGGCTCGTTCTTAGAGACC -3'. PCR products were then cloned into
605 the *pPD95.77* vector via the *SalI* and *XbaI* sites. The resulting plasmid, pPW02 (*clec-208p::clec-*
606 *208::SL2::gfp*), was microinjected into wild-type animals at 50ng/μL with *unc-122p::mCherry* at
607 20ng/μl as a co-injection marker to generate strain JRS61 (Table S3). The *clec-208* genomic
608 rescue strain JRS64 (Table S3) was generated by crossing JRS61 with *nmur-1(ok1387)*
609 animals.
610
611 A *clec-263* genomic DNA fragment (5,389 bp) was amplified by PCR from mixed stage wild-type
612 *C. elegans* using the following primers: 5'- TTTGTCGACGCGATGGGTGGTTGTATTATTC -3'
613 and 5'- CAAGGATCCAAATCGTATTTCCGTCGTTGGTC -3'. PCR products were then cloned
614 into the *pPD95.77* vector via the *SalI* and *BamHI* sites. The resulting plasmid, pPW03 (*clec-*
615 *263p::clec-263::SL2::gfp*), was microinjected into wild-type animals at 30ng/μL with *unc-*
616 *122p::mCherry* at 20ng/μl as a co-injection marker to generate strain JRS62 (Table S3). The
617 *clec-263* genomic rescue strain JRS65 (Table S3) was generated by crossing JRS62 with *nmur-*
618 *1(ok1387)* animals.
619
620 **Statistical analysis**

621 Survival curves were plotted using GraphPad PRISM (version 8) computer software. Survival
622 was considered different from the appropriate control indicated in the main text when $P < 0.05$.
623 PRISM uses the product limit or Kaplan-Meier method to calculate survival fractions and the log-
624 rank test, which is equivalent to the Mantel-Haenszel test, to compare survival curves.
625 Occupancy assays, CFU assays, and qRT-PCR results were analyzed using two-sample *t*-tests
626 for independent samples; *P*-values <0.05 are considered significant. All experiments were
627 repeated at least three times, unless otherwise indicated.
628

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633 *OG1RF::gfp* strain. Some worm strains were provided by the *Caenorhabditis* Genetics Center,
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641

642 **AUTHOR CONTRIBUTIONS**

643 P.W., S.W., J.W., C.C., D.S., Y.L., and I.B. designed and performed experiments and analyzed
644 data. J.S. designed experiments and analyzed data. Y.L., P.W., and J.S. wrote the paper.
645

646 **COMPETING INTERESTS**

647 The authors declare that they have no competing interests.

648

649 **DATA AND MATERIALS AVAILABILITY**

650 The RNA-seq data have been deposited in NCBI's SRA database through the GEO. The
651 processed gene quantification files and differential expression files have been deposited in the
652 GEO. All of the data can be accessed through the GEO with the accession number
653 GSE154324. All data needed to evaluate the conclusions in the paper are present in the paper
654 and/or the Supplementary Materials. Additional data related to this paper may be requested
655 from the authors. The *C. elegans* strains and plasmids constructed by us and primer sequences
656 used for their construction are available upon request.

657

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793

794 **FIGURE LEGENDS**

795 **Fig. 1. Functional loss of NMUR-1 differentially affects *C. elegans* survival against *E. coli***
796 **HT115, *E. faecalis* and *S. enterica*.** Wild-type (WT) and *nmur-1(ok1387)* animals were
797 exposed to *E. coli* HT115 **(A)**, *E. faecalis* **(B)**, or *S. enterica* **(C)** and scored for survival over
798 time. Each graph is a combination of three independent experiments. Each experiment included
799 *N* = 60 animals per strain. *P*-values represent the significance level of mutant survival relative to
800 WT, *P* = 0.8706 in (A), *P* = 0.0004 in (B), and *P* < 0.0001 in (C).

801

802 **Fig. 2. Functional loss of NMUR-1 does not affect pathogen avoidance behavior.** WT and
803 *nmur-1(ok1387)* animals were subjected to lawn occupancy assays in which the animals were
804 placed on a small lawn of *E. faecalis* **(A)** or *S. enterica* **(B)** in a 3.5cm plate and monitored over
805 time for their presence on the lawn. Each graph is a combination of three independent
806 experiments. Each experiment included *N* = 60 animals per strain. Error bars represent the
807 standard error of the mean (SEM).

808

809 **Fig 3. Functional loss of NMUR-1 changes intestinal accumulation of *E. faecalis* and *S.***
810 ***enterica*. (A)** WT and *nmur-1(ok1387)* animals were exposed to GFP-expressing *E. faecalis* for
811 24 hours and then visualized using a Zeiss Axio Imager M2 fluorescence microscope. The scale
812 bar indicates 500 µm. **(B)** WT and *nmur-1(ok1387)* animals were exposed to *E. faecalis* for 24
813 hours, and the Colony Forming Units (CFU) of live bacterial cells recovered from their intestines
814 were quantified. The graph represents the combined results of three independent experiments.
815 *N* = 10 animals per strain were used for each experiment. Error bars represent SEM. *P*-value
816 represents the significance level of mutant gut colonization relative to WT, *P* = 0.0296. **(C)** WT
817 and *nmur-1(ok1387)* animals were exposed to *E. faecalis* for 24 hours. Pharyngeal pumping
818 rates of animals were counted as the number of pumps per 30 sec. Counting was conducted in

819 triplicate and averaged to give a pumping rate. The measurements included *N* = 10 adult
820 animals per strain. Error bars represent SEM. *P* = 0.1068. **(D)** WT and *nmur-1(ok1387)* animals
821 were exposed to *E. faecalis* for 24 hours. Defecation rates of animals were measured as the
822 average time of 10 intervals between two defecation cycles. The measurements included *N* =
823 10 animals per strain. *P* = 0.1379. **(E)** WT and *nmur-1(ok1387)* animals were exposed to GFP-
824 expressing *S. enterica* for 24 hours and then visualized using a Zeiss Axio Imager M2
825 fluorescence microscope. The scale bar indicates 500 μm. **(F)** WT and *nmur-1(ok1387)* animals
826 were exposed to GFP-expressing *S. enterica* for 24 hours, and the CFU of live bacterial cells
827 recovered from their intestines were quantified. The graph represents the combined results of
828 three independent experiments. *N* = 10 animals per strain were used for each experiment. Error
829 bars represent SEM. *P* = 0.0290. **(G)** WT and *nmur-1(ok1387)* animals were exposed to *S.*
830 *enterica* for 24 hours. Pharyngeal pumping rates of animals were counted as the number of
831 pumps per 30 sec. Counting was conducted in triplicate and averaged to give a pumping rate.
832 The measurements included *N* = 10 adult animals per strain. Error bars represent SEM. *P* =
833 0.0006. **(H)** WT and *nmur-1(ok1387)* animals were exposed to *S. enterica* for 24 hours.
834 Defecation rates of animals were measured as the average time of 10 intervals between two
835 defecation cycles. The measurements included *N* = 10 animals per strain. *P* = 0.6310.
836
837 **Fig. 4. NMUR-1 regulates transcriptional activity. (A)** *In vitro* transcription assays were
838 performed using nuclear extract from WT or *nmur-1(ok1387)* animals. After transcription, RNA
839 was purified, reverse transcribed, and amplified by PCR, followed by gel analysis. Lane 1,
840 transcription with WT nuclear extract; lane 2, transcription with *nmur-1(ok1387)* nuclear extract;
841 lane 3, transcriptional positive control (with HeLa nuclear extract); lane 4, transcriptional
842 negative control (without any nuclear extract); lane 5, PCR positive control; lane 6, PCR
843 negative control (without DNA template). **(B)** WT and mutant nuclear extracts were titrated using

844 the *in vitro* transcription system. The RNA copy number generated from transcription was
845 plotted against the amount of nuclear extract used in the reaction, followed by fitting with the
846 Michaelis-Menten model and the non-linear least-squares method to generate the titration
847 curves and calculate titration parameters. **(C)** qRT-PCR analysis of GFP expression after two-
848 hour heat shock in *CL2070(dvls70)* and *nmur-1(ok1387);CL2070(dvls70)* animals. Asterisk (*)
849 denotes a significant difference between *CL2070(dvls70)* and *nmur-1(ok1387);CL2070(dvls70)*
850 animals.
851
852 **Fig. 5. NMUR-1-regulated C-type lectin genes are required for *C. elegans* defense against**
853 ***E. faecalis*. (A)** WT and **(B)** *nmur-1(ok1387)* animals grown on dsRNA for C-type lectin genes
854 or the empty vector (EV) control were exposed to *E. faecalis* and scored for survival over time.
855 The graphs are a combination of three independent replicates. Each experiment included N =
856 60 animals per strain. P-values in (A) represent the significance level of RNAi treatment relative
857 to WT+EV: WT+*clec-94*, $P < 0.0001$; WT+*clec-137*, $P = 0.0220$; WT+*clec-157*, $P = 0.3728$;
858 WT+*clec-208*, $P < 0.0001$; WT+*clec-263*, $P < 0.0001$. P-values in (B) represent the significance
859 level of the RNAi treatment relative to *nmur-1(ok1387)*+EV: *nmur-1(ok1387)+clec-94*, P =
860 0.3633; *nmur-1(ok1387)+clec-137*, $P = 0.1841$; *nmur-1(ok1387)+clec-157*, $P = 0.8617$; *nmur-*
861 *1(ok1387)+clec-208*, $P = 0.1348$; *nmur-1(ok1387)+clec-263*, $P = 0.1320$. **(C)** WT and *nmur-*
862 *1(ok1387)* animals in which C-type lectin expression was rescued were exposed to *E. faecalis*
863 and scored for survival over time. All C-type lectin genes were rescued using their own
864 promoters. The graph is a combination of three independent experiments. Each experiment
865 included N = 60 adult animals per strain. P-values represent the significance of the survival of
866 rescue strains relative to *nmur-1(ok1378)* animals: WT, $P < 0.0001$; nmur-1(ok1387)+*clec-94*, P
867 < 0.0001; *nmur-1(ok1387)+clec-208*, $P = 0.0025$; *nmur-1(ok1387)+clec-263*, $P = 0.9799$.
868

869 **Fig. 6. NMUR-1-regulated UPR genes are required for *C. elegans* defense against *S.***
870 ***enterica*. (A)** *TU3401* and **(B)** *nmur-1(ok1387);TU3401* animals grown on dsRNA for UPR
871 genes or the empty vector (EV) control were exposed to *S. enterica* and scored for survival over
872 time. The graphs are a combination of three independent replicates. Each experiment included
873 *N* = 60 animals per strain. *P*-values in (A) represent the significance level of RNAi treatment
874 relative to *TU3401*+EV: *TU3401+T20D4.3*, *P* = 0.4224; *TU3401+T20D4.5*, *P* = 0.6102;
875 *TU3401+C17B7.5*, *P* = 0.5905; *TU3401+npl-4.2*, *P* < 0.0001. *P*-values in (B) represent the
876 significance level of RNAi treatment relative to *nmur-1(ok1387)*;*TU3401*+EV: *nmur-*
877 *1(ok1387);TU3401+T20D4.3*, *P* = 0.0007; *nmur-1(ok1387);TU3401+T20D4.5*, *P* = 0.0993;
878 *nmur-1(ok1387);TU3401+C17B7.5*, *P* = 0.1216; *nmur-1(ok1387);TU3401+npl-4.2*; *P* < 0.0001.
879

880 **Fig. 7. CAPA-1 is required for *C. elegans* defense against *E. faecalis* but not *S. enterica*.**
881 **(A)** WT, *nmur-1(ok1387)*, *capa-1(ok3065)*, and *capa-1(ok3650);nmur-1(ok1387)* animals were
882 exposed to *E. faecalis* and scored for survival over time. The graph is representative of three
883 independent experiments. Each experiment included *N* = 60 animals per strain. *P*-values
884 represent the significance level of mutant survival relative to WT: *nmur-1(ok1387)*, *P* = 0.0005;
885 *capa-1(ok3065)*, *P* = 0.0134; *capa-1(ok3065);nmur-1(ok1387)*, *P* = 0.0028. **(B)** WT, *nmur-*
886 *1(ok1387)*, *capa-1(ok3065)*, and *capa-1(ok3650);nmur-1(ok1387)* animals were exposed to *E.*
887 *faecalis* on plates containing either 0 or 1μg/mL synthetic ligand and scored for survival over
888 time. The graph is a combination of three independent experiments. Each experiment included
889 *N* = 60 animals per strain. *P*-values represent the significance level of the survival of
890 mutant+1μg/mL relative to WT+1μg/mL: *nmur-1(ok1387)*+1μg/mL, *P* < 0.0001; *capa-*
891 *1(ok3065)*+1μg/mL, *P* = 0.6758; *capa-1(ok3065);nmur-1(ok1387)*+1μg/mL, *P* = 0.0024. **(C)** WT,
892 *nmur-1(ok1387)*, *capa-1(ok3065)*, and *capa-1(ok3065);nmur-1(ok1387)* animals were exposed
893 to *S. enterica* and scored for survival over time. The graph is representative of three

894 independent experiments. Each experiment included *N* = 60 animals per strain. *P*-values
895 represent the significance level of mutant survival relative to WT: *nmur-1(ok1387)*, *P* < 0.0001;
896 *capa-1(ok3065)*, *P* = 0.2323; *capa-1(ok3065);nmur-1(ok1387)*, *P* < 0.0001. **(D)** WT, *nmur-*
897 *1(ok1387)*, *capa-1(ok3065)*, and *capa-1(ok3065);nmur-1(ok1387)* animals were exposed to *S.*
898 *enterica* on plates contain either 0 or 1µg/mL of synthetic ligand and scored for survival over
899 time. The graph is a combination of three independent experiments. Each experiment included
900 *N* = 60 animals per strain. *P*-values represent the significance level of the survival of
901 mutant+1µg/mL relative to WT+1µg/mL: *nmur-1(ok1387)*+1µg/mL, *P* < 0.0001; *capa-*
902 *1(ok3065)*+1µg/mL, *P* = 0.1643; *capa-1(ok3065);nmur-1(ok1387)*+1µg/mL, *P* < 0.0001.
903
904 **Table 1. Gene ontology (GO) analysis of downregulated genes in *nmur-1(ok1387)* animals**
905 **relative to wild-type animals.**
906
907 **Table 2. GO analysis of downregulated genes in *nmur-1(ok1387)* animals relative to wild-**
908 **type animals exposed to *E. faecalis*.**
909
910 **Table 3. Enrichment of biological process and molecular function revealed by GO**
911 **analysis of upregulated genes in *nmur-1(ok1387)* animals relative to wild-type animals**
912 **exposed to *S. enterica*.**
913
914 **SUPPLEMENTARY MATERIALS**
915 **Fig. S1. Functional loss of NMUR-1 differentially affects *C. elegans* survival against**
916 **various bacteria.** WT and *nmur-1(ok1387)* animals were exposed to *E. coli* OP50 **(A)**, *P.*
917 *aeruginosa* **(B)**, *Y. pestis* **(C)**, *M. nematophilum* **(D)**, or *S. aureus* **(E)** and scored for survival
918 over time. Each graph is a representation of three independent experiments. Each experiment
919 included *N* = 60 animals per strain. *P*-values represent the significance level of mutant survival

920 relative to WT, $P = 0.0011$ in (A), $P = 0.8084$ in (B), $P < 0.0001$ in (C), $P = 0.2401$ in (D), and P
921 $= 0.0678$ in (E).
922
923 **Fig. S2. Functional loss of NMUR-1 does not affect *C. elegans* survival against heat-killed**
924 ***E. faecalis* or heat-killed *S. enterica*.** WT and *nmur-1(ok1387)* animals were exposed to heat-
925 killed *E. faecalis* **(A)** or heat-killed *S. enterica* **(B)** and scored for survival over time. Each graph
926 is a representation of three independent experiments. Each experiment included $N = 60$ animals
927 per strain. *P*-value represents the significance level of the survival of mutants relative to WT, P
928 $= 0.2191$.
929
930 **Fig. S3. Functional loss of NMUR-1 differentially affects *C. elegans* survival against *E.***
931 ***faecalis* and *S. enterica* in full-lawn assays.** WT and *nmur-1(ok1387)* animals were exposed
932 to a full lawn of *E. faecalis* **(A)** or *S. enterica* **(B)** and scored for survival over time. Each graph is
933 a combination of three independent experiments. Each experiment included $N = 60$ animals per
934 strain. *P*-values represent the significance level of the survival of mutants relative to WT, $P <$
935 0.0001 in (A); $P < 0.0001$ in (B).
936
937 **Table S1. Enrichment of molecular functions revealed by GO analysis of upregulated**
938 **genes in *nmur-1(ok1387)* animals relative to wild-type animals.**
939
940 **Table S2. Enrichment of biological processes revealed by GO analysis of upregulated**
941 **genes in *nmur-1(ok1387)* animals relative to wild-type animals exposed to *E. faecalis*.**
942
943 **Table S3. List of transgenic *C. elegans* strains generated in this study.**
944

945 **FIGURES AND TABLES**
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949 **Fig. 1**
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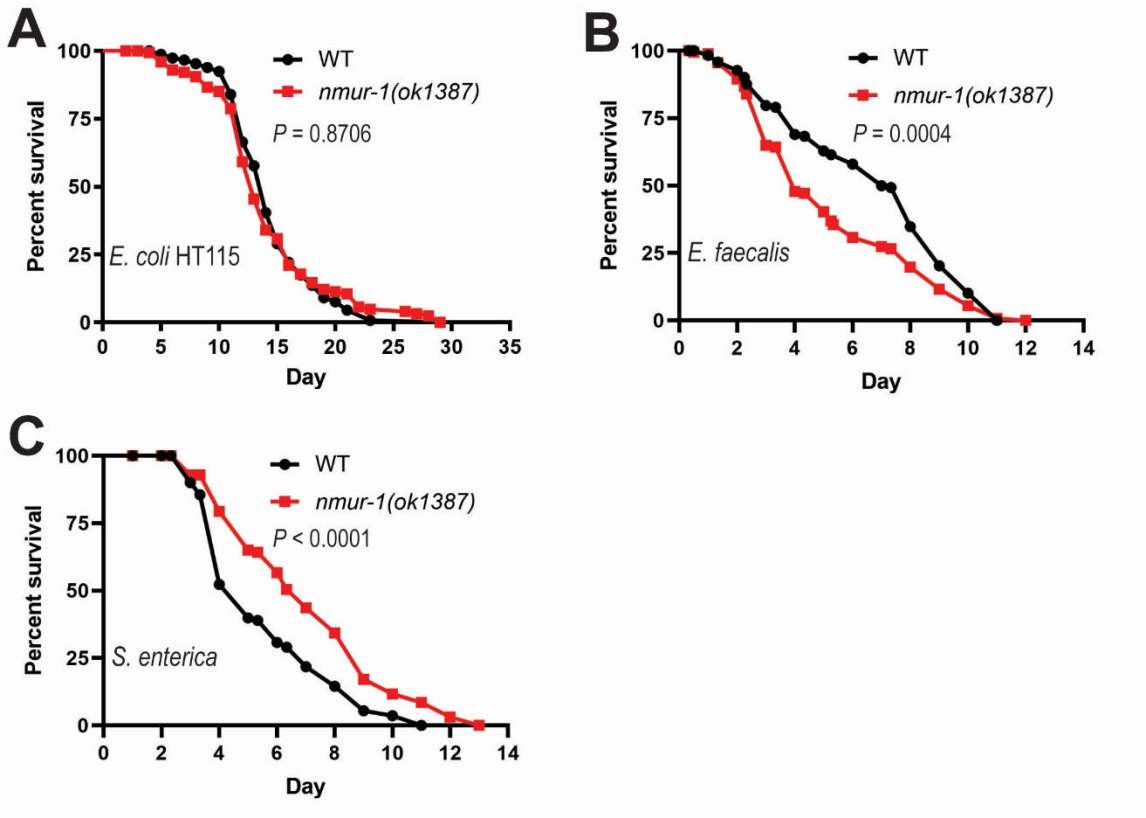

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953
954 **Fig. 1. Functional loss of NMUR-1 differentially affects *C. elegans* survival against *E. coli***
955 **HT115, *E. faecalis* and *S. enterica*.** Wild-type (WT) and *nmur-1(ok1387)* animals were
956 exposed to *E. coli* HT115 **(A)**, *E. faecalis* **(B)**, or *S. enterica* **(C)** and scored for survival over
957 time. Each graph is a combination of three independent experiments. Each experiment included
958 N = 60 animals per strain. P-values represent the significance level of mutant survival relative to
959 WT, P = 0.8706 in (A), P = 0.0004 in (B), and P < 0.0001 in (C).
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962 **Fig. 2**
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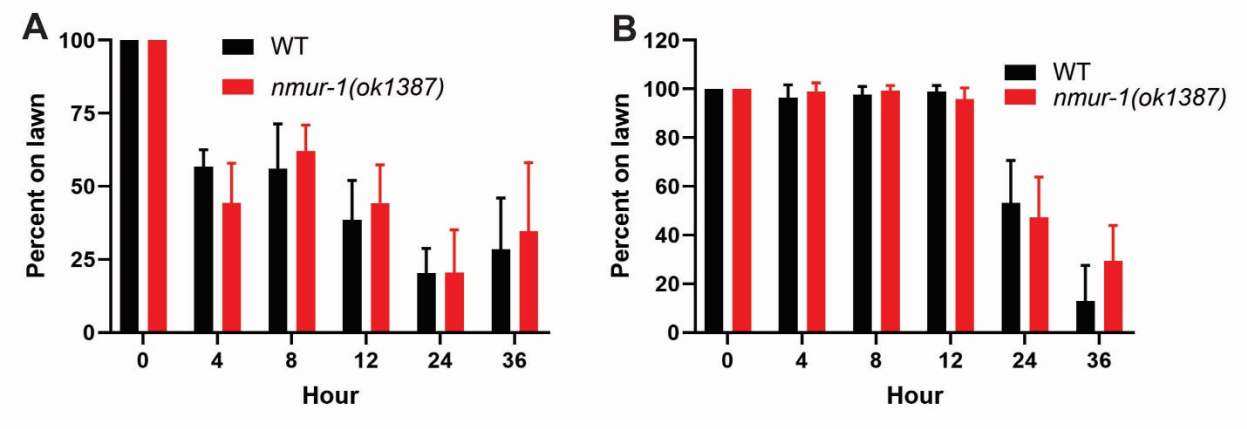

965
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967 **Fig. 2. Functional loss of NMUR-1 does not affect pathogen avoidance behavior.** WT and
968 *nmur-1(ok1387)* animals were subjected to lawn occupancy assays in which the animals were
969 placed on a small lawn of *E. faecalis* **(A)** or *S. enterica* **(B)** in a 3.5cm plate and monitored over
970 time for their presence on the lawn. Each graph is a combination of three independent
971 experiments. Each experiment included *N* = 60 animals per strain. Error bars represent the
972 standard error of the mean (SEM).
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976 **Fig. 3**
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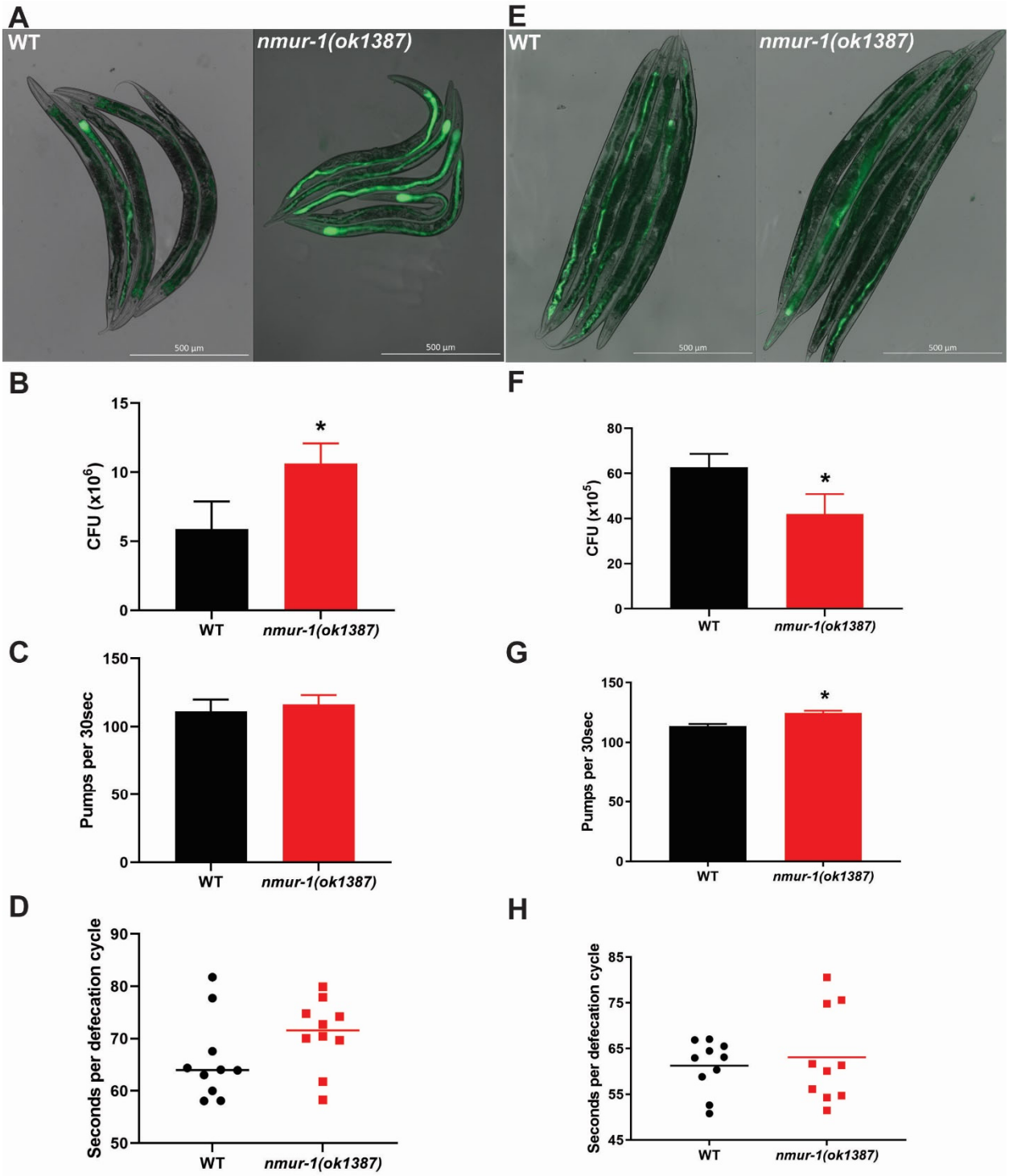

979
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981
982 **Fig 3. Functional loss of NMUR-1 changes intestinal accumulation of *E. faecalis* and *S.***
983 ***enterica*. (A)** WT and *nmur-1(ok1387)* animals were exposed to GFP-expressing *E. faecalis* for

984 24 hours and then visualized using a Zeiss Axio Imager M2 fluorescence microscope. The scale
985 bar indicates 500 µm. **(B)** WT and *nmur-1(ok1387)* animals were exposed to *E. faecalis* for 24
986 hours, and the Colony Forming Units (CFU) of live bacterial cells recovered from their intestines
987 were quantified. The graph represents the combined results of three independent experiments.
988 *N* = 10 animals per strain were used for each experiment. Error bars represent SEM. *P*-value
989 represents the significance level of mutant gut colonization relative to WT, *P* = 0.0296. **(C)** WT
990 and *nmur-1(ok1387)* animals were exposed to *E. faecalis* for 24 hours. Pharyngeal pumping
991 rates of animals were counted as the number of pumps per 30 sec. Counting was conducted in
992 triplicate and averaged to give a pumping rate. The measurements included *N* = 10 adult
993 animals per strain. Error bars represent SEM. *P* = 0.1068. **(D)** WT and *nmur-1(ok1387)* animals
994 were exposed to *E. faecalis* for 24 hours. Defecation rates of animals were measured as the
995 average time of 10 intervals between two defecation cycles. The measurements included *N* =
996 10 animals per strain. *P* = 0.1379. **(E)** WT and *nmur-1(ok1387)* animals were exposed to GFP-
997 expressing *S. enterica* for 24 hours and then visualized using a Zeiss Axio Imager M2
998 fluorescence microscope. The scale bar indicates 500 µm. **(F)** WT and *nmur-1(ok1387)* animals
999 were exposed to GFP-expressing *S. enterica* for 24 hours, and the CFU of live bacterial cells
1000 recovered from their intestines were quantified. The graph represents the combined results of
1001 three independent experiments. *N* = 10 animals per strain were used for each experiment. Error
1002 bars represent SEM. *P* = 0.0290. **(G)** WT and *nmur-1(ok1387)* animals were exposed to *S.*
1003 *enterica* for 24 hours. Pharyngeal pumping rates of animals were counted as the number of
1004 pumps per 30 sec. Counting was conducted in triplicate and averaged to give a pumping rate.
1005 The measurements included *N* = 10 adult animals per strain. Error bars represent SEM. *P* =
1006 0.0006. **(H)** WT and *nmur-1(ok1387)* animals were exposed to *S. enterica* for 24 hours.
1007 Defecation rates of animals were measured as the average time of 10 intervals between two
1008 defecation cycles. The measurements included *N* = 10 animals per strain. *P* = 0.6310.
1009
1010

1011 **Fig. 4**
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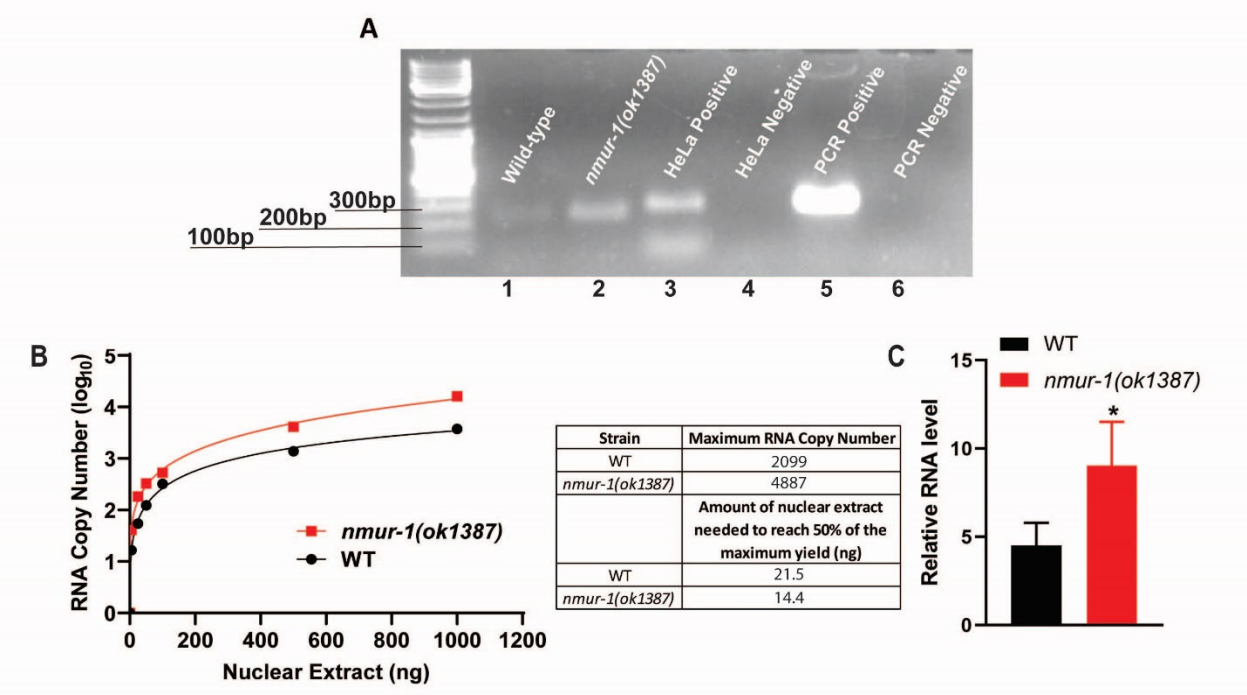

Strain	Maximum RNA Copy Number
WT	2099
nmur-1(ok1387)	4887
	Amount of nuclear extract needed to reach 50% of the maximum yield (ng)
WT	21.5
nmur-1(ok1387)	14.4

1014
1015
1016 **Fig. 4. NMUR-1 regulates transcriptional activity. (A)** *In vitro* transcription assays were
1017 performed using nuclear extract from WT or *nmur-1(ok1387)* animals. After transcription, RNA
1018 was purified, reverse transcribed, and amplified by PCR, followed by gel analysis. Lane 1,
1019 transcription with WT nuclear extract; lane 2, transcription with *nmur-1(ok1387)* nuclear extract;
1020 lane 3, transcriptional positive control (with HeLa nuclear extract); lane 4, transcriptional
1021 negative control (without any nuclear extract); lane 5, PCR positive control; lane 6, PCR
1022 negative control (without DNA template). **(B)** WT and mutant nuclear extracts were titrated using
1023 the *in vitro* transcription system. The RNA copy number generated from transcription was
1024 plotted against the amount of nuclear extract used in the reaction, followed by fitting with the
1025 Michaelis-Menten model and the non-linear least-squares method to generate the titration
1026 curves and calculate titration parameters. **(C)** qRT-PCR analysis of GFP expression after two-
1027 hour heat shock in *CL2070(dvls70)* and *nmur-1(ok1387);CL2070(dvls70)* animals. Asterisk (*)
1028 denotes a significant difference between *CL2070(dvls70)* and *nmur-1(ok1387);CL2070(dvls70)*
1029 animals
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1033 **Fig. 5**
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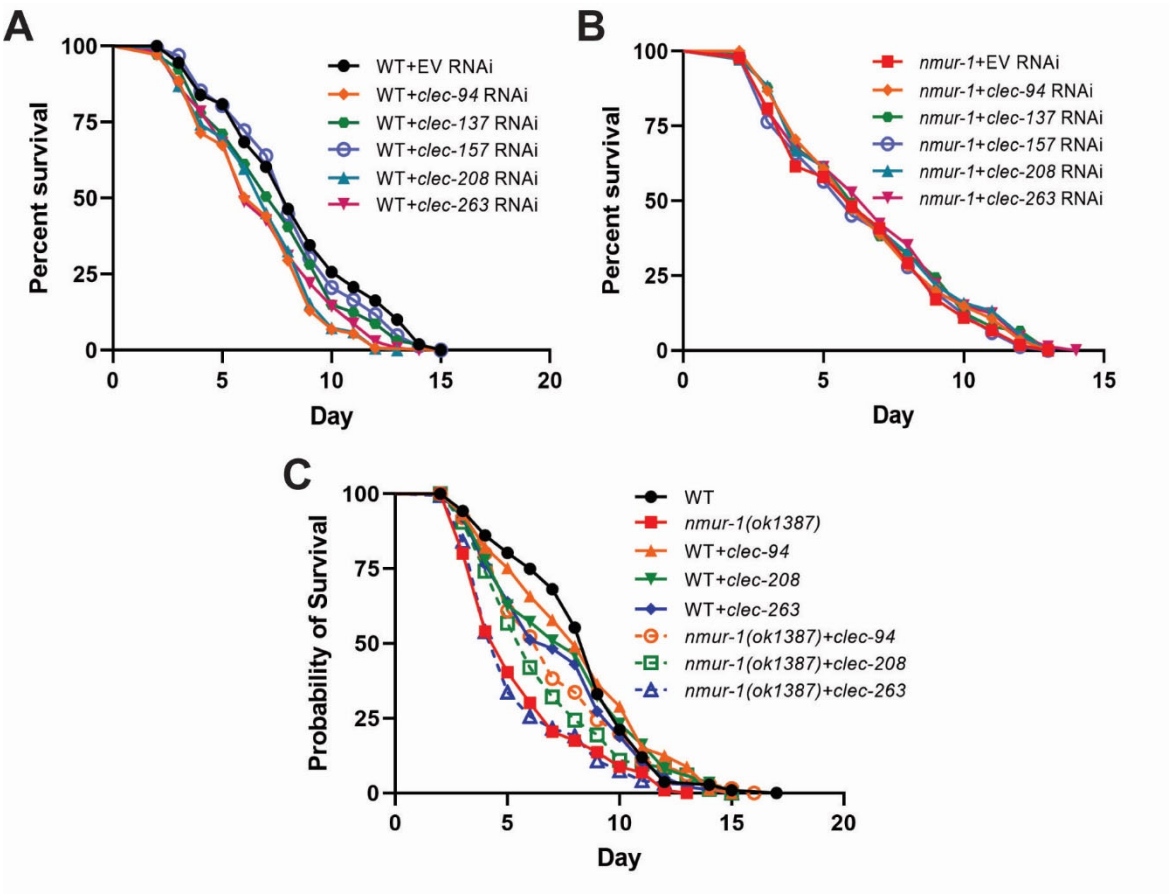

1036
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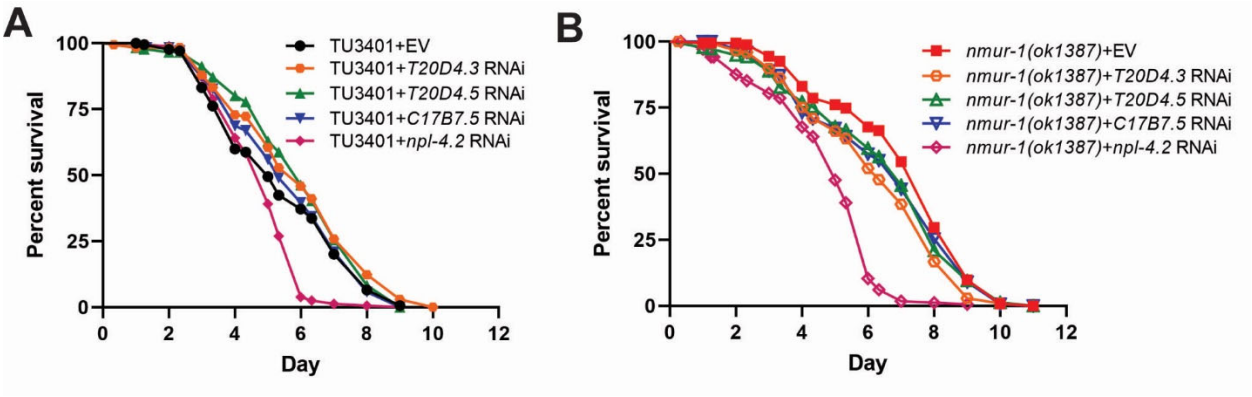
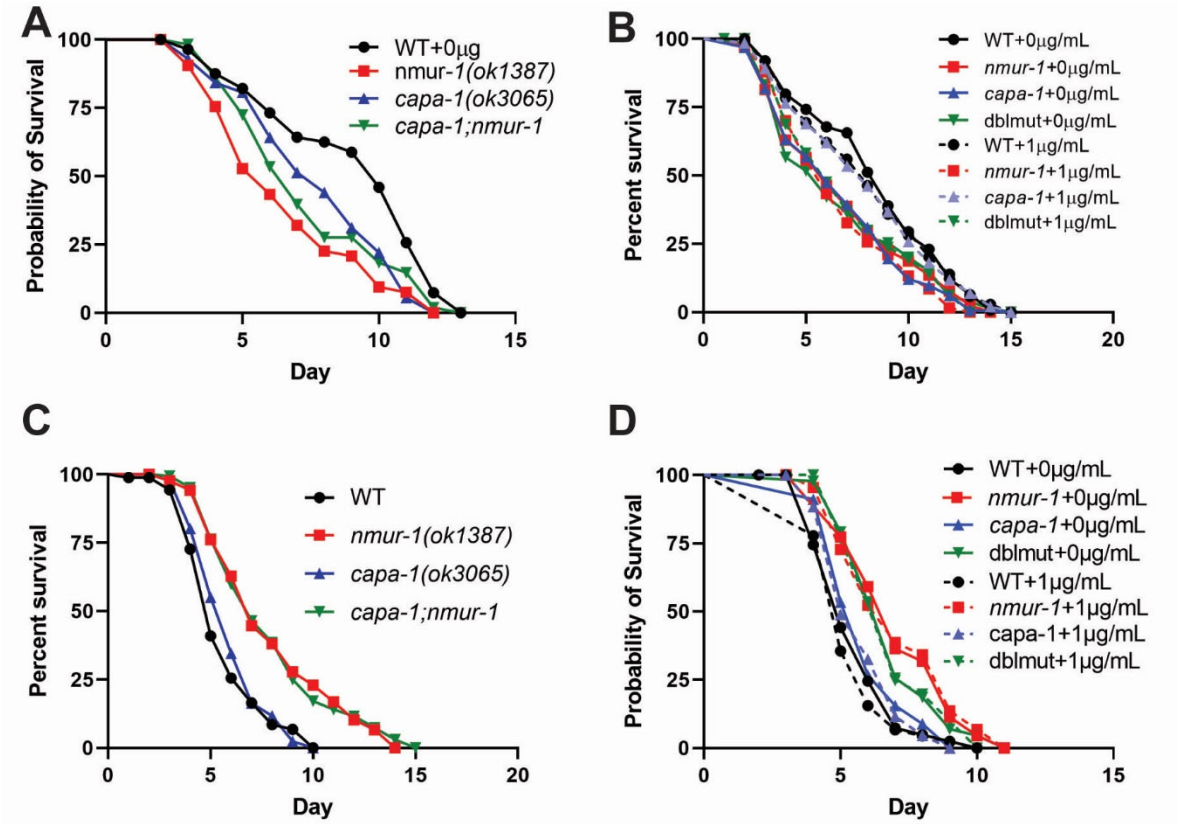
1038
1039 **Fig. 5. NMUR-1-regulated C-type lectin genes are required for *C. elegans* defense against**
1040 ***E. faecalis*. (A)** WT and **(B)** *nmur-1(ok1387)* animals grown on dsRNA for C-type lectin genes
1041 or the empty vector (EV) control were exposed to *E. faecalis* and scored for survival over time.
1042 The graphs are a combination of three independent replicates. Each experiment included N =
1043 60 animals per strain. P-values in (A) represent the significance level of RNAi treatment relative
1044 to WT+EV: WT+*clec-94*, $P < 0.0001$; WT+*clec-137*, $P = 0.0220$; WT+*clec-157*, $P = 0.3728$;
1045 WT+*clec-208*, $P < 0.0001$; WT+*clec-263*, $P < 0.0001$. P-values in (B) represent the significance
1046 level of the RNAi treatment relative to *nmur-1(ok1387)*+EV: *nmur-1(ok1387)+clec-94*, P =
1047 0.3633; *nmur-1(ok1387)+clec-137*, $P = 0.1841$; *nmur-1(ok1387)+clec-157*, $P = 0.8617$; *nmur-*
1048 *1(ok1387)+clec-208*, $P = 0.1348$; *nmur-1(ok1387)+clec-263*, $P = 0.1320$. **(C)** WT and *nmur-*
1049 *1(ok1387)* animals in which C-type lectin expression was rescued were exposed to *E. faecalis*
1050 and scored for survival over time. All C-type lectin genes were rescued using their own
1051 promoters. The graph is a combination of three independent experiments. Each experiment
1052 included N = 60 adult animals per strain. P-values represent the significance of the survival of
1053 rescue strains relative to *nmur-1(ok1378)* animals: WT, $P < 0.0001$; nmur-1(ok1387)+*clec-94*, P
1054 < 0.0001; *nmur-1(ok1387)+clec-208*, $P = 0.0025$; *nmur-1(ok1387)+clec-263*, $P = 0.9799$.
1055
1056
1057

1058 **Fig. 6**
1059
1060



1061
1062
1063 **Fig. 6. NMUR-1-regulated UPR genes are required for *C. elegans* defense against *S.***
1064 ***enterica*. (A)** *TU3401* and **(B)** *nmur-1(ok1387);TU3401* animals grown on dsRNA for UPR
1065 genes or the empty vector (EV) control were exposed to *S. enterica* and scored for survival over
1066 time. The graphs are a combination of three independent replicates. Each experiment included
1067 N = 60 animals per strain. P-values in (A) represent the significance level of RNAi treatment
1068 relative to *TU3401*+EV: *TU3401+T20D4.3*, P = 0.4224; *TU3401+T20D4.5*, P = 0.6102;
1069 *TU3401+C17B7.5*, P = 0.5905; *TU3401+npl-4.2*, P < 0.0001. P-values in (B) represent the
1070 significance level of RNAi treatment relative to *nmur-1(ok1387)*;*TU3401*+EV: *nmur-*
1071 *1(ok1387);TU3401+T20D4.3*, P = 0.0007; *nmur-1(ok1387);TU3401+T20D4.5*, P = 0.0993;
1072 *nmur-1(ok1387);TU3401+C17B7.5*, P = 0.1216; *nmur-1(ok1387);TU3401+npl-4.2*; P < 0.0001.
1073
1074

1075 **Fig. 7**
1076



1077
1078 **Fig. 7. CAPA-1 is required for *C. elegans* defense against *E. faecalis* but not *S. enterica*.**
1079 **(A)** WT, *nmur-1(ok1387)*, *capa-1(ok3065)*, and *capa-1(ok3650);nmur-1(ok1387)* animals were
1080 exposed to *E. faecalis* and scored for survival over time. The graph is representative of three
1081 independent experiments. Each experiment included *N* = 60 animals per strain. *P*-values
1082 represent the significance level of mutant survival relative to WT: *nmur-1(ok1387)*, *P* = 0.0005;
1083 *capa-1(ok3065)*, *P* = 0.0134; *capa-1(ok3065);nmur-1(ok1387)*, *P* = 0.0028. **(B)** WT, *nmur-*
1084 *1(ok1387)*, *capa-1(ok3065)*, and *capa-1(ok3650);nmur-1(ok1387)* animals were exposed to *E.*
1085 *faecalis* on plates containing either 0 or 1μg/mL synthetic ligand and scored for survival over
1086 time. The graph is a combination of three independent experiments. Each experiment included
1087 *N* = 60 animals per strain. *P*-values represent the significance level of the survival of
1088 mutant+1μg/mL relative to WT+1μg/mL: *nmur-1(ok1387)*+1μg/mL, *P* < 0.0001; *capa-*
1089 *1(ok3065)*+1μg/mL, *P* = 0.6758; *capa-1(ok3065);nmur-1(ok1387)*+1μg/mL, *P* = 0.0024. **(C)** WT,
1090 *nmur-1(ok1387)*, *capa-1(ok3065)*, and *capa-1(ok3065);nmur-1(ok1387)* animals were exposed
1091 to *S. enterica* and scored for survival over time. The graph is representative of three
1092 independent experiments. Each experiment included *N* = 60 animals per strain. *P*-values
1093 represent the significance level of mutant survival relative to WT: *nmur-1(ok1387)*, *P* < 0.0001;
1094 *capa-1(ok3065)*, *P* = 0.2323; *capa-1(ok3065);nmur-1(ok1387)*, *P* < 0.0001. **(D)** WT, *nmur-*
1095 *1(ok1387)*, *capa-1(ok3065)*, and *capa-1(ok3065);nmur-1(ok1387)* animals were exposed to *S.*
1096 *enterica* on plates contain either 0 or 1μg/mL of synthetic ligand and scored for survival over
1097 time. The graph is a combination of three independent experiments. Each experiment included
1098 *N* = 60 animals per strain. *P*-values represent the significance level of the survival of
1099 mutant+1μg/mL relative to WT+1μg/mL: *nmur-1(ok1387)*+1μg/mL, *P* < 0.0001; *capa-*
1100 *1(ok3065)*+1μg/mL, *P* = 0.1643; *capa-1(ok3065);nmur-1(ok1387)*+1μg/mL, *P* < 0.0001.

1101 **Table 1. Gene ontology (GO) analysis of downregulated genes in *nmur-1(ok1387)* animals**
1102 **relative to wild-type animals.**
1103
1104 **(A) Enriched molecular functions**

GO term	Description	P-value#	FDR q-value*	Enrichment (N, B, n, b)§
GO:0003690	double-stranded DNA binding	2.37E-08	5.83E-05	5.59 (9751,169,165,16)
GO:0000976	transcription regulatory region sequence-specific DNA binding	3.29E-08	4.05E-05	6.41 (9751,129,165,14)
GO:0000981	DNA-binding transcription factor activity, RNA polymerase II-specific	3.66E-08	3.01E-05	7.79 (9751,91,165,12)
GO:1990837	sequence-specific double-stranded DNA binding	5.88E-08	3.62E-05	6.13 (9751,135,165,14)
GO:0001067	regulatory region nucleic acid binding	3.08E-07	1.52E-04	5.37 (9751,154,165,14)
GO:0044212	transcription regulatory region DNA binding	3.08E-07	1.26E-04	5.37 (9751,154,165,14)
GO:0003700	DNA-binding transcription factor activity	1.10E-06	3.86E-04	3.35 (9751,371,165,21)
GO:0140110	transcription regulator activity	1.97E-06	6.07E-04	3.01 (9751,451,165,23)
GO:0000977	RNA polymerase II regulatory region sequence-specific DNA binding	4.31E-06	1.18E-03	5.56 (9751,117,165,11)
GO:0001012	RNA polymerase II regulatory region DNA binding	5.51E-06	1.36E-03	5.42 (9751,120,165,11)
GO:0043565	sequence-specific DNA binding	7.12E-05	1.59E-02	2.81 (9751,379,165,18)

1105
1106 **(B) Downregulated genes related to enriched molecular functions**

Genes	Fold change	Adjusted *P* valueΨ	Genes	Fold change	Adjusted *P* valueΨ
dmd-5	3.7	3.09E-05	nhr-25	2.6	4.47E-13
unc-39	3.6	5.68E-09	ceh-43	2.5	1.35E-13
ceh-27	3.6	2.49E-11	tbx-8	2.4	1.64E-04
egl-46	3.6	9.25E-18	unc-130	2.3	1.84E-11
ces-2	3.4	1.14E-19	efl-3	2.3	2.26E-05
cnd-1	3.4	1.32E-05	sepa-1	2.1	4.00E-03
fkh-2	3.3	6.61E-07	ham-1	2.1	7.64E-13
zip-8	3.2	1.13E-06	ham-2	2.0	6.06E-08
zip-7	2.8	5.01E-11	tbx-11	2.0	6.53E-05
ref-2	2.8	1.97E-06	hbl-1	2.0	6.38E-07
eya-1	2.7	3.20E-13	ceh-32	2.0	1.20E-04
hlh-14	2.6	1.66E-07			

1107
1108 # P-value is computed according to the mHG model (Eden *et al.* 2007 PLoS Comp Bio 3(3):e39).
1109 * FDR q-value is the correction of the above p-value for multiple testing using the Benjamini and
1110 Hochberg method (Benjamini and Hochberg 1995 J R Statist Soc B 57(1):289-300).
1111 § Enrichment (N, B, n, b) is defined as follows: N - total number of genes; B - total number of genes
1112 associated with a specific GO term; n - number of genes in the target set; b - number of genes in the
1113 intersection;Enrichment = (b/n) / (B/N).
1114 ΨAdjusted *P* value is the correction of the P value for multiple testing using the Benjamini and Hochberg
1115 method (Benjamini and Hochberg 1995 J R Statist Soc B 57 (1):289–300).
1116

1117 **Table 2. GO analysis of downregulated genes in *nmur-1(ok1387)* animals relative to wild-**
1118 **type animals exposed to *E. faecalis*.**
1119
1120 **(A) Enriched molecular functions**

GO term	Description	P-value#	FDR q-value*	Enrichment (N, B, n, b)§
GO:0030246	carbohydrate binding	2.29E-15	5.65E-12	17.45 (10161,182,48,15)

1121
1122 **(B) Downregulated genes related to the enriched molecular functions**

Genes	Fold change	Adjusted *P* valueΨ	Genes	Fold change	Adjusted *P* valueΨ
clec-207	5.4	4.16E-15	clec-161	2.4	1.10E-04
clec-94	4.9	1.50E-12	clec-95	2.4	2.91E-04
clec-137	4.3	3.68E-10	clec-99	2.4	6.31E-05
clec-263	3.7	1.68E-08	clec-197	2.4	7.91E-04
clec-208	3.4	2.01E-07	clec-136	1.9	2.03E-02
clec-157	2.9	3.25E-06	clec-174	1.9	2.50E-03
pqn-84	2.8	2.76E-06	clec-190	1.8	1.29E-04
clec-219	2.5	2.92E-04			

1123
1124 # P-value is computed according to the mHG model (Eden *et al.* 2007 PLoS Comp Bio 3(3):e39).
1125 * FDR q-value is the correction of the above p-value for multiple testing using the Benjamini and
1126 Hochberg method (Benjamini and Hochberg 1995 J R Statist Soc B 57(1):289-300).
1127 § Enrichment (N, B, n, b) is defined as follows: N - total number of genes; B - total number of genes
1128 associated with a specific GO term; n - number of genes in the target set; b - number of genes in the
1129 intersection;Enrichment = (b/n) / (B/N).
1130 ΨAdjusted *P* value is the correction of the P value for multiple testing using the Benjamini and Hochberg
1131 method (Benjamini and Hochberg 1995 J R Statist Soc B 57 (1):289–300).
1132
1133

1134 **Table 3. Enrichment of biological process and molecular function revealed by GO**
1135 **analysis of upregulated genes in *nmur-1(ok1387)* animals relative to wild-type animals**
1136 **exposed to *S. enterica*.**
1137
1138 **(A) Enriched biological processes and molecular functions**

GO term	Description	P-value[#]	FDR q-value*	Enrichment (N, B, n, b)[§]
Biological process				
GO:0006515	protein quality control for misfolded or incompletely synthesized proteins	1.35E-8	7.89E-5	137.71 (9812,19,15,4)
GO:0006516	glycoprotein catabolic process	3.45E-7	1.01E-3	196.24 (9812,10,15,3)
GO:0006517	protein deglycosylation	1.04E-6	2.03E-3	140.17 (9812,14,15,3)
GO:0009100	glycoprotein metabolic process	2E-5	2.92E-2	54.51 (9812,36,15,3)
Molecular function				
GO:0000224	peptide-N4-(N-acetyl-beta-glucosaminyl)asparagine amidase activity	2.41E-7	5.96E-4	218.04 (9812,9,15,3)
GO:0016811	hydrolase activity, acting on carbon-nitrogen (but not peptide) bonds, in linear amides	2.98E-5	3.67E-2	47.86 (9812,41,15,3)

1139
1140 **(B) Upregulated genes related to the enriched biological process protein quality control**
1141

Genes	Fold change	Adjusted *P* value[Ψ]
C17B7.5	2.9	2.57E-14
T20D4.5	2.8	2.40E-15
npl-4.2	2.8	1.22E-22
T20D4.3	2.6	1.60E-12

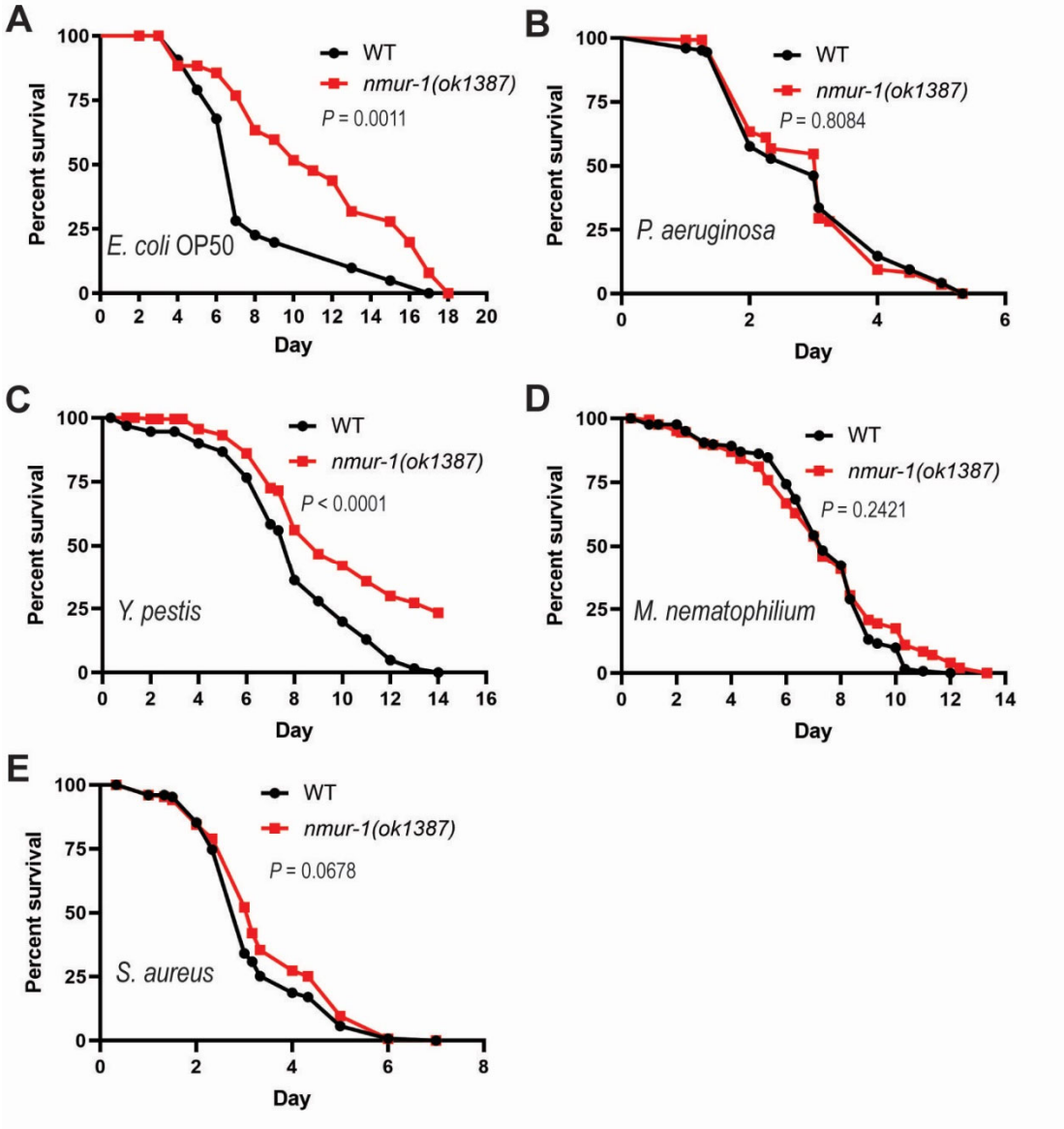
1142
1143 [#] P-value is computed according to the mHG model (Eden *et al.* 2007 PLoS Comp Bio 3(3):e39).
1144 * FDR q-value is the correction of the above p-value for multiple testing using the Benjamini and
1145 Hochberg method (Benjamini and Hochberg 1995 J R Statist Soc B 57(1):289-300).
1146 [§] Enrichment (N, B, n, b) is defined as follows: N - total number of genes; B - total number of genes
1147 associated with a specific GO term; n - number of genes in the target set; b - number of genes in the
1148 intersection;Enrichment = (b/n) / (B/N).
1149 [Ψ]Adjusted *P* value is the correction of the P value for multiple testing using the Benjamini and Hochberg
1150 method (Benjamini and Hochberg 1995 J R Statist Soc B 57 (1):289–300).
1151

1152 **SUPPLEMENTARY MATERIALS**

1153

1154 **Fig. S1**

1155



1156

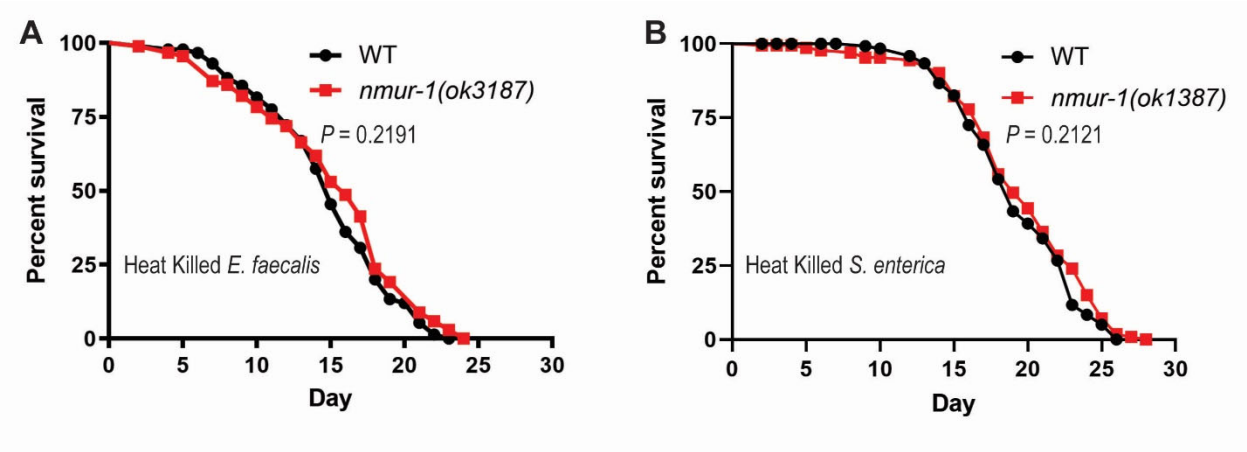
1157

1158 **Fig. S1. Functional loss of NMUR-1 differentially affects *C. elegans* survival against**
1159 **various bacteria.** WT and *nmur-1(ok1387)* animals were exposed to *E. coli* OP50 **(A)**, *P.*
1160 *aeruginosa* **(B)**, *Y. pestis* **(C)**, *M. nematophilum* **(D)**, or *S. aureus* **(E)** and scored for survival
1161 over time. Each graph is a representation of three independent experiments. Each experiment
1162 included N = 60 animals per strain. P-values represent the significance level of mutant survival
1163 relative to WT, P = 0.0011 in (A), P = 0.8084 in (B), P < 0.0001 in (C), P = 0.2401 in (D), and P
1164 = 0.0678 in (E).

1165

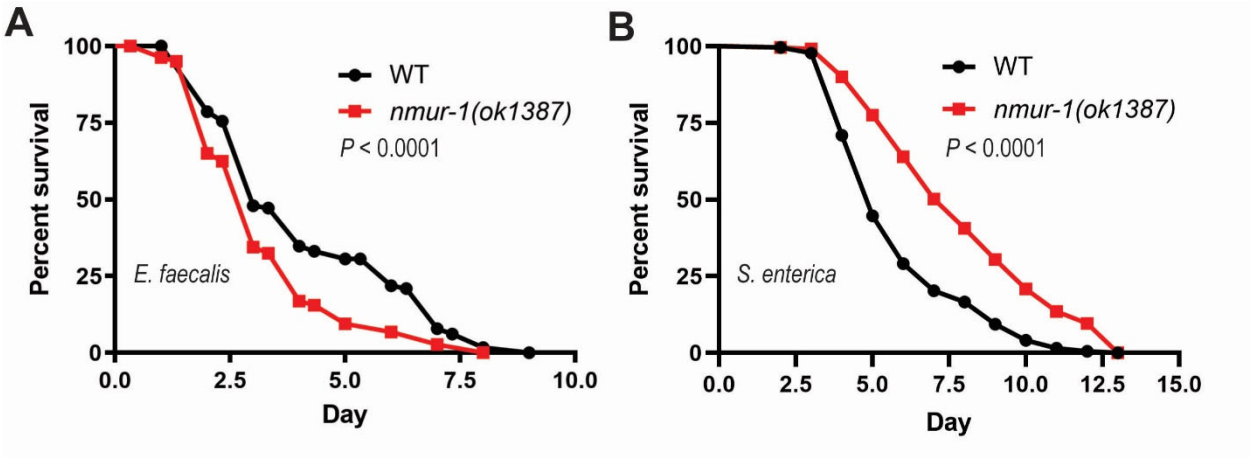
1166

1167 **Fig. S2**
1168



1169
1170
1171 **Fig. S2. Functional loss of NMUR-1 does not affect *C. elegans* survival against heat-killed**
1172 ***E. faecalis* or heat-killed *S. enterica*.** WT and *nmur-1(ok1387)* animals were exposed to heat-
1173 killed *E. faecalis* **(A)** or heat-killed *S. enterica* **(B)** and scored for survival over time. Each graph
1174 is a representation of three independent experiments. Each experiment included *N* = 60 animals
1175 per strain. *P*-value represents the significance level of the survival of mutants relative to WT, *P*
1176 = 0.2191.
1177
1178

1179 **Fig. S3**
1180
1181



1182
1183
1184 **Fig. S3. Functional loss of NMUR-1 differentially affects *C. elegans* survival against *E.***
1185 ***faecalis* and *S. enterica* in full-lawn assays.** WT and *nmur-1(ok1387)* animals were exposed
1186 to a full lawn of *E. faecalis* **(A)** or *S. enterica* **(B)** and scored for survival over time. Each graph is
1187 a combination of three independent experiments. Each experiment included $N = 60$ animals per
1188 strain. *P*-values represent the significance level of the survival of mutants relative to WT, $P <$
1189 0.0001 in (A); $P < 0.0001$ in (B).
1190
1191
1192

1193 **Table S1. Enrichment of molecular functions revealed by GO analysis of upregulated**
1194 **genes in *nmur-1(ok1387)* animals relative to wild-type animals.**
1195

GO term	Description	P-value[#]	FDR q-value*	Enrichment (N, B, n, b)[§]
GO:0004721	phosphoprotein phosphatase activity	1.21E-40	3.08E-37	5.37 (9948,161,921,80)
GO:0004725	protein tyrosine phosphatase activity	6.28E-37	7.97E-34	6.95 (9948,87,921,56)
GO:0042302	structural constituent of cuticle	1.05E-34	8.86E-32	5.62 (9948,125,921,65)
GO:0140096	catalytic activity, acting on a protein	1.97E-33	1.25E-30	2.17 (9948,1133,921,228)
GO:0004672	protein kinase activity	2.29E-30	1.16E-27	3.19 (9948,376,921,111)
GO:0016791	phosphatase activity	4.24E-30	1.79E-27	4.08 (9948,212,921,80)
GO:0042578	phosphoric ester hydrolase activity	3.37E-25	1.22E-22	3.53 (9948,245,921,80)
GO:0016773	phosphotransferase activity, alcohol group as acceptor	9.08E-25	2.88E-22	2.78 (9948,431,921,111)
GO:0004715	non-membrane spanning protein tyrosine kinase activity	2.07E-21	5.83E-19	7.36 (9948,44,921,30)
GO:0004674	protein serine/threonine kinase activity	2.35E-21	5.96E-19	3.12 (9948,277,921,80)
GO:0016301	kinase activity	2.42E-21	5.59E-19	2.52 (9948,485,921,113)
GO:0016772	transferase activity, transferring phosphorus-containing groups	1.34E-15	2.84E-13	2.13 (9948,579,921,114)
GO:0016788	hydrolase activity, acting on ester bonds	1.19E-14	2.33E-12	2.22 (9948,471,921,97)
GO:0004713	protein tyrosine kinase activity	9.57E-13	1.74E-10	4.19 (9948,80,921,31)
GO:0004722	protein serine/threonine phosphatase activity	2.86E-11	4.84E-09	4.63 (9948,56,921,24)
GO:0005198	structural molecule activity	4.50E-09	7.14E-07	2.08 (9948,342,921,66)
GO:0005524	ATP binding	5.16E-08	7.71E-06	1.61 (9948,798,921,119)
GO:0032559	adenyl ribonucleotide binding	1.31E-07	1.85E-05	1.58 (9948,820,921,120)
GO:0030554	adenyl nucleotide binding	1.60E-07	2.14E-05	1.57 (9948,823,921,120)
GO:0008144	drug binding	1.52E-06	1.93E-04	1.49 (9948,912,921,126)
GO:0035639	purine ribonucleoside triphosphate binding	7.87E-05	9.51E-03	1.38 (9948,960,921,123)
GO:0032555	purine ribonucleotide binding	1.46E-04	1.68E-02	1.36 (9948,983,921,124)
GO:0017076	purine nucleotide binding	1.74E-04	1.92E-02	1.36 (9948,987,921,124)
GO:0005102	signaling receptor binding	1.89E-04	1.99E-02	1.92 (9948,186,921,33)
GO:0032553	ribonucleotide binding	2.89E-04	2.94E-02	1.34 (9948,999,921,124)
GO:0042329	structural constituent of collagen and cuticulin-based cuticle	3.51E-04	3.43E-02	5.40 (9948,12,921,6)
GO:0030246	carbohydrate binding	4.64E-04	4.37E-02	1.94 (9948,156,921,28)

1196
1197 [#] P-value is computed according to the mHG model (Eden *et al.* 2007 PLoS Comp Bio 3(3):e39).
1198 * FDR q-value is the correction of the above p-value for multiple testing using the Benjamini and
1199 Hochberg method (Benjamini and Hochberg 1995 J R Statist Soc B 57(1):289-300).
1200 [§] Enrichment (N, B, n, b) is defined as follows:
1201 N - total number of genes
1202 B - total number of genes associated with a specific GO term
1203 n - number of genes in the target set
1204 b - number of genes in the intersection
1205 Enrichment = (b/n) / (B/N)
1206

1207 **Table S2. Enrichment of biological processes revealed by GO analysis of upregulated**
1208 **genes in *nmur-1(ok1387)* animals relative to wild-type animals exposed to *E. faecalis*.**
1209

GO term	Description	P-value[#]	FDR q-value*	Enrichment (N, B, n, b)[§]
GO:0050830	defense response to Gram-positive bacterium	1.24E-11	7.49E-08	15.32 (10357,61,133,12)
GO:0042742	defense response to bacterium	3.97E-09	1.20E-05	7.57 (10357,144,133,14)
GO:0009617	response to bacterium	3.97E-09	7.98E-06	7.57 (10357,144,133,14)
GO:0098542	defense response to other organism	2.70E-08	4.07E-05	6.53 (10357,167,133,14)
GO:0043207	response to external biotic stimulus	2.91E-08	3.52E-05	6.49 (10357,168,133,14)
GO:0009607	response to biotic stimulus	2.91E-08	2.93E-05	6.49 (10357,168,133,14)
GO:0051707	response to other organism	2.91E-08	2.51E-05	6.49 (10357,168,133,14)
GO:0051704	multi-organism process	2.02E-07	1.52E-04	5.56 (10357,196,133,14)
GO:0009605	response to external stimulus	9.53E-06	6.39E-03	4.02 (10357,271,133,14)
GO:0042338	cuticle development involved in collagen and cuticulin-based cuticle molting cycle	1.13E-05	6.83E-03	16.22 (10357,24,133,5)
GO:0006952	defense response	3.61E-05	1.98E-02	3.38 (10357,346,133,15)

1210
1211 [#] P-value is computed according to the mHG model (Eden *et al.* 2007 PLoS Comp Bio 3(3):e39).
1212 * FDR q-value is the correction of the above p-value for multiple testing using the Benjamini and
1213 Hochberg method (Benjamini and Hochberg 1995 J R Statist Soc B 57(1):289-300).
1214 [§] Enrichment (N, B, n, b) is defined as follows:
1215 N - total number of genes
1216 B - total number of genes associated with a specific GO term
1217 n - number of genes in the target set
1218 b - number of genes in the intersection
1219 Enrichment = (b/n) / (B/N)
1220
1221

1222 **Table S3. List of transgenic *C. elegans* strains generated in this study.**
1223

Strain	Plasmid and genetic background	Host	Note
JRS60	pPW01 *clec-94p(4.2kb)::clec-94 gDNA::SL2::gfp*	*N2*	*clec-94* gene over-expression in *N2* animals
JRS61	pPW02 *clec-208p(3.6kb)::clec-208 gDNA::SL2::gfp*	*N2*	*clec-208* gene over-expression in *N2* animals
JRS62	pPW03 *clec-263p(5.4kb)::clec-263 gDNA::SL2::gfp*	*N2*	*clec-263* gene over-expression in *N2* animals
JRS63	pPW01 *clec-94p(4.2kb)::clec-94 gDNA::SL2::gfp*	*nmur-1(ok1387)*	*clec-94* genomic rescue driven by *clec-94* promoter
JRS64	pPW02 *clec-208p(3.6kb)::clec-208 gDNA::SL2::gfp*	*nmur-1(ok1387)*	*clec-208* genomic rescue driven by *clec-208* promoter
JRS65	pPW03 *clec-263p(5.4kb)::clec-263 gDNA::SL2::gfp*	*nmur-1(ok1387)*	*clec-263* genomic rescue driven by *clec-263* promoter

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