File S1

SUPPLEMENTARY MATERIALS AND METHODS

RNA isolation and qRT-PCR

Prior to RNA isolation, wild type and *skpo-1* mutant animals were grown on *cdc-25.1* dsRNA expressing *E. coli* HT115 from the egg to L4 larval stage. L4 animals were then exposed to either *cdc-25.1* RNAi or *E. faecalis* OG1RF for 18 hours. The RNA was isolated using Trizol (Invitrogen) according to the manufacturer. RNA samples were treated with DNase I to eliminate contaminating DNA by the Turbo DNA free kit (Applied Biosystem) according to the manufacturer. qRT-PCR was performed as described (VAN DER HOEVEN *et al.* 2011). Primers used are listed in Table S6 (*act-1* served as the reference gene).

Bacterial Colonization

The CFU analysis was conducted in a manner similar to past work (GARSIN *et al.* 2001). Briefly, L4 wild type and *skpo-1* mutant animals grown on *E. coli* OP50 were exposed on 100 mm plates containing BHI-agar with gentamycin (10 μg/mL) seeded with 100 μl of *E. faecalis* OG1RF for either 12 or 36 hours at 25˚ C. Worms were washed 3 times with M9 buffer at 1.4 rpm. Worms were then washed twice with 25 mM tetramisole hydrochloride to prevent ingestion of the antibiotic treatment. Worms were incubated at room temperature for 60 minutes in 25 mM tetramisole hydrochloride supplemented with ampicillin and kanamycin, both at 1 mg/mL, to kill surface-attached *E. faecalis.* Worms were collected at 1.4 rpm and washed twice with 25 mM tetramisole hydrochloride prior to grinding. Ten worms in 10 μl were transferred to 200 μl of M9 and ground for 1 minute using a motorized pestle (Kontes cordless cat# K749540-0000 and pestles cat# K749521-1590). Serial dilutions were performed and 100 μl of each dilution plated onto 100 mm BHI gentamycin 10 μg/mL plates for 24 hours at 37˚ C.

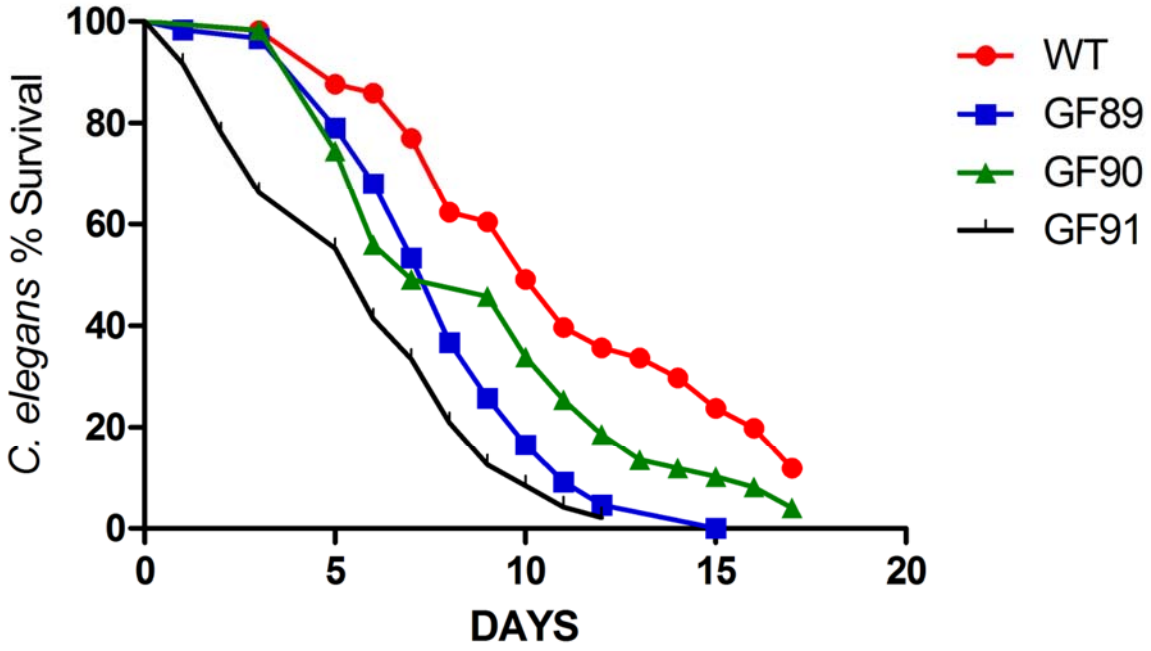

Figure S1 Survival of *cdc-25.1* RNAi-treated *skpo-1* animal lines on *E. faecalis.* Wild type and *skpo-1* worms were grown on *cdc-25.1* dsRNA expressing *E. coli* HT115 from L1 through L4 larval stage prior to exposure to *E. faecalis* OG1RF. The *skpo-1* worms (GF89, GF90 and GF91) were more susceptible to *E. faecalis* relative to wild type (*P*-values < 0.0001, 0.0110, and < 0.0001, respectively).

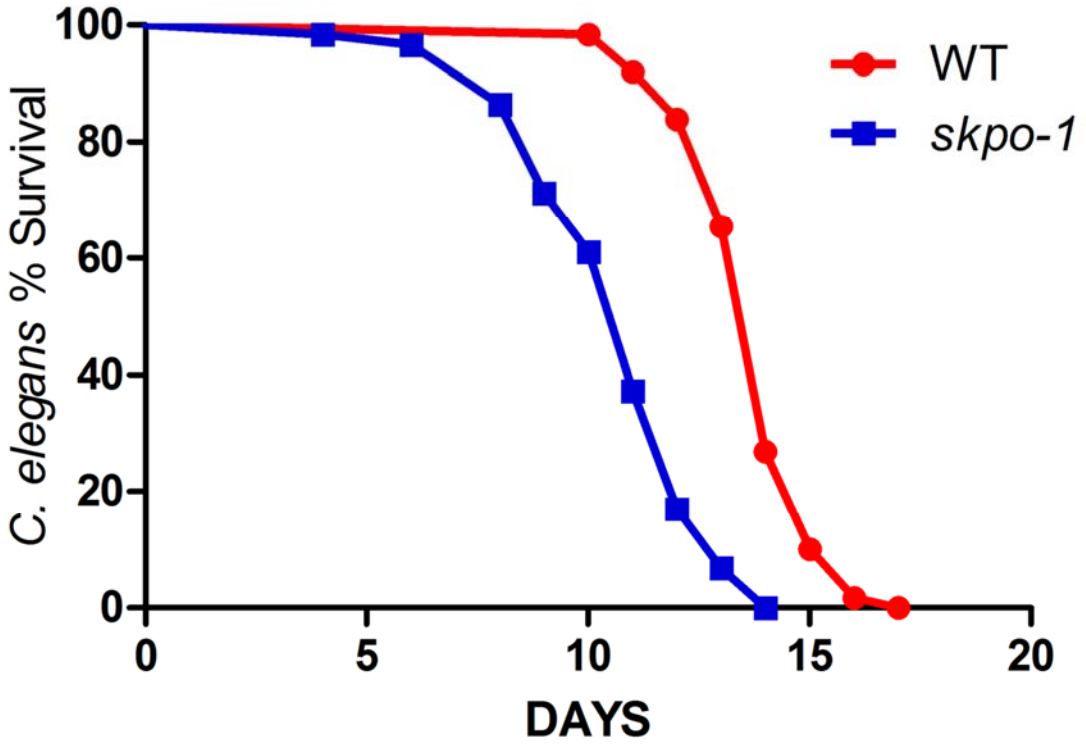

Figure S2 Lifespan defect of *skpo-1* mutants on *E. coli* OP50 without prior *cdc-25.1* exposure. Wild type and *skpo-1* worms were grown on *E. coli* OP50 from L1 through L4 larval stage prior to the longevity assay in which they were transferred to NGM plates with FUDR (100 µg/ml) seeded with concentrated (20X) *E. coli* OP50. The average lifespan of the *skpo-1* worms was significantly shorter than their wild type, counterparts (*P*-value < 0.0001).

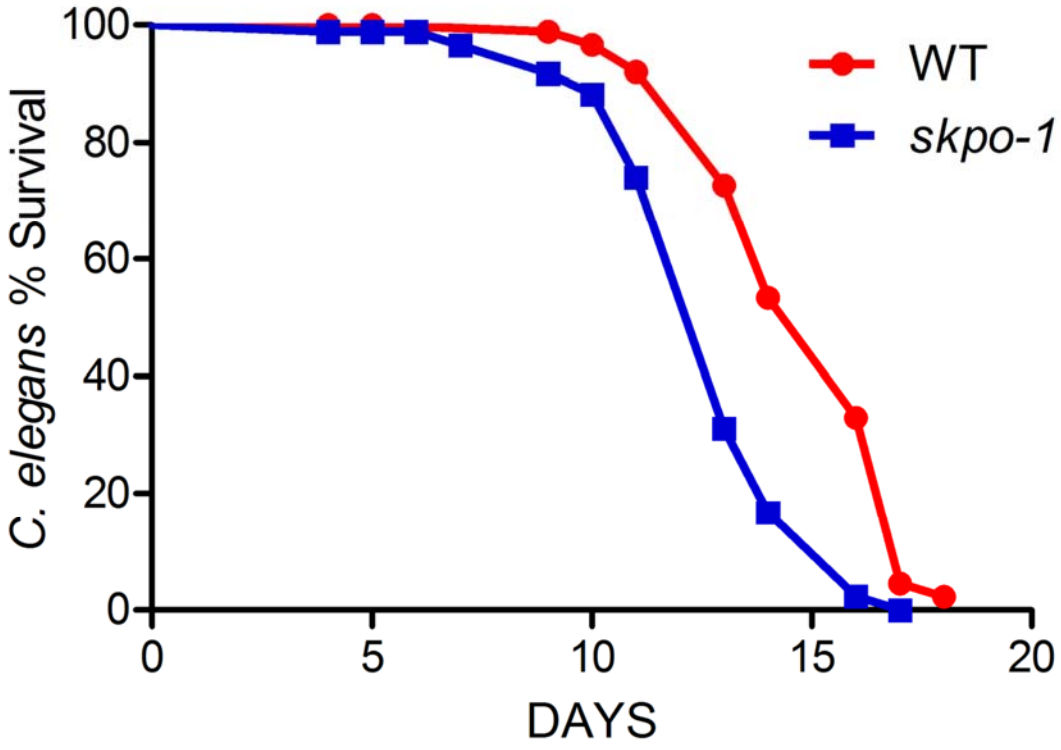

Figure S3 *skpo-1* mutants display a lifespan defect on heat-killed *E. coli* OP50. Wild type and *skpo-1* worms were grown on *cdc-25.1* dsRNA expressing *E. coli* HT115 from L1 through L4 larval stage prior to the longevity assay. The worms were transferred to NGM plates like in Figure S2; however, the *E. coli* OP50 had been heat-killed. We observed that the *skpo-1* worms displayed a longevity defect on heat-killed OP50, relative to wild type worms (*P*-value <0.0001).

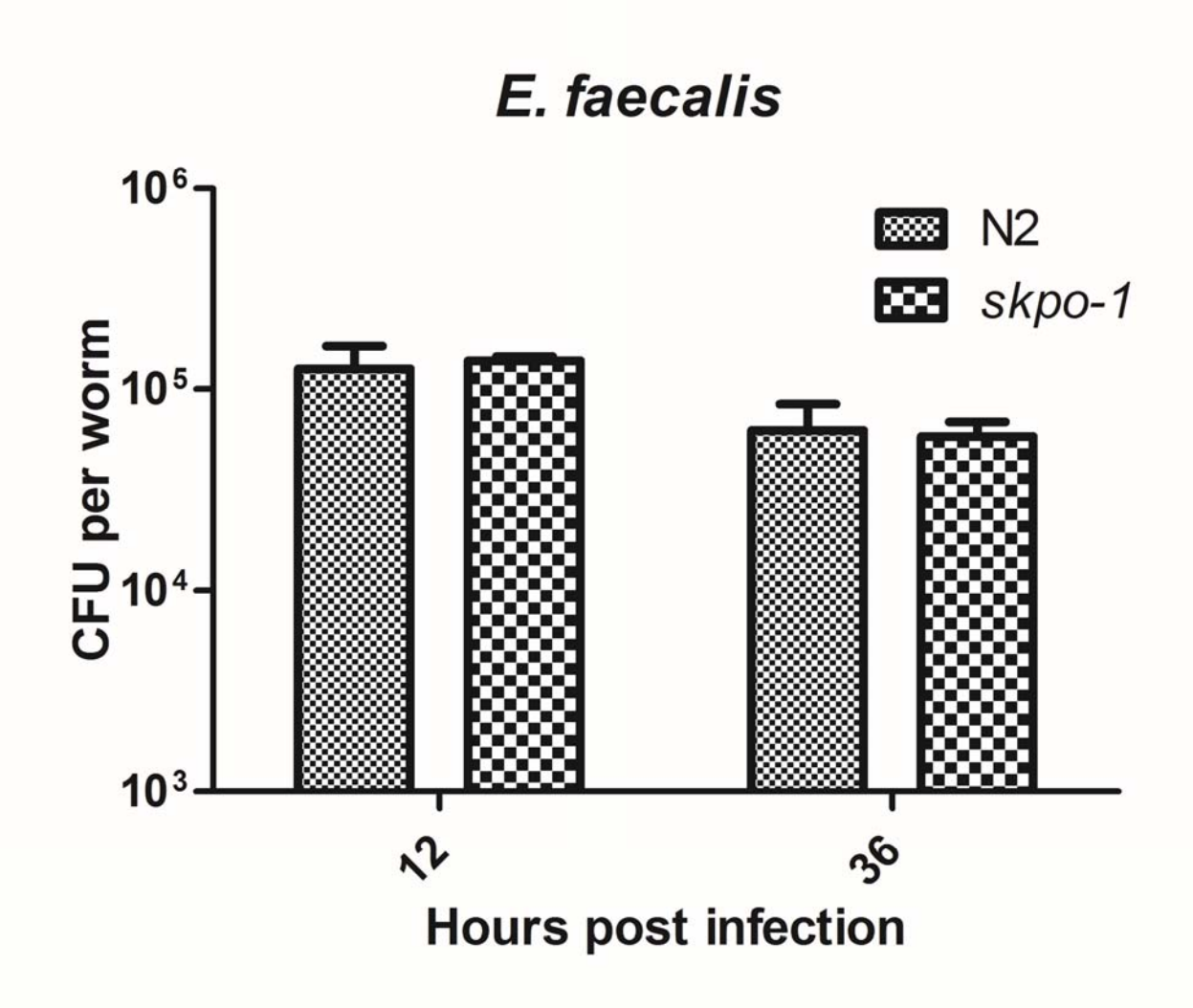

Figure S4 The *skpo-1* mutant does not have increased intestinal bacterial load during *E. faecalis* infection. L4 wild type and *skpo-1* mutant animals were exposed to *E. faecalis* at 25˚ C for 12 or 36 hours, respectively. CFU values for both wild type and *skpo-1* animals indicated that there is no significant difference in intestinal colonization by *E. faecalis*. Three biological replicates for each strain were used per time point and the experiment repeated twice—*i.e* a total n = 60 per strain. *P*-values were 0.3913 and 0.3592 for 12 and 36 hours, respectively.

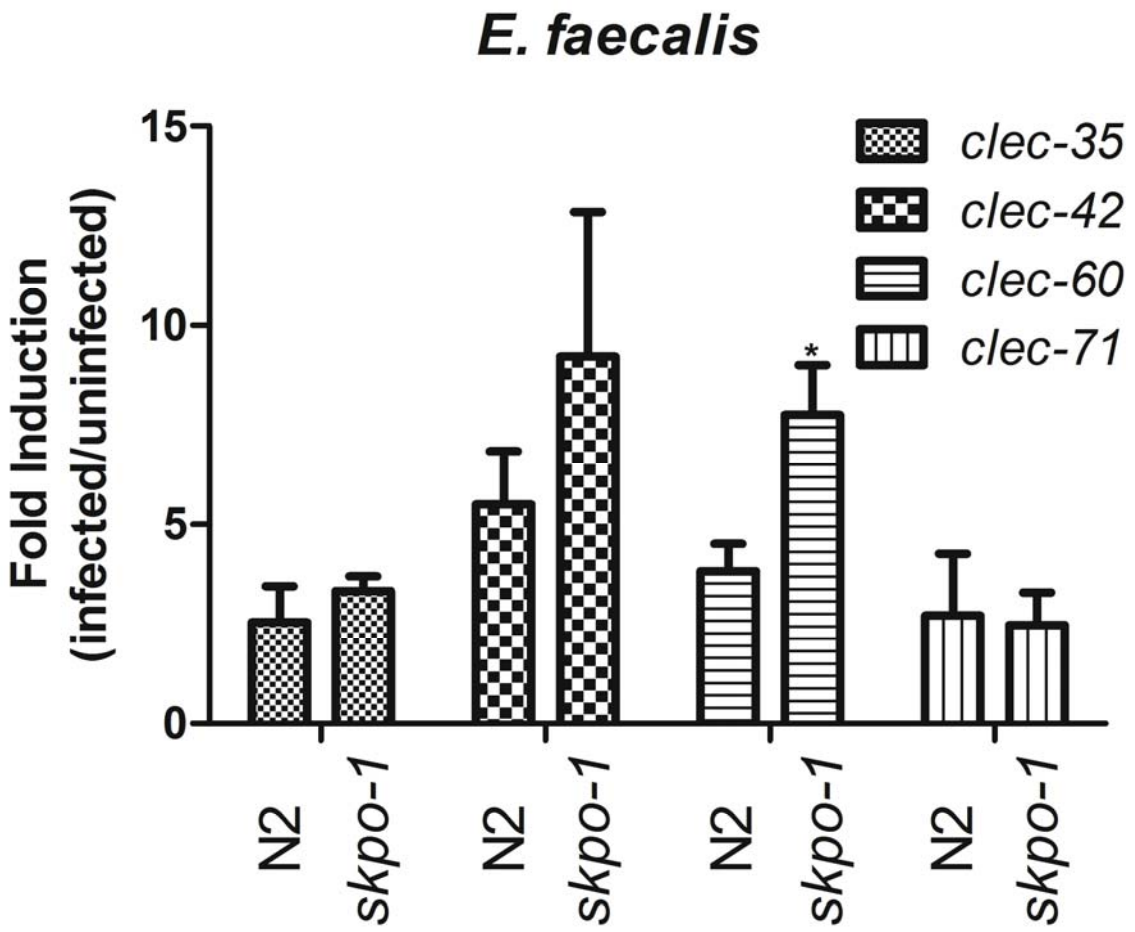

Figure S5 *clec-60* expression is elevated in *skpo-1* mutant animals, relative to wild type, in response to *E. faecalis* infection. L4 wild type and *skpo-1* mutant animals were exposed to *E. faecalis* for 18 hours at 25˚ C. Three technical replicates per strain per gene were assayed for relative expression of innate immune response genes and the experiment was repeated, independently, twice. These genes were previously found to display increased expression in wild type animals in response to *E. faecalis* (ENGELMANN *et al.* 2011). Of the genes surveyed, *clec-60* was the only gene to display a significant increase in expression during infection, relative to *act-1*. *P*-values were 0.1912, 0.1768, 0.04432, and 0.4684 for *clec-35, 42, 60* and *71*, respectively.

Table S1 Median survival and *P*-values of *E. faecalis* OG1RF killing assays

Figure number	Experiment number	Strain, exposure conditions during development	Median survival (days)	*P*-value
1A	1	wild type, VC RNAi	8	C
		wild type, *skpo-1* RNAi	7	=0.0404
	2	wild type, VC RNAi	7	C
		wild type, *skpo-1* RNAi	6	=0.0331
	3	*eri-1*, VC RNAi	8	C
		eri-1, skpo-1 RNAi	7	=0.001
	4	*eri-1*, VC RNAi	7	C
		eri-1, skpo-1 RNAi	5	=0.0053
	5	*eri-1*, VC RNAi	9	C
		eri-1, skpo-1 RNAi	8	=0.0037
1C	1	wild type, *cdc-25.1* RNAi	10	C
		skpo-1, *cdc-25.1* RNAi	6	<0.0001
	2	wild type, *cdc-25.1* RNAi	10	C
		skpo-1, *cdc-25.1* RNAi	8	<0.0001
	3	wild type, *cdc-25.1* RNAi	13	C
		skpo-1, *cdc-25.1* RNAi	8	=0.0016
	4	wild type, *cdc-25.1* RNAi	13	C
		skpo-1, *cdc-25.1* RNAi	8	=0.0005
	5	wild type, *cdc-25.1* RNAi	13	C
		skpo-1, *cdc-25.1* RNAi	7	<0.0001
4A	1	*wrt-2*, VC RNAi	5	C
		wrt-2, *skpo-1* RNAi	4	=0.0002
	2	*wrt-2*, VC RNAi	4	C
		wrt-2, *skpo-1* RNAi	3	=0.0176
	3	*wrt-2*, VC RNAi	5	C
		wrt-2, *skpo-1* RNAi	4	=0.0376
	4	*wrt-2*, VC RNAi	4	C
		wrt-2, *skpo-1* RNAi	4	=0.0058
4C	1	*vha-6*, VC RNAi	11	C
		vha-6, *skpo-1* RNAi	11	=0.6379
	2	*vha-6*, VC RNAi	11	C
		vha-6, *skpo-1* RNAi	11	=0.735
S1	1	wild type, OP50	8	C
		*skpo-1**, OP50	3	<0.0001
	2	wild type, OP50	8	C
		*skpo-1**, OP50	3	<0.0001

C = control, *Data only shown for GF89 *skpo-1* strain.

Table S2 Median survival and *P*-values of *E. coli* OP50 longevity assays

Figure number	Experiment number	Strain, exposure conditions during development	Median survival (Days)	*P*-value
1B	1	*eri-1*, VC RNAi	10	C
		eri-1, *skpo-1* RNAi	10	=0.3772
	2	*eri-1*, VC RNAi	10	C
		eri-1, *skpo-1* RNAi	10	=0.8948
1D	1	wild type, *cdc-25.1* RNAi	10	C
		skpo-1, *cdc-25.1* RNAi	8	<0.0001
	2	wild type, *cdc-25.1* RNAi	14	C
		skpo-1, *cdc-25.1* RNAi	11	<0.0001
	3	wild type, *cdc-25.1* RNAi	14	C
		skpo-1, *cdc-25.1* RNAi	11	<0.0001
4B	1	*vha-6*, VC RNAi	10	C
		vha-6, *skpo-1* RNAi	10	=0.9997
	2	*vha-6*, VC RNAi	11	C
		vha-6, *skpo-1* RNAi	10	=0.4484
4D	1	*wrt-2*, VC RNAi	11	C
		wrt-2, *skpo-1* RNAi	11	=0.6379
	2	*wrt-2*, VC RNAi	11	C
		wrt-2, *skpo-1* RNAi	11	=0.7235
S2	1	wild type, OP50	14	C
		skpo-1, OP50	11	<0.0001
	2	wild type, OP50	14	C
		skpo-1, OP50	11	<0.0001
S3*	1	N2, *cdc-25.1* RNAi*	16	C
		skpo-1, *cdc-25.1* RNAi*	13	<0.0001
	2	N2, *cdc-25.1* RNAi*	14	C
		skpo-1, *cdc-25.1* RNAi*	12	<0.0001

C = control, *Assayed on heat-killed OP50

Table S3 Data for Relative Mortality Calculation

Figure number	Experiment number	Strain	Relative mortality	*P*-value
1F	Average of	N2	1	
	experiments 1-4		1	
			1	
			1	
			Avg = 1	C
		skpo-1	1.312	
			1.452	
			1.654	
			1.253	
			Avg = 1.418	=0.0091

C = control

Table S4 Median survival and *P*-values of *P. aeruginosa* PA14 killing assays

Figure Number	Experiment number	Strain, exposure conditions during development	Median survival (Hours)	*P*-value
1E	1	N2, *cdc-25.1* RNAi	91	C
		skpo-1, *cdc-25.1* RNAi	88	=0.3783
	2	N2, *cdc-25.1* RNAi	89	C
		skpo-1, *cdc-25.1* RNAi	69	=0.0639
	3	N2, *cdc-25.1* RNAi	72	C
		skpo-1, *cdc-25.1* RNAi	72	=0.2613

Table S5 Primers for cDNA inserts ligated into pL4440 construct for dsRNA production in *E. coli*

Oligo name	Sequence
F32A52.A 5F	5’ TTG CGG CCG CAA TTC ACC AGG GAA TTT ACA CC 3’
F32A52.A 3R	5’ CCT CGA GGG TGG TCT TTT CAC ATT CTG G 3’
F32A52.B 5F	5’ TTG CGG CCG CAA CAA CCA CTC ATT TCA CCA GG 3’
F32A52.B 3R	5’ CCT CGA GGC TGT AGT TGT TAC TCT TTG TGG 3’
pxn-1 5F	5’ TTG CGG CCG CAA GGA CTC TGG AAG GTA CAC 3’
pxn-1 3R	5’ CCT CGA GGA TAC TTG GCA CTT CCA CTC T 3’

Table S6 qRT-PCR primers for assessing expression of innate immune response genes in response to *E. faecalis*

Oligo name	Sequence
clec-35 5F	5’ AGA TGC TGG ACA GTG GAA AAG 3’
clec-35 3R	5’ GTG CGG AGT ATT GTA GCG TAG 3’
clec-42 5F	5’ GTA ACT CCG TAT TGG CTG GG 3’
clec-42 3R	5’ GTA AAC GCA GCT TCC AAT CTC 3’
clec-60 5F	5’ TGT AAG AGA ACA GTT GGA ACC C 3’
clec-60 3R	5’ TAT GTG CAT GGG TAC TGA TCG 3’
clec-71 5F	5’ ACG ACA GGA AGT GAT GTA TTG G 3’
clec-71 3R	5’ TTG ACG GAC TTT AGC CAC TG 3’
act-1 5F	5’ ACC ATG TAC CCA GGA ATT GC 3’
act-1 3R	5’ TGG AAG GTG GAG AGG GAA G 3’

SUPPLEMENTARY LITERATURE CITED

Engelmann, I., A. Griffon, L. Tichit, F. Montanana-Sanchis, G. Wang *et al.*, 2011 A comprehensive analysis of gene expression changes provoked by bacterial and fungal infection in C. elegans. PLoS One 6**:** e19055.

Garsin, D. A., C. D. Sifri, E. Mylonakis, X. Qin, K. V. Singh *et al.*, 2001 A simple model host for identifying Gram-positive virulence factors. Proceedings of the National Academy of Sciences of the United States of America 98**:** 10892-10897.

van der Hoeven, R., K. C. McCallum, M. R. Cruz and D. A. Garsin, 2011 Ce-Duox1/BLI-3 Generated Reactive Oxygen Species Trigger Protective SKN-1 Activity via p38 MAPK Signaling during Infection in C. elegans. PLoS Pathog 7**:** e1002453.