Supplemental Material

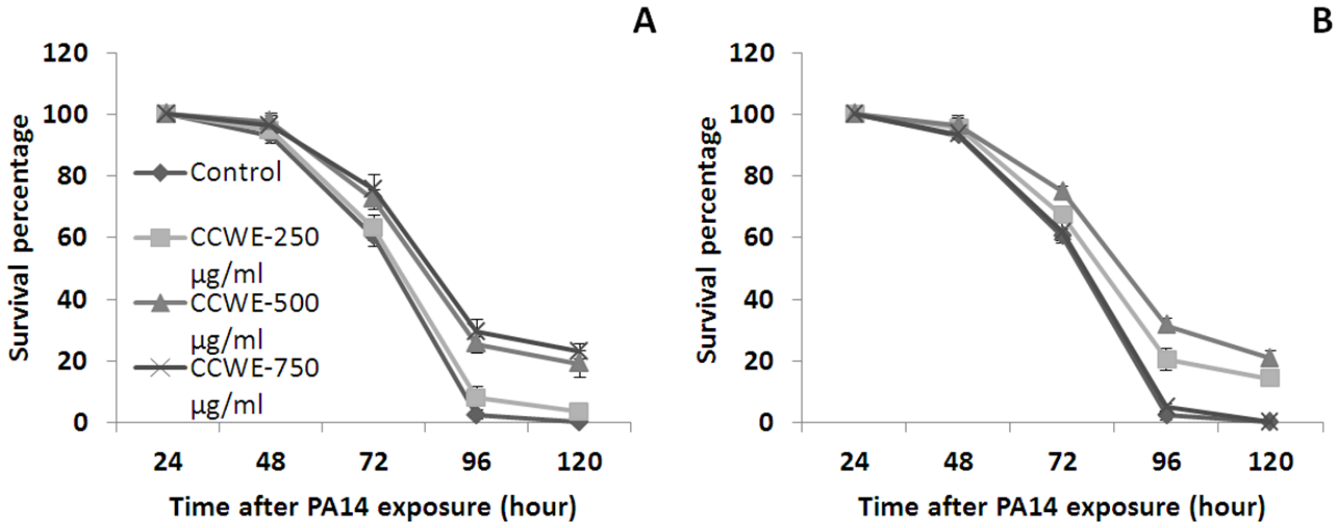

Fig. S1. Effect of CCWE on PA14 killing of C. elegans. Three concentrations of CCWE, 250 µg/ml, 500 µg/ml, and 750 µg/ml, were tested in comparison of water as a negative control. **A.** Wild type N2 worms grown with CCWE as a food supplement were exposed to PA14 lawn. **B.** Worms raised without food supplement were exposed to PA14 cultured in the presence of CCWE. The experiments were repeated three times. Survival curves were generated with data from the sum of three biological replicates. N=60-90 for each treatment. Data are presented as mean ± SD. See Tab.S1 for statistical analysis. CCWE, *Chondrus crispus* water extract; PA14, *Pseudomonas aeruginosa* strain PA14.

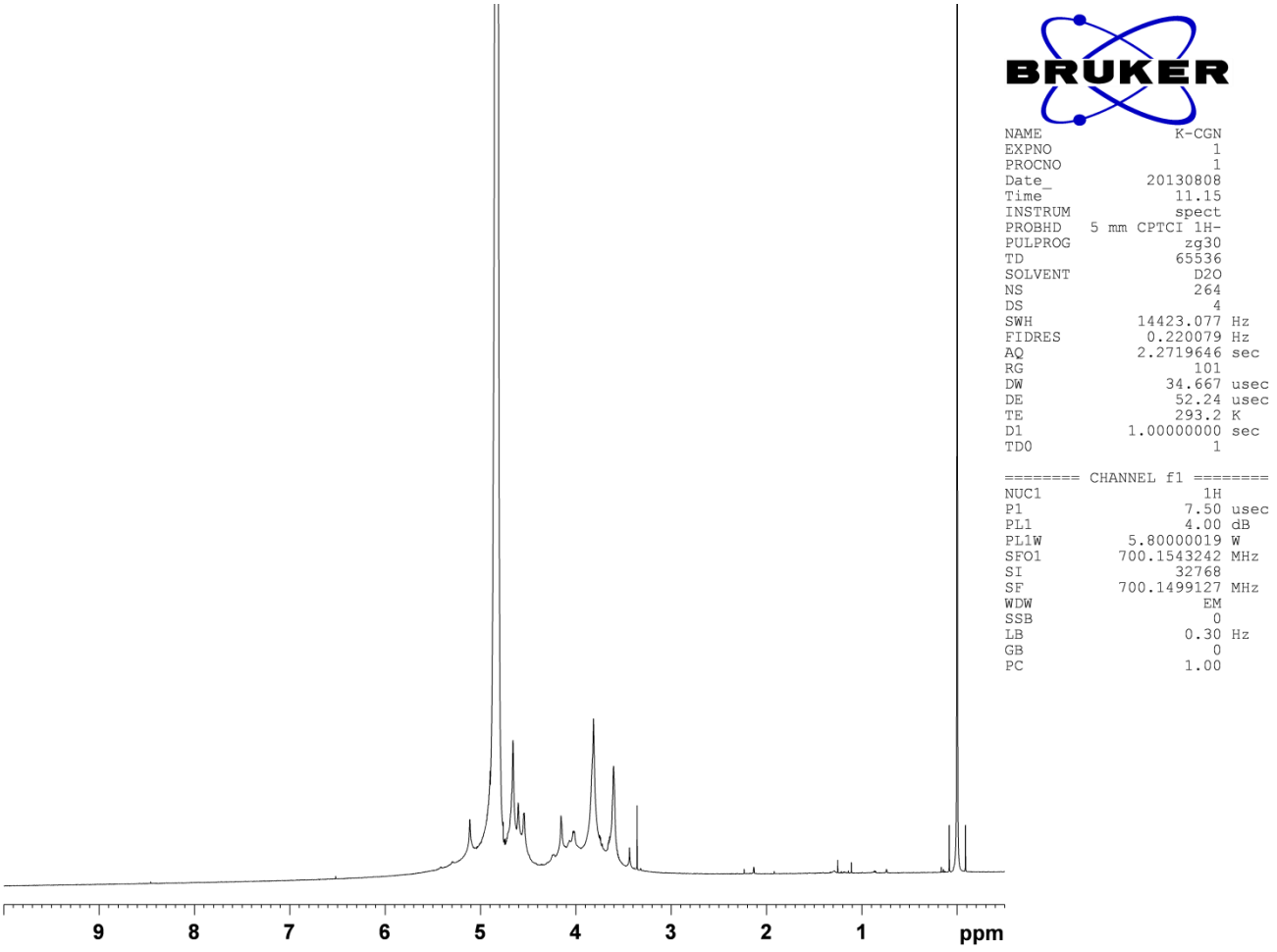

Fig. S2. Quality control of the K-CGN sample by NMR analysis. The NMR spectra were measured on a Bruker 700 MHz spectrometer with deuterated water. The proton NMR spectrum of the sample was compared to previously reported data of K-CGN (1). K-CGN denotes kappa carrageenan.

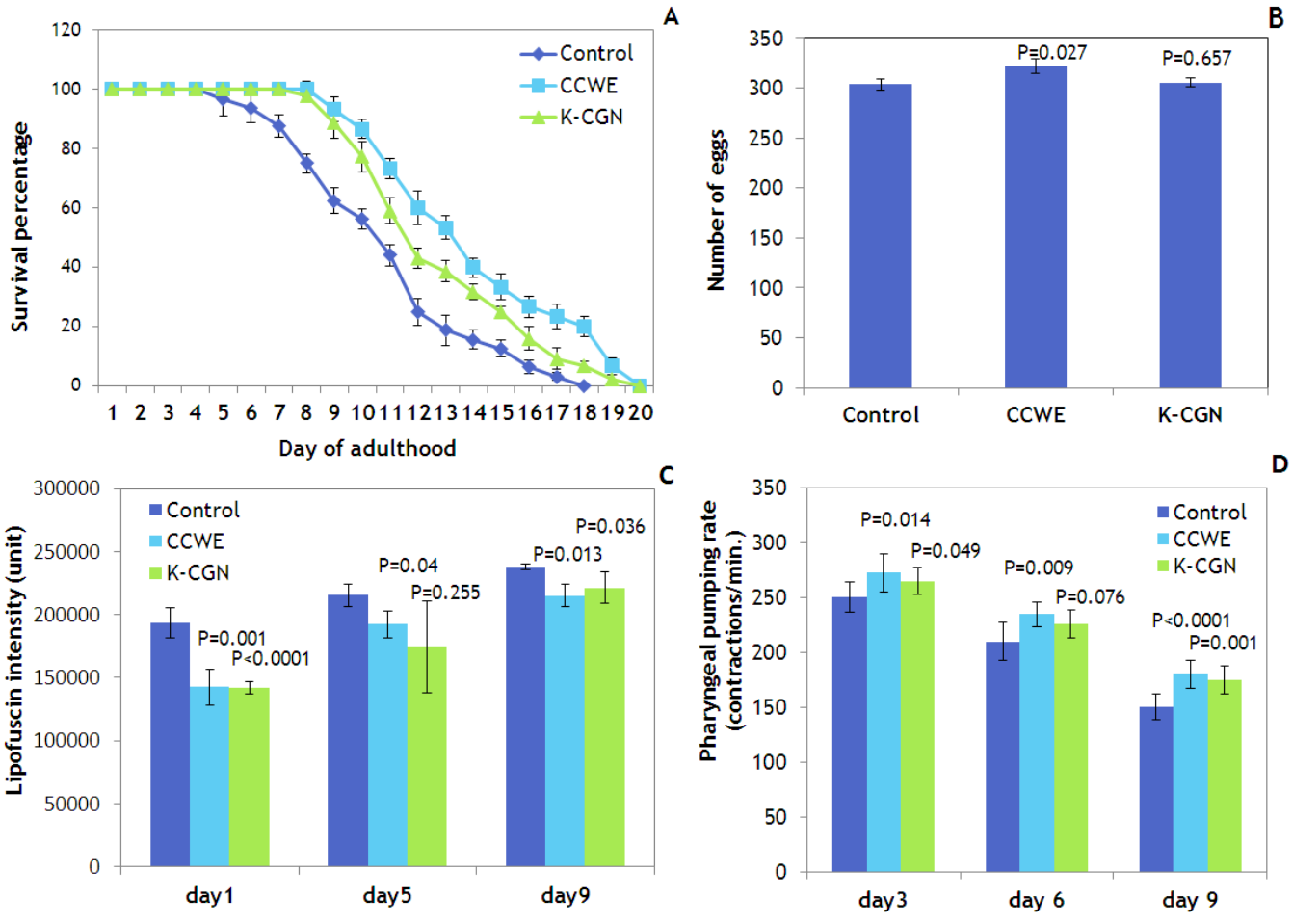

Fig. S3. Effect of CCWE and K-CGN on the health of *C. elegans*. The experiments were performed with synchronized wild type N2 worms which were raised at 20 °C with (treatment) or without (control) CCWE or K-CGN in the food source from the egg stage. Concentrations of CCWE and K-CGN were 500 μg/ml and 200 μg/ml, respectively. **A.** CCWE and K-CGN extended the lifespan. Adult worms (N=87 for CCWE; N=79 for K-CGN; N=92 for control) were transferred to fresh treatment plates, scored daily for survival, and transferred to fresh treatment plates every two days during their reproductive period. Thereafter, worms were transferred to fresh treatment plates as needed to ensure sufficient food source. CCWE vs. control, p=0.001; K-CGN vs. control, p=0.037 (log-rank test). **B.** Effect on the productivity of *C. elegans*. At late L4 stage, two worms were transferred to a fresh treatment plate (n=6 for each treatment). During the reproductive period, eggs were counted daily under a dissection microscope at 8 x magnification after the hermaphrodites being transferred to new treatment

plates. **C.** Effect on pharyngeal pumping rate. On days 3, 6, and 9 of adulthood, worms were observed under a microscope at 100 x magnification for pharyngeal pumping at room temperature. N=18-30 for each treatment. **D.** Effect on lipofuscin accumulation. Age-matched N2 worms were transferred to fresh treatment plates every 3 days. Fluorescence image of lipofuscin were taken on days 1, 5, and 9 of adulthood on a Leica DMIRB microscope at 100 x magnification with blue excitation light. N=18-30 for each treatment. The intensity of lipofuscin was quantified using ImageJ. All experiments were repeated three times. Data are presented as mean ± SD. P values are for treatment vs. control. CCWE, *Chondrus crispus* water extract; K-CGN, kappa carrageenan.

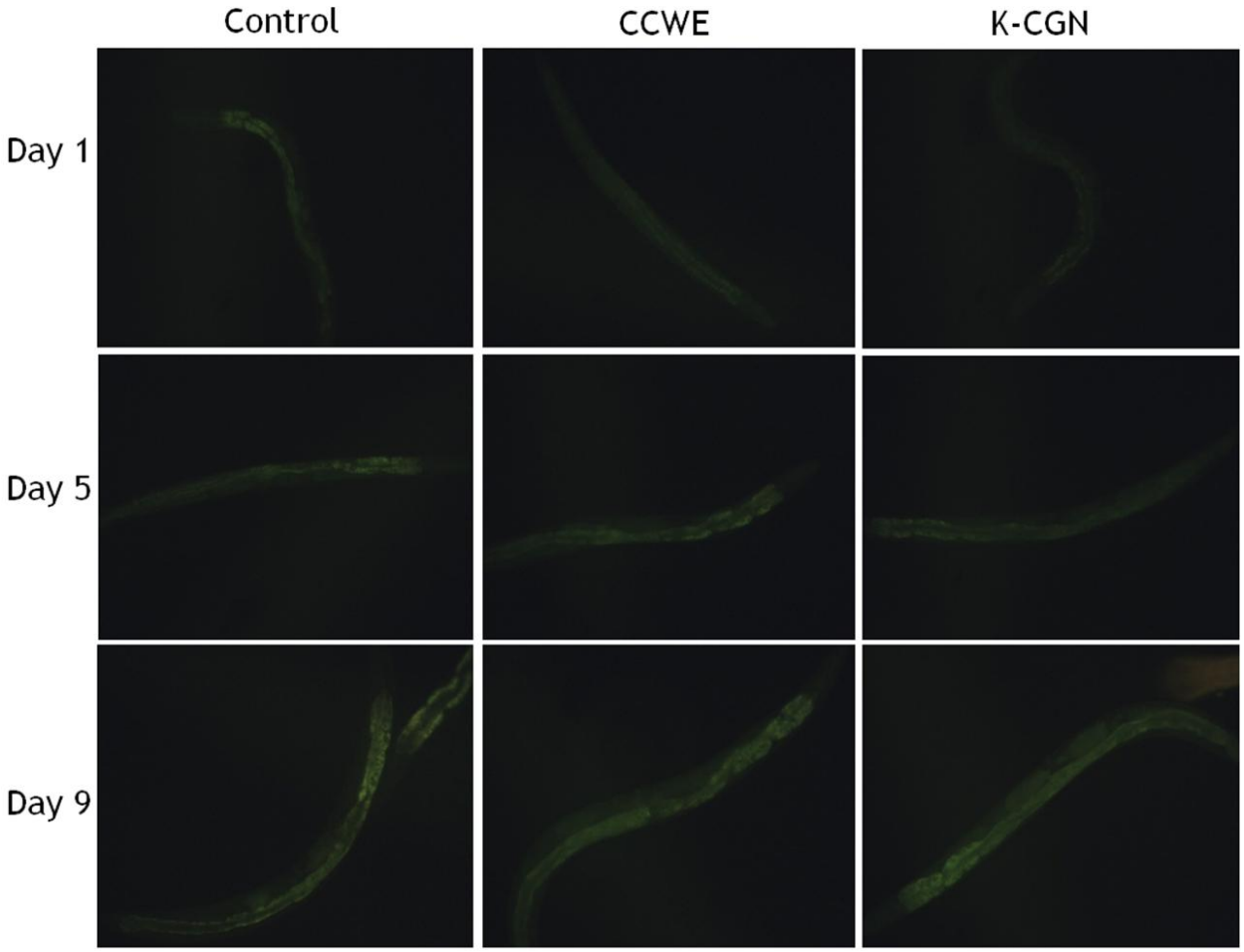

Fig. S4. Representative images of the aging pigment lipofuscin in *C. elegans*. Age-matched wild type N2 worms were raised with or without (for control) CCWE or K-CGN in their food source, and transferred to fresh treatment plates every 3 days. Fluorescence images were taken on days 1, 5, and 9 of adulthood

on a Leica DMIRB microscope at 100 x magnification with blue excitation light. N=18-30 for each treatment. The experiment was repeated three times. CCWE, *Chondrus crispus* water extract; K-CGN, kappa carrageenan.

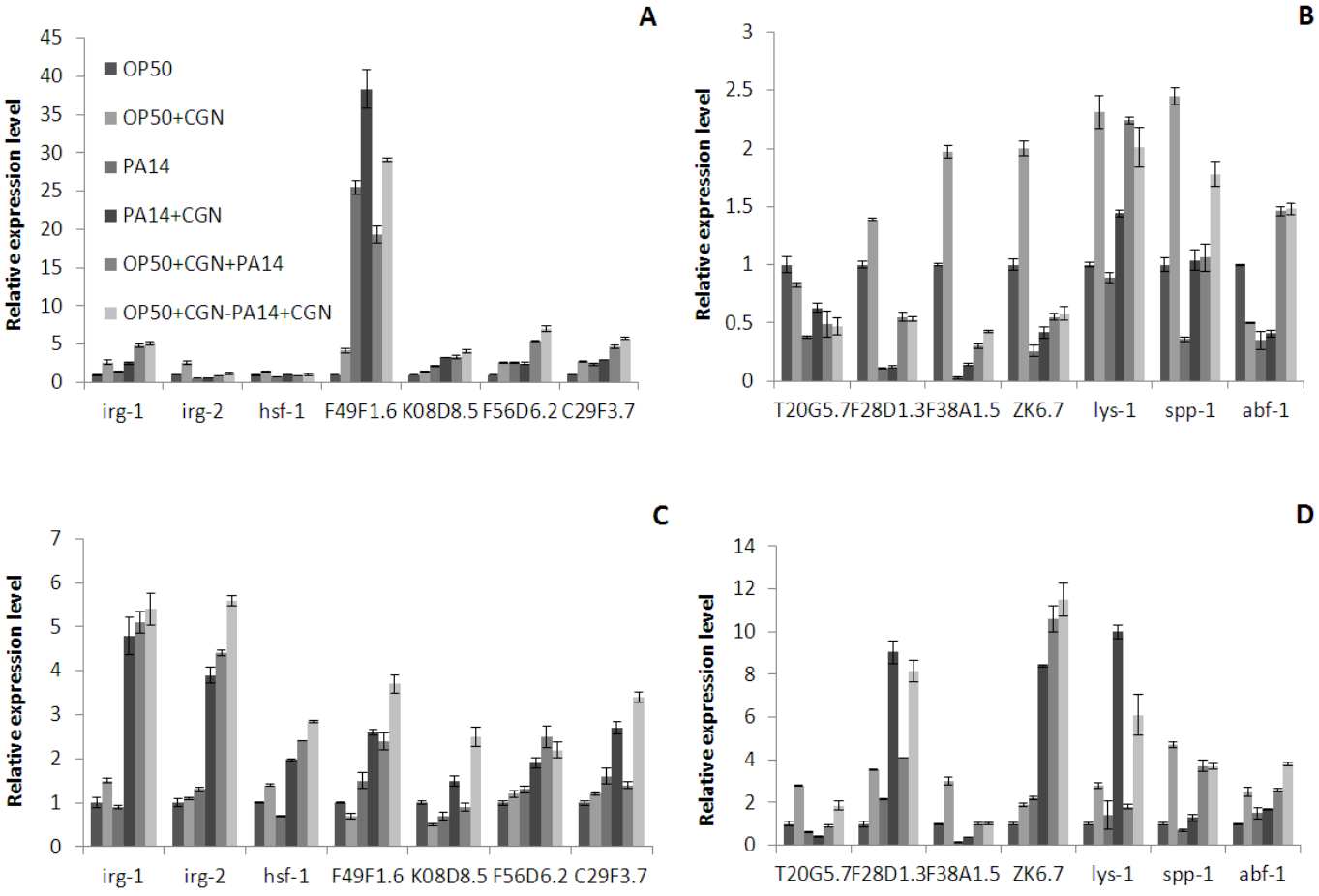

Fig. S5. Effect of K-CGN on expression of immune response genes. Expression of early response genes at 6 h- (A) or 24 h- (C) post exposure, or late response genes at 3 h- (B) or 48 h- (D) post exposure was analyzed by Q-PCR, with three biological replicates and three technical replicates. Data are presented as mean ± SD. N2 worms were grown with or without dietary supplementation of K-CGN (200 μg/ml) and expression of immune genes were analyzed by Q-PCR at 6 h or 24 h of adulthood without PA14 exposure, or at 6 h or 24 h post exposure to a PA14 lawn which was pre-established with or without K-CGN. Worms fed OP50 were used as a control for non-infection conditions and OP50-fed PA14-infected

worms as a control for infection conditions. OP50, worms fed *Escherichia coli* strain OP50, the laboratory standard food source; OP50+CGN, worms fed OP50 with K-CGN as food supplement; PA14, worms grown with OP50 and exposed to PA14; PA14+CGN, worms grown with OP50 and exposed to PA14 which was cultured in the presence of K-CGN; OP50+CGN+PA14, worms raised on OP50 with K-CGN as food supplement and exposed to PA14; OP50+CGN-PA14+CGN, worms raised on OP50 with K-CGN as food supplement were exposed to PA14 cultured in the presence of K-CGN. At 6 h adulthood under non-infection condition, dietary supplementation of CGN was associated with up-regulation of all early response genes (A) and all late response genes but *T20G5.7* and *abf-1*(B) ($p<0.05$, OP50 vs. OP50+CGN); while under infection conditions, at least one of the three CGN treatments resulted in up-regulation of three early response genes, *irg-1*, *F49F1.6*, and *F56D6.2* (A), as well as one of the late response genes, *F38A1.5* (B) ($p<0.05$, PA14 vs. PA14+CGN, OP50+CGN+PA14, or OP50+CGN-PA14+CGN). At 24 h adult hood, under non-infection condition, dietary supplementation of CGN was associated with up-regulation of all early response genes but *irg-2*, *F49F1.6*, and *K08D8.5* (C) and all late response genes (D) ($p<0.05$, OP50 vs. OP50+CGN); while under infection conditions, at least one of the three CGN treatments resulted in up-regulation of all early response genes (C) and all late response genes (D) ($p<0.05$, PA14 vs. PA14+CGN, OP50+CGN+PA14, or OP50+CGN-PA14+CGN). CCWE, *Chondrus crispus* water extract; K-CGN, kappa carrageenan; PA14, *Pseudomonas aeruginosa* strain PA14.

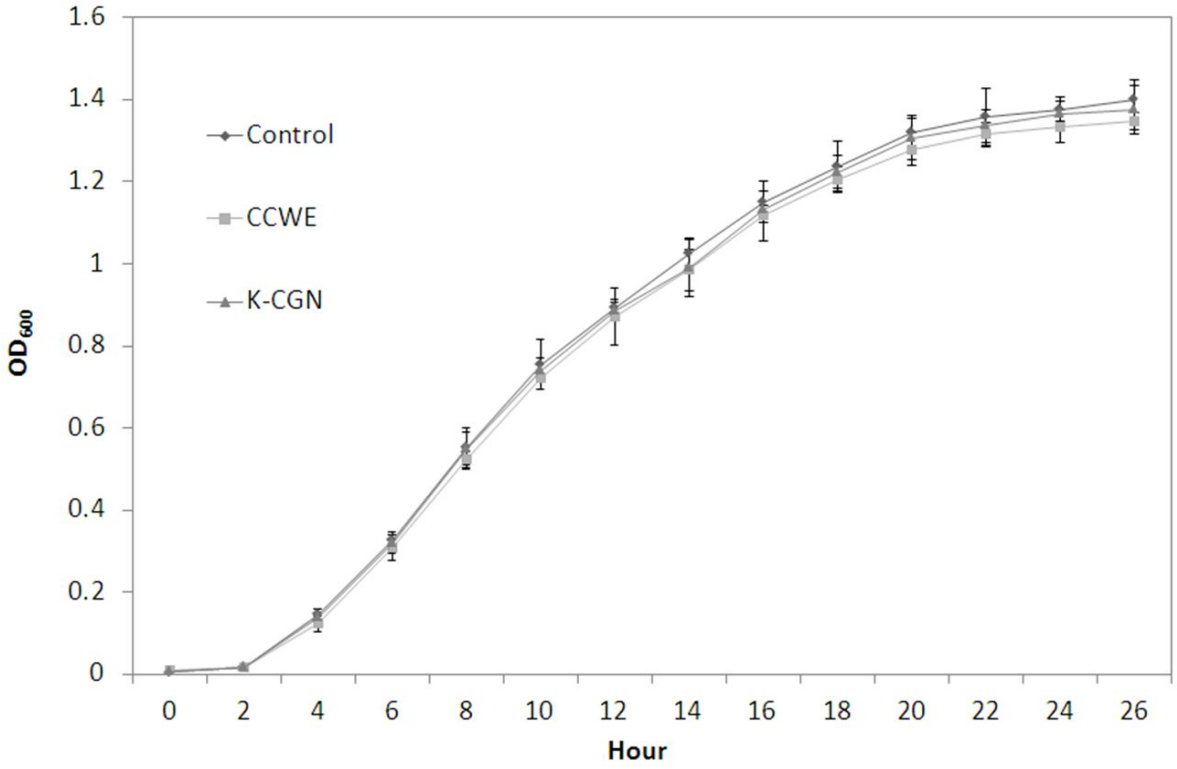

Fig. S6. CCWE and K-CGN did not abolish the growth of PA14. Fifty µl of a fresh overnight culture of PA14 was inoculated to 20 ml of freshly autoclaved LB media in the absence (control) or presence of 500 µg/ml of CCWE. The cultures were incubated at 37 °C with gentle shaking (150 rpm). Absorbance at 600 nm was measured every two hours till the stationary phase was reached. The experiment was performed with three biological replicates and three technical replicates. Mean ± SD is presented in the growth curves. P>0.05 (control vs. CCWE or control vs. K-CGN). CCWE, *Chondrus crispus* water extract; K-CGN, kappa carrageenan; PA14, *Pseudomonas aeruginosa* strain PA14.

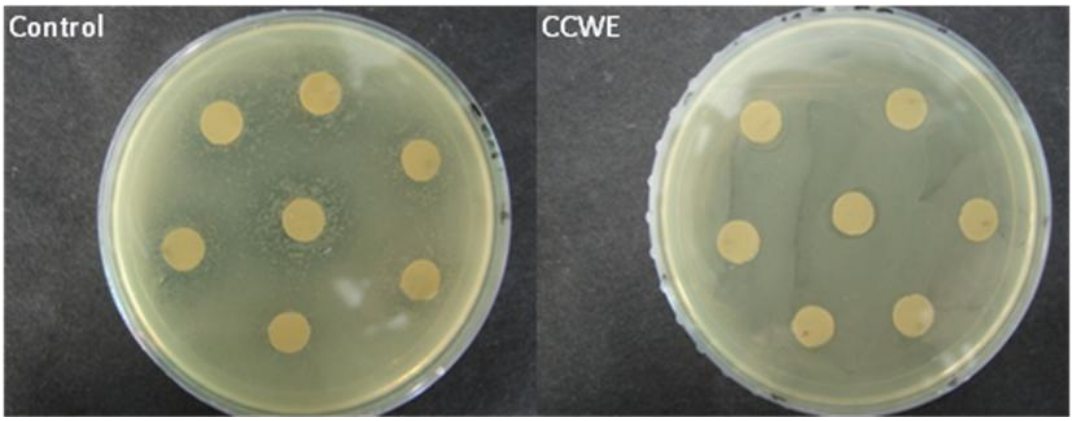

Fig. S7. CCWE exhibited no direct antimicrobial activity against PA14. Control, discs saturated with spectinomycin; CCWE, discs saturated with various concentrations of *Chondrus crispus* water extract. Photos were taken with the same settings of camera parameters. PA14, *Pseudomonas aeruginosa* strain PA14.

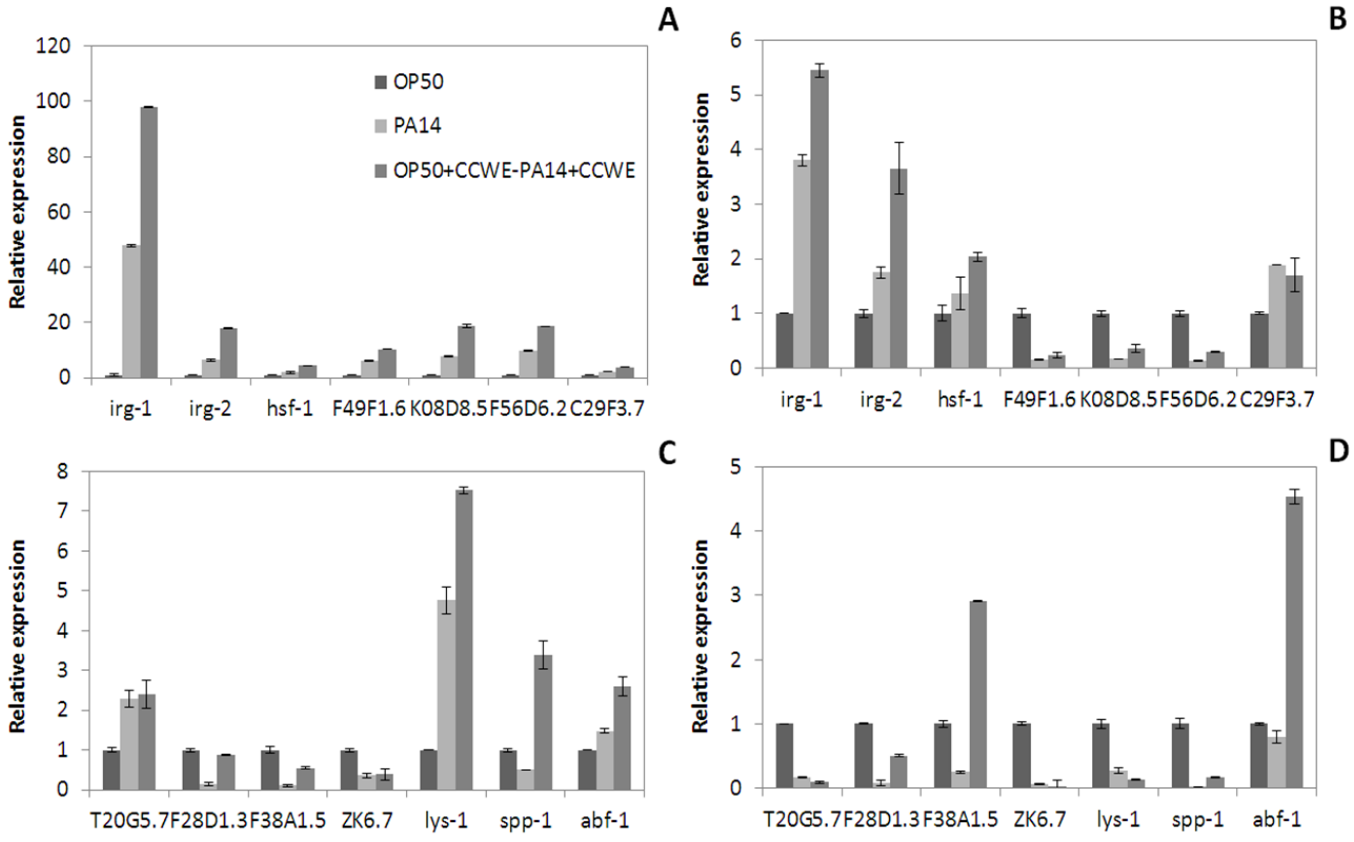

Fig. S8. CCWE modulated the expression of immune response genes in *C. elegans* against PA14 infection. Expression of early response genes at 3 h- (A) or 48 h- (B) post exposure, or late response

genes at 3 h- (C) or 48 h- (D) post exposure was analyzed by Q-PCR, with three biological replicates and two technical replicates. Data are presented as mean ± SD. P =0.06 (OP50 vs. PA14) for hsf-1; $p<0.05$ (OP50 vs. PA14) for irg-1, irg-2, T20G5.7, and lys-1; $p\leq0.01$ (OP50 vs. PA14) for other genes in panels S4A and S4C. P=0.62 or p=0.67 (PA14 vs. OP50+CCWE-PA14+CCWE) for TG205.7 or ZK6.7, respectively; $p<0.05$ (PA14 vs. OP50+CCWE-PA14+CCWE) for all other genes in panels S4A and S4C. P =0.15 and p=0.21(OP50 vs. PA14) for hsf-1 and abf-1, respectively; $p\leq0.01$ (OP50 vs. PA14) for other genes in panels S4B and S4D. P=0.065 or p=0.17 (PA14 vs. OP50+CCWE-PA14+CCWE) for KD08D8.5, and C29F3.7, respectively; $p<0.05$ (PA14 vs. OP50+CCWE-PA14+CCWE) for other genes in panels S4B and S4D. CCWE, *Chondrus crispus* water extract; PA14, *Pseudomonas aeruginosa* strain PA14.

Tab. S1. CCWE protected wild type *C. elegans* against PA14 infection.

Treatment	Total (N)	Censored (N)	Survival hours (Mean, SE)	P value vs. control (Log-rank)
Control	93	0	85.2, 1.66	
CCWE250-S1A	87	3	90.2, 1.75	0.0015
CCWE500-S1A	99	19	94.8, 1.90	<0.0001
CCWE750-S1A	105	24	96.2, 1.91	<0.0001
CCWE250-S1B	95	13	92.0, 1.96	0.002
CCWE500-S1B	90	19	96.5, 2.08	<0.0001
CCWE750-S1B	89	0	86.3. 1.75	0.618

CCWE250, 500, 750-S1A (or -S1B) denotes 250, 500, or 750 μg/ml of CCWE which corresponds to the survival data presented in Fig. S1A (or Fig. S1B). SE stands for standard error. CCWE, *Chondrus crispus* water extract. PA14, *Pseudomonas aeruginosa* strain PA14.

Tab. S2. Primer list.

No.	Primer label	Sequence (5'-3')
1	ZK6.7-F	CGAATTCCTCCCAAACAACT
2	ZK6.7-R	GAATAGGACGTTGTCGCAGA
3	lys-1-F	TTCGGATCTTTCAAGAAGGC
4	lys-1-R	TGGGATTCCAACAACGTAAA
5	spp-1-F	TGAACATCGGAACTCTTTGC
6	spp-1-R	TCAGCTCTTCCTCACACTCG
7	F28D1.3-F	AATCTGGATGCCTCGGATAC
8	F28D1.3-R	CATCTGAGCAGTTGCAGAGC
9	T20G5.7-F	ATGTTCTCCCTCAAGACCGT
10	T20G5.7-R	CGGAAGTGTAAACGACGAAG
11	abf-1-F	TGCCTTCTCCTTGTTCTCCT
12	abf-1-R	ATCCTCTGCATTACCGGAAC
13	F38A1.5-F	CTGGGCCGGTATTAATTTGT
14	F38A1.5-R	GTCTTCTTCGTCACGCACAT
15	ama-1-F	CTGACCCAAAGAACACGGTGA
16	ama-1-R	TCCAATTCGATCCGAAGAAGC
17	nhr_23_108F	GATTCTTGACACTGCGACGA
18	nhr_23_108R	TGAATTCGGTGAATCGTGTC
19	irg_1_96F	AAGCAGCATGCGTATTTTCA
20	irg_1_96R	GCAGCTTCTCCTTTTTCTCC
21	irg_2_119F	CAAGTTACTGGGCATCAGCA
22	irg_2_119R	TCACTATGTCCAACGCGAAA
23	F49F1.6_87F	TGCACTACTACATCCTGCCTATTC
24	F49F1.6_87R	CCGGACATGTGATCATTGAG
25	hsf_1_96F	CAGCCAACAGGGAATCAAAT
26	hsf_1_96R	TGCTGCTCCAGAAACTGAAA
27	K08D8.5_86F	TTACGATGGTGATTCCGTGA
28	K08D8.5_86R	GCTTGTTGCCAGTTGAGACA
29	F56D6.2_110F	GCCCGGACAGTAATGACAAG
30	F56D6.2_110R	GCCGACAGGATTCTGGTAGT
31	C29F3.7_86F	GATCGGCAACTTTACCTCCA
32	C29F3.7_86R	AATTGTGGCGGATATTCTGG
33	lasI-F	GCTCCTTGAACACTTGAGCA
34	lasI-R	GCGCGAAGAGTTCGATAAAA
35	lasR-F	CCGCCGAATATTTCCCATA
36	lasR-R	GATATCGGTTATCTGCAACTGCT
37	rhlI-F	GGAGCGCTATTTCGTTCG
38	rhlI-R	GTCTCGCCCTTGACCTTCT
39	rhlR-F	TGCGTTGCATGATCGAGT
40	rhlR-R	CGGGTTGGACATCAGCAT
41	hcnC-F	GCCTGGACAGTTGGTAGGC

42	hcnC-R	GAACAGAACCTATGACATCGTGA
43	aroE-F	TTCTTCGAGCAGGGCAAG
44	aroE-R	CAATTCGTCCACCAGACGAT
45	rpoN-F	ATACCTTCATGCGCAACCA
46	rpoN-R	GGCTCTGCAGGCTCTTGAT
47	sbe-F	CTCGTTGGTCTCCTCGAGTT
48	sbe-R	CCATCTACCAGCGTGAAGG
49	sodB-F	GTTCAAGGAAGAGTTCACCAAGA
50	sodB-R	GTCGGCCTTCTTCACCAG
51	16S rRNA-F	GATTAACGCTTGCACCCTTC
52	16S rRNA-R	TAAGCACCGGCTAACTTCGT
53	*nadB*-F	CTACCTGGACATCAGCCACA
54	*nadB*-R	GGTAATGTCGATGCCGAAGT
55	*rpl*U-F	CGCAGTGATTGTTACCGGTG
56	*rpl*U-R	CAACCGCAATGGGCGCTATTGC

Sequences for primers No. 1-16 and 33-52 were adapted from previously published work (2); sequences for No. 53-54 and No. 55-56 were from published work (3) and (4), respectively; primers No. 17-32 were designed using Primer3.

References

1. **Yu G, Guan H, Ioanoviciu AS, et al.** 2002. Structural studies on k-carrageenan derived oligosaccharides. Carbohydr Res. 337:433–440.

2. **Kandasamy S, Khan W, Evans F, Critchley AT, Prithiviraj B.** 2012. Tasco®: A product of *Ascophyllum nodosum* enhances immune response of *Caenorhabditis elegans* against *Pseudomonas aeruginosa* infection. Mar. Drugs **10**: 84-105.

3. **Tremblay J, Déziel E.** 2010. Gene expression in *Pseudomonas aeruginosa* swarming motility. BMC Genomics. 11:587 doi:10.1186/1471-2164-11-587.

4. **Kuchma SL, Brothers KM, George A. O'Toole et al.** 2007. BifA, a Cyclic-Di-GMP Phosphodiesterase, Inversely Regulates Biofilm Formation and Swarming Motility by *Pseudomonas aeruginosa* PA14. J. Bacteriol. 189(22): 8165-78.