Supplemental Information

Microbial colonization activates an immune fight-and-flight response via neuroendocrine signaling

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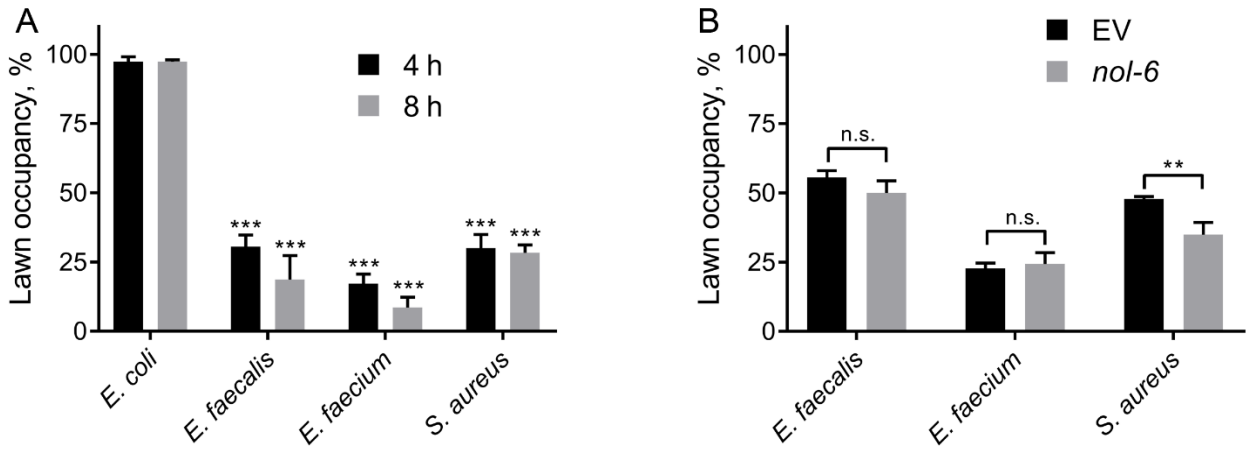

Figure S1. Microbial Pathogens Elicit Different Patterns of Avoidance in *C. elegans*, Related to Figures 1 and 2

(A) Occupancy of different bacterial lawns by wild-type N2 *C. elegans* at 4 hours and 8 hours after transfer to the lawns. p-values for *E. faecalis*, *E. faecium* and *S. aureus* are relative to *E. coli* for the corresponding time points. ***$p < 0.001$ via the t-test.

(B) Occupancy of different bacterial lawns by wild-type N2 animals grown on *nol-6* as well as RNAi control bacteria at 2 hours after transfer to the lawns. EV, empty vector RNAi control. **$p < 0.01$ via the t-test. n.s., non-significant.

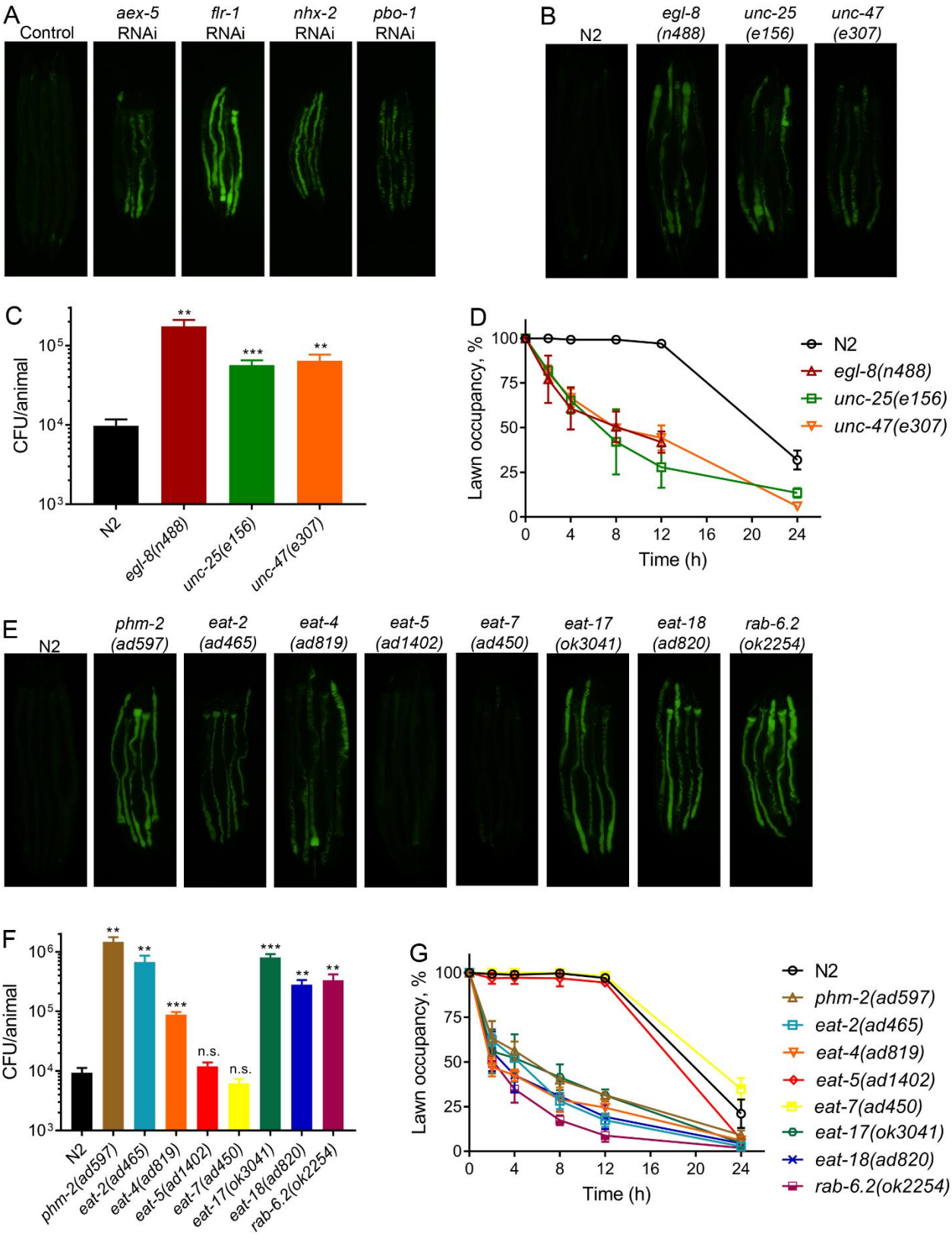
A
Control
aex-5 RNAi
flr-1 RNAi
nhx-2 RNAi
pbo-1 RNAi
B
N2
egl-8 (n488)
unc-25 (e156)
unc-47 (e307)
C
CFU/animal
N2
egl-8(n488)
unc-25(e156)
unc-47(e307)
D
Lawn occupancy, %
Time (h)
N2
egl-8(n488)
unc-25(e156)
unc-47(e307)
E
N2
phm-2 (ad597)
eat-2 (ad465)
eat-4 (ad819)
eat-5 (ad1402)
eat-7 (ad450)
eat-17 (ok3041)
eat-18 (ad820)
rab-6.2 (ok2254)
F
CFU/animal
n.s.
n.s.
N2
phm-2(ad597)
eat-2(ad465)
eat-4(ad819)
eat-5(ad1402)
eat-7(ad450)
eat-17(ok3041)
eat-18(ad820)
rab-6.2(ok2254)
G
Lawn occupancy, %
Time (h)
N2
phm-2(ad597)
eat-2(ad465)
eat-4(ad819)
eat-5(ad1402)
eat-7(ad450)
eat-17(ok3041)
eat-18(ad820)
rab-6.2(ok2254)

Figure S2. *P. aeruginosa* Colonization of the Intestine Induces Pathogen Avoidance, Related to Figure 3

(A) Representative photomicrographs of animals incubated on *P. aeruginosa*-GFP for 5 hours after growth on RNAi bacteria.

(B) Representative photomicrographs of young hermaphroditic adult N2, *egl-8(n488)*, *unc-25(e156)*, and *unc-47(e307)* animals incubated on *P. aeruginosa*-GFP for 5 hours at 25^{o}C.

(C) Colony forming units per animal for young hermaphroditic adult N2, *egl-8(n488)*, *unc-25(e156)*, and *unc-47(e307)* animals exposed to *P. aeruginosa*-GFP for 5 hours at 25^{o}C. ***$p < 0.001$, and **$p < 0.01$ via the t-test.

(D) Percent occupancy of *P. aeruginosa* lawns over time by N2, *egl-8(n488)*, *unc-25(e156)*, and *unc-47(e307)* animals.

(E) Representative photomicrographs of young hermaphroditic adult N2, *phm-2(ad597)*, *eat-2(ad465)*, *eat-4(ad819)*, *eat-5(ad1402)*, *eat-7(ad450)*, *eat-17(ok3041)*, *eat-18(ad820)*, and *rab-6.2(ok2254)* animals incubated on *P. aeruginosa*-GFP for 5 hours at 25^{o}C.

(F) Colony forming units per animal for young hermaphroditic adult N2, *phm-2(ad597)*, *eat-2(ad465)*, *eat-4(ad819)*, *eat-5(ad1402)*, *eat-7(ad450)*, *eat-17(ok3041)*, *eat-18(ad820)*, and *rab-6.2(ok2254)* animals exposed to *P. aeruginosa*-GFP for 5 hours at 25^{o}C. ***$p < 0.001$, and **$p < 0.01$ via the t-test. n.s., non-significant.

(G) Percent occupancy of *P. aeruginosa* lawns over time by N2, *phm-2(ad597)*, *eat-2(ad465)*, *eat-4(ad819)*, *eat-5(ad1402)*, *eat-7(ad450)*, *eat-17(ok3041)*, *eat-18(ad820)*, and *rab-6.2(ok2254)* animals.

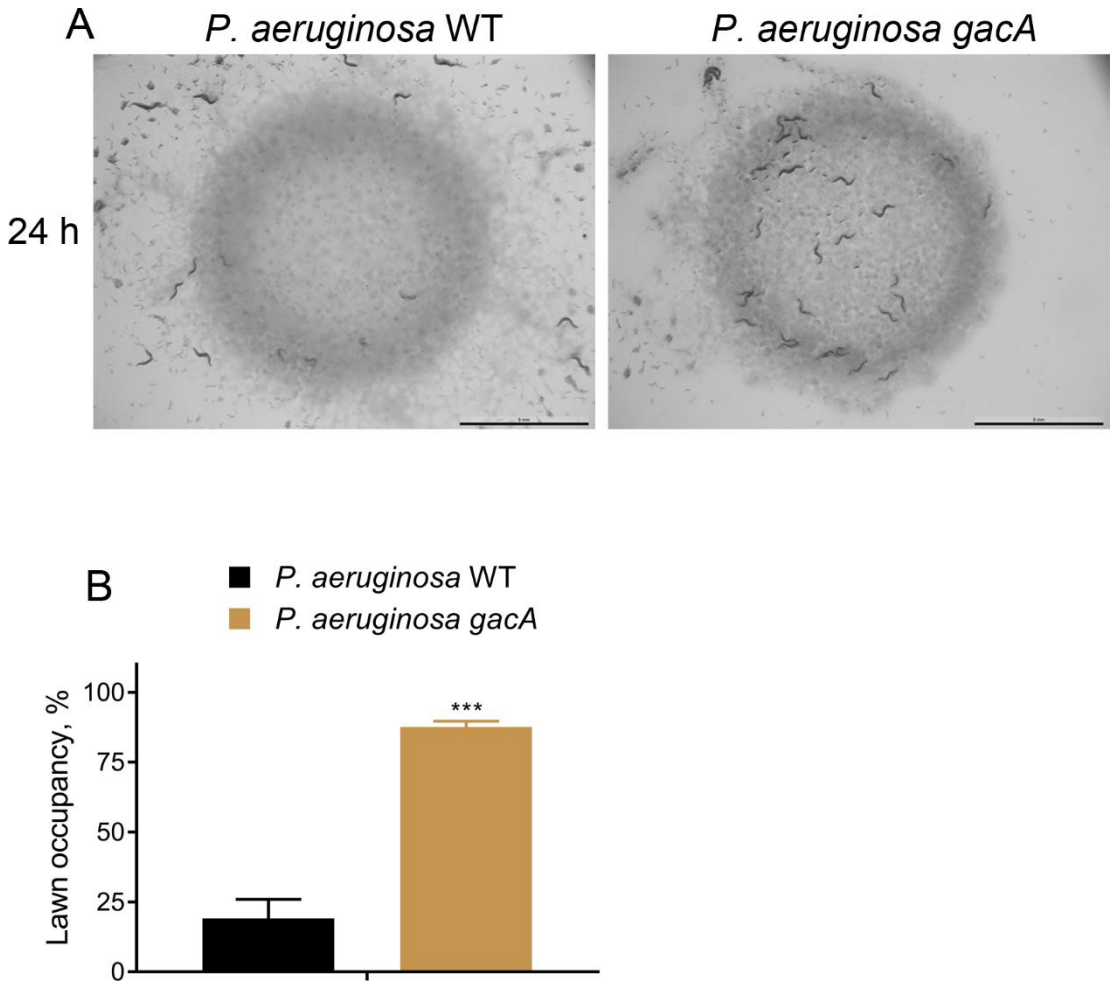

Figure S3. Avoidance Behavior of N2 animals on Different *P. aeruginosa* Variants, Related to Figure 3

(A) Representative photomicrographs of N2 animals on *P. aeruginosa* WT and *P. aeruginosa gacA* mutant after 24 hours of incubation.

(B) Percent occupancy of N2 animals on *P. aeruginosa* WT and the *P. aeruginosa gacA* mutant after 24 hours of incubation. ***$p < 0.001$ via the t-test.

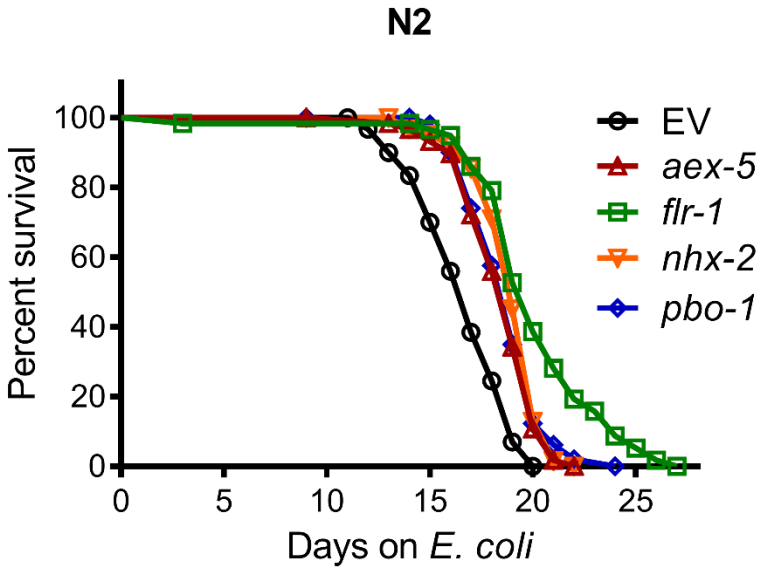

Figure S4. Intestinal Lumen Bloating Leads to an Increased Lifespan, Related to Figure 4

Survival of N2 animals grown on bacteria for RNAi against *aex-5*, *flr-1*, *nhx-2*, and *pbo-1* along with the empty vector control at 20°C. Day 0 represents young adults. The p values for *aex-5*, *flr-1*, *nhx-2*, and *pbo-1* RNAi relative to the values for the empty vector, EV control are ***p < 0.0001.

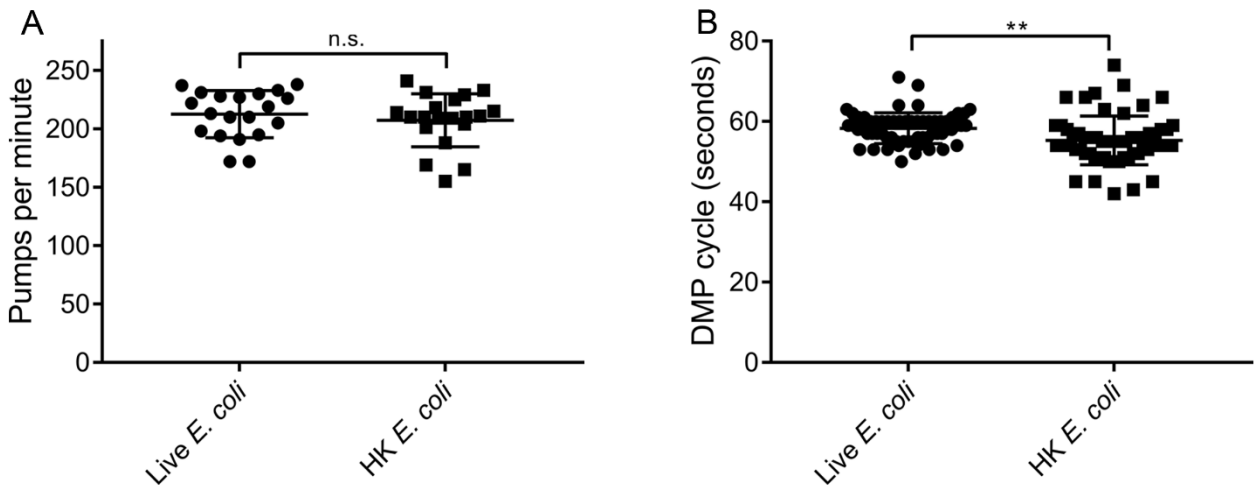

Figure S5. Pharyngeal Pumping Rate and DMP Cycle Length of Animals Fed Heat-Killed *E. coli*, Related to Figure 4

(A) Pharyngeal pumps per minute of N2 animals incubated on heat-killed (HK) *E. coli* for 24 hours at 20ºC. The animals were grown on live *E. coli* until the young adult stage before incubation on HK *E. coli*. The control animals were maintained on live *E. coli*. n.s., non-significant.

(B) The DMP cycle length of N2 animals incubated on HK *E. coli* for 24 hours at 20 ºC. The animals were grown on live *E. coli* until the young adult stage before incubation on HK *E. coli*. The control animals were maintained on live *E. coli*. **$p < 0.01$ via the t-test.

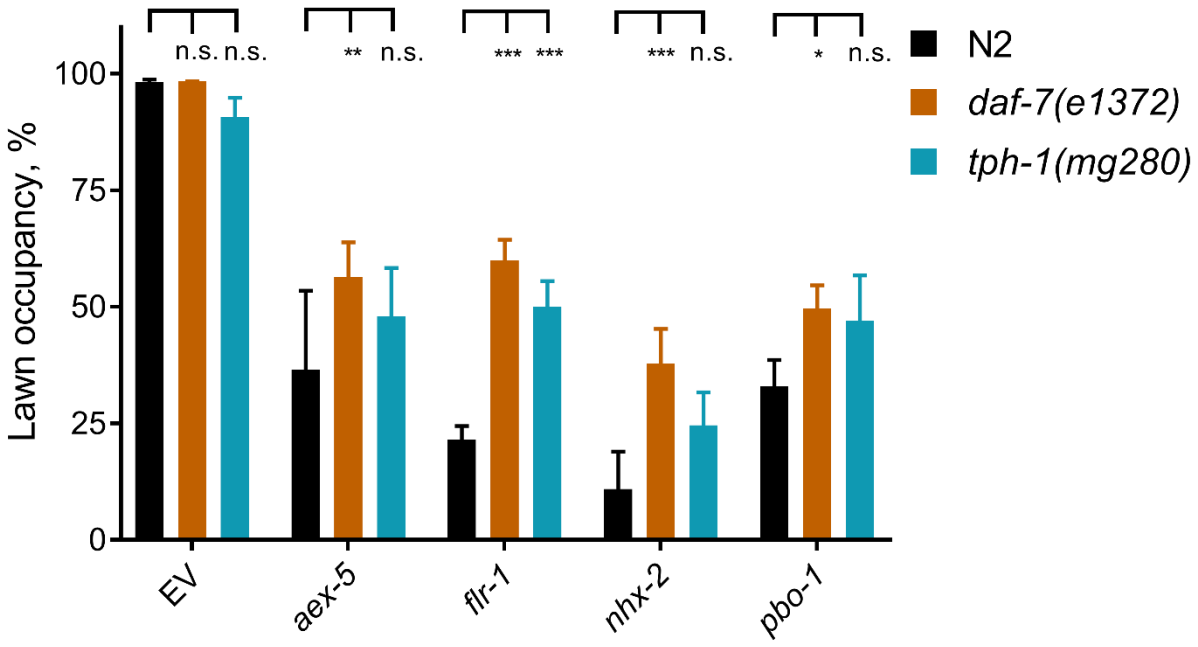

Figure S6. Role of the DAF-7 and TPH-1 Neural Pathways in Pathogen Avoidance Induced by Intestinal Bloating, Related to Figure 6

Percent lawn occupancy after 12 hours on *P. aeruginosa* of N2, *daf-7(e1372)*, and *tph-1(mg280)* animals. ***p < 0.001, **p < 0.01, and *p < 0.05. n.s., non-significant.

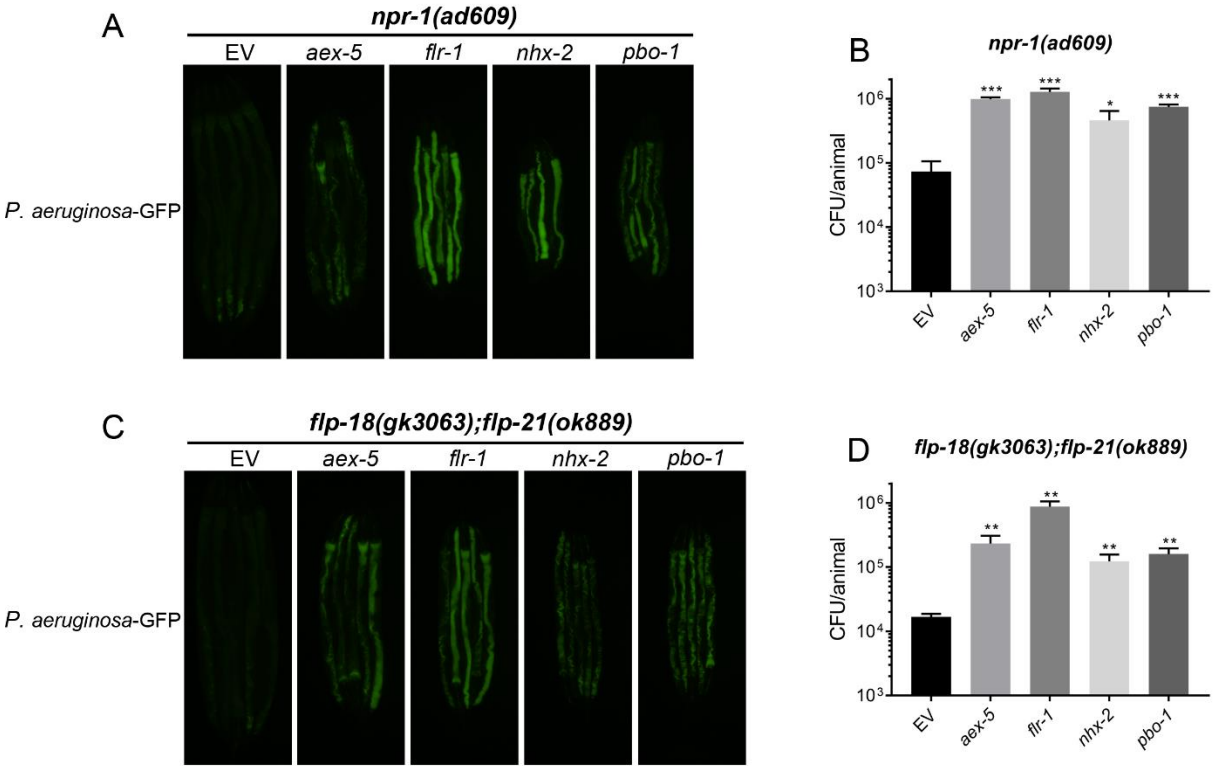

Figure S7. DMP Gene Knockdown Leads to Enhanced *P. aeruginosa* Colonization in *npr-1(ad609)* and *flp-18(gk3063);flp-21(ok889)* Animals, Related to Figure 6

(A) Representative photomicrographs of *npr-1(ad609)* animals exposed to *P. aeruginosa*-GFP for 5 hours at 25ºC after growth on RNAi bacteria.

(B) Colony forming units per animal for *npr-1(ad609)* animals exposed to *P. aeruginosa*-GFP for 5 hours at 25ºC after growth on RNAi bacteria. ***$p < 0.001$, and *$p < 0.05$ via the t-test.

(C) Representative photomicrographs of *flp-18(gk3063);flp-21(ok889)* animals exposed to *P. aeruginosa*-GFP for 5 hours at 25ºC after growth on RNAi bacteria.

(D) Colony forming units per animal for *flp-18(gk3063);flp-21(ok889)* animals exposed to *P. aeruginosa*-GFP for 5 hours at 25ºC after growth on RNAi bacteria. **$p < 0.01$ via the t-test.

Table S1. Primer used in the study, Related to Figures 4 and 5

Primer for quantitative reverse transcription-PCR		
Gene name	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')
Pan-*act*	TCGGTATGGGACAGAAGGAC	CATCCCAGTTGGTGACGATA
clec-60	CTGCAGTCTGGATGGCAGAA	CGCAAGATGCCGTTTCACAA
cpr-2	ATCAAAGGGAGGACACGCTG	AAGAGTTGACAGCGAGCCAA
F53A9.8	CAAGAACACGGACACGGAGA	ATGTTGGGTGTCGCAGTGAT
ilys-3	AAGAGGTGCGCGAATGATCT	CCTGTCCAGTTCCAGCACAT
lys-4	CTAGAGCAGCTGGCCTCAC	GCAGCAGATGGGCAGTTAGA
lys-5	GCTCCATTCGGAGGATGGTC	GACTCCACACGGACCAGTAG
ugt-18	GGGTTGGGCAGTACAATGGA	ACTGAAGACATCCTCCGTGC
daf-7	GGGTGCTGCTTGTATGACCT	CCAAGTTGAAGTGGTGTGCG
flp-18	GTTCGACAAGCGCAGTGAAG	CCCTGCGTAATGCTCTTCCA
flp-21	GCGGCTGTTCATCTTGCTTT	TAAACTCTGTCACTGCCGGG
npr-1	ACTCTATGCGTGGCTGAACC	TCACTTTTACGCCGGTCTCC
tph-1	TCAGATGGCATCCGCAATGA	CCACTTCGACGAAAACGACG
Primer for construction of RNAi clone		
Gene name	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')
pbo-1	GCTCCCGGGATGGGCCAAAATTCGTCTCA	AGGTCTAGATGTCAAAAAACGGAAGGACATC