Microbial colonization induces histone acetylation critical for inherited gut-germline-neural signaling

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Abstract

The gut-neural axis plays a critical role in the control of several physiological processes, including the communication of signals from the microbiome to the nervous system, which affects learning, memory, and behaviors. However, the pathways involved in gut-neural signaling of gut-governed behaviors remain unclear. We found that the intestinal distension caused by the bacterium *Pseudomonas aeruginosa* induces histone H4 Lys8 acetylation (H4K8ac) in the germline of *Caenorhabditis elegans*, which is required for both a bacterial aversion behavior and its transmission to the next generation. We show that induction of H4K8ac in the germline is essential for bacterial aversion and that a 14-3-3 chaperone protein family member, PAR-5, is required for H4K8ac. Our findings highlight a role for H4K8ac in the germline not only in the inheritance of pathogen avoidance but also in the transmission of pathogenic cues that travel through the gut-neural axis to control the aversive behavior.

Introduction

Increasing evidence suggests that the intestine plays an important role in response to environmental changes, which ultimately affect behaviors by communicating with neurons [1-5]. While there is also evidence indicating that microbial cues sensed by the intestine can be transmitted to the offspring and affect their behavior [6, 7], the pathways involved in gut-neural communication and the inheritability of gut-governed behaviors remain unclear. The germline can transmit epigenetic information from the environment to the next generation through communication with other tissues [8, 9], and it may regulate behaviors in response to environmental stress [10, 11]. However, a potential role of the germline in the gut-neural axis has not been established.

To provide insights into the gut-neural circuits that regulate behaviors in response to microbial colonization of the intestine, we have taken advantage of the nematode *Caenorhabditis elegans*, which has evolved behavioral responses that allow the animal to avoid potentially pathogenic bacteria. Upon exposure to *Pseudomonas aeruginosa*, *C elegans* exhibits a pathogen-aversive behavior, which is governed by distinct groups of neurons [12-15]. Moreover, recent studies indicate that *P. aeruginosa* colonization of the intestine causes a distension that regulates behavior and learning via neuroendocrine signaling [16, 17].

In this study, we show that the germline is part of the gut-neural axis involved in pathogen avoidance. The mechanism by which intestinal colonization by bacteria induces pathogen avoidance specifically requires histone H4 Lys8 acetylation (H4K8ac) in the germline. H4K8ac is also needed for the transmission of the pathogen aversive behavior to the next generation. Chromatin immunoprecipitation-mass spectrometry (ChIP-MS) identified a 14-3-3 chaperone protein family member, PAR-5, as essential in the germline for H4K8ac and gut-

neural signaling of pathogen avoidance. These results suggest that H4K8ac in the germline participates in a circuit that receives inputs from the infected gut and transmits the information to the nervous system to elicit pathogen avoidance.

Results and Discussion

Microbial colonization of the intestine induces histone acetylation H4K8ac in the germline

Histone post-translational modifications (PTM) are the most common epigenetic mechanisms, and different modifications have been demonstrated to be involved in diverse biological processes [18, 19]. A systematic study revealed that histone PTMs also widely occur among different developmental stages of *C elegans* [20]. To study whether histone PTMs play a role in the control of the pathogen-aversive behavior elicited by microbial colonization of the *C elegans* intestine, we first determined different histone modifications in young adult animals that were exposed to *P. aeruginosa* for 24 hrs. To ensure that the histone PTMs studied do not correspond to the progeny of the animals, we used *fer-1* animals, which are infertile at 25°C due to a mutation that prevents the sperm from penetrating the oocyte [21]. Out of the three PTMs studied, including histone H3 lysine 4 tri-methylation (H3K4me3), histone H3 lysine 4 methylation (H3K4me1), and histone H4 lysine 8 acetylation (H4K8ac), only H4K8ac increased (Fig 1A and S1 Fig), suggesting that *P. aeruginosa* colonization specifically induces histone H4K8 acetylation in the infected animals.

Because colonization by *P. aeruginosa* causes distension of the *C. elegans* intestinal lumen, which triggers bacterial aversion [16, 22], we reasoned that H4K8ac may also increase by intestinal distension. To test this hypothesis, we studied H4K8ac in *aex-5* and *eat-2* RNAi animals. Inhibition of genes *aex-5* and *eat-2*, which alters the defecation motor program (DMP) of the animals, results in pathogen avoidance triggered by intestinal distension [16]. Consistent with the idea that intestinal distension alone induces histone H4K8ac, inhibition of *aex-5* and *eat-2* resulted in induced histone H4K8ac in uninfected animals (Fig 1B, S2 Fig).

To determine where histone acetylation occurs, we performed whole animal fluorescent immunohistochemistry using an anti-H4K8ac antibody. We found that H4K8ac increases mainly in the germline upon exposure to *P. aeruginosa* or *aex-5* and *eat-2* inhibition by RNAi (Fig 1C, S3 Fig). These results highlight an important role of the germline in communicating danger signals from the intestine to the nervous system to elicit pathogen avoidance.

Gut-germline-neural signaling is required for pathogen avoidance

We sought to address whether the germline was part of the gut-neural signaling required for the elicitation of pathogen avoidance. To study a potential role of the germline in gut-neural signaling, we used *glp-1* animals, which lack most germline cells due to defects in mitotic and meiotic division [23, 24]. As shown in Fig 2A and S4 Fig, H4K8ac was not observed in *glp-1* animals. We also confirmed the lack of H4K8ac by immunohistochemistry (S5 Fig), which also indicates that histone acetylation indeed occurs in the germline in response to intestinal distension caused by microbial colonization.

Because H4K8ac is induced in the germline of DMP defective animals, which exhibit rapid pathogen avoidance, and *glp-1* animals lack most germline cells and exhibit no H4K8ac, we hypothesized that intestinal distention in *glp-1* animals would fail to elicit avoidance to *P. aeruginosa*. As shown in Fig 2B, inhibition of *aex-5* and *eat-2* did elicit pathogen avoidance in *glp-1* animals. It is known that *glp-1* animals exhibit enhanced resistance to a wide array of microbes, including *P. aeruginosa* [25], which results in a slow microbial colonization [26].

Thus, it was not clear whether the inability of *glp-1* animals to avoid *P. aeruginosa* was due to the absence of the germline or the enhanced resistance to infection and colonization by the pathogen. To distinguish between these two possibilities, we tested pathogen avoidance after 48hrs, when bacterial colonization is comparable in wild-type and *glp-1* animals deficient in *aex-5* and *eat-2* (Fig 2C, S6 Fig). We did not observe any difference in the pathogen avoidance of *glp-1* animals compared to that of *glp-1* animals deficient in *aex-5* and *eat-2* (Fig 2D), even at times when they were similarly colonized by *P. aeruginosa* (Fig 2C, S6 Fig). Taken together, these results indicate that intestinal distension caused by *P. aeruginosa* infection elicits H4K8ac activation in the germline that is required for pathogen avoidance.

PAR-5 is required for H4K8ac in the germline

To identify potential interacting partners that may affect H4K8ac in response to *P. aeruginosa* colonization, we performed chromatin immunoprecipitation-mass spectrometry (ChIP-MS). A total of 25 H4K8ac-interacting candidate proteins that were upregulated more than 3-fold in infected animals were identified (S1 Table). We further studied PAR-5 because it is the only candidate that is expressed in the germline and in neurons, from where it could also be involved in the control of pathogen avoidance. Another reason why we focused on PAR-5 is that it belongs to a 14-3-3 family of chaperones that, through interactions with different proteins, can regulate PTMs such as H4K8ac. Consistent with this idea, we found that the induction of H4K8ac by *P. aeruginosa* infection is inhibited by *par-5* RNAi (Fig 3B, S7 Fig). We also found that *par-5* RNAi inhibited H4K8ac in the germline (Fig 3A and S8 Fig).

To further confirm the relationship between H4K8ac and pathogen avoidance, we asked whether animals fail to avoid *P. aeruginosa* when *par-5* is inhibited. As shown in Fig 3C, animals did not avoid *P. aeruginosa* when *par-5* is inhibited by RNAi. Because the *par-5* homolog gene, *ftt-2*, shares ~78.2% sequence identity at the nucleotide level and ~85.9% sequence identity at the amino acid level [27], we studied the specificity of *par-5* RNAi. First, we investigated the pathogen avoidance of *ftt-2* mutants and found that unlike *par-5* RNAi animals, *ftt-2* animals were capable of avoiding *P. aeruginosa* (S9 Fig).

We also investigated the expression of the two proteins using anti-FTT-2 and anti-PAR-5 antibodies and found that PAR-5 but not FTT-2 diminished upon *par-5* RNAi (S10 Fig). Our results indicate that enhanced H4K8ac in the germline is required for pathogen avoidance. Thus, we employed strains capable of tissue-specific RNAi to evaluate the tissue-specific contributions of *par-5* RNAi responsible for the inhibition of pathogen avoidance. As shown in Fig 3D, *par-5* RNAi in the germline significantly reduced pathogen avoidance, which is consistent with our previous results indicating that H4K8ac occurs in the germline in response to infection.

Enhanced H4K8ac in the germline induces a transgenerational pathogen avoidance behavior

We hypothesized that if the intestinal distension caused by bacterial colonization or inhibition of DMP genes induces H4K8ac in the germline, the signal may be transmitted to the progeny. Thus, we asked whether the offspring of animals exposed to *P. aeruginosa* could also exhibit increased H4K8ac in the germline. Because the effect of RNAi is transgenerationally transmitted, we cannot investigate whether H4K8ac induced by inhibition of DMP genes is also passed to the progeny. Therefore, we used heat-killed *E. coli* that induces intestinal distension, similar to that of DMP defective animals, and also elicits a similar pathogen avoidance behavior [16]. We exposed L4 animals to *P. aeruginosa* or heat-killed *E. coli* for 24 hrs, two conditions which can

induce bloating in the intestinal lumen and result in bacterial aversion [16]. As shown in Fig 4A and S11 Fig, the F1 offspring from infected P0 animals exhibited higher H4K8ac in the germline than control animals, indicating that bloating of the intestine induces H4K8ac in the germline that is transgenerationally transmitted.

Intestinal distension caused by bacterial colonization or inhibition of DMP genes induces pathogen avoidance [16, 17] and H4K8ac in the germline (Fig 1B). Moreover, the enhanced H4K8ac is transmitted to the F1 offspring (Fig 4A, S11 Fig). Thus, we investigated whether the F1 offspring also exhibits higher pathogen avoidance. The progeny of animals infected with *P. aeruginosa* or fed heat-killed *E. coli* to induce intestinal distension exhibited significantly higher pathogen avoidance than the progeny of animals fed control live *E. coli*, which does not cause intestinal distension (Fig 4B). Taken together, these results demonstrate that enhanced H4K8ac in the germline is required for the transgenerational pathogen avoidance induced by bloating caused by bacterial colonization of the intestine.

In summary, we have shown that histone H4K8ac increased in the germline upon exposure to *P. aeruginosa*, suggesting that the intestinal distension caused by microbial colonization induces histone H4K8ac in the germline. Indeed, we observed that H4K8ac increases in animals that exhibit distended guts due to *aex-5* or *eat-2* inhibition. The rapid pathogen avoidance elicited by *aex-5* or *eat-2* inhibition is suppressed by the absence of the germline. Furthermore, found that PAR-5 regulates pathogen avoidance induced by intestinal distension by stabilizing H4K8ac and that reduction of H4K8ac in the germline by *par-5* inhibition suppressed pathogen avoidance (Fig 4C). Our results highlight a critical role for the germline in the control of the gut-neural axis. Further studies will be required to identify the downstream signals involved in germline-neural communication.

Materials and methods

Bacterial strains

The following bacterial strains were used: *Escherichia coli* OP50, *Pseudomonas aeruginosa* PA14 and *P. aeruginosa* PA14-GFP culture were grown in Luria-Bertani (LB) broth at 37 ℃.

Nematode strains and growth conditions

C. elegans hermaphrodites were maintained on *E. coli* OP50 at 20℃ unless otherwise indicated. Bristol N2 was used as the wild-type control. Germline specific RNAi strain DCL569 (*mkcSi13 II;rde-1(mkc36) V*), neuron-specific RNAi strain TU3401 (*sid-1(pk3321) V;uIs69 V*), gut-specific RNAi strain MGH171 (*sid-1(qt9) V;alxIs9*), HH142 *fer-1(b232)*, CB4037 *glp-1(e2141)* and MT14355 *ftt-2*(*n4426*) strains were obtained from the *Caenorhabditis elegans* Genetics Center (University of Minnesota, Minneapolis, MN).

Bacterial lawn avoidance assay

The bacterial lawns were prepared by picking individual *P. aeruginosa* PA14 colonies into 3 mL of the LB and growing them at 37℃ for 12 hours on a shaker. Then, a 20 uL culture was seeded onto the center of a 3.5-cm modified NGM plate and incubated at 37℃ for 12 hrs. Twenty synchronized hermaphroditic animals grown on *E. coli* HT115(DE3) carrying a control vector or an RNAi clone targeting a gene were transferred into the bacterial lawns, and the number of animals on and off the lawn were counted at the indicated times for each experiment. Experiments were performed at 25℃ except for *aex-5* and *eat-2* RNAi animals, which are hypersusceptible to *P. aeruginosa* at 25℃ [16]. The percent occupancy was calculated as (N_{on} lawn/N_{total}) ×100.

P. aeruginosa-GFP Colonization Assay

Bacterial lawns were prepared by inoculating individual bacterial colonies into 3 mL of LB with 50 mg/mL kanamycin and growing them at 37℃ for 12 hours on a shaker. For the colonization assays, bacterial lawns of *P. aeruginosa*-GFP were prepared by spreading 200 uL of the culture on the entire surface of 3.5-cm-diameter modified NGM plates. The plates were incubated at 37℃ for 12 hrs and then cooled to room temperature before the animals were transferred. The assays were performed at 25℃ except otherwise mentioned. At the indicated times for each experiment, the animals were transferred from *P. aeruginosa*-GFP plates to fresh *E. coli* OP50 plates and visualized under a fluorescence microscope. Quantification of fluorescence intensity was done using image J.

RNA Interference

The preparation of RNAi experiments has been explained in previous studies[28]. Briefly, *E. coli*, with the appropriate vectors, was grown in LB broth containing ampicillin (100 mg/mL) at 37℃ overnight and plated onto NGM plates containing 100 mg/mL ampicillin and 3 mM isopropyl β-d-1-thiogalactopyranoside (IPTG) (RNAi plates). RNAi-expressing bacteria was grown overnight at 37℃. Synchronized L4-stage animals were transferred to RNAi plates and grown for 24 hrs before the subsequent experiments. All RNAi clones except *eat-2* were from the Ahringer RNAi library.

Chromatin Immunoprecipitation-Mass Spectrometry

Total protein extracts from *fer-1(b232)* animals were obtained by sonication in FA buffer after crosslinking with formaldehyde for 30 mins. Anti-H4K8ac antibody was used to precipitate proteins bound to H4K8ac by incubation overnight followed with Magnetic beads incubation at 4℃ for 1 hr to pull down the proteins. Proteins were eluted with sample buffer and resolved on a 4-12% NuPage Novex gel (Invitrogen) and stained with Imperial Protein Stain (Thermo Scientific). Gel was run for 10 minutes. Cut bands were reduced, alkylated with iodoacetamide, and in-gel digested with trypsin (Promega) prior to MS analysis.

Western Blotting

Synchronized *fer-1(b232)* or *glp-1(e2141)* animals were transferred to plates with *P. aeruginosa* for 24 hrs with *E. coli* as control. Worms were collected and washed three times with M9 to remove the bacteria. The cell lysates were obtained by sonication. The samples were mixed with sample loading buffer for gel electrophoresis. Proteins were transferred from the gel to the membrane at 300 mA for 50 minutes. After one hour blocking at room temperature, the membrane was incubated at 4 ℃ overnight with anti-H4K8ac, anti-FTT-2, and anti-PAR-5 antibodies followed with one-hour anti-Rabbit antibody at room temperature. β-actin serves as internal control. The densities of the protein bands were quantified using Image J.

Whole Mount Fluorescent Immunohistochemistry

Synchronized worms were transferred to plates with *P. aeruginosa* for 24 hrs with *E. coli* as control. Worms were washed three times with M9 to remove the bacteria and resuspended in fixing solution (160 mM KCl, 100 mM Tris-HCl pH 7.4, 40 mM NaCl, 20 mM Na_2EGTA, 1 mM EDTA, 10 mM spermidine HCl, 30 mM PIPES pH 7.4, 1% Triton X-100, 50% methanol, 2% formaldehyde) and subjected to snap freezing in liquid nitrogen. The worms were fixed on ice for 4 hrs and washed briefly in T buffer (100 mM Tris-HCl pH 7.4, 1 mM EDTA, 1% Triton X-100) before a 15 min incubation in T buffer supplemented with 1% β-mercaptoethanol at 37 °C. The worms were washed with borate buffer (25 mM H_3BO_3, 12.5 mM NaOH pH 9.5) and then incubated in borate buffer containing 10 mM DTT for 15 mins, followed by H_2O_2 incubation for another 30 mins. Worms were blocked in PBST (PBS pH 7.4, 0.5% Triton X-100, 1 mM EDTA)

containing 1% BSA for 30 mins and incubated overnight with anti-H4K8ac antibody (1:100) and with Alexa Fluor 594 secondary antibody (1:100). 4',6-diamidino-2-phenylindole (DAPI; 20 ug/ml) was added to visualize nuclei. The worms were mounted on a microscope slide and visualized using a Leica plus fluorescence microscope. The intensity was quantified using Image J.

Statistical Analysis

Two-sample t-test for independent samples was used to analyze the data. All the experiments were repeated at least 3 times, unless otherwise indicated. All error bars represent the standard deviation. The data was judged to be statistically significant when $P < 0.05$.

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Figure Legends

Fig 1. *P. aeruginosa* infection and intestinal distension induce H4K8ac in the germline.

(A) Western blots of extracts from *fer-1(b232)* animals exposed to *E. coli* (*E. C*) or *P. aeruginosa* (*P. A*) for 24 hrs (representative of 3 independent experiments). (B) Western blots on extracts from *fer-1(b232)* animals exposed to *E. coli* (*E. C*) or *P. aeruginosa* (*P. A*) following *aex-5* and *eat-2* RNAi for 24 hrs (representative of 3 independent experiments). (C) Whole-mount immunofluorescence of wild-type animals stained with an anti-H4K8ac antibody after exposure to *E. coli* (*E. C*) or *P. aeruginosa* (*P. A*) following *aex-5* and *eat-2* RNAi for 24 hrs.

Fig 2. The germline is part of the gut-neural axis involved in pathogen avoidance.
(A) Western blot of extracts from *fer-1(b232)* and *glp-1(e2141)* animals exposed to *E. coli* (*E. C*) or *P. aeruginosa* (*P. A*) (representative of 3 independent experiments). (B) Lawn occupancy of wild-type N2 (WT) or *glp-1(e2141)* animals at 24 hrs of exposure to *P. aeruginosa* at 20°C following *aex-5* and *eat-2* RNAi (n=20). (C) Bacterial colonization of wild-type N2 (WT) and *glp-1(e2141)* animals after 48 hrs of exposure to *P. aeruginosa* at 20°C following *aex-5* and *eat-2* RNAi (n=4). (D) Lawn occupancy of wild-type N2 (WT) or *glp-1(e2141)* animals at 48 hrs of exposure to *P. aeruginosa* at 20°C following *aex-5* and *eat-2* RNAi (n=20).

Fig 3. PAR-5 is required for *P. aeruginosa* H4K8ac in the germline.

(A) Whole-mount immunofluorescence of wild-type N2 animals stained with an anti H4K8ac antibody after exposure to *E. coli* or *P. aeruginosa* for 24 hrs following *par-5* RNAi at 25°C. (B) Western blot of extracts from *fer-1(b232)* animals exposed to *E. coli* (*E. C*) or *P. aeruginosa* (*P. A*) for 24 hrs following *par-5* RNAi at 25°C (representative of 3 independent experiments). (C) Lawn occupancy of wild-type N2 animals at 24 hrs of exposure to *P. aeruginosa* following *par-5* RNAi at 25°C (n=20). (D) Lawn occupancy of tissue-specific RNAi animals at 24 hrs of exposure to *P. aeruginosa* following *par-5* RNAi in the germline, neurons, or the intestine at 25°C (n=20).

Fig 4. Enhanced H4K8ac in the germline is required for the transgenerational pathogen avoidance.
(A) Whole-mount immunofluorescence using an anti-H4K8ac antibody to strain F1 wild-type N2 young adults from P0 animals exposed to *E. coli* (*E. C*), *P. aeruginosa* (*P. A*), or heat-killed *E. coli* (HK *E. C*) starting at the L4 stage for 24 hrs. (B) Lawn occupancy of F1 wild-type N2 animals at 12 hrs from P0 animals exposed to *E. coli* (*E. C*), (*P. A*), or heat-killed *E. coli* (HK *E. C*) starting at the L4 stage for 24 hrs (n=20). (C) *C. elegans* exposure to *P. aeruginosa* causes an intestinal colonization and distention that leads to H4K8 acetylation in the germline. Both H4K8 acetylation in the germline and the germline itself are required for a transgenerational pathogen avoidance.

S1 Fig. Quantification of western blots of extracts from *fer-1(b232)* animals exposed to *E. coli* (*E. C*) or *P. aeruginosa* (*P. A*). Three independent experiments were performed.

S2 Fig. Quantification of western blot of extracts from *fer-1(b232)* animals exposed to *E. coli* (*E. C*) or *P. aeruginosa* (*P. A*) for 24 hrs following *aex-5* and *eat-2* RNAi. Three independent experiments were performed.

S3 Fig. Quantification of immunofluorescence of wild-type N2 animals stained with an anti-H4K8ac antibody after exposure to *E. coli* (*E. C*) or *P. aeruginosa* (*P. A*) for 24 hrs following *aex-5* and *eat-2* RNAi (n=5). Three independent experiments were performed.

S4 Fig. Quantification of western blot on extracts from *fer-1(b232)* and *glp-1(e2141)* animals exposed to *E. coli* (*E. C*) or *P. aeruginosa* (*P. A*). Three independent experiments were performed.

S5 Fig. Quantification of immunofluorescence of wild-type N2 and *glp-1(e2141)* animals stained with an anti-H4K8ac antibody after exposure to *P. aeruginosa* (*P. A*) for 24 hrs (n=5). Three independent experiments were performed

S6 Fig. Quantification of t he fluorescence emitted by *P. aeruginosa* in wild-type N2 and *glp-1(e2141)* animals after 48 hrs of exposure at 20°C following *aex-5* and *eat-2* RNAi (n=4). Three independent experiments were performed.

S7 Fig. Quantification of western blot of extracts from *fer-1(b232)* animals exposed to *E. coli* (*E. C*) or *P. aeruginosa* (*P. A*) at 24 hrs following *par-5* RNAi. Three independent experiments were performed.

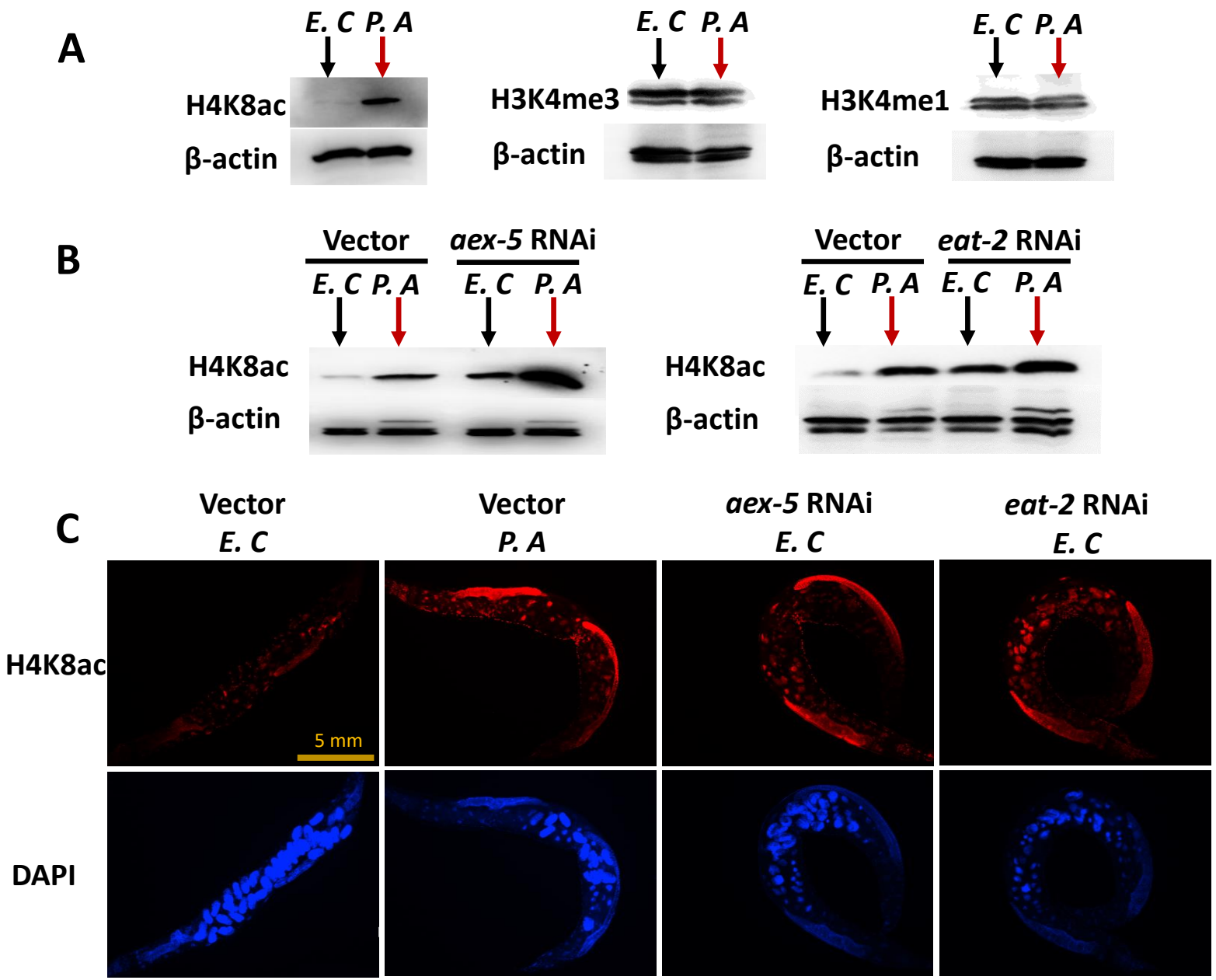
S8 Fig. Quantification of the immunofluorescence of wild-type N2 animals stained with an anti H4K8ac antibody after exposure to *E. coli* (*E. C*) or *P. aeruginosa* (*P. A*) following *par-5* RNAi (n=5). Three independent experiments were performed.

S9 Fig. Lawn occupancy of wild-type N2 or *ftt-2*(*n4426*) animals at 24 hrs following *par-5* RNAi at 25 °C (n=20). Three independent experiments were performed.

S10 Fig. Western blot and its quantification of extracts from *fer-1(b232)* animals exposed to *E. coli* (*E. C*) or *P. aeruginosa* (*P. A*) at 24 hrs following *par-5* RNAi. Two independent experiments were performed.

S11 Fig. Quantification of immunofluorescence that uses an anti-H4K8ac antibody to strain F1 wild-type animals from P0 animals exposed to *E. coli* (*E. C*), *P. aeruginosa* (*P. A*), or heat-killed *E. coli* (HK *E. C*) (n=20). Three independent experiments were performed.

Fig 1


A
E. C P. A
H4K8ac
β-actin
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H3K4me3
β-actin
E. C P. A
H3K4me1
β-actin
B
Vector
aex-5 RNAi
E. C P. A
E. C P. A
H4K8ac
β-actin
Vector
eat-2 RNAi
E. C P. A
E. C P. A
H4K8ac
β-actin
C
Vector
E. C
Vector
P. A
aex-5 RNAi
E. C
eat-2 RNAi
E. C
H4K8ac
DAPI
5 mm

Fig 2

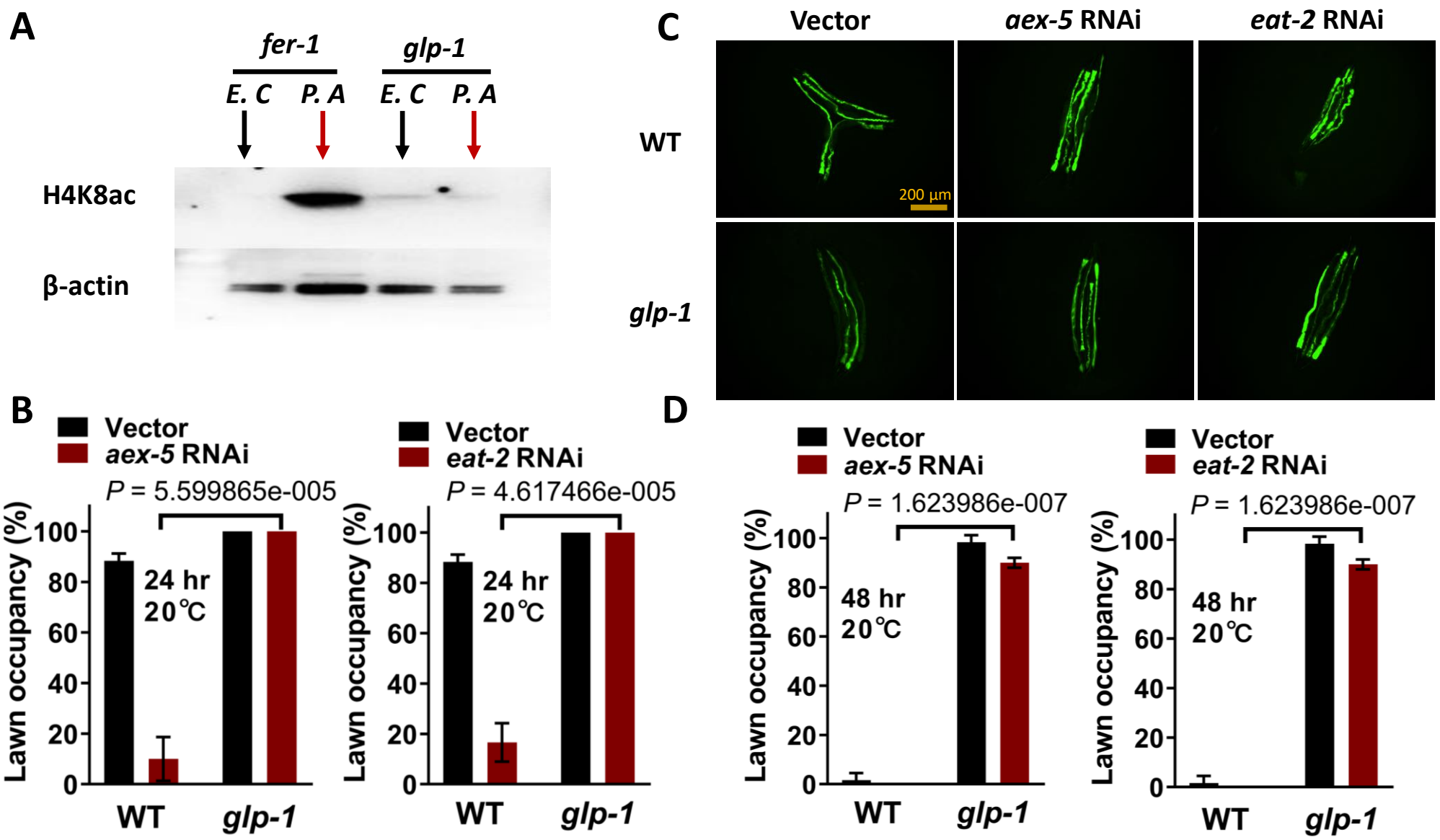

A
fer-1
glp-1
E. C
P. A
E. C
P. A
H4K8ac
β-actin
C
Vector
aex-5 RNAi
eat-2 RNAi
WT
glp-1
200 μm
B
Vector
aex-5 RNAi
P = 5.599865e-005
24 hr
20℃
Lawn occupancy (%)
WT
glp-1
Vector
eat-2 RNAi
P = 4.617466e-005
24 hr
20℃
Lawn occupancy (%)
WT
glp-1
D
Vector
aex-5 RNAi
P = 1.623986e-007
48 hr
20℃
Lawn occupancy (%)
WT
glp-1
Vector
eat-2 RNAi
P = 1.623986e-007
48 hr
20℃
Lawn occupancy (%)
WT
glp-1

Fig 3

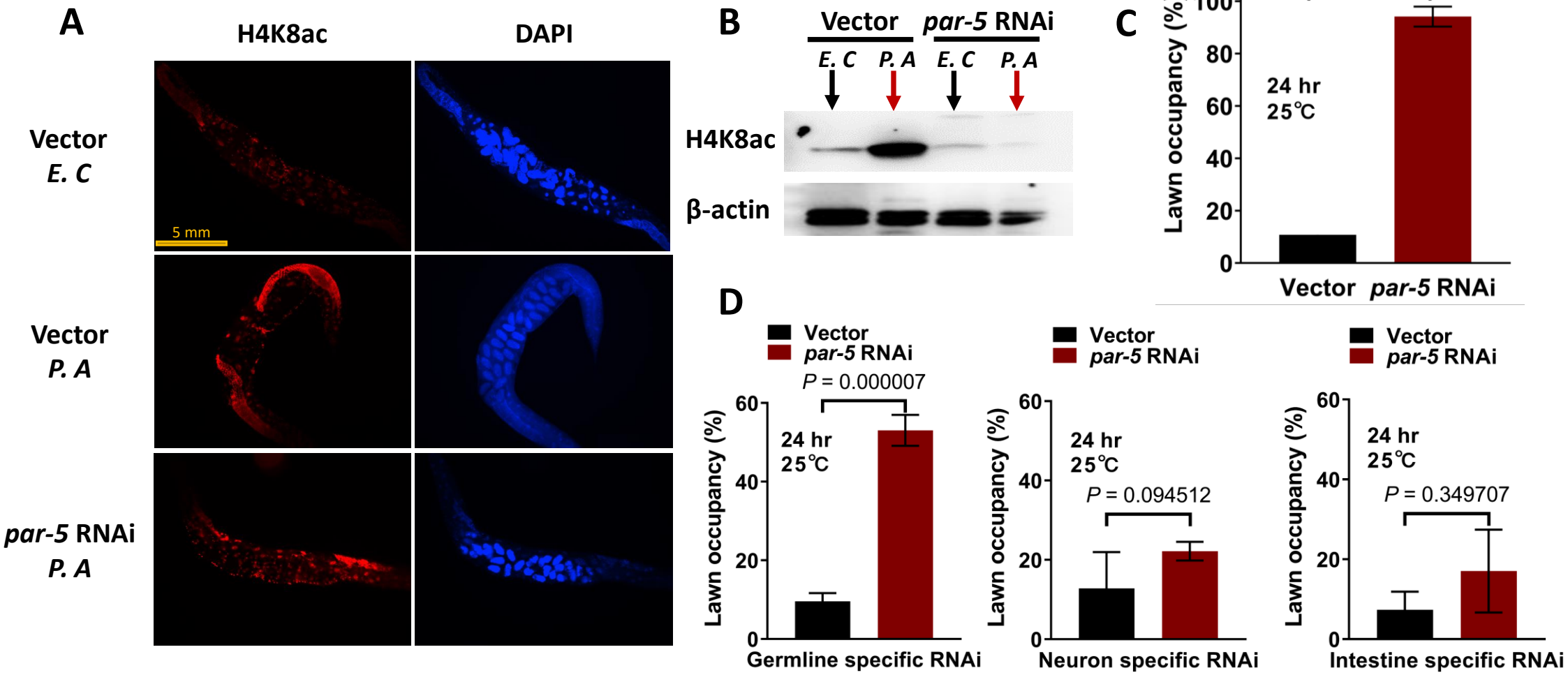
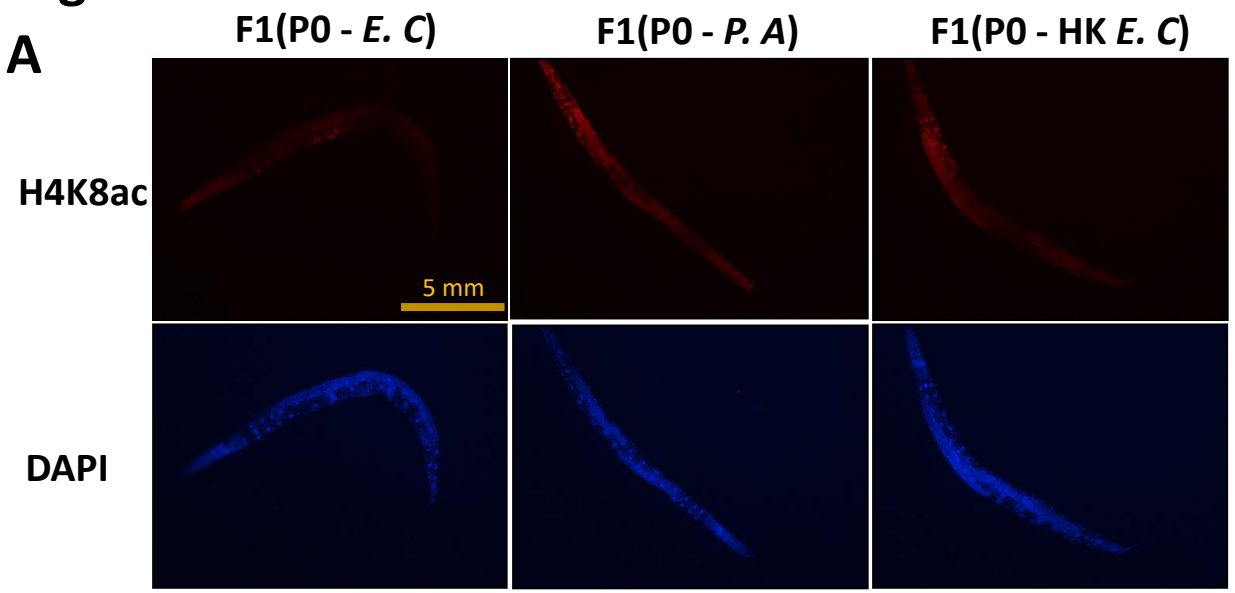
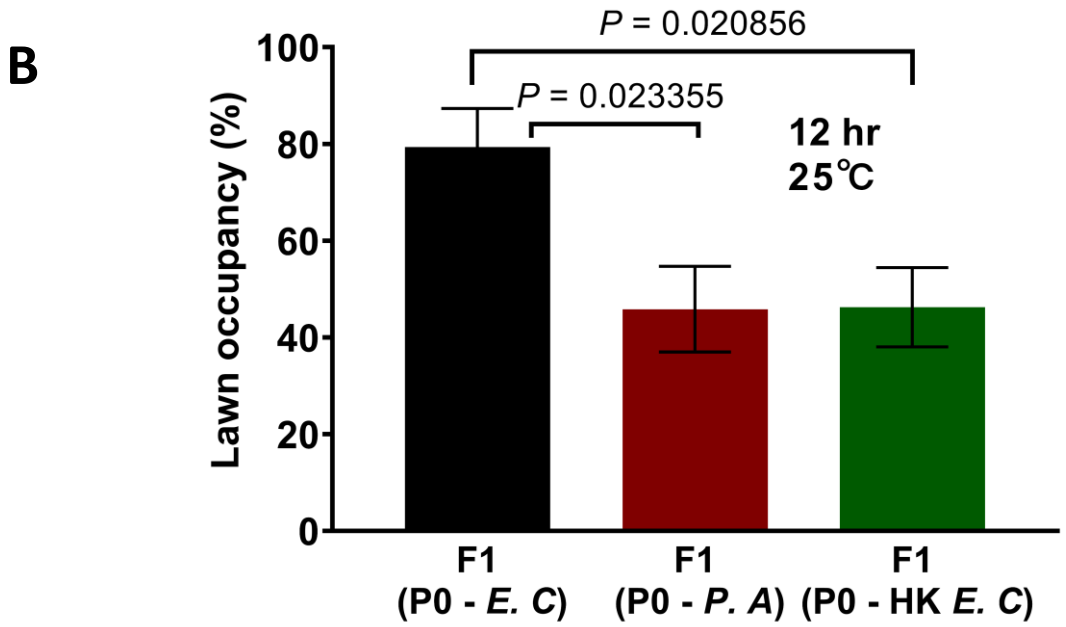
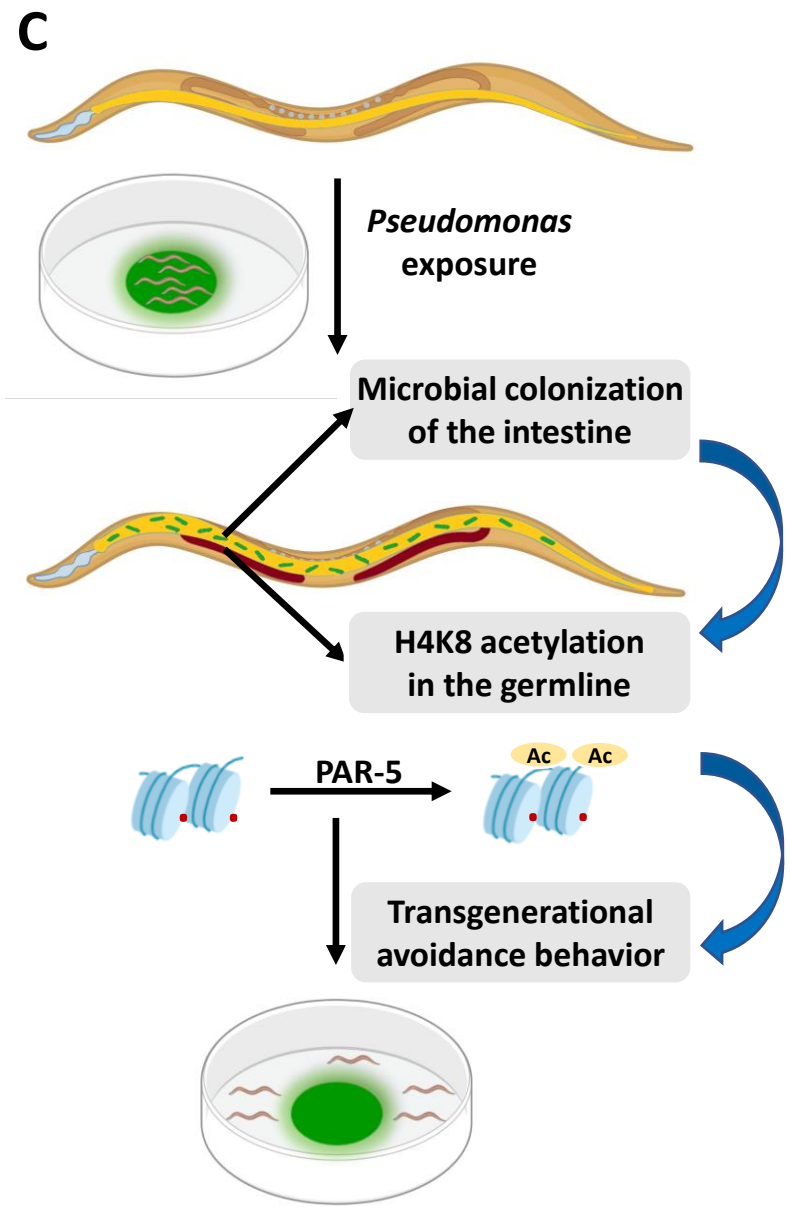

A
H4K8ac
DAPI
Vector
E. C
Vector
P. A
par-5 RNAi
P. A
5 mm
B
Vector
par-5 RNAi
E. C
P. A
E. C
P. A
H4K8ac
β-actin
C
P = 0.0016
Lawn occupancy (%)
100
80
60
40
20
0
24 hr
25℃
Vector
par-5 RNAi
D
Vector
par-5 RNAi
P = 0.000007
Lawn occupancy (%)
60
40
20
0
24 hr
25℃
Germline specific RNAi
Vector
par-5 RNAi
P = 0.094512
Lawn occupancy (%)
60
40
20
0
24 hr
25℃
Neuron specific RNAi
Vector
par-5 RNAi
P = 0.349707
Lawn occupancy (%)
60
40
20
0
24 hr
25℃
Intestine specific RNAi

Fig 4


A
F1(P0 - E. C)
F1(P0 - P. A)
F1(P0 - HK E. C)
H4K8ac
DAPI
5 mm


B
P = 0.020856
P = 0.023355
12 hr
25℃
Lawn occupancy (%)
100
80
60
40
20
0
F1 (P0 - E. C)
F1 (P0 - P. A)
F1 (P0 - HK E. C)


C
Pseudomonas exposure
Microbial colonization of the intestine
H4K8 acetylation in the germline
PAR-5
Ac
Ac
Transgenerational avoidance behavior