**Materials and Methods**

*C. elegans strains*

C. elegans strains were maintained on nematode growth media (NGM) plates seeded with *Escherichia coli* strain OP50 or HT115(DE3)/pL4440-*unc-62* or /pL4440-empty vector at 20°C or room temperature using standard methods (Brenner, 1974). The *hpo-9(tm3719)* strain lacks nucleotides 12131/12132 to 12892/12893 (761 bp deletion) of cosmid C05C8. PCR was used to genotype the *tm3719* allele (KO1: 5’-ACAAATCTGTTGTACAACATCTT-3’ and KO2: 5’-GATAGTGTGGAATTATATTCGTGT-3’) and the wild-type (WT1: 5’-CAGCAATAAGCGAATATTTCAAGG-3’ and WT2: 5-AATCTCTCGCAAGAAGCTCC-3’).

*Egg retention assay*

Larval stage 4 (L4) worms were isolated and allowed to mature at 20° C for 22 hrs. Next, young adult worms were placed individually in a solution of sodium hypochlorite, which dissolves the worm but leaves the eggs intact. The number of eggs sustained was counted within 10 to 20 min.

*RNA interference*

Adult worms laid eggs on a plate covered with HT115 containing the *unc-62 RNAi* or the empty vector. Then the adult worms were removed. The progeny was allowed to grow on that plate until the L4 stage and then was transferred to another plate seeded with the HT115 containing *unc-62* RNAi or empty vector. After 22 hrs, egg retention assay was performed as described above.

*Mice*

All experiments were carried out in compliance with the USPHS Guide for Care and Use of Laboratory Animals and approved by the IACUC at the University of Florida (#201405757).

*Wheel running*

The mice were maintained on a 12-hour light and 12-hour dark cycle for 7 days. Wheel-running activity was recorded as the number of wheel revolutions occurring during 5 min bins and analyzed using Lafayette Instrument Activity Wheel Monitor software. The activities from the 3rd to the 7th day were included in the data analysis, grouped by light phase and dark phase. Data collected for each 5 min bin were averaged and analyzed.

*Tail-flick test*

Each mouse was placed in an acrylic restrainer with the distal end of its tail protruding under a heat lamp. The lamp, together with a timer, was turned on, both of which stopped automatically when the mouse flicked its tail away from the light. The latency to respond was limited to 20 s to prevent injury to the mouse.

*Western blot*

The striata were dissected and homogenized in 200 µl of ice-cold RIPA buffer (Santa Cruz) containing protease inhibitor cocktail (Roche). The homogenate was incubated for 30 min on ice and centrifuged at 10,000 × g for 10 min at 4°C. The supernatant was used as samples for Western blot and the protein concentration was measured by protein assay reagent (Bio-Rad). After being boiled for 5 min, chilled on ice for 1 min and spun down, the proteins were separated on 10% SDS-PAGE gel and transferred to Millipore Immobilon –FL transfer membranes (PVDF). The PVDF membranes were washed in 0.1M PBS for 5 min and blocked with LICOR Odyssey blocking buffer for 1 hr. The membranes were incubated overnight at 4°C with goat polyclonal Meis1/2 antibody (Santa Cruz, sc-10599) or mouse β-actin antibody (Santa Cruz, sc-47778) at 1:1000 dilution in the blocking buffer. The membranes were washed with 0.1M PBS containing 0.1% Tween 20 for 5 min four times, then treated for 1 hr with LI-COR IRDye 680RD rabbit anti-goat IgG (H+L) or LI-COR IRDye 800CW goat anti-mouse IgG (H+L) at 1:15,556 dilution, respectively. After washing with 0.1M PBS containing 0.1% Tween 20 for 5 min four times and 0.1M PBS for 5 min three times, the membranes were dried and the signals were detected by an LI-COR Odyssey imaging system.

*Quantitative real-time RT-PCR*

The striata were dissected and kept in RNAlater RNA stabilization reagent (Qiagen). The RNA was isolated by the RNeasy mini kit. The RNA concentration was measured using NanoDrop 2000c (Thermo Scientific). The cDNA was synthesized using SuperScript III First-strand Synthesis SupeMix for the qRT-PCR kit (Invitrogen). Quantitative PCR was performed using CFX real-time PCR detection system (Bio-Rad) with SYBR Select Master Mix for CFX (Life Technologies) and the following primer sets: *Btbd9* forward: 5’-GACTCTTGTCTCCGGATGCT-3’ and *Btbd9* reverse: 5’-TCACAACCTGAGCCCCATAC-3’ for Btbd9 cDNA; *β-actin* forward: 5’-CACCCGCGAGCACAGCTTCTTTG-3’ and *β-actin* reverse: 5’-AATACAGCCCGGGGAGCATCGTC- 3’ for β-actin cDNA. The relative quantity of cDNA for *Btbd9* to that for *β-actin* was calculated by the ΔCT method.

*Statistics*

Data were processed with SPSS to test for normality. Egg retention assay, Western blot and RT-PCR were analyzed by the Student’s t-test. The tail-flick experiment was analyzed by repeated measurement ANOVA. The wheel-running experiment was analyzed by SAS generalized linear model (GENMOD) with a negative binomial distribution. Significance was assigned at *p* < 0.05.

Brenner S (1974) The genetics of Caenorhabditis elegans. Genetics 77:71-94.