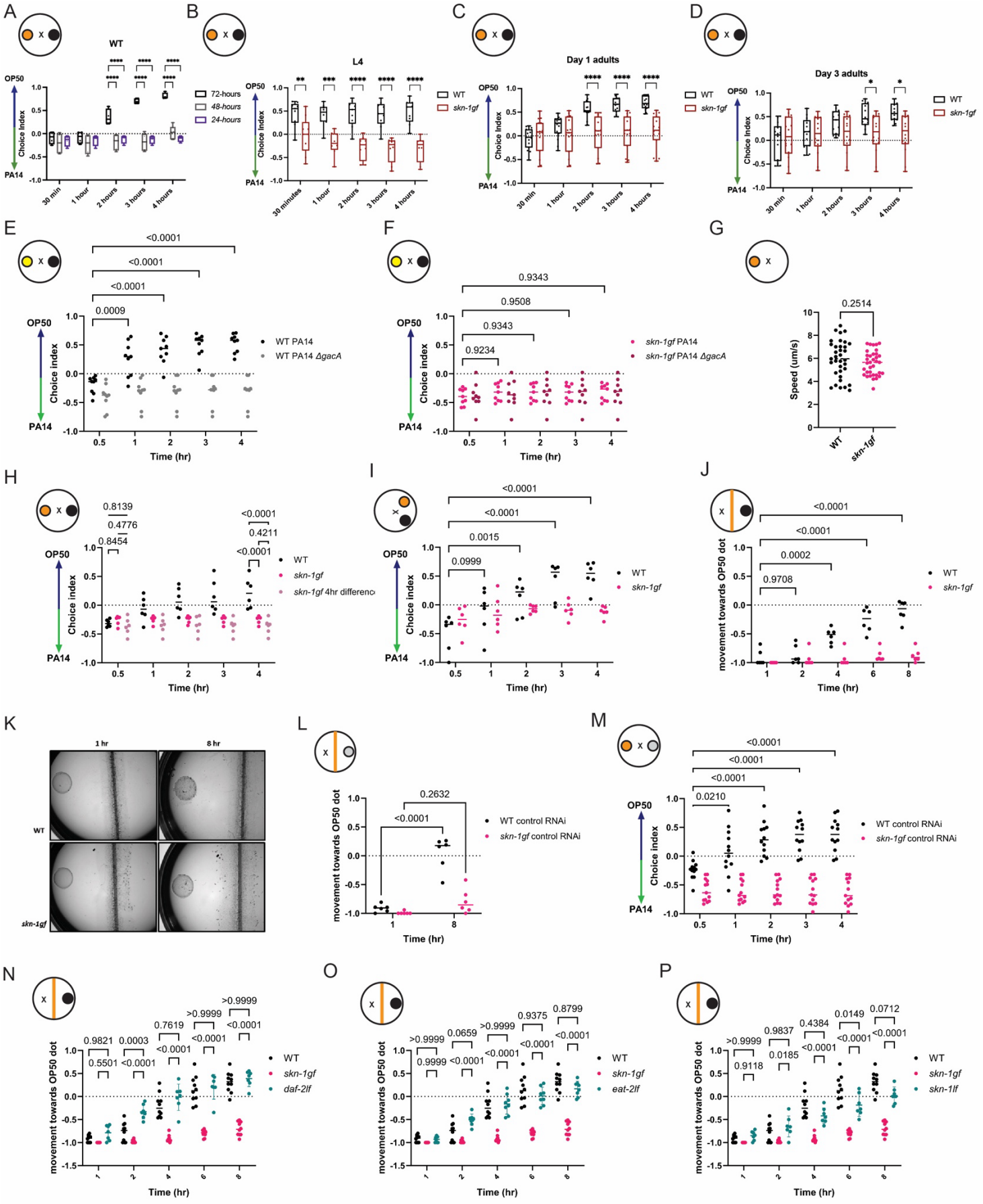
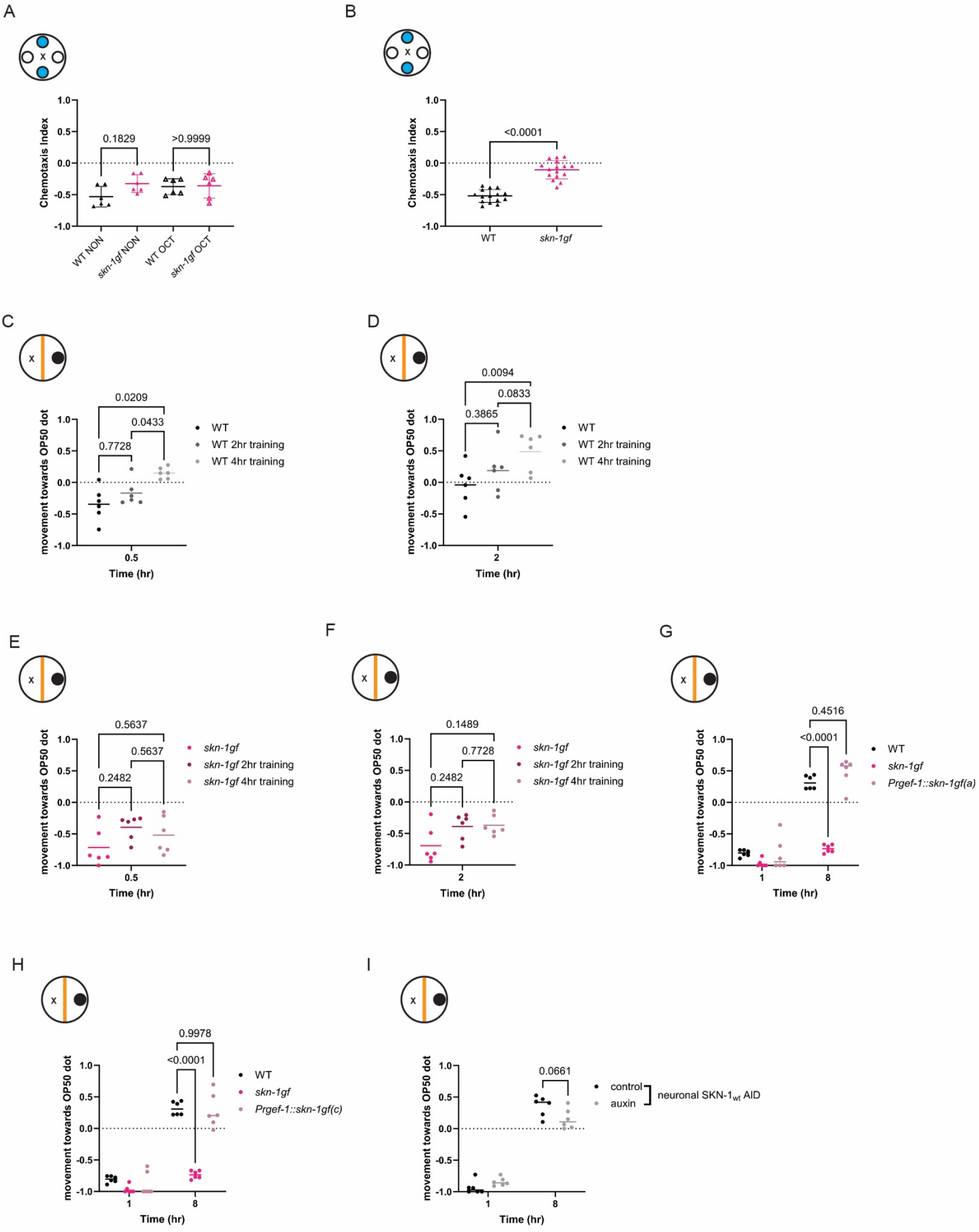
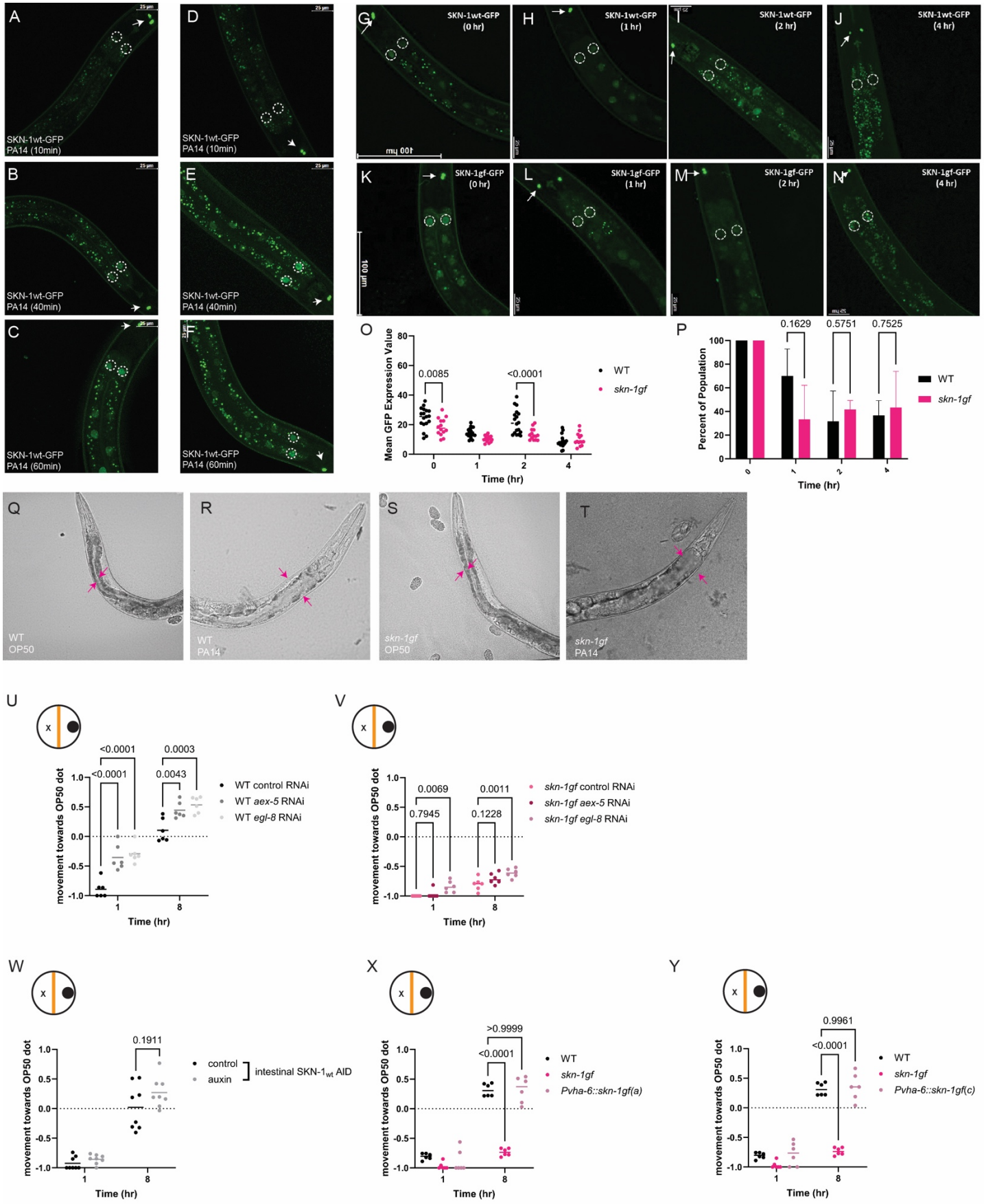

A
WT
OP50
PA14
Choice Index
72-hours
48-hours
24-hours
30 min
1 hour
2 hours
3 hours
4 hours
B
L4
30 minutes
C
Day 1 adults
WT
skn-1gf
D
Day 3 adults
E
WT PA14
WT PA14 ΔgacA
Time (hr)
F
skn-1gf PA14
skn-1gf PA14 ΔgacA
G
Speed (um/s)
H
skn-1gf 4hr difference
I
J
movement towards OP50 dot
K
1 hr
8 hr
L
WT control RNAi
skn-1gf control RNAi
M
N
daf-2lf
O
eat-2lf
P
skn-1lf

**Supplemental Figure S1. Factors that affect SKN-1gf driven pathogen apathy.** (**A**) Pathogen response in WT animals across various PA14 cultures: 72 hr (37°C for 24 hours, followed by 48 hours at 25°C), 48 hr (37°C for 24 hours, followed by 24 hours at 25°C), and 24 hr at 37°C. (**B-D**) Apathy response in WT and *skn-1gf* worms across developmental stages (L4, Day 1 adulthood, and Day 3 adulthood). Apathy response of (**E**) WT and (**F**) *skn-1gf* animals toward non-virulent PA14 *ΔgacA* mutants. (**G**) WT and *skn-1gf* animals display similar movement speed towards the first chosen diet. (**H**) 4hr difference in developmental timing between WT and *skn-1gf* animal does not alter pathogen apathy response in *skn-1gf* animals. (**I**) Changes in the distance between the two diets does not alter the pathogen apathy response in WT and *skn-1gf* animals. (**J**) "Forced" choice assay, where *skn-1gf* mutant animals reach the PA14 line within 30min like WT animals but remain on PA14, whereas the WT animals move toward the *E. coli* OP50 option over the 8-hour time course. (**K**) Images of the apathy response of *skn-1gf* worms at the 1hr and 8hr time points reveals ample food throughout the assay. (**L-M**) *skn-1gf* animals raised on control RNAi (*E. coli* HT115) until L4 stage still display apathy toward PA14 in the (**L**) traditional food choice and (**M**) forced food choice assays. (**N-P**) *daf-2lf* (**N**), *eat-2lf* (**O**), and *skn-1lf* (**P**) animals do not display pathogen apathy behavior. Each of the food choice assay comprised of N≥3 (with ≥150 worms per biological replicate per strain/condition) and analyzed via two-way ANOVA test; **($p<0.01$) ***($p<0.001$) ****($p<0.0001$). Individual "p" value numbers for the comparisons within each graph is placed above the respective comparison bars in the graph. Source data for all behavioral responses are provided as a Source Data Table 1.

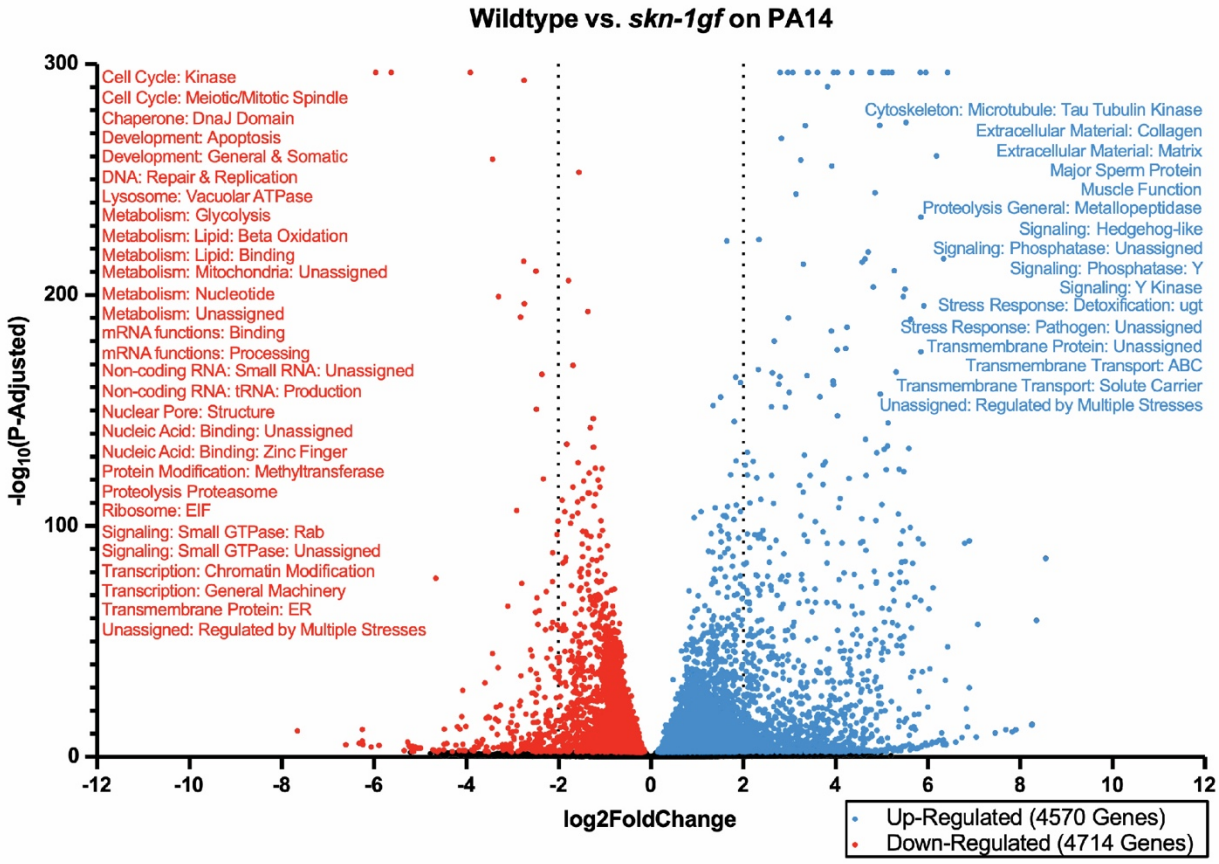

A
Chemotaxis Index
0.1829
>0.9999
WT NON
skn-1gf NON
WT OCT
skn-1gf OCT
B
Chemotaxis Index
<0.0001
WT
skn-1gf
C
movement towards OP50 dot
0.0209
0.0433
0.7728
WT
WT 2hr training
WT 4hr training
0.5
Time (hr)
D
movement towards OP50 dot
0.0094
0.0833
0.3865
WT
WT 2hr training
WT 4hr training
2
Time (hr)
E
movement towards OP50 dot
0.5637
0.5637
0.2482
skn-1gf
skn-1gf 2hr training
skn-1gf 4hr training
0.5
Time (hr)
F
movement towards OP50 dot
0.1489
0.7728
0.2482
skn-1gf
skn-1gf 2hr training
skn-1gf 4hr training
2
Time (hr)
G
movement towards OP50 dot
0.4516
<0.0001
WT
skn-1gf
Prgef-1::skn-1gf(a)
1
8
Time (hr)
H
movement towards OP50 dot
0.9978
<0.0001
WT
skn-1gf
Prgef-1::skn-1gf(c)
1
8
Time (hr)
I
movement towards OP50 dot
0.0661
control
auxin
neuronal SKN-1wt AID
1
8
Time (hr)

**Supplemental Figure S2. Training and neuronal responses of *skn-1gf*.** Chemorepulsion away from (**A**) octanol (OCT) and nonanone (NON) is similar between WT and *skn-1gf* mutants but not (**B**) 1-undecene. Pathogen leaving responses in WT (**C-D**) and *skn-1gf* (**E-F**) worms post-pathogen training (0hr, 2hr, and 4hr exposure). (**G**) Pan-neuronal (*rgef-1p*) degradation of SKN-1wt does not alter pathogen leaving behavior. Pan-neuronal (*rgef-1p*) expression of SKN-1gf (**H**) isoform a or (**I**) isoform c in the neurons does not elicit an apathy response like *skn-1gf* worms. Each of the chemotaxis assay comprised of N≥3 (with ≥150 worms per biological replicate per strain/condition) and analyzed via one-way ANOVA test data is presented as mean values +/- SEM. Each of the food choice assay comprised of N≥3 (with ≥150 worms per biological replicate per strain/condition) and analyzed via two-way ANOVA test; **($p<0.01$) ***($p<0.001$) ****($p<0.0001$). Individual "p" value numbers for the comparisons within each graph is placed above the respective comparison bars in the graph. Source data for all behavioral responses are provided as a Source Data Table 1.


A
SKN-1wt-GFP
PA14 (10min)
B
SKN-1wt-GFP
PA14 (40min)
C
SKN-1wt-GFP
PA14 (60min)
D
SKN-1wt-GFP
PA14 (10min)
E
SKN-1wt-GFP
PA14 (40min)
F
SKN-1wt-GFP
PA14 (60min)
G
SKN-1wt-GFP
(0 hr)
H
SKN-1wt-GFP
(1 hr)
I
SKN-1wt-GFP
(2 hr)
J
SKN-1wt-GFP
(4 hr)
K
SKN-1gf-GFP
(0 hr)
L
SKN-1gf-GFP
(1 hr)
M
SKN-1gf-GFP
(2 hr)
N
SKN-1gf-GFP
(4 hr)
O
Mean GFP Expression Value
0.0085
<0.0001
WT
skn-1gf
Time (hr)
P
Percent of Population
0.1629
0.5751
0.7525
WT
skn-1gf
Time (hr)
Q
WT
OP50
R
WT
PA14
S
skn-1gf
OP50
T
skn-1gf
PA14
U
movement towards OP50 dot
<0.0001
<0.0001
0.0003
0.0043
WT control RNAi
WT aex-5 RNAi
WT egl-8 RNAi
Time (hr)
V
movement towards OP50 dot
0.0069
0.7945
0.0011
0.1228
skn-1gf control RNAi
skn-1gf aex-5 RNAi
skn-1gf egl-8 RNAi
Time (hr)
W
movement towards OP50 dot
0.1911
control
auxin
intestinal SKN-1wt AID
Time (hr)
X
movement towards OP50 dot
>0.9999
<0.0001
WT
skn-1gf
Pvha-6::skn-1gf(a)
Time (hr)
Y
movement towards OP50 dot
0.9961
<0.0001
WT
skn-1gf
Pvha-6::skn-1gf(c)
Time (hr)

**Supplemental Figure S3. Nuclear accumulation of SKN-1 and intestinal bloating in response to PA14.** (**A-F**) Intestinal accumulation and stabilization of SKN-1gf-GFP and SKN-1wt-GFP upon PA14 exposure in comparison to OP50 (time points: 10 mins, 40 mins, and 60 mins); (**G-N**) Kinetics of loss of intestinal SKN-1 nuclear localization over 4 hours of post PA14 exposure when transferred to OP50 - the first two intestinal nuclei of each worm are outlined by white-dotted circles and the constitutive expression SKN-1 in the ASI neurons is marked with white arrows; quantification of the (**O**) intensity and the **(P**) percent of the population. (**Q-T**) DIC images of intestinal lumen in (**Q, R**) WT and (**S, T**) *skn-1gf* worms post-exposure to pathogen for 24 hours. *skn-1wt*-GFP and *skn-1gf*-GFP on OP50 (**Q, S**) or PA14 (**R, T**). (**U-V**) RNAi of *aex-5* or *egl-8* in WT animals causes enhanced pathogen leaving even in the absence of PA14, however only *egl-8* RNAi was capable of marginally inducing leaving behavior of the *skn-1gf* mutant. (**W**) Intestinal-specific (*ges-1p*) degradation of SKN-1wt does not alter pathogen leaving behavior in SKN-1wt mutants. Intestinal-specific (*ges-1p*) expression of SKN-1gf isoforms a (**X**) or c (**Y**) is not sufficient to elicit the pathogen apathy behavior as observed in *skn-1gf* worms. For the population studies a total of 60 (N=3; n=20) animals were counted per time point per condition. For quantification studies a minimum of three animals per time point per condition was taken into consideration. Each of the nuclear accumulation time points comprised of N≥3 (with n≥3 per biological replicate per strain/condition) and analyzed via two-way ANOVA test; **($p<0.01$) ***($p<0.001$) ****($p<0.0001$). Each of the food choice assay comprised of N≥3 (with ≥150 worms per biological replicate per strain/condition) and analyzed via two-way ANOVA test; **($p<0.01$) ***($p<0.001$) ****($p<0.0001$). Individual "p" value numbers for the comparisons within each graph is placed above the respective comparison bars in the graph. All experiments were conducted at 25°C. Source data for all nuclear activation responses are provided as a Source Data Table 2. Source data for all behavioral responses are provided as a Source Data Table 1.

A




B

## Tissue Enrichment Analysis

**WT (Up) vs. *skn-1gf* (Down)**

| Term | Enrichment | P-value |
|---|---|---|
| germ line | 1.6-fold | 2.3e-07 |
| reproductive system | 1.4-fold | 1.6e-05 |
| pharynx | 1.4-fold | 8.6e-04 |

**WT (Down) vs. *skn-1gf* (Up)**

| Term | Enrichment | P-value |
|---|---|---|
| amphid socket | 2.6-fold | 0.0002 |
| excratory socket | 2.7-fold | 0.0024 |
| phasmid socket | 2.4-fold | 0.0079 |

C

## Phenotype Enrichment Analysis

**WT (Up) vs. *skn-1gf* (Down)**

| Term | Enrichment | P-value |
|---|---|---|
| eggshell variant | 5.4-fold | 1.1e-06 |
| shortened lifespan | 2.1-fold | 0.0013 |
| intestinal cell uptake | 4.5-fold | 0.0048 |

**WT (Down) vs. *skn-1gf* (Up)**

| Term | Enrichment | P-value |
|---|---|---|
| molt | 3.8-fold | 7.8e-05 |
| intestinal vacuole | 6.6-fold | 3.7e-04 |
| behavior | 1.5-fold | 0.002 |

D

## Gene Ontology (GO) Enrichment Analysis

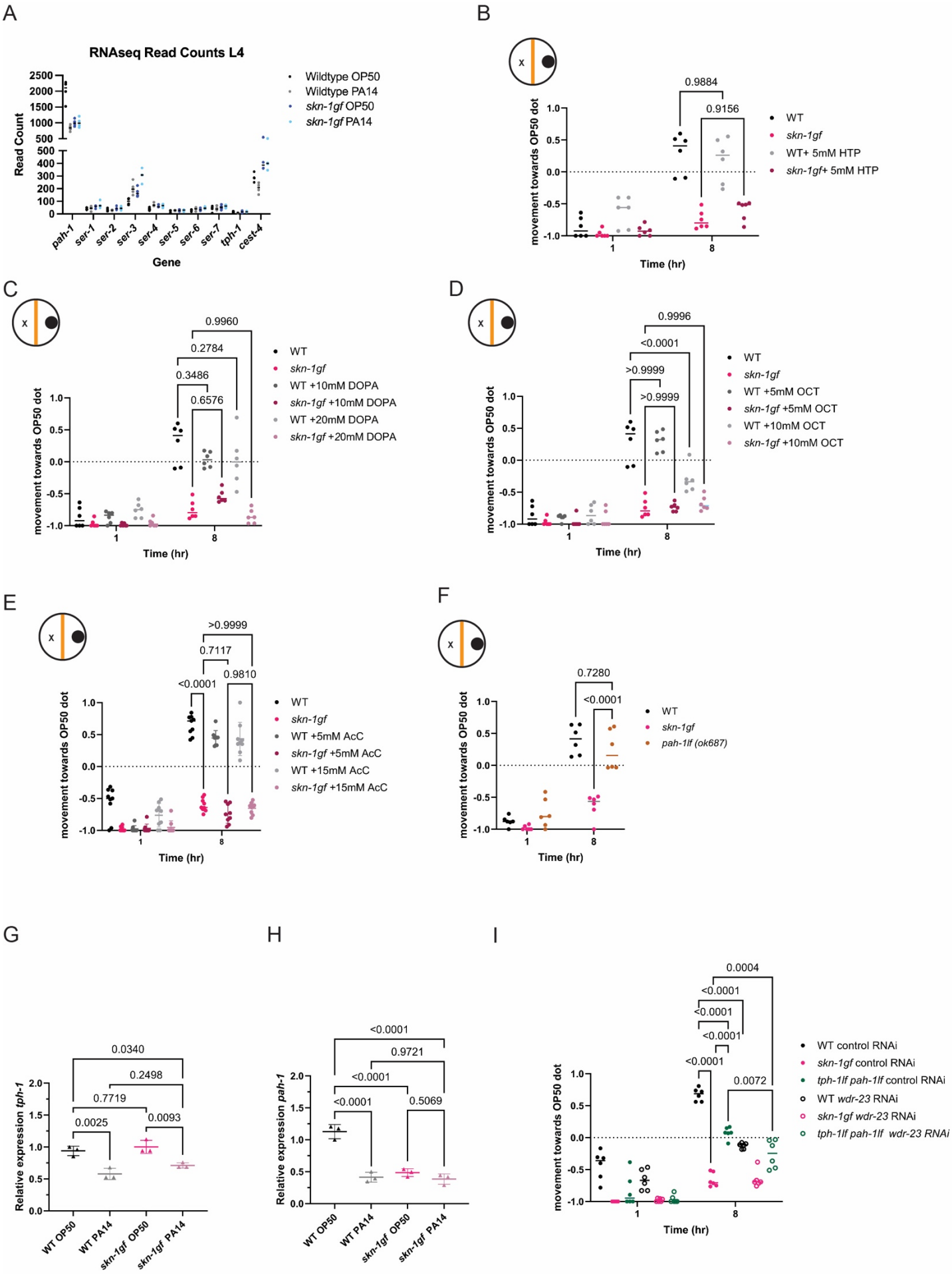
**WT (Up) vs. *skn-1gf* (Down)**

| Term | Enrichment | P-value |
|---|---|---|
| catabolic process | 3.9-fold | 3.0e-05 |
| proton transport | 6.4-fold | 4.8e-05 |
| serine hydrolase | 7.5-fold | 5.7e-05 |

**WT (Down) vs. *skn-1gf* (Up)**

| Term | Enrichment | P-value |
|---|---|---|
| cuticle structure | 10-fold | 1.1e-13 |
| adherens junction | 9-fold | 8.3e-05 |
| amino acid biosynthetic function | 6.9-fold | 2.9e-04 |

**Supplemental Figure S4. Gene set enrichment analysis (GSEA) of context specific transcriptional signature of PA14 exposure.** (**A**) RNAseq of differential transcriptional responses to PA14 exposure in WT and *skn-1gf* animal (WT vs *skn-1gf* up-regulated in blue to the right and down-regulated in red to the left). Gene set enrichment analysis (GSEA) tools in WormBase were used for the RNAseq data in Supplemental Table S3 to identify (**B**) tissue enrichment, (**C**) phenotype enrichment, and (**D**) gene ontology (GO) term enrichment of transcripts that differentially respond to PA14 in WT and *skn-1gf* animals. RNAseq data is available at the NIH (GEO) Gene Expression Omnibus (GSE251677). Source data for all Genotype-specific transcriptional responses are provided as a Source Data Table 3.


A
RNAseq Read Counts L4
Read Count
Gene
pah-1
ser-1
ser-2
ser-3
ser-4
ser-5
ser-6
ser-7
tph-1
cest-4
Wildtype OP50
Wildtype PA14
skn-1gf OP50
skn-1gf PA14
B
movement towards OP50 dot
Time (hr)
0.9884
0.9156
WT
skn-1gf
WT+ 5mM HTP
skn-1gf+ 5mM HTP
C
movement towards OP50 dot
Time (hr)
0.9960
0.2784
0.3486
0.6576
WT
skn-1gf
WT +10mM DOPA
skn-1gf +10mM DOPA
WT +20mM DOPA
skn-1gf +20mM DOPA
D
movement towards OP50 dot
Time (hr)
0.9996
<0.0001
>0.9999
>0.9999
WT
skn-1gf
WT +5mM OCT
skn-1gf +5mM OCT
WT +10mM OCT
skn-1gf +10mM OCT
E
movement towards OP50 dot
Time (hr)
>0.9999
0.7117
<0.0001
0.9810
WT
skn-1gf
WT +5mM AcC
skn-1gf +5mM AcC
WT +15mM AcC
skn-1gf +15mM AcC
F
movement towards OP50 dot
Time (hr)
0.7280
<0.0001
WT
skn-1gf
pah-1lf (ok687)
G
Relative expression tph-1
0.0340
0.2498
0.7719
0.0025
0.0093
WT OP50
WT PA14
skn-1gf OP50
skn-1gf PA14
H
Relative expression pah-1
<0.0001
0.9721
<0.0001
<0.0001
0.5069
WT OP50
WT PA14
skn-1gf OP50
skn-1gf PA14
I
movement towards OP50 dot
Time (hr)
0.0004
<0.0001
<0.0001
<0.0001
<0.0001
0.0072
WT control RNAi
skn-1gf control RNAi
tph-1lf pah-1lf control RNAi
WT wdr-23 RNAi
skn-1gf wdr-23 RNAi
tph-1lf pah-1lf wdr-23 RNAi

**Supplemental Figure S5. Pathogen apathy with constitutive SKN-1 is associated exclusively with serotonin.** (**A**) The GO-terms enrichment analysis of serotonin pathway genes that differentially respond to genetic and dietary conditions in WT and *skn-1gf* animal upon exposure to PA14, in comparison to OP50. Supplementation of (**B**) 5mM 5-HTP, (**C**) 10-20mM dopamine, (**D**) or 5-10mM octopamine, or (**E**) 5-15mM acetylcholine (AcC) does not alleviate the pathogen apathy response in *skn-1gf* animals. (**F**) Pathogen leaving behavior of *pah-1lf* (*ok687*) is like WT. Quantitative PCR analysis of expression levels of *tph-1* (**G**) and *pah-1* (**H**) in WT and *skn-1gf* mutants with and without PA14 exposure. Data is represented as mean values for (N=3; n=3 replicates) (**I**) Pathogen leaving behavior of *tph-1lf pah-1lf* animals with constitutive SKN-1 activation resulting from *wdr-23* RNAi. Each of the food choice assay comprised of N≥3 (with ≥150 worms per biological replicate per strain/condition) and analyzed via two-way ANOVA test; **($p<0.01$) ***($p<0.001$) ****($p<0.0001$). Individual "p" value numbers for the comparisons within each graph is placed above the respective comparison bars in the graph. Source data for all Genotype-specific transcriptional responses are provided as a Source Data Table 3. Source data for all behavioral responses are provided as a Source Data Table 1.

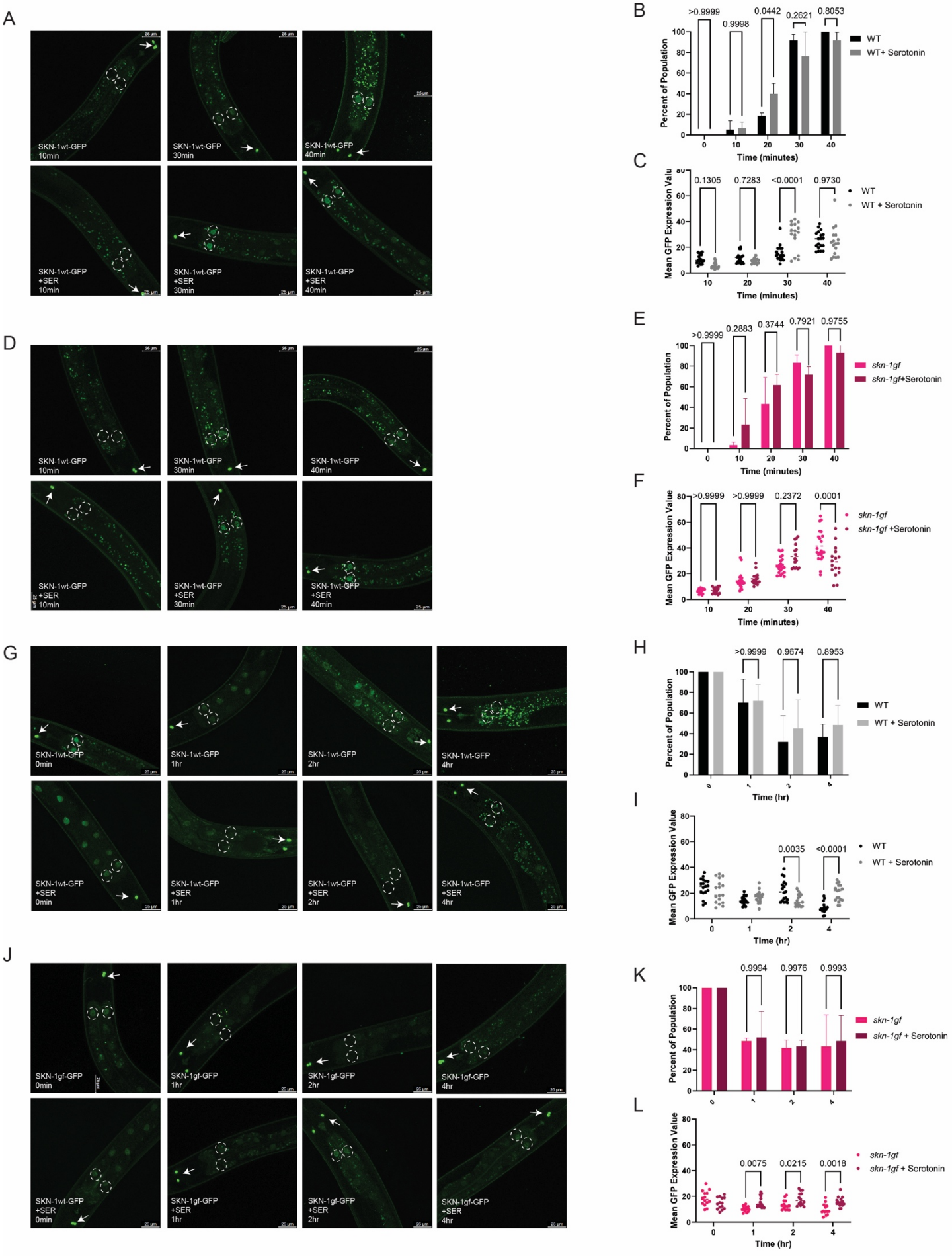

A
SKN-1wt-GFP 10min
SKN-1wt-GFP 30min
SKN-1wt-GFP 40min
SKN-1wt-GFP +SER 10min
SKN-1wt-GFP +SER 30min
SKN-1wt-GFP +SER 40min
B
>0.9999
0.9998
0.0442
0.2621
0.8053
Percent of Population
Time (minutes)
WT
WT+ Serotonin
C
0.1305
0.7283
<0.0001
0.9730
Mean GFP Expression Valu
Time (minutes)
WT
WT + Serotonin
D
SKN-1wt-GFP 10min
SKN-1wt-GFP 30min
SKN-1wt-GFP 40min
SKN-1wt-GFP +SER 10min
SKN-1wt-GFP +SER 30min
SKN-1wt-GFP +SER 40min
E
>0.9999
0.2883
0.3744
0.7921
0.9755
Percent of Population
Time (minutes)
skn-1gf
skn-1gf+Serotonin
F
>0.9999
>0.9999
0.2372
0.0001
Mean GFP Expression Value
Time (minutes)
skn-1gf
skn-1gf +Serotonin
G
SKN-1wt-GFP 0min
SKN-1wt-GFP 1hr
SKN-1wt-GFP 2hr
SKN-1wt-GFP 4hr
SKN-1wt-GFP +SER 0min
SKN-1wt-GFP +SER 1hr
SKN-1wt-GFP +SER 2hr
SKN-1wt-GFP +SER 4hr
H
>0.9999
0.9674
0.8953
Percent of Population
Time (hr)
WT
WT + Serotonin
I
0.0035
<0.0001
Mean GFP Expression Value
Time (hr)
WT
WT + Serotonin
J
SKN-1gf-GFP 0min
SKN-1gf-GFP 1hr
SKN-1gf-GFP 2hr
SKN-1gf-GFP 4hr
SKN-1wt-GFP +SER 0min
SKN-1gf-GFP +SER 1hr
SKN-1gf-GFP +SER 2hr
SKN-1gf-GFP +SER 4hr
K
0.9994
0.9976
0.9993
Percent of Population
Time (hr)
skn-1gf
skn-1gf + Serotonin
L
0.0075
0.0215
0.0018
Mean GFP Expression Value
Time (hr)
skn-1gf
skn-1gf + Serotonin

**Figure S6. Activation of intestinal SKN-1 in response to PA14 with and without serotonin supplementation**. (**A**) Intestinal accumulation of SKN-1wt-GFP upon PA14 exposure when prior grown on OP50 with and without serotonin supplementation (time point: 10-40 mins). Quantification of the (**B**) intensity and the (**C**) percent of the population of nuclear localization over the time course of PA14 exposure. (**D**) Intestinal accumulation of SKN-1-gf-GFP upon PA14 exposure when prior grown on OP50 with and without serotonin supplementation (time point: 10-40 mins). Quantification of the (**E**) intensity and the (**F**) percent of the population with nuclear localization over the time course of PA14 exposure. (**G**) Measuring the change in intestinal nuclear localization of SKN-1wt-GFP upon removal from PA14 and return to *E. coli* (time points: 1hr-4hr) with and without serotonin supplementation throughout (prior and post PA14 exposure); Quantification of the (**H**) intensity and the (**I**) percent of the population with nuclear localization over the time course. (**J**) Measuring the change in intestinal nuclear localization of SKN-1gf-GFP upon removal from PA14 and return to *E. coli* (time points: 1hr-4hr) with and without serotonin supplementation throughout (prior and post PA14 exposure). Quantification of the (**K**) intensity the (**L**) percent of the population of nuclear localization over the time course post PA14 exposure. White-dotted circles outline the first two intestinal nuclei of each worm; the constitutive expression in the ASI neurons is marked with white arrows. For the population studies, a total of 60 (N=3; n=20) animals were counted per time point per condition. For quantification studies a minimum of three animals per time point per condition was considered with three biological replicates i.e. N=3 (with n≥3 per biological replicate per strain/condition). The data have been analyzed via a two-way ANOVA test: **($p<0.01$) ***($p<0.001$) ****($p<0.0001$). Individual “p” value numbers for the comparisons within each graph is placed above the respective comparison bars in the graph. Source data for all nuclear activation responses are provided as a Source Data Table 2.