SUPPLEMENTARY INFORMATION:

Supplemental Methods

Additional C. elegans and bacterial strains used and culturing methods

For experiments involving reproductive adults, progeny worms were separated from parents by washing adult worms daily onto new plates using M9 + 0.01% Triton X-100. For staging synchronous animals, hours post feeding (hpf) designates the time lapsed after starvation-mediated synchronized L1 larvae were placed onto food and allowed to grow. *E. coli* strain OP50 was used for all experiments unless otherwise stated. OP50 bacteria was cultured overnight at 37°C in LB + streptomycin and 250 μL of the culture was seeded on NGM plates the following day. Plates were allowed to dry, and bacteria allowed to grow on NGM plates for at least 2 days before use. For experiments using FUdR, 10 μL at a concentration of 100 mg/mL of the drug was dropped on regular NGM plates. Killed OP50 was attained by growing the bacteria in normal culture conditions for 12 hours and subsequently adding both kanamycin and ampicillin to the culture at 1:25 concentration and incubating while shaking for an additional 12 hours. The treated OP50 was then spun down and resuspended to 5X concentration with M9 and was exposed to 12500 μJ of UV and subsequently seeded on NGM plates and allowed to dry overnight.

Lifespan Experiment

Synchronized L1-stage larval worms were dropped directly onto OP50 bacterial lawn and allowed to grow. Once worms reach adulthood, around 72 hours post feeding, they were transferred daily to a fresh plate using a platinum wire to separate parental worms from progeny. Transferring was done until no more progeny were observed on the plate. Scoring for dead worms was done by gentle prodding with a platinum wire, and worms were considered dead either when they were unresponsive to touch or internal progeny hatching occurred. Each genotype was assayed in biological triplicate using >50 animals in each independent lifespan experiment. Animals that crawled off the plate were censored.

Cold stress assay

Age-synchronized worms, either at 48 or 144 hours post feeding, were placed at 2°C for 48 hours. Following cold exposure, worms were removed from 2°C and allowed to recover at room temperature for 1 hour. Worms were then scored for survival and were considered dead if they were unresponsive to a gentle prodding from a platinum tip wire.

Paraquat Exposure Experiment

Paraquat (PQ) exposure was done by supplementing NGM plates with 75 μM paraquat. A 10 mM paraquat stock solution was made by dissolving paraquat (methyl viologen dichloride hydrate; Sigma Aldrich) in ddH_2O, which can then be stored for several months at 4°C. 7.5 mL of 10 mM paraquat solution was added to 1 L of liquid NGM media at 55°C, and then plates were poured and seeded as normal with OP50. Synchronized L1 larval worms were dropped on to 75 μM paraquat treatment plates. Worms are washed daily to new treatment plates to separate parents from progeny, until the stated age. At that time point, they were collected and stained with ORO and scored for fat distribution.

Chromatin immunoprecipitation (ChIP)

Synchronous populations of ~700,000 animals in triplicate were grown to day 2 of adulthood (120 hpf) on NGM plates or PQ treatment plates and treated with 1.1% formaldehyde to crosslink SKN-1 or SKN-1gf protein to chromatin. Worms were pelleted and flash frozen in FA Buffer

(WormBook). Samples were thawed and chromatin isolated by sonication in a Diagenode Bioruptor as previously described (1). SKN-1 or SKN-1gf bound to chromatin was immunoprecipitated and the associated DNA released by heating to 65°C overnight. Enrichment was assessed by qPCR of the promoter region of each gene in the ChIP DNA relative to the total starting material. All comparisons of enrichment at the promoter region were normalized to a qPCR of the 3'UTR region of the same gene.

RNA interference (RNAi) Experiment

OP50 RNAi strains were used for RNAi experiments (2). OP50 RNAi was grown overnight in LB + ampicillin and seeded the following day on NGM plates with IPTG and allowed to express dsRNA overnight. Synchronized L1 worms were dropped onto plates containing appropriate OP50 RNAi and cultured until stated age. Animals were then collected and used for experiments.

Nile Red Staining and Quantification

A working Nile Red solution was made by diluting 6 μL of stock solution (0.5 mg/mL) in 1 mL 40% isopropanol and adding 10 μL of DAPI per samples being stained. Synchronized worms at the stated age were collected using 1 mL of 1X PBS + 0.01% Triton X-100 (PBST) into a microcentrifuge tube and allowed to gravity settle. Supernatant was aspirated and washed three additional times with 1 mL PBST. After the final wash, supernatant was removed until 100 μL was left and then 600 μL of 60% isopropanol was added, and samples were rocked for 3 minutes at room temperature. Samples were spun down at 25x*g* and supernatant was removed until 100 μL was left. Worms were then stained with 600 μL of Nile Red working solution and left in the dark for 2 hours. Following staining, 600 μL of the supernatant was removed, and the samples were de-stained with 600 μL of PBST for 30 minutes in the dark. Samples were placed on glass slides and images were taken using the green fluorescence channel of a Zeiss Axiocam MRm. The intensity of lipid droplets was quantified using ImageJ.

RNA sequencing

At least 1000 age synchronized day 2 adult worms were washed off plates in triplicate with 1.5 mL of M9 buffer + 0.01% Triton X-100 (M9T) into a microcentrifuge tube and spun down at 1000x*g* The supernatant was removed, and the pellet was washed and spun down twice more to remove any residual bacteria from the sample. After the last wash, the supernatant was aspirated and 500 μL of TRI reagent was added. The sample was then frozen at -80°C overnight. Subsequently, the sample was thawed and lysed using a syringe and needle. RNA extraction was then performed following Zymo Direct-zol RNA isolation kit protocol. RNA samples were sequenced and analyzed by Novogene.

SUPPLEMENTARY FIGURES:

Fig. S1. Categorization of fat levels for wild-type and *skn-1gf* worms.
Representative images of lipid level distribution for ORO stained wild-type (**A**) and *skn-1gf* (**B**) worms, along with population of worms scored and proportion of population displaying each phenotype at 120 hours post feeding. At this time point, *skn-1gf* worms display a strong loss of somatic fat phenotype, while a majority of wild-type worms retain fat throughout their body. The criteria for lipid level categorization is described in the methods. All experiments were performed in a minimum of three biological replicates and lipid distribution was assessed in at least 300 animals. Scale bar = 50μm.

Fig. S2. RNAi screen of chromatin modifiers effect on Asdf levels in *skn-1gf*
RNAi screen of *skn-1gf* worms done using OP50 RNAi clones. Chromatin modifiers associated with H3K4me3 complex, *wdr-5* and *rbbp-5,* suppressed the Asdf phenotype in *skn-1gf* worms. The RNAi screen was performed in biological triplicate and 50 worms were scored for each experiment. Two independent *wdr-5* RNAi clones tested positive. *set-2* RNAi animals were developmentally delayed and asynchronous at time of scoring.

Fig. S3. Loss of *wdr-5* suppresses pleiotropic phenotypes of *skn-1gf* animals
(**A**) Representative images of lipid level distribution for ORO stained *wdr-5lf;skn-1gf* worms, along with population of worms scored and proportion of population displaying each phenotype at 120 hours post feeding. Loss of *wdr-5* suppresses fat loss in *skn-1gf* worms. (**B**) *wdr-5lf* abolishes H3K4me3 chromatin marks. (**C**) *skn-1gf* worms have a shortened lifespan, which is partially rescued by loss of *wdr-5.* Graph shows the combination of three biological replicates of the lifespan assay with >50 worms in each replicate (after censoring – WT n=127; *skn-1gf* n=241; *wdr-5lf; skn-1gf* n=304). (**D-E**) *skn-1gf* mutants are resistant to oxidative stress early in adulthood (80hpf) (**D**) that is lost later in life (120hpf) (**E**). This increased sensitivity is abolished in the absence of WDR-5. (**F-G**) Worms at 48 hours post feeding display similar survival levels following cold stress assay except for *wdr-5lf;skn-1gf*, which has an increased survival percentage, possibly due to the slower growth rate of the double mutant and reflecting the younger developmental stage of the animal at the time of the assay (**F**). (**G**) At 144 hours post feeding, *skn-1gf* worms display a decreased survival to cold stress, which may be due to the loss of fat phenotype seen during this age. This decreased survival is suppressed by the *wdr-5lf(ok1417)* mutation. (**H**) *wdr-5lf(ok1417)* animals reach egg laying adulthood at a slower rate (less than 24 hours) than wild type animals. (**I**) *wdr-5lf;skn-1gf* display suppressed levels of Asdf. ****P<0.0001 Fisher's exact two-tailed test used to compare stress survival percentage and log-rank test for lifespan analysis. All experiments were performed in a minimum of three biological replicates and lipid distribution was assessed in at least 300 animals. Scale bar = 50μm.

Fig. S4. Comparison of transcript levels in *wdr-5lf;skn-1gf* relative to *skn-1gf* animals at 120 hours post feeding using RNA-seq
(**A-B**) RNA-seq analysis of *wdr-5lf;skn-1gf* worms reveals genes associated with innate immune responses (**A**) are downregulated in *wdr-5lf;skn-1gf* worms when compared to *skn-1gf* worms, but oxidative stress genes (**B**) remain at the same. This suggests that the pleiotropic consequences suppressed in the *wdr-5lf;skn-1gf* double mutant may be due to the suppression of innate immune response genes. (**C**) Model of the impact of loss of Histone H3 trimethylation evokes on the transcriptional activity of SKN-1 and physiological responses. ** $P<0.01$, *** $P<0.001$. **** $P<0.0001$

Fig. S5. Representative images of fat level categorization for WT and *skn-1gf* worms treated with paraquat

Representative images of lipid level distribution for ORO stained (**A**) wild-type and (**B**) *skn-1gf* worms treated with 75 μM paraquat (PQ), along with the population of worms scored and proportion of population displaying each phenotype at 120 hours post feeding. Exposure to 75 μM of PQ results in a relatively unchanged lipid level distribution in wild-type worms, while *skn-1gf* worms show a suppression of the Asdf phenotype and an overall increase in lipid levels compared to untreated *skn-1gf* worms. (**C-D**) PQ treatment does not abolish OP50 bacteria growth. OP50 was inoculated in either LB (**C**) or LB + 75uM PQ (**D**) and grown overnight with shaking. Cultures were then diluted and 5μl plated on LB and allowed to grow to assess culture viability by colony count. All experiments were performed in a minimum of three biological replicates and lipid distribution was assessed in at least 200 animals. Scale bar = 50μm.

Fig. S6. Comparison of transcript levels in *skn-1gf* worms that were untreated or treated with paraquat at 120 hours post feeding.

RNA-seq analysis of *skn-1gf* worms treated with paraquat (PQ) reveals that oxidative stress genes (**A**) remain unchanged or increased when compared to untreated *skn-1gf* worms. Read counts for genes associated with the innate immune response (**B**) are significantly lower in PQ-treated *skn-1gf* worms compared to untreated *skn-1gf* worms. This trend in gene expression remains consistent with *wdr-5lf;skn-1gf*, another suppressor of the Asdf phenotype in *skn-1gf* worms. **(C**) Depiction of overall change in transcriptional focus of SKN-1gf activity in response to oxidative stress. **P*<0.05, ***P*<0.01, *****P*<0.0001

Fig. S7. *nsy-1gf* worms display a similar age-dependent fat loss as *skn-1gf* worms

(**A**) Representative images of lipid level distribution for ORO stained *nsy-1gf* worms, along with the population of worms scored and the proportion of the population displaying each phenotype at 144 hours post feeding. Constitutive activation of *nsy-1*, a MAP3K involved in the innate immune pathway, causes worms to undergo a similar age-dependent depletion of lipids in somatic tissues at 144 hours post feeding. (**B**) This loss of fat at 144hpf is *skn-1* dependent as RNAi of *nsy-1gf* worms for *skn-1* seems to result in more non-Asdf worms when compared to L4440 RNAi. All experiments were performed in a minimum of three biological replicates and lipid distribution was assessed in at least 250 animals. *****P*<0.0001 Fisher's exact two-tailed test used to compare survival percentage. Scale bar = 50μm.

Fig. S8. Exposure to the pathogen *Pseudomonas aeruginosa* results in SKN-1 dependent fat loss and is attenuated by non-virulent strains.

Representative images of the lipid level distribution for wild-type worms exposed for 4 hours to *P. aeruginosa* (**A**) and 2 other non-virulent mutants *gacA* (**B**), or *rhlR* (**C**). Worms were stained with ORO 72 hours post feeding. The population of worms scored and the percentage displaying each phenotype are provided. Wild-type worms exposed to pathogens exhibited a similar fat loss phenotype to that seen in *skn-1gf* and *nsy-1gf* worms. This phenotype is not observed in wild-type worms exposed to non-virulent mutant pathogens. (**D-E**) The loss of somatic lipids in response to *P. aeruginosa* exposure is attenuated in *vit-5* RNAi treated animals (**D**) and enhanced in SKN-1gf mutants (**E**). (**F-G**) RNAi of *skn-1* delays somatic fat loss in response to pathogen. At least two biological replicates were performed and a minimum of 100 animals were analyzed in each replicate. *P<0.05, ****P<0.0001 Fisher's exact two-tailed test used to compare non-Asdf to Asdf worms

Fig. S9. Adult animals are resistant to *P. aeruginosa* fast-killing. Wild type (black), *skn-1gf(lax188)* mutants (red), *wdr-5lf(ok1417)* mutants (blue), or *wdr-5lf(ok1417);skn-1gf(lax188)* double mutants (green) were exposed to *Pseudomonas aeruginosa* "fast kill" as young adults (YA). As previously described, post-developmental animals are less sensitive to *P. aeruginosa* fast-kill exposure as compared to larval stage 4 (L4) animals.

Fig. S10. Metabolic phenotypes of SKN-1gf activity and subsequent redirection. (A-B) Analysis of the mRNA reads of the indicated genes related to metabolism. **(C)** Representative images of Nile Red (NR) stained wildtype and *skn-1gf(lax188)* mutants reveals a 60% reduction in total lipids at day 2 of adulthood. See also tables S1 thru S4 for all RNA-seq measurements. $^{*}P < 0.05$; $^{***}P < 0.001$; $^{****}P < 0.0001$ by two-tailed t-test. Scale bar = 50μm.

REFERENCES:

1. Cecere G & Grishok A (2014) RNA Chromatin Immunoprecipitation (RNA-ChIP) in Caenorhabditis elegans. *Bio-protocol* 4(24):e1358.
2. Xiao R*, et al.* (2015) RNAi Interrogation of Dietary Modulation of Development, Metabolism, Behavior, and Aging in C. elegans. *Cell Rep* 11(7):1123-1133.

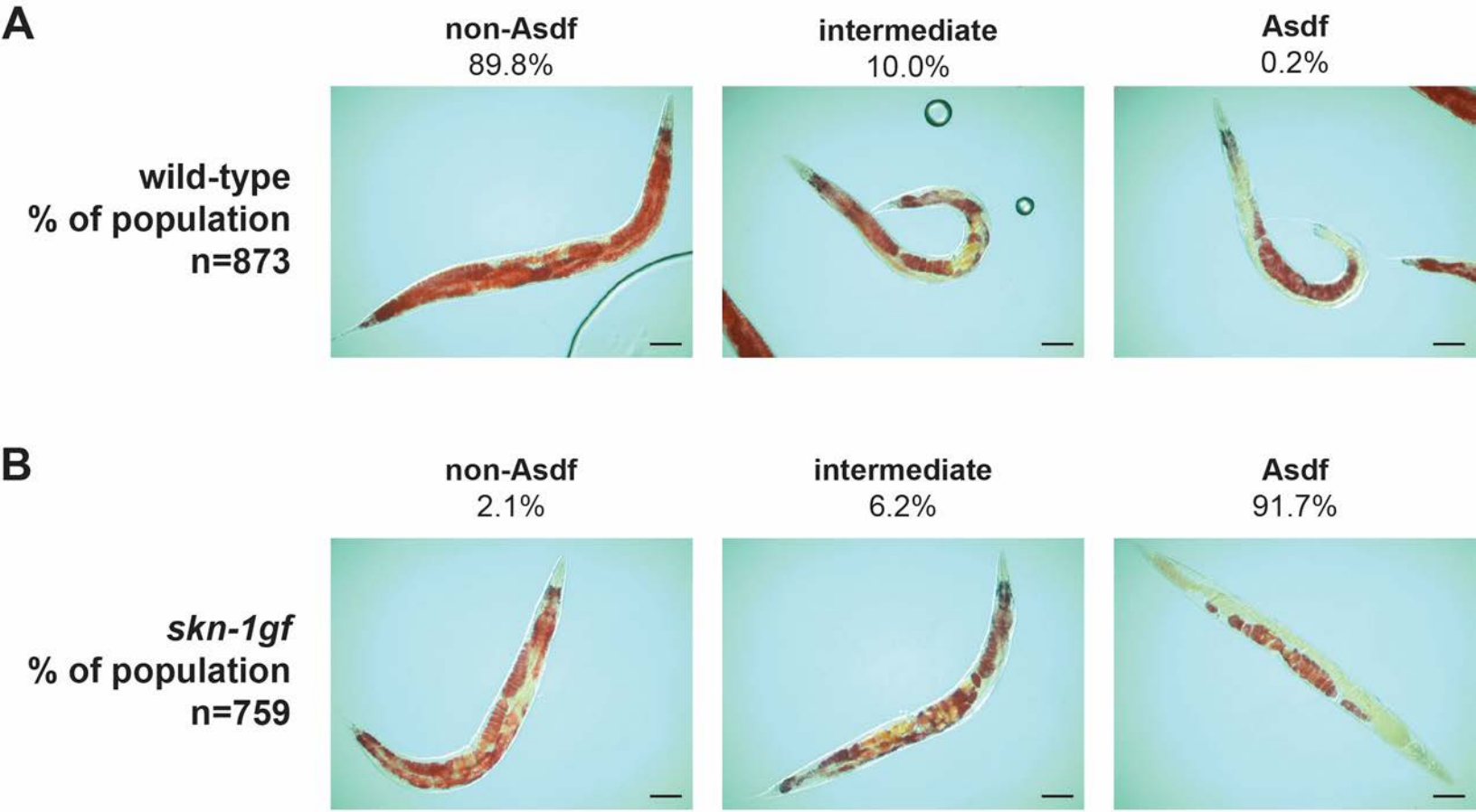

A
non-Asdf
89.8%
intermediate
10.0%
Asdf
0.2%
wild-type
% of population
n=873
B
non-Asdf
2.1%
intermediate
6.2%
Asdf
91.7%
skn-1gf
% of population
n=759

Figure S1

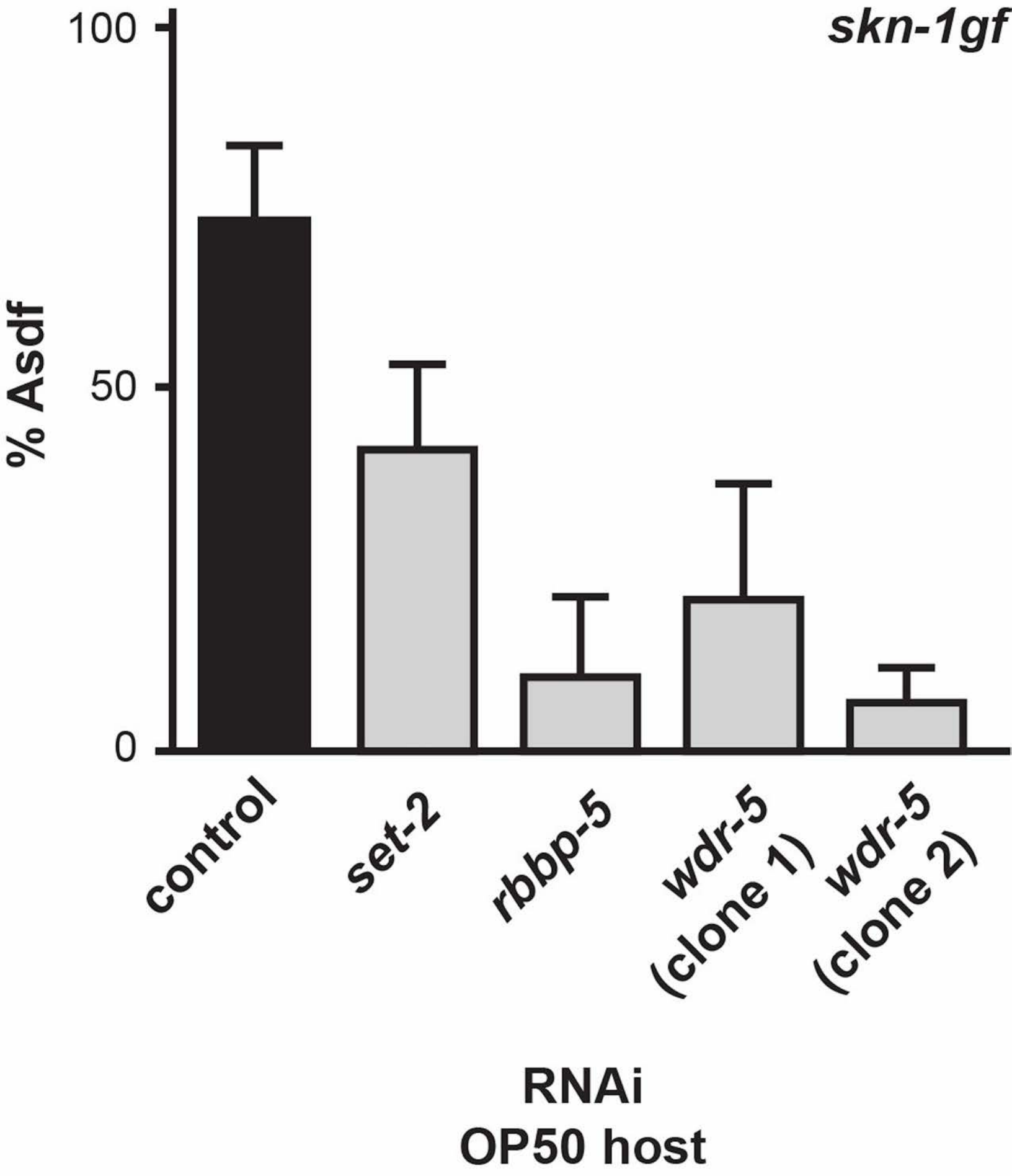

skn-1gf
100
50
0
% Asdf
control
set-2
rbbp-5
wdr-5 (clone 1)
wdr-5 (clone 2)
RNAi
OP50 host

Figure S2

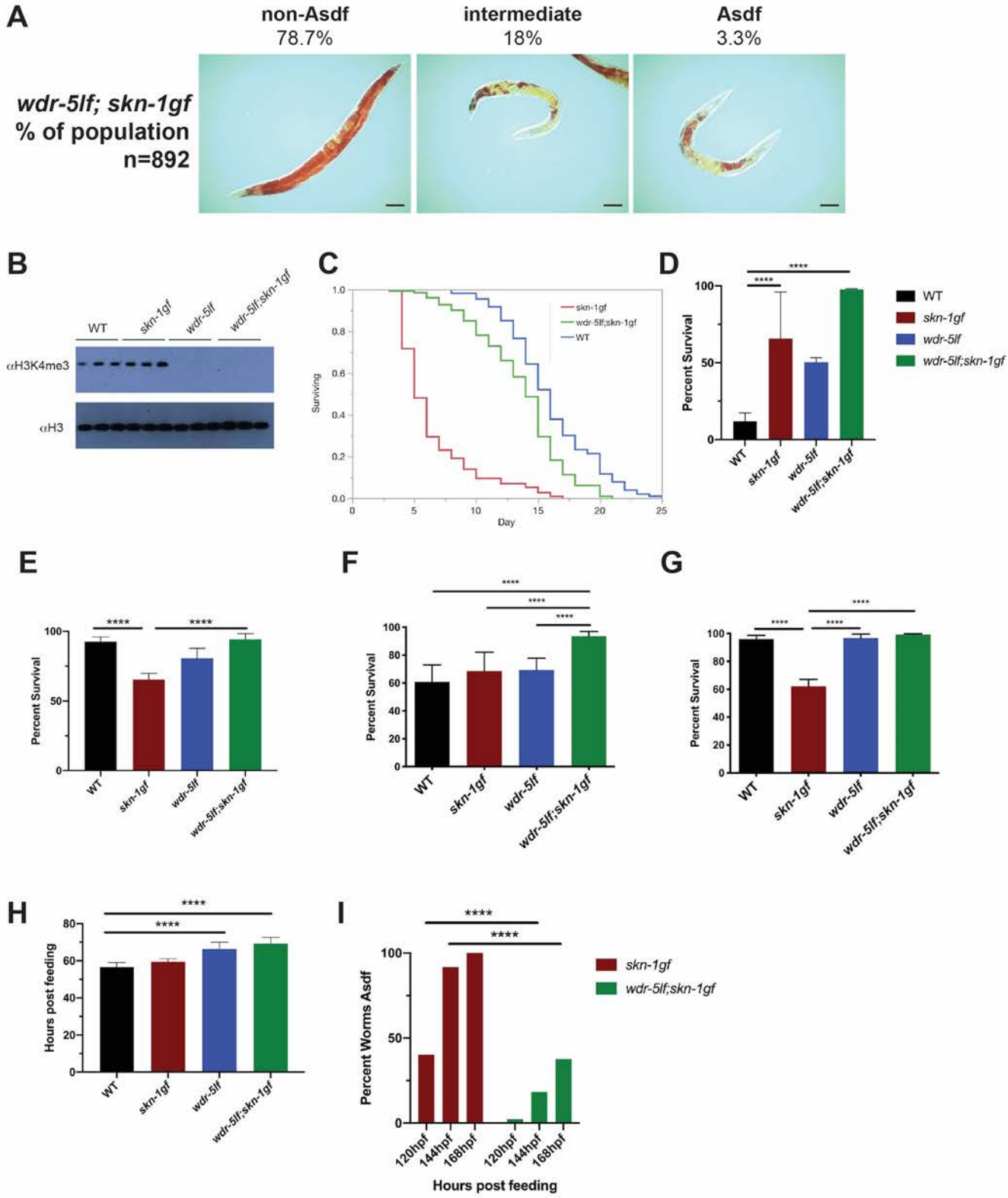
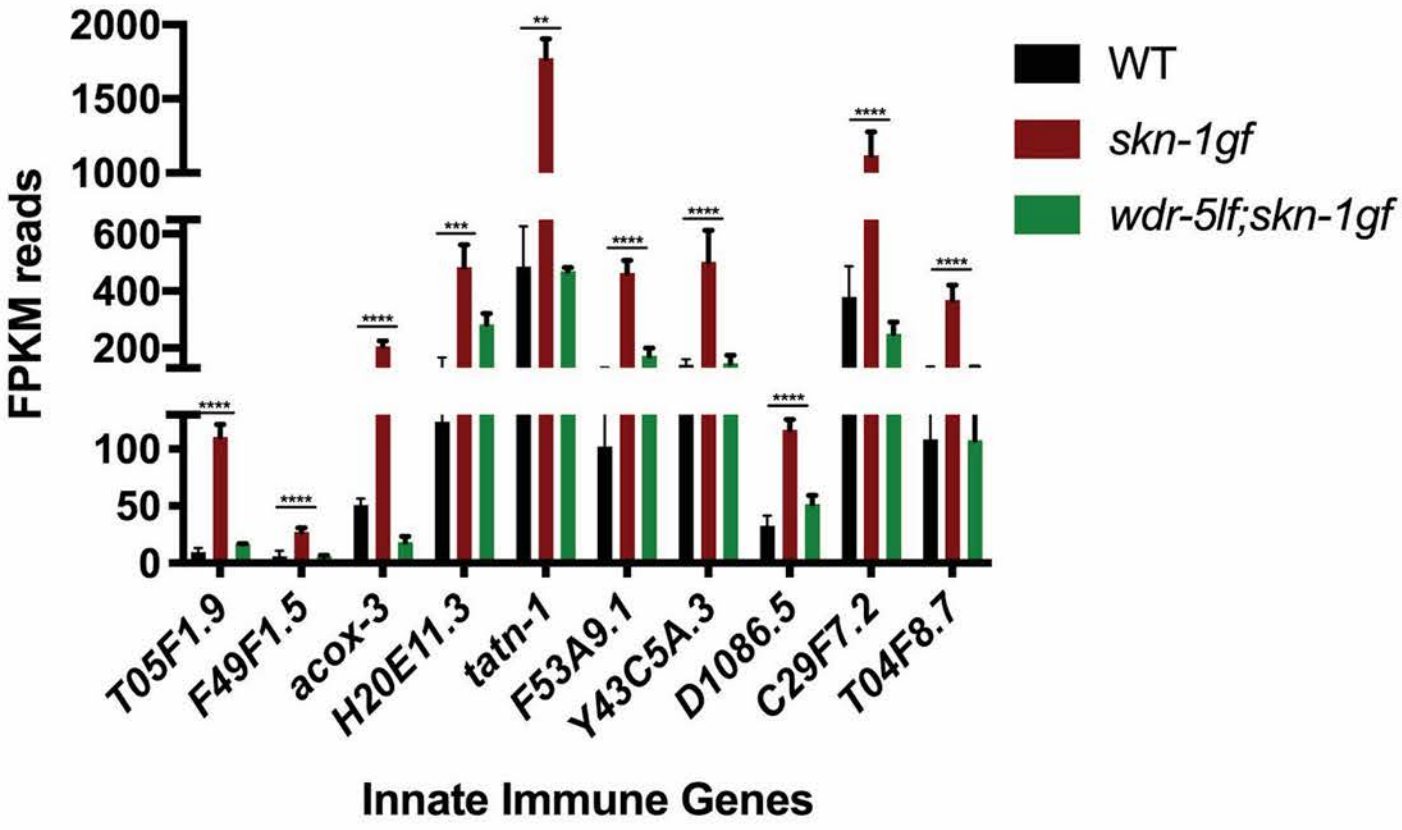
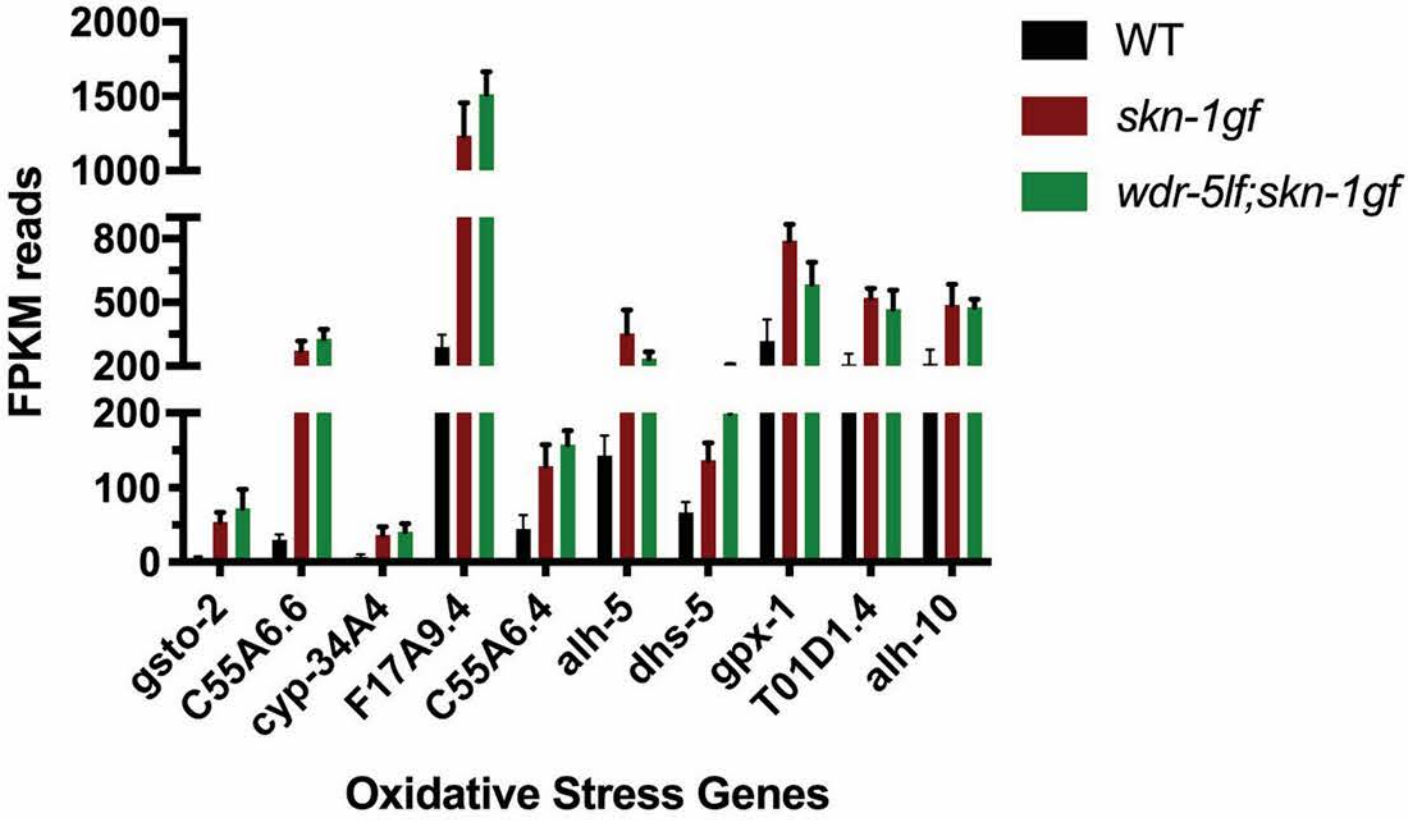

A
non-Asdf
78.7%
intermediate
18%
Asdf
3.3%
wdr-5lf; skn-1gf
% of population
n=892
B
WT
skn-1gf
wdr-5lf
wdr-5lf;skn-1gf
αH3K4me3
αH3
C
skn-1gf
wdr-5lf;skn-1gf
WT
Surviving
Day
D
Percent Survival
WT
skn-1gf
wdr-5lf
wdr-5lf;skn-1gf
E
Percent Survival
F
Percent Survival
G
Percent Survival
H
Hours post feeding
I
Percent Worms Asdf
skn-1gf
wdr-5lf;skn-1gf
120hpf
144hpf
168hpf
Hours post feeding

Figure S3

A



B



C

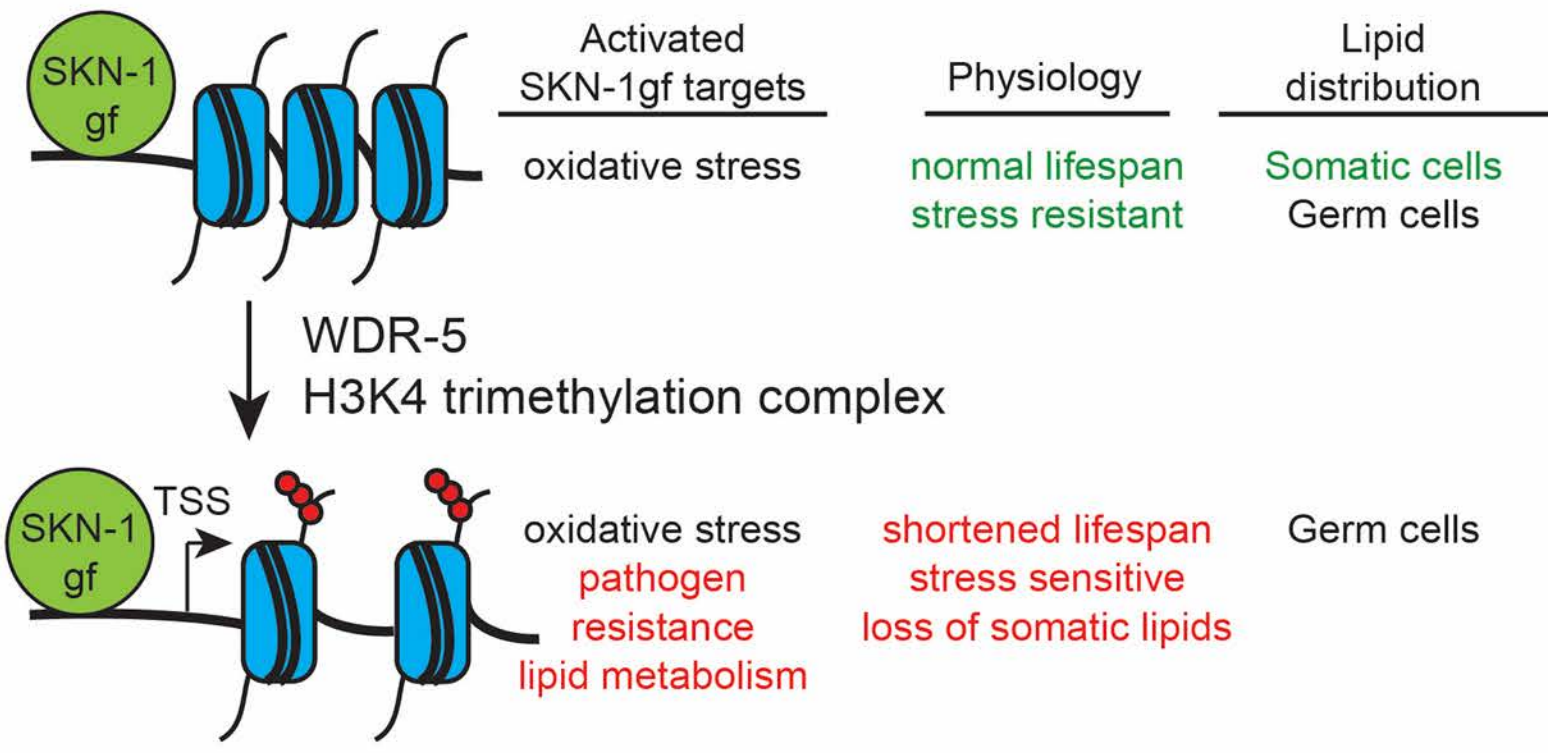


Figure S4

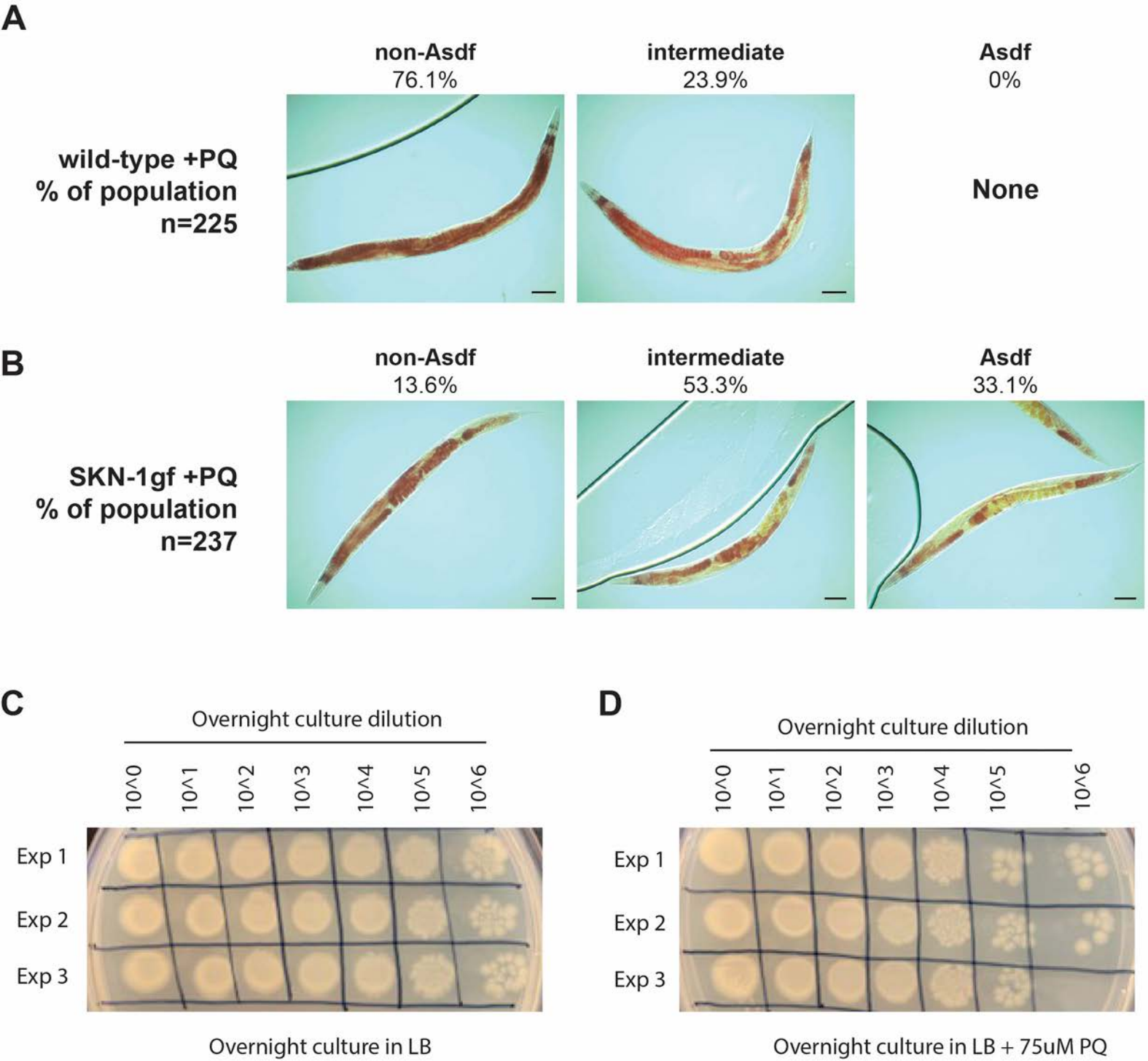

A
non-Asdf
76.1%
intermediate
23.9%
Asdf
0%
wild-type +PQ
% of population
n=225
None
B
non-Asdf
13.6%
intermediate
53.3%
Asdf
33.1%
SKN-1gf +PQ
% of population
n=237
C
Overnight culture dilution
10^0
10^1
10^2
10^3
10^4
10^5
10^6
Exp 1
Exp 2
Exp 3
Overnight culture in LB
D
Overnight culture dilution
10^0
10^1
10^2
10^3
10^4
10^5
10^6
Exp 1
Exp 2
Exp 3
Overnight culture in LB + 75uM PQ

Figure S5

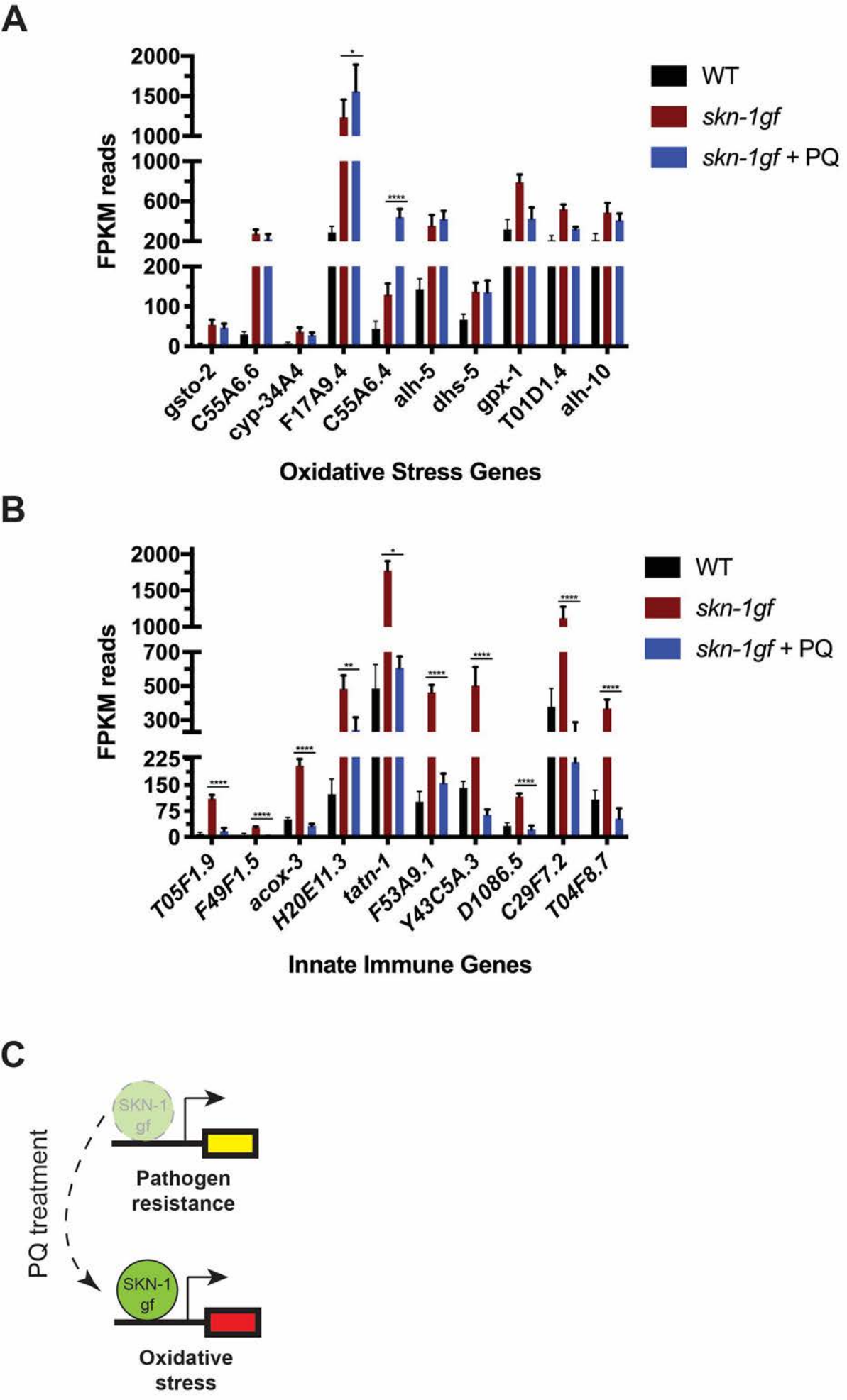
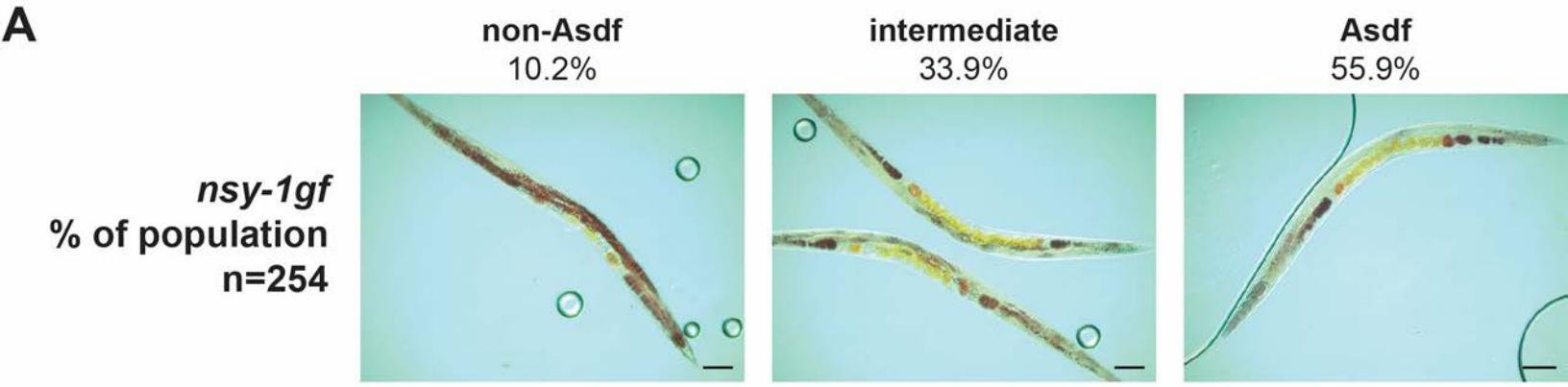

A
FPKM reads
2000
1500
1000
1000
600
200
200
100
0
WT
skn-1gf
skn-1gf + PQ
gsto-2
C55A6.6
cyp-34A4
F17A9.4
C55A6.4
alh-5
dhs-5
gpx-1
T01D1.4
alh-10
Oxidative Stress Genes
B
FPKM reads
2000
1500
1000
700
500
300
225
150
75
0
WT
skn-1gf
skn-1gf + PQ
T05F1.9
F49F1.5
acox-3
H20E11.3
tatn-1
F53A9.1
Y43C5A.3
D1086.5
C29F7.2
T04F8.7
Innate Immune Genes
C
PQ treatment
SKN-1 gf
Pathogen resistance
SKN-1 gf
Oxidative stress

Figure S6

A


non-Asdf
10.2%
intermediate
33.9%
Asdf
55.9%
nsy-1gf
% of population
n=254

B

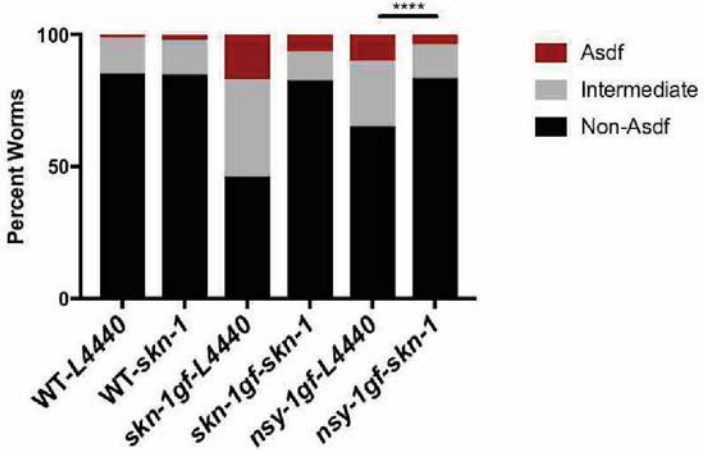
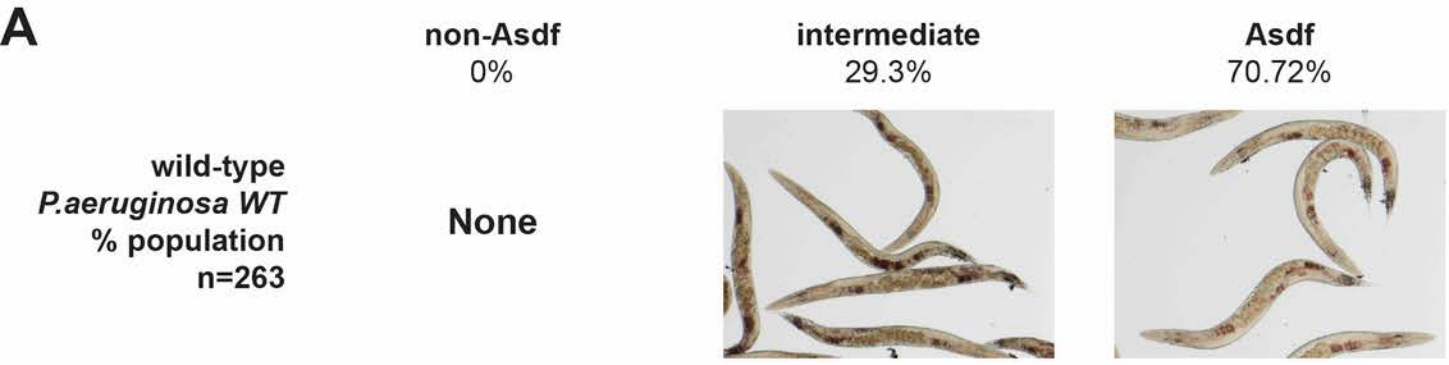
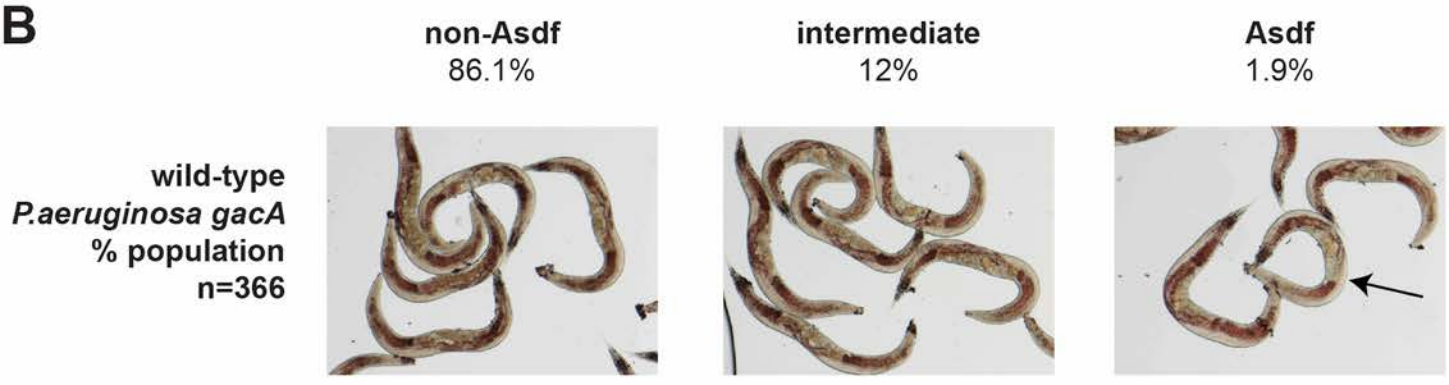
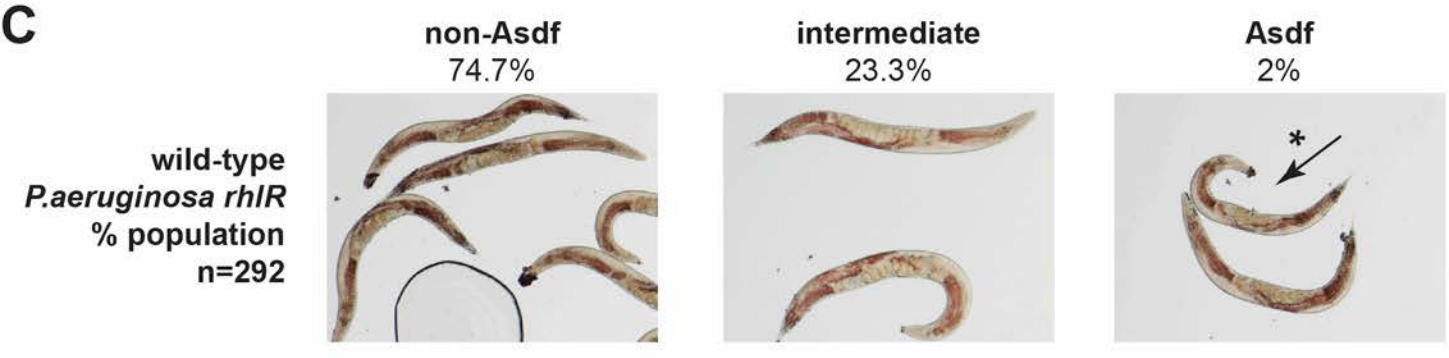
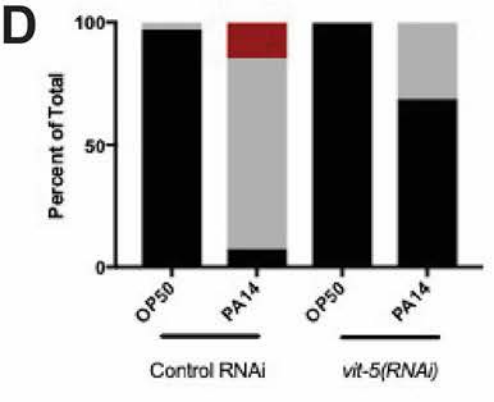

****
100
50
0
Percent Worms
Asdf
Intermediate
Non-Asdf
WT-L4440
WT-skn-1
skn-1gf-L4440
skn-1gf-skn-1
nsy-1gf-L4440
nsy-1gf-skn-1

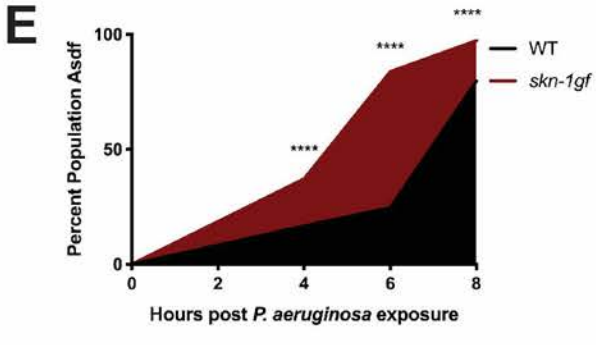
Figure S7

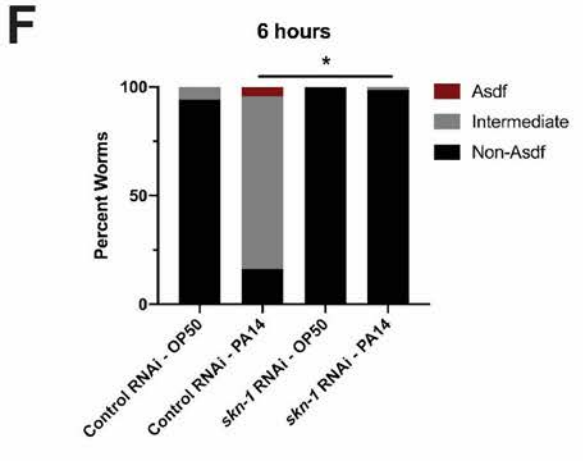

A
non-Asdf
0%
intermediate
29.3%
Asdf
70.72%
wild-type
P.aeruginosa WT
% population
n=263
None


B
non-Asdf
86.1%
intermediate
12%
Asdf
1.9%
wild-type
P.aeruginosa gacA
% population
n=366


C
non-Asdf
74.7%
intermediate
23.3%
Asdf
2%
wild-type
P.aeruginosa rhlR
% population
n=292


D
Percent of Total
100
50
0
OP50
PA14
OP50
PA14
Control RNAi
vit-5(RNAi)


E
Percent Population Asdf
100
50
0
0
2
4
6
8
****
****
****
WT
skn-1gf
Hours post P. aeruginosa exposure


F
6 hours
*
Percent Worms
100
50
0
Asdf
Intermediate
Non-Asdf
Control RNAi - OP50
Control RNAi - PA14
skn-1 RNAi - OP50
skn-1 RNAi - PA14

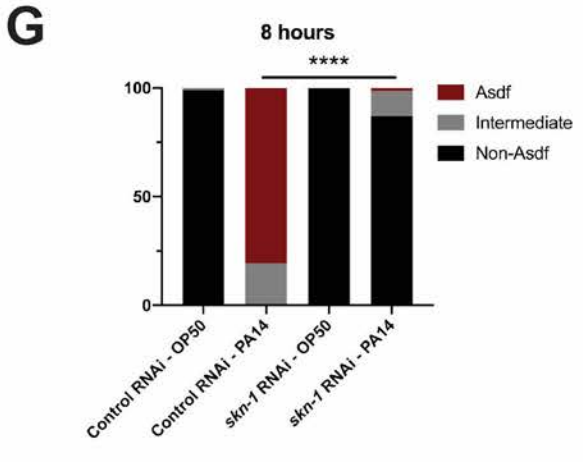

G
8 hours
****
Percent Worms
100
50
0
Asdf
Intermediate
Non-Asdf
Control RNAi - OP50
Control RNAi - PA14
skn-1 RNAi - OP50
skn-1 RNAi - PA14

Figure S8

A

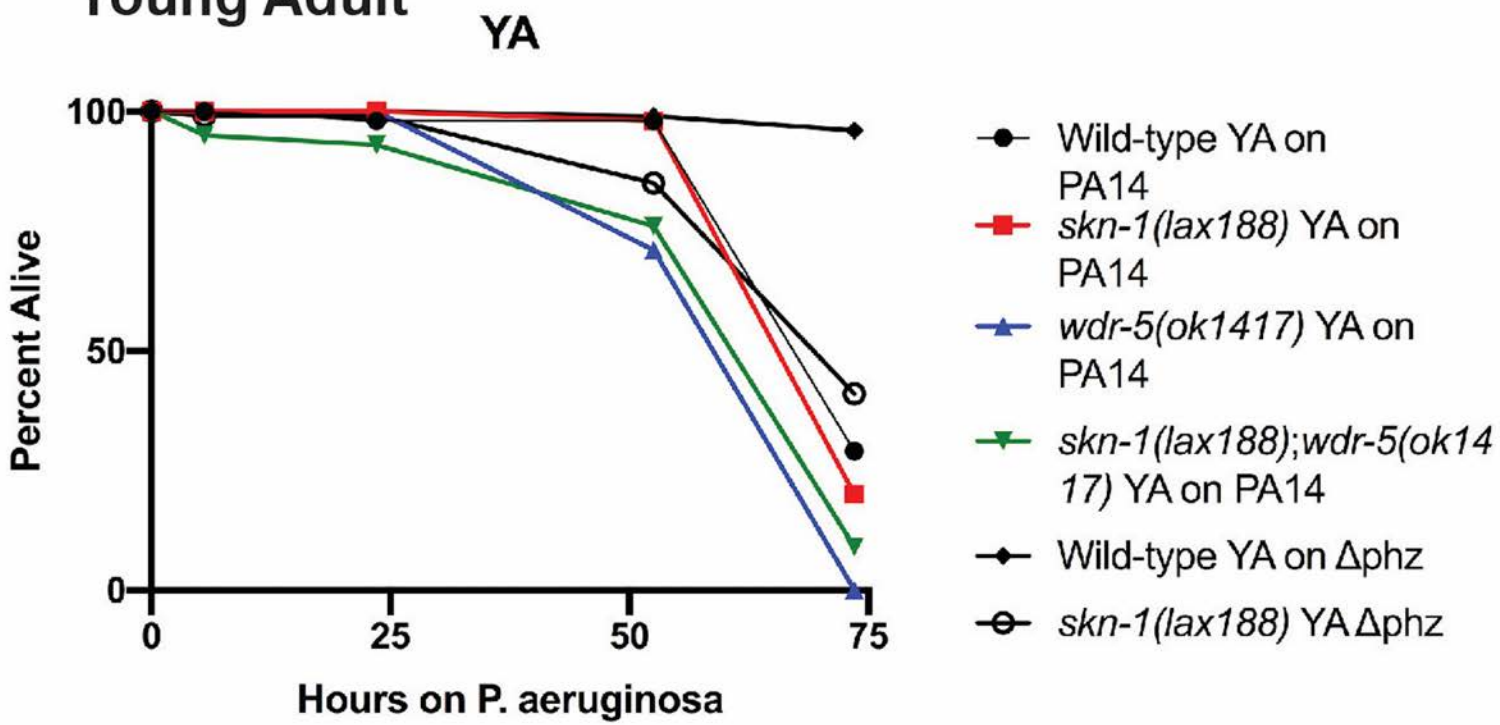
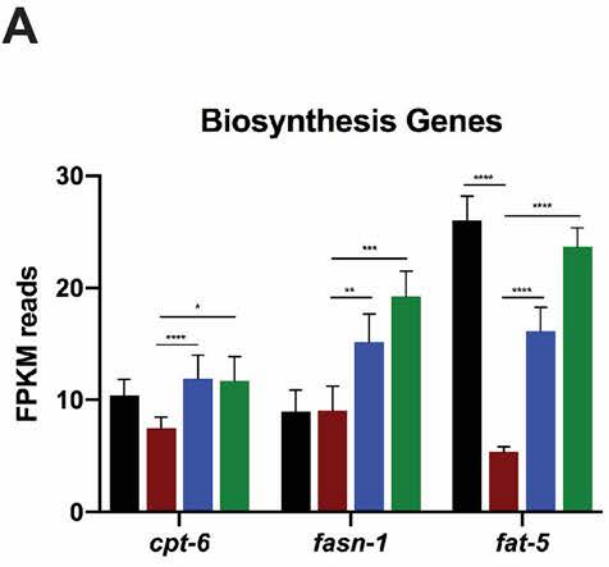
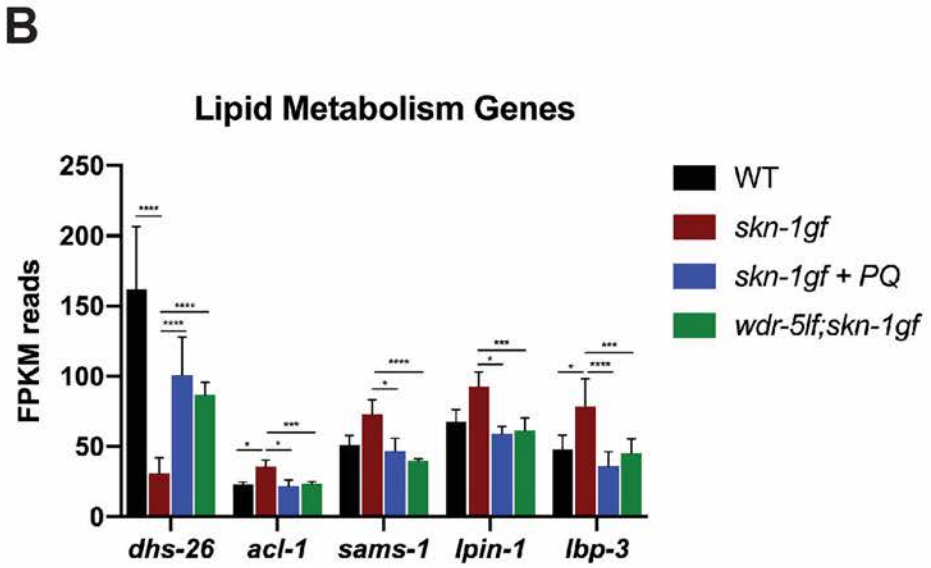
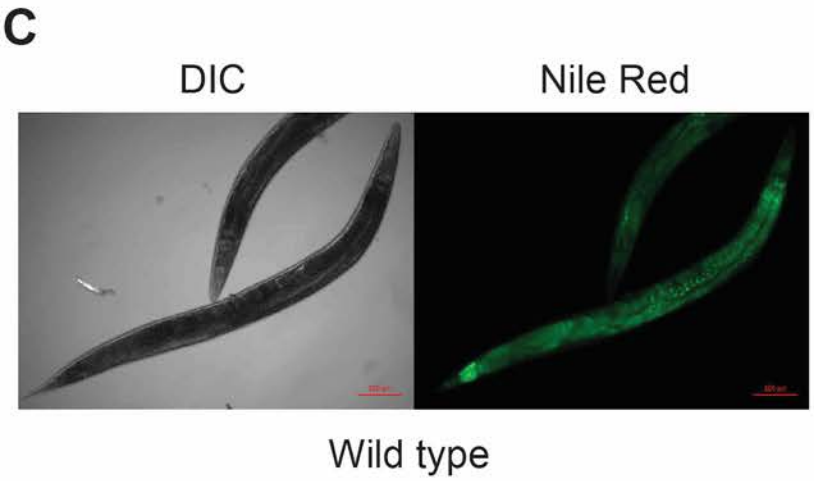

Fast Kill
Young Adult
YA
100
50
0
Percent Alive
0
25
50
75
Hours on P. aeruginosa
Wild-type YA on PA14
skn-1(lax188) YA on PA14
wdr-5(ok1417) YA on PA14
skn-1(lax188);wdr-5(ok1417) YA on PA14
Wild-type YA on Δphz
skn-1(lax188) YA Δphz

Figure S9


A
Biosynthesis Genes
FPKM reads
30
20
10
0
cpt-6
fasn-1
fat-5


B
Lipid Metabolism Genes
FPKM reads
250
200
150
100
50
0
dhs-26
acl-1
sams-1
lpin-1
lbp-3
WT
skn-1gf
skn-1gf + PQ
wdr-5lf;skn-1gf


C
DIC
Nile Red
Wild type

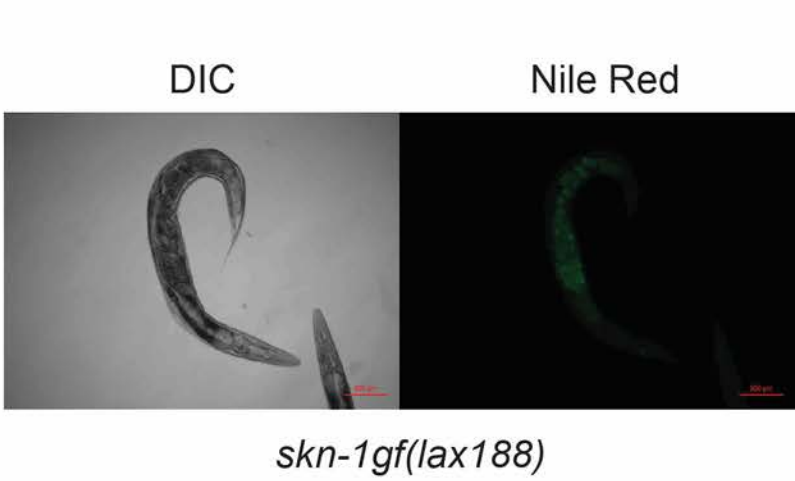

DIC
Nile Red
skn-1gf(lax188)

Figure S10