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Supplemental information

Quantification of tissue-specific protein translation in whole *C. elegans* using O-propargyl-puromycin labeling and fluorescence microscopy

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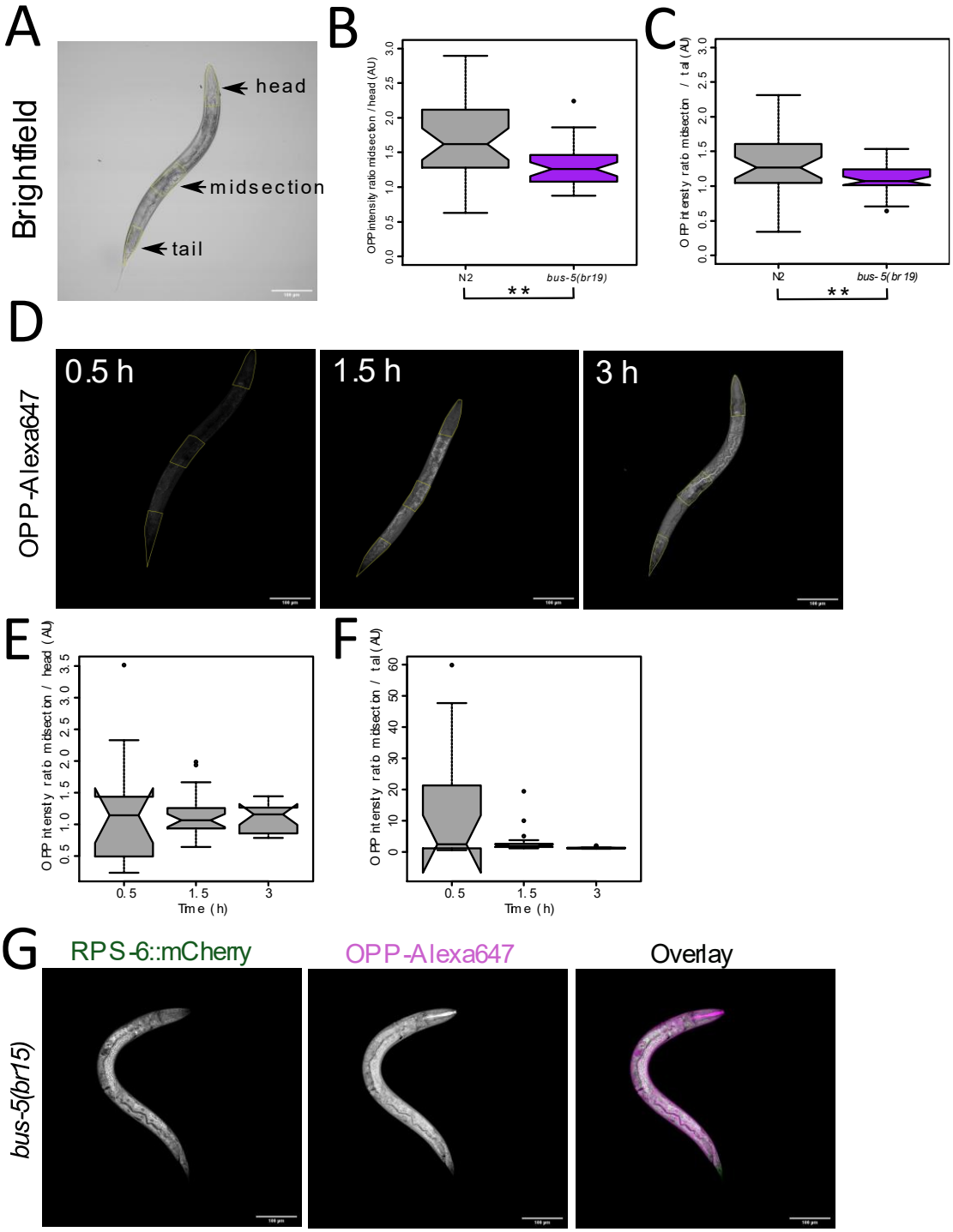
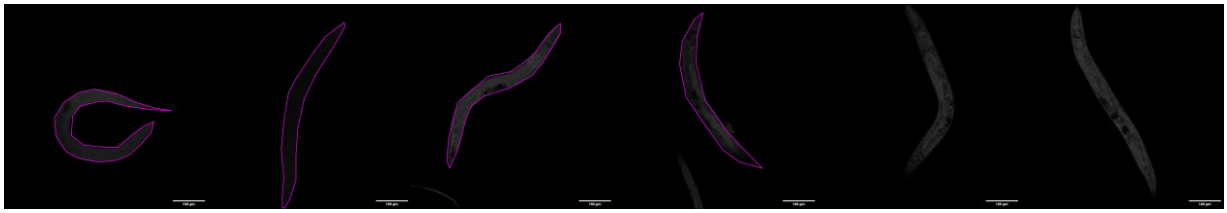

A
Brightfield
head
midsection
tail
B
OPP intensity ratio midsection / head (AU)
N2
bus-5(br19)
**
C
OPP intensity ratio midsection / tail (AU)
N2
bus-5(br19)
**
D
OPP-Alexa647
0.5 h
1.5 h
3 h
E
OPP intensity ratio midsection / head (AU)
Time (h)
F
OPP intensity ratio midsection / tail (AU)
Time (h)
G
bus-5(br15)
RPS-6::mCherry
OPP-Alexa647
Overlay

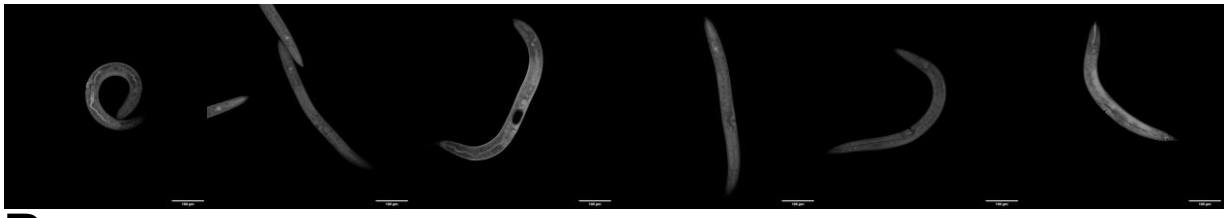
Supplemental Figure S1. Uptake of OPP in distal tissues between N2 and *bus-5(br15)* and with time, related to Figure 1. A) Representative brightfield image of *bus-5(br15)* mutant with areas used to quantify OPP signal intensity in the head, tail, and midsection indicated in yellow. Quantification of the OPP intensity between B) midsection and head and between C) the midsection and the tail in N2 and *bus-5(br15)*. n=33 summed across three independent replicates. D) Representative fluorescent images of *bus-5(br15)* mutant treated with OPP for 0.5 (left), 1.5 (middle), or 3 hr (right). The head, tail, and midsection are indicated in yellow. Quantification of the OPP intensity after incubation for 0.5, 1.5 and 3 hr between E) midsection and head and between F) the midsection and the tail in the *bus-5(br15)* mutant. n=33 summed across 3 independent replicates. G) Representative image of RPS-6::mCherry (left), OPP-Alexa 647 (middle), and the overlay of the two channels in *bus-5(br15);rps-6::mCherry* (right). In the overlay RPS-6::mCherry is green, OPP is magenta. Notches in box plots indicate the estimated 95% confidence interval of the median value (black line). ** p-value < 0.01. Wilcoxon rank sum test.

A

N2

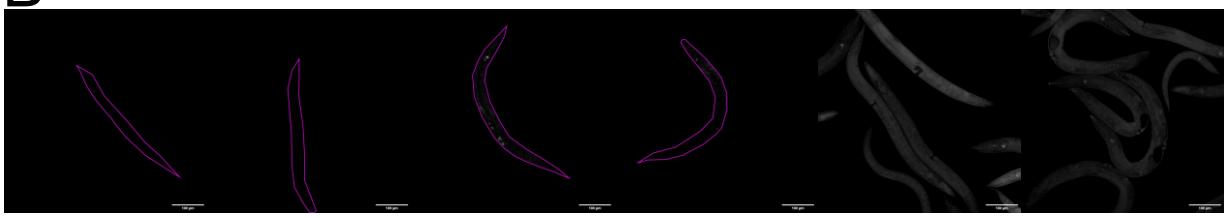


DC19

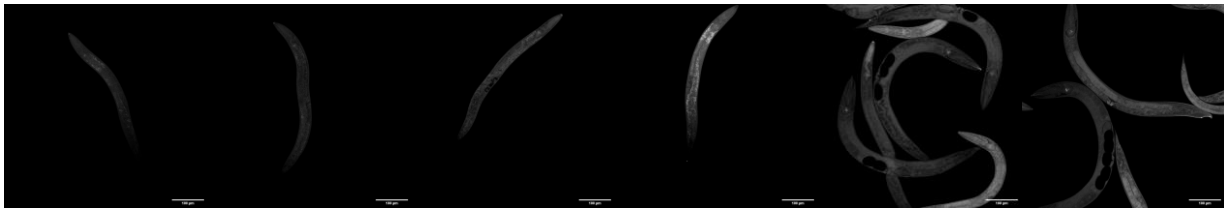


B

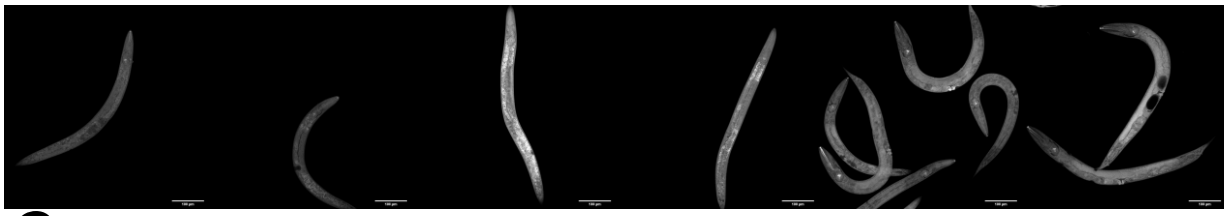
0.5 hr



1.5 hr

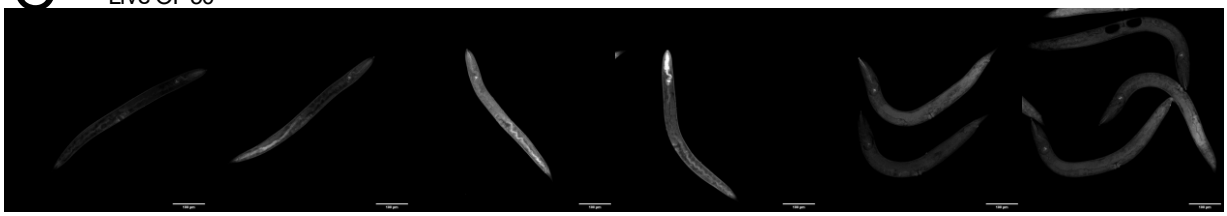


3 hr

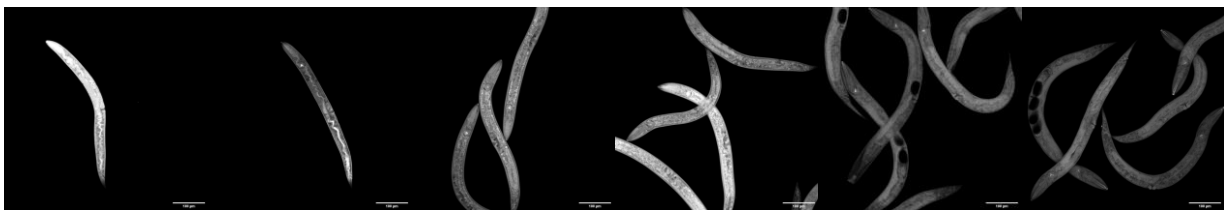


C

Live OP50



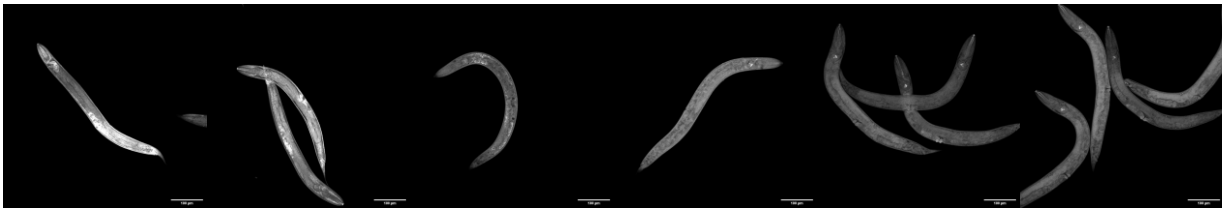
PFA killed OP50



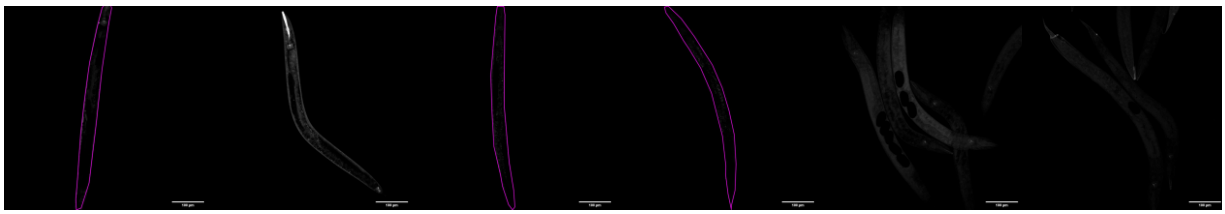
Supplemental Figure S2. Additional representative images of worms from OPP assays, related to Figures 1 and 2. A) Additional representative images from the three independent replicates of N2 vs DC19 OPP assay shown in Figure 1C and D. The N2 images are shown in the first row, and DC19 images are shown in the second row. B) Additional representative images from the three independent replicates of the OPP incubation time assay quantified in Figure 1F. The 0.5 hr incubation images are shown in the first row, the 1.5 hr incubation images are shown in the second row, and the 3 hr incubation are show in the third row. C) Additional representative images from the three independent replicates of the OPP assay comparing live OP50 vs PFA killed OP50 shown in Figure 2A. The live OP50 images are shown in the first row, and the PFA killed OP50 images are shown in the second row.

A

PFA Fixed

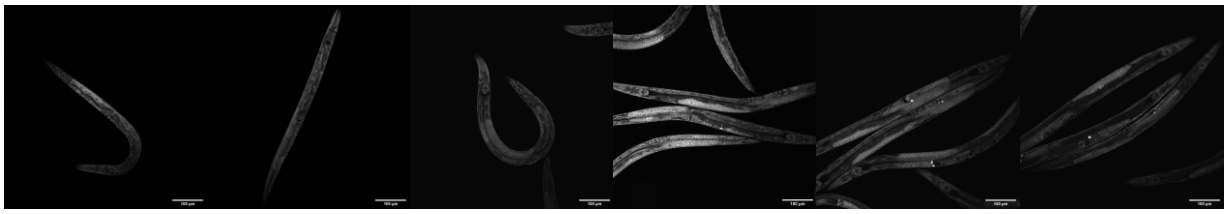


Killed with PFA

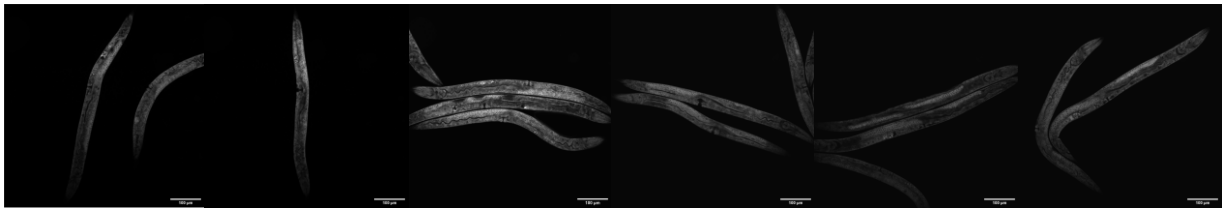


B

rps-6::mCherry live imaging

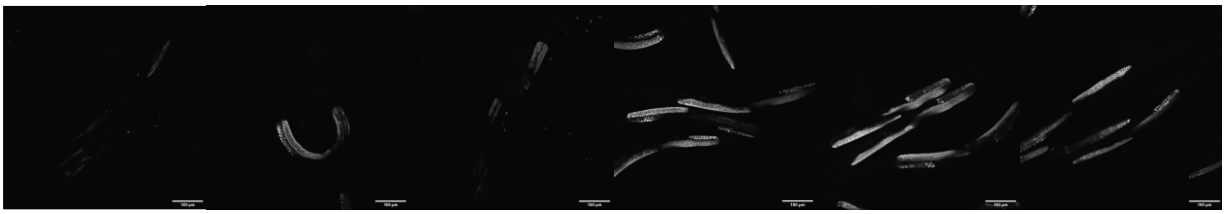


rps-6::mCherry fixation imaging

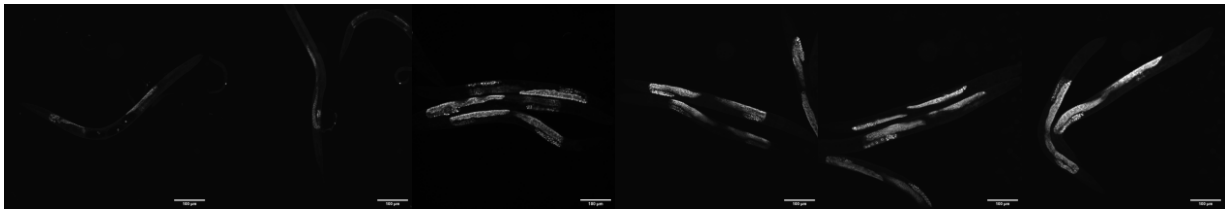


C

glh-1::GFP live imaging



glh-1::GFP fixation imaging

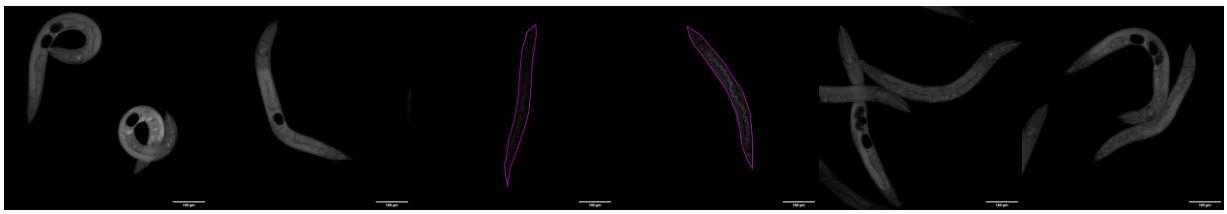


Supplemental Figure S3. Additional representative images of fixed RPS-6::mCherry and GLH-1::GFP worms, related to Figure 2. A) Additional representative images from the three independent replicates of the PFA fixation time assay quantified in Figure 2B. The first row shows the worms fixed normally for 1 hr in PFA, the second row shows worms killed in PFA by exposing them for 5 mins. B) Additional representative images from the three independent replicates of the RPS-6::mCherry fluorescence in live worms, and after PFA fixation shown in Figure 2C and D. The first row shows the RPS-6::mCherry fluorescence in live worms, the second row shows RPS-6::mCherry fluorescence after PFA fixation. C) Additional representative images from the three independent replicates of the GLH-1::GFP fluorescence in live worms, and after PFA fixation shown in Figure 2F and G. The first row shows the GLH-1::GFP fluorescence in live worms, the second row shows GLH-1::GFP fluorescence after PFA fixation.

A

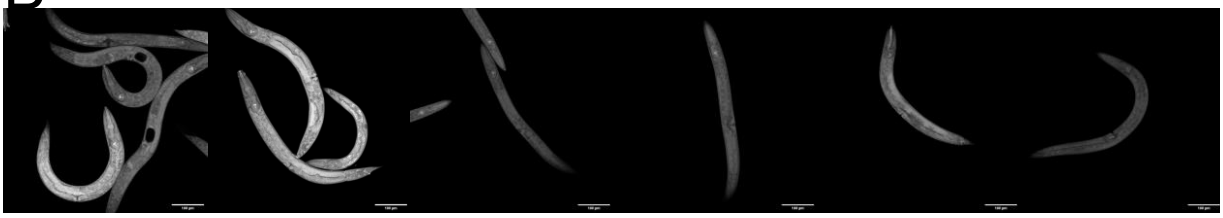
Bortezomib chase

M9 chase

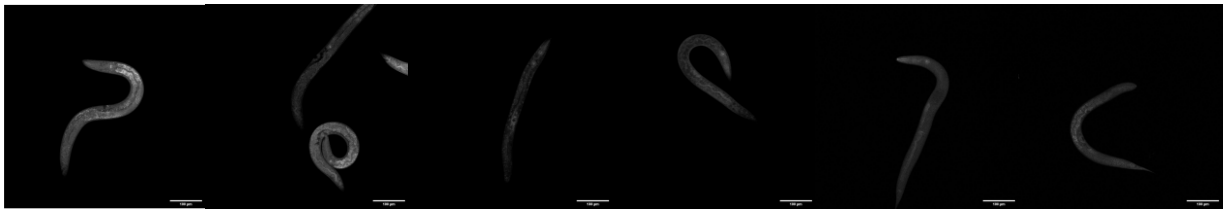


B

OPP Control

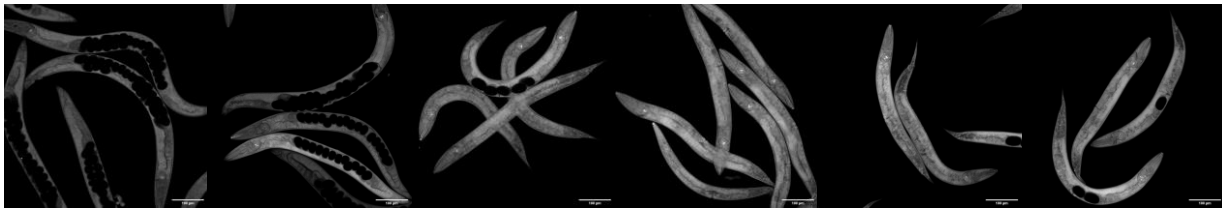


Heat-shocked

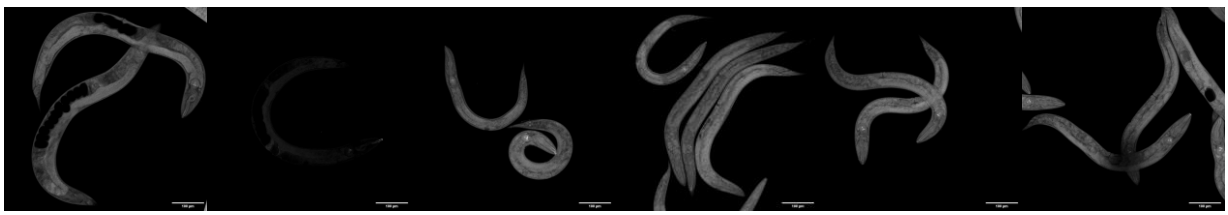


C

OPP Control

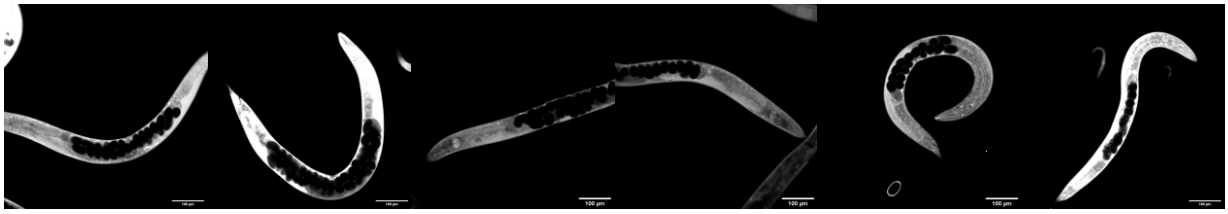


Cycloheximide treated

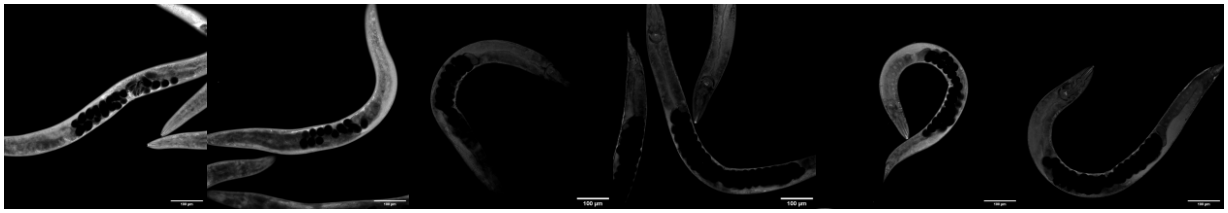


Supplemental Figure S4. Additional representative images of worms from OPP assays that were treated with bortezomib, heat shock or cycloheximide, related to Figure 3. A) Additional representative images from the three independent replicates of the OPP Bortezomib chase assay quantified in Figure 3A. The first row includes worms chased with Bortezomib following the OPP incubation, the second row shows worms chased in M9 following the OPP incubation. B) Additional representative images from the three independent replicates of the OPP heat shock assay shown in Figure 3D and E. The first row shows OPP treated control worms; the second row shows heat shocked worms treated with OPP. C) Additional representative images from the three independent replicates of the OPP cycloheximide treatment assay quantified in Figure 3C. The first row shows OPP treated control worms, the second row shows worms treated with cycloheximide prior to and during OPP incubation.

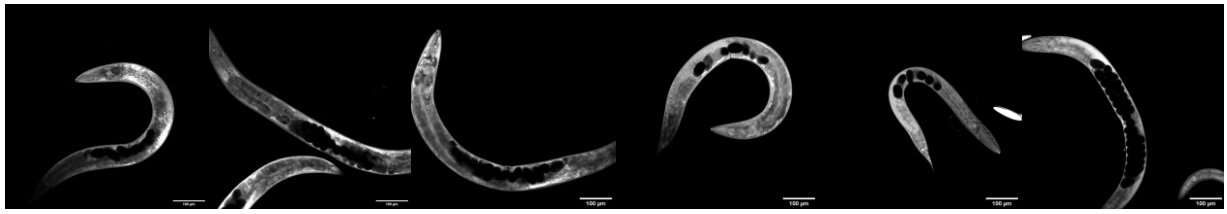
A L4440 RNAi treated



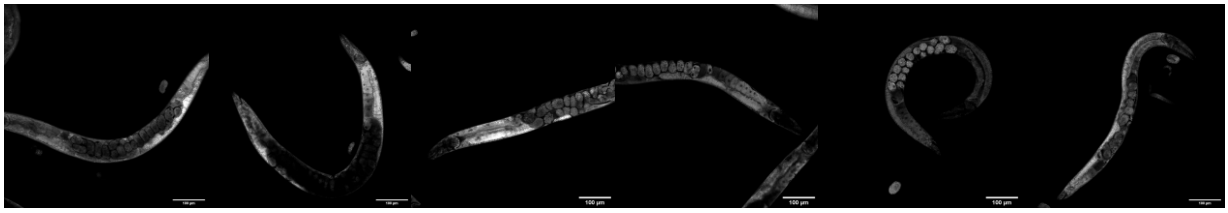
iff-1 RNAi treated



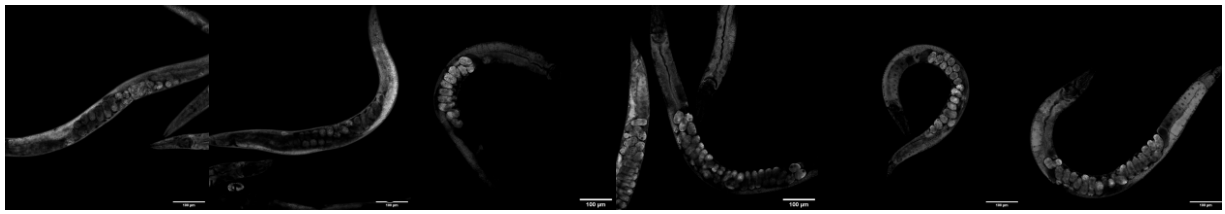
ifg-1 RNAi treated



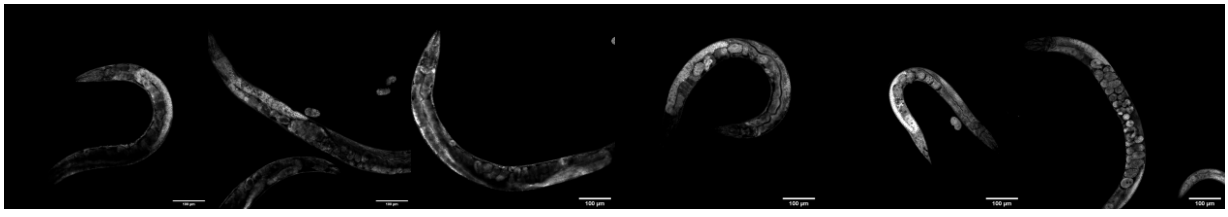
B L4440 RNAi treated rps -6 signal



iff-1 RNAi treated rps-6 signal



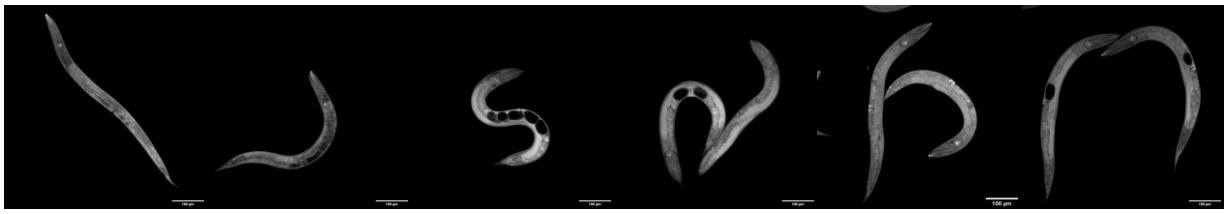
ifg-1 RNAi treated rps-6 signal



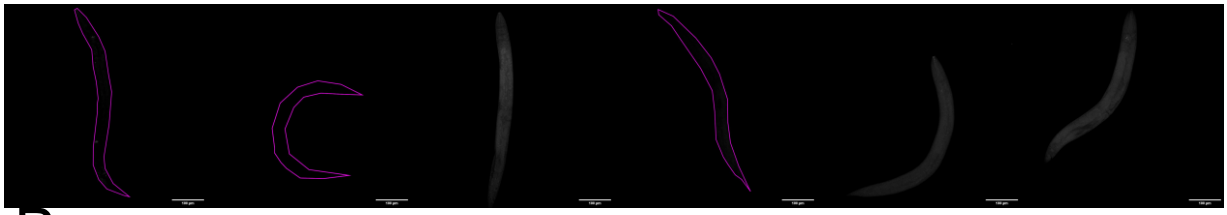
Supplemental Figure S5. Additional representative images of worms from OPP assays and RPS-6::mCherry that were treated with ifg-1 or iff-1 RNAi, related to Figure 3. A) Additional representative images from the three independent replicates of the OPP assay of L4440 control, *iff-1* and *ifg-1* RNAi treated worms shown in Figure 3G. The first row shows L4440 treated worms, the second row shows *iff-1* treated worms and the third row shows *ifg-1* treated worms. B) Additional representative images from the three independent replicates of the RPS-6::mCherry fluorescence under L4440 control, *iff-1* and *ifg-1* RNAi treatment shown in Figure 3I. The first row shows L4440 treated worms, the second row shows *iff-1* treated worms and the third row shows *ifg-1* treated worms.

A

OPP Control

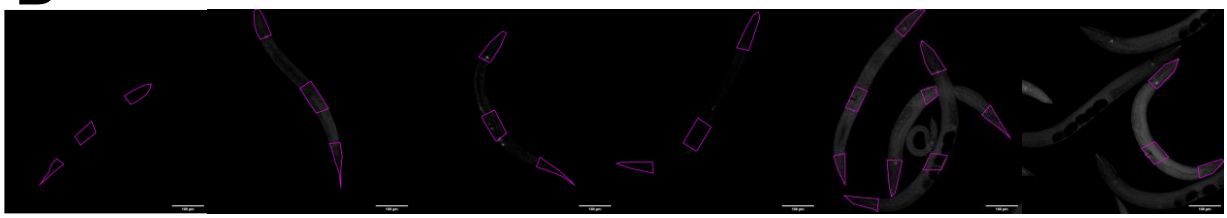


No puromycin

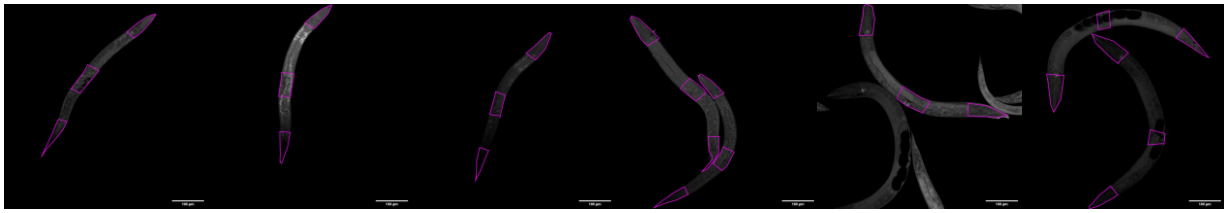


B

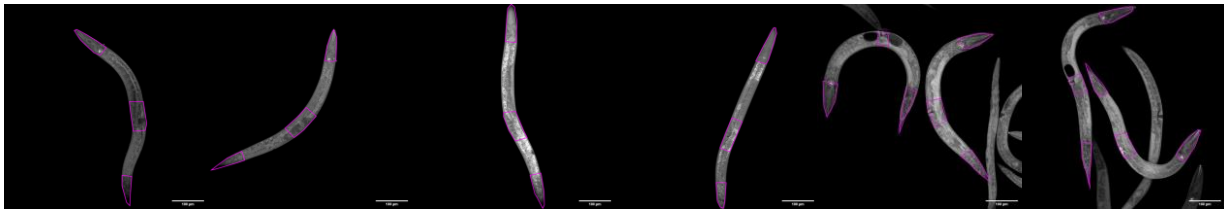
0.5 hr puromycin incubation, head tail and vulva comparison



1.5 hr puromycin incubation, head tail and vulva comparison

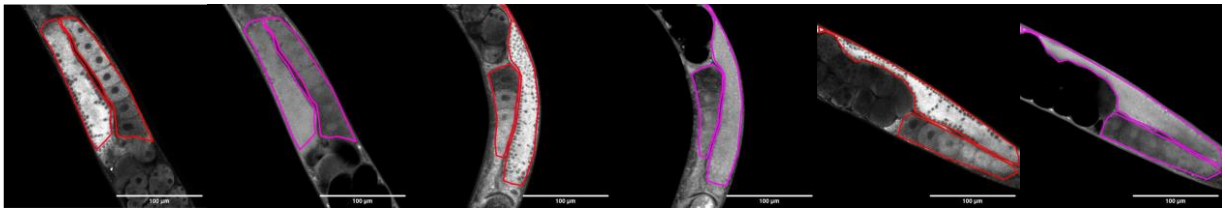


3 hr puromycin incubation, head tail and vulva comparison



C

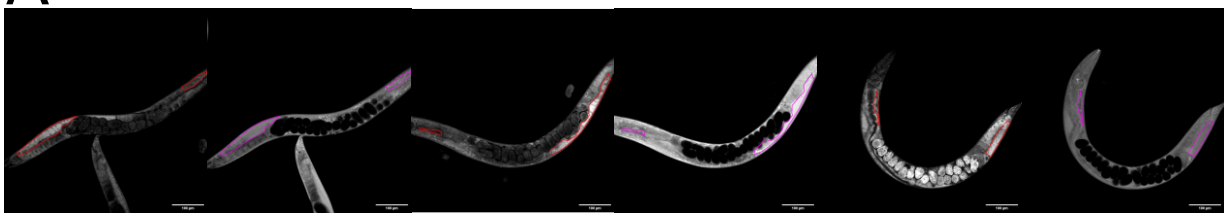
2D Germline L4440 RNAi treated worms quantification



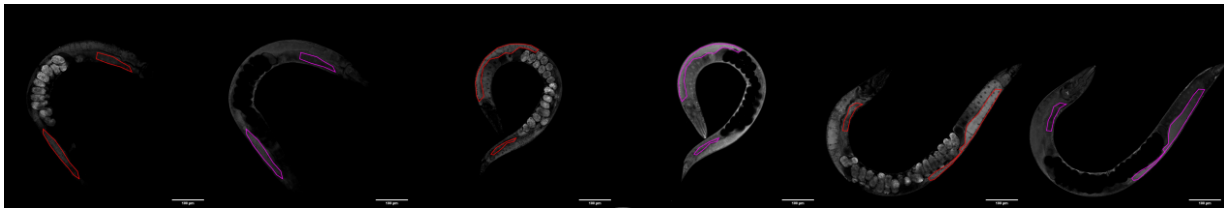
Supplemental Figure S6. Additional representative images of worms from OPP assays in which specific tissues were quantified, related to Figures 1, 3, and 4. A) Additional representative images from the three independent replicates of OPP-Alexa 647 and only Alexa 647 azide assay quantified in Figure 3B. The first row shows control OPP treated worms, the second row shows worms incubated in M9 with no OPP followed by incubation in the Alexa 647 Azide. B) Additional representative images from the three independent replicates of the OPP incubation time assay, areas selected for comparison highlighted in magenta as shown in Figure S1D. The first row shows the 0.5 hr OPP incubation, the second row shows the 1.5 hr OPP incubation and the third row shows the 3 hr incubation. C) Additional representative images from the three independent replicates of L4440 treated worms' germline specific RPS-6::mCherry (selected in red) and OPP (selected in magenta) measurements shown in Figure 4A. The distal and proximal sections of the germline are highlighted separately.

A

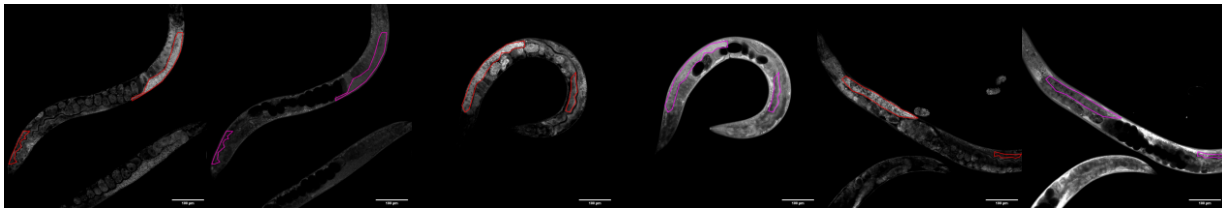
L4440 RNAi treated germline vs intestine rps-6::mCherry (red) OP-Puro (magenta)



iff-1 RNAi treated germline vs intestine rps-6::mCherry (red) OP-Puro (magenta)

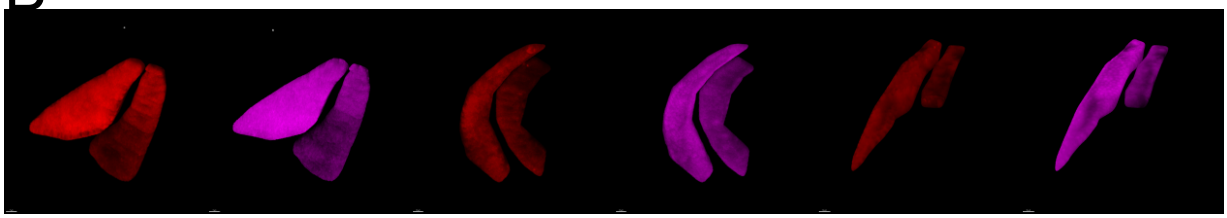


ifg-1 RNAi treated germline vs intestine rps -6::mCherry (red) OP-Puro (magenta)

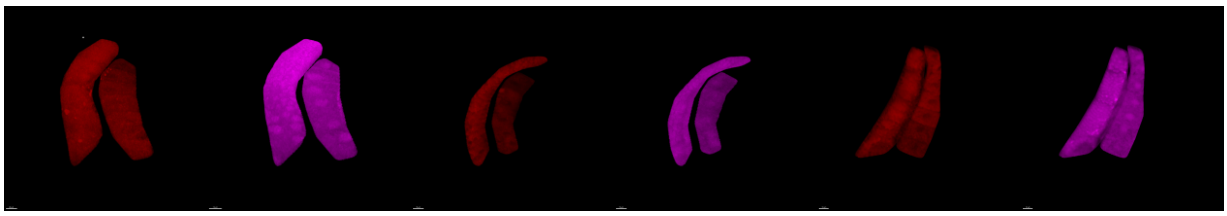


B

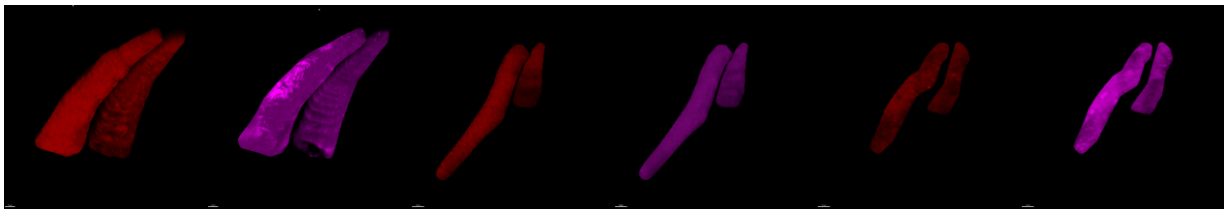
L4440 RNAi treated 3D germline rps-6::mCherry (red) OP-Puro (magenta)



iff-1 RNAi treated 3D germline rps -6::mCherry (red) OP-Puro (magenta)



ifg-1 RNAi treated 3D germline rps-6::mCherry (red) OP-Puro (magenta)



Supplemental Figure S7. Additional representative images of worms from tissue specific OPP assays and RPS-6::mCherry that were treated with ifg-1 or iff-1 RNAi, related to Figure 4 and Figure 5. A) Additional representative images from the three independent replicates of the OPP assay of L4440 RNAi treated worms, *iff-1* RNAi treated worms and *ifg-1* treated worms shown in Figure 4C. The intestine and the distal portion of the germline are selected in the images, RPS-6::mCherry (highlighted in red) and OPP (highlighted in magenta) are included for each rep and treatment. The first row shows L4440 treated worms, the second row shows *iff-1* treated worms and the third row shows *ifg-1* treated worms. B) Additional representative images from the three independent replicates of 3D distal and proximal germline selections of the OPP assay of L4440 RNAi treated worms, *iff-1* RNAi treated worms and *ifg-1* treated worms shown in Figure 5A and C. The distal and proximal portions of the germline are highlighted in RPS-6 (red) and OPP (magenta). The first row shows L4440 treated worms, the second row shows *iff-1* treated worms and the third row shows *ifg-1* treated worms.