Cell Reports

Supplemental Information

C. elegans Body Cavity Neurons Are Homeostatic Sensors that Integrate Fluctuations in Oxygen Availability and Internal Nutrient Reserves

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Figure S1

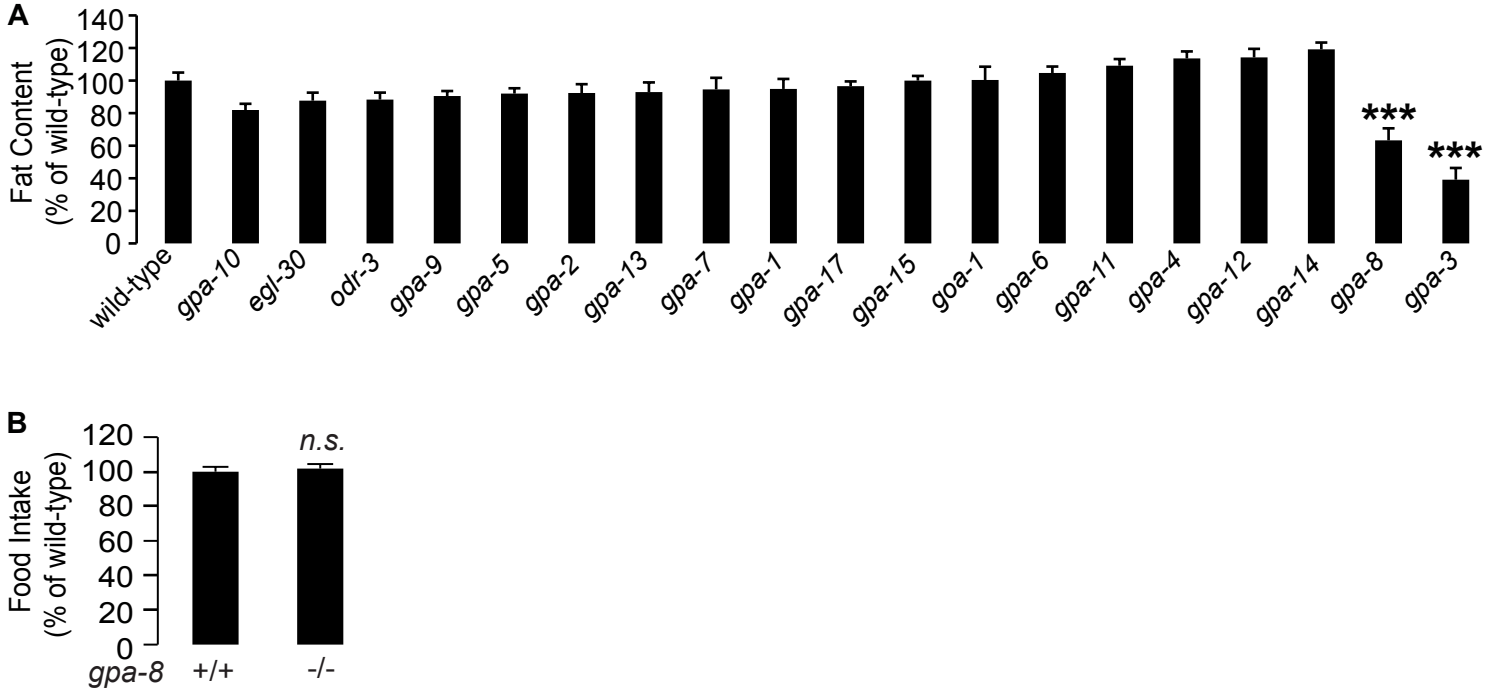
A
Fat Content
(% of wild-type)
140
120
100
80
60
40
20
0
***
***
wild-type
gpa-10
egl-30
odr-3
gpa-9
gpa-5
gpa-2
gpa-13
gpa-7
gpa-1
gpa-17
gpa-15
goa-1
gpa-6
gpa-11
gpa-4
gpa-12
gpa-14
gpa-8
gpa-3
B
Food Intake
(% of wild-type)
120
100
80
60
40
20
0
n.s.
gpa-8
+/+
-/-

Figure S1. GPA-8 is required to maintain peripheral fat levels, related to Figure 1.

(A) All viable *C. elegans* Gα mutants were fixed and stained with oil Red O. Fat content was quantified for each genotype and is expressed as a percentage of wild-type animals ± SEM (n=10–20). ***, $p<0.001$ by Student's t-test.

(B) Food intake was measured by counting the rhythmic contractions of the pharyngeal bulb over a 10 s period using a Zeiss M2-Bio microscope at 10X magnification. Data are expressed as a percentage of wild-type animals ± SEM. n.s., not significant by Student's t-test.

Figure S2

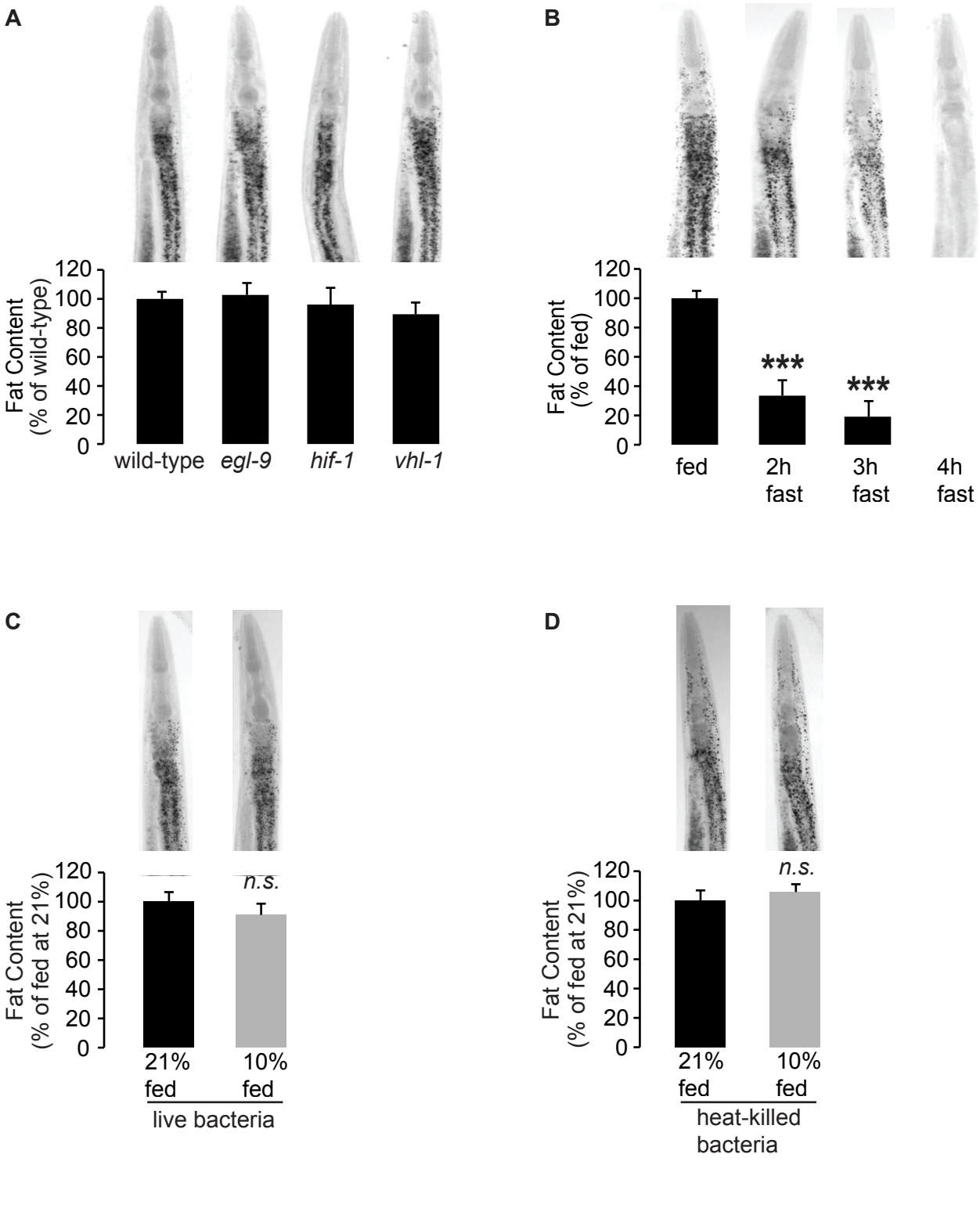
A
Fat Content (% of wild-type)
120
100
80
60
40
20
0
wild-type
egl-9
hif-1
vhl-1
B
Fat Content (% of fed)
***
***
fed
2h fast
3h fast
4h fast
C
Fat Content (% of fed at 21%)
n.s.
21% fed
10% fed
live bacteria
D
Fat Content (% of fed at 21%)
n.s.
21% fed
10% fed
heat-killed bacteria

Figure S2. Oxygen-dependent fat regulation is not a function of the hypoxia-sensing pathway, related to Figure 3.

(A) Representative images are shown of animals fixed and stained with oil Red O (upper panels). Fat content was quantified for each genotype and is expressed as a percentage of wild-type animals ± SEM (lower panels; n=8-10). No significant differences were observed by one-way ANOVA.

(B) Wild-type adult animals were fasted for either 2, 3, or 4 h. Representative images are shown of animals fixed and stained with oil Red O (upper panels). Fat content was quantified for each condition and is expressed as a percentage of fed animals ± SEM (lower panels; n=11-18). ***, $p<0.001$ by Student's t-test.

(C) Wild-type adult animals on live bacteria were exposed to either 21% or 10% oxygen for a period of 2.5 h. Representative images are shown of animals fixed and stained with oil Red O (upper panels). Fat content was quantified and is expressed as a percentage of animals exposed to 21% oxygen ± SEM (lower panels; n=17-19). n.s., not significant by Student's t-test.

(D) Wild-type adult animals raised on live bacteria were washed once and transferred to plates containing heat-killed bacteria. Animals were exposed to either 21% or 10% oxygen for a period of 2.5 h. Representative images are shown of animals fixed and stained with oil Red O (upper panels). Fat content was quantified and is expressed as a percentage of animals exposed to 21% oxygen ± SEM (lower panels; n=20-23). n.s., not significant by Student's t-test.

Figure S3

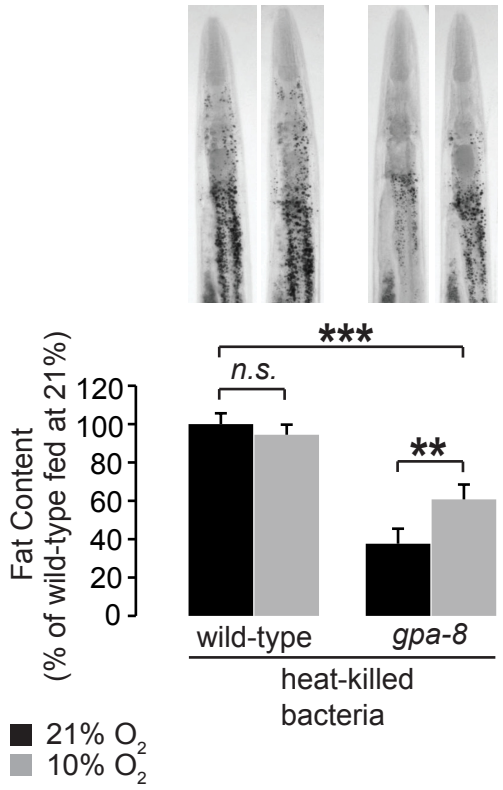
***
n.s.
**
120
100
80
60
40
20
0
Fat Content
(% of wild-type fed at 21%)
wild-type
gpa-8
heat-killed
bacteria
21% O2
10% O2

Figure S3. *gpa-8* mutants show oxygen-dependent fat regulation on heat-killed bacteria, related to Figure 3.

Adult wild-type adult animals and *gpa-8* mutants raised on live bacteria were washed once and transferred to plates containing heat-killed bacteria. Animals were exposed to either 21% or 10% oxygen for a period of 2.5 h. Representative images are shown of animals fixed and stained with oil Red O (upper panels). Fat content was quantified and is expressed as a percentage of wild-type animals exposed to 21% oxygen ± SEM (lower panels; n=20-21). **, $p<0.01$ and ***, $p<0.001$ by two-way ANOVA.

Figure S4

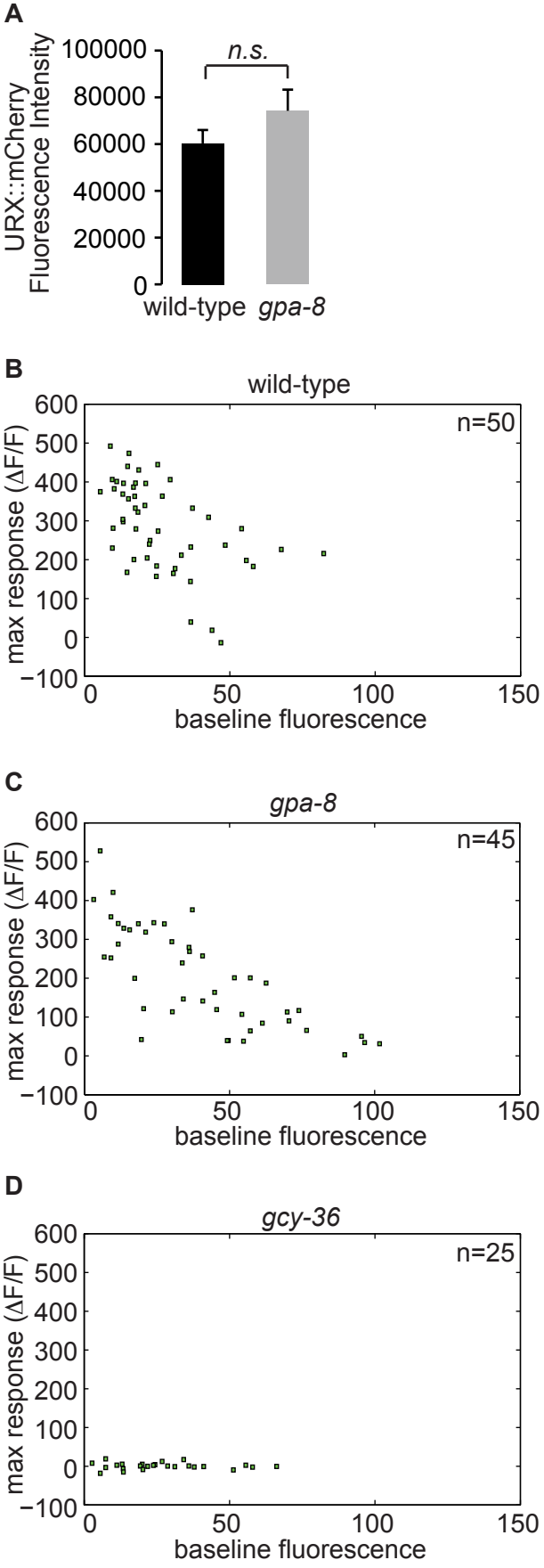
A
URX::mCherry Fluorescence Intensity
100000
80000
60000
40000
20000
0
n.s.
wild-type
gpa-8
B
wild-type
n=50
max response (ΔF/F)
baseline fluorescence
C
gpa-8
n=45
D
gcy-36
n=25

Figure S4. Properties of URX neurons in wild-type, *gpa-8* mutants and *gcy-36* mutants, related to Figure 4.

(A) We imaged mCherry fluorescence in wild-type and *gpa-8* animals expressing both GCaMP5k and mCherry under the control of the *flp-8* promoter. Images were taken in animals exposed to 21% oxygen. For each genotype, the fluorescence intensity was imaged at the same exposure, determined to be within the linear range. Fluorescence intensity was quantified and expressed as an average ± SEM (n=34). n.s., not significant by Student's t-test.

(B-D) Maximum $\Delta F/F_0$ values at 21% oxygen are plotted against the baseline fluorescence at 10% oxygen (F_0) for each animal in the wild-type, *gpa-8*, and *gcy-36* backgrounds (n=25-50).

Figure S5

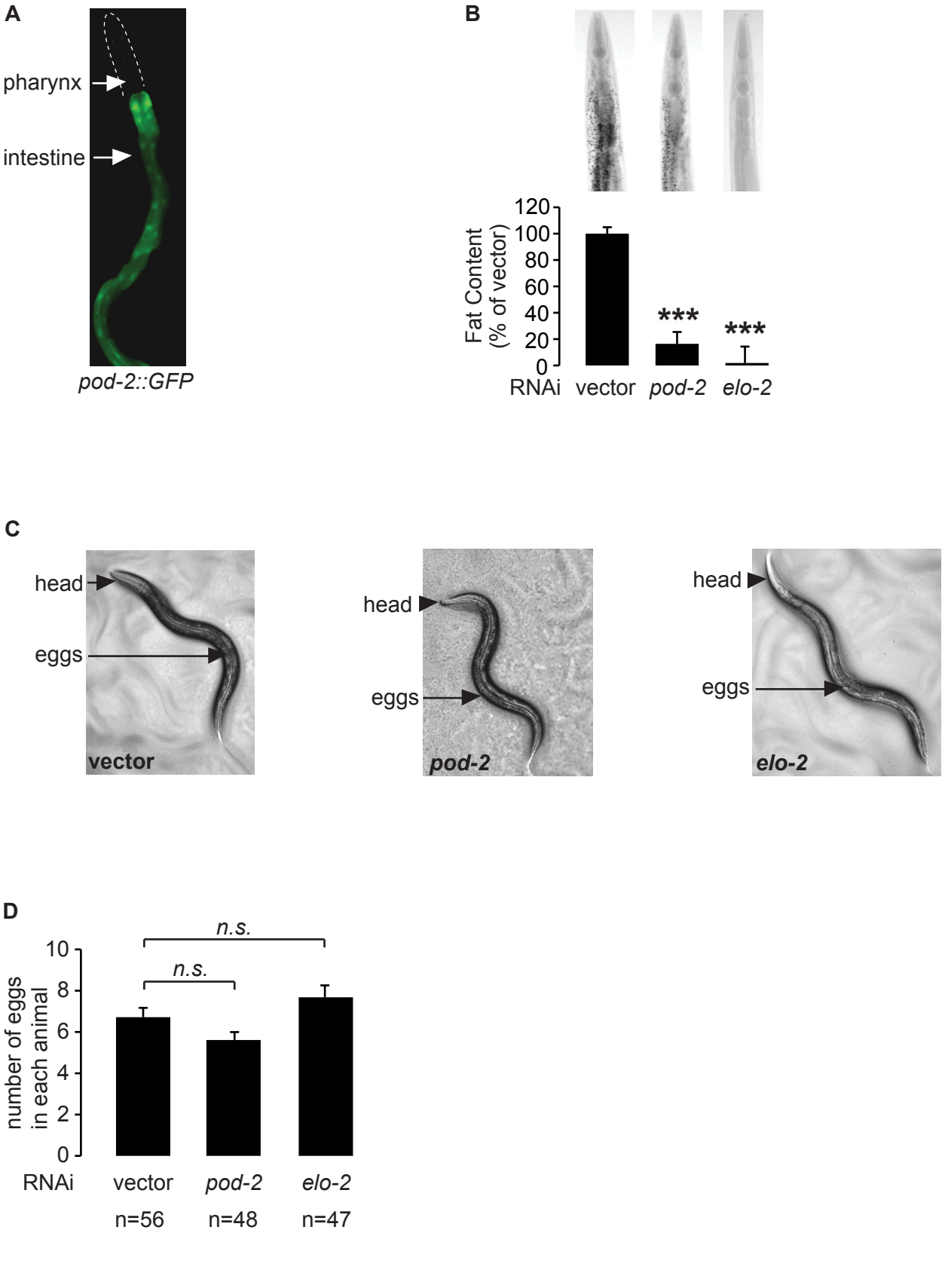
A
pharynx
intestine
pod-2::GFP
B
Fat Content (% of vector)
120
100
80
60
40
20
0
***
***
RNAi
vector
pod-2
elo-2
C
head
eggs
vector
head
eggs
pod-2
head
eggs
elo-2
D
n.s.
n.s.
number of eggs in each animal
10
8
6
4
2
0
RNAi
vector
pod-2
elo-2
n=56
n=48
n=47

Figure S5. Inactivation of ACC/*pod-2* and *elo-2* robustly decreases body fat without overt developmental effects, related to Figure 5.

(A) We generated transgenic animals expressing GFP under the control of the ACC/*pod-2* promoter. A representative animal is shown. White arrowheads indicate the pharynx and the intestine. We observed GFP expression solely in the intestine.

(B) Representative images are shown of vector, *pod-2* and *elo-2* RNAi-treated wild-type animals fixed and stained with oil Red O (upper panels). Fat content was quantified for each condition and is expressed as a percentage of vector RNAi ± SEM (lower panels; n=8-20). ***, $p<0.001$ by Student's t-test.

(C) Representative images are shown of living day 1 adult vector, *pod-2* and *elo-2* RNAi-treated wild-type animals.

(D) The number of eggs within each wild-type animal treated with vector, *pod-2* or *elo-2* RNAi was counted at the time of URX imaging. Data are expressed as average ± SEM (n=47-56). n.s., not significant by one-way ANOVA.

Figure S6

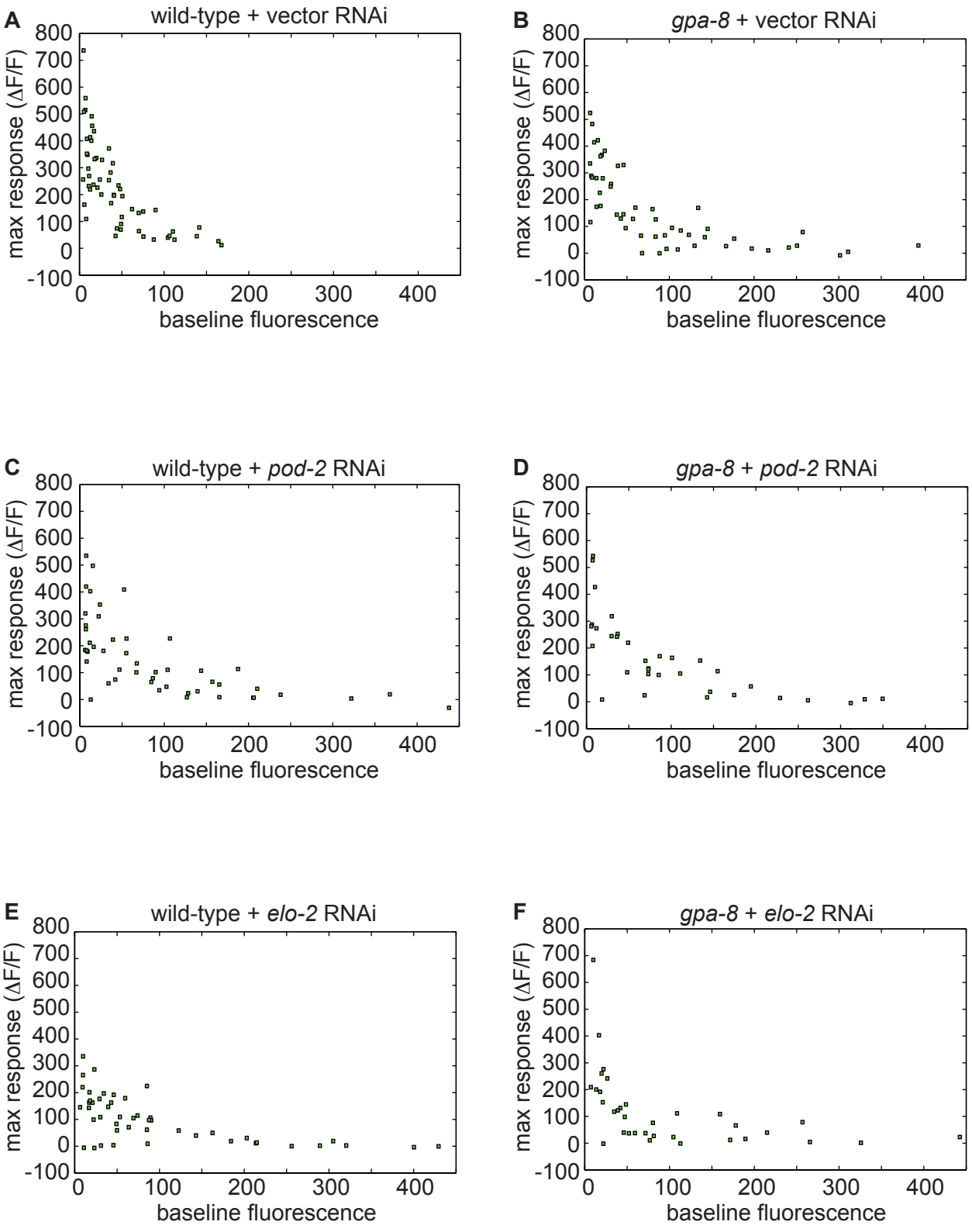
A
wild-type + vector RNAi
B
gpa-8 + vector RNAi
C
wild-type + pod-2 RNAi
D
gpa-8 + pod-2 RNAi
E
wild-type + elo-2 RNAi
F
gpa-8 + elo-2 RNAi
max response (ΔF/F)
baseline fluorescence
800
700
600
500
400
300
200
100
0
-100
0
100
200
300
400

Figure S6. RNAi-dependent body fat loss modulates URX neuronal function in a GPA-8-dependent manner, related to Figure 6.

(A-F) Maximum $\Delta F/F_0$ values at 21% oxygen are plotted against the baseline fluorescence at 10% oxygen (F_0) for individual wild-type and *gpa-8* animals treated with the denoted RNAi (n=34-56).