SUPPLEMENTAL INFORMATION

An Integrated Serotonin and Octopamine Neuronal Circuit Directs The Release of An Endocrine Signal to Control *C. elegans* Body Fat

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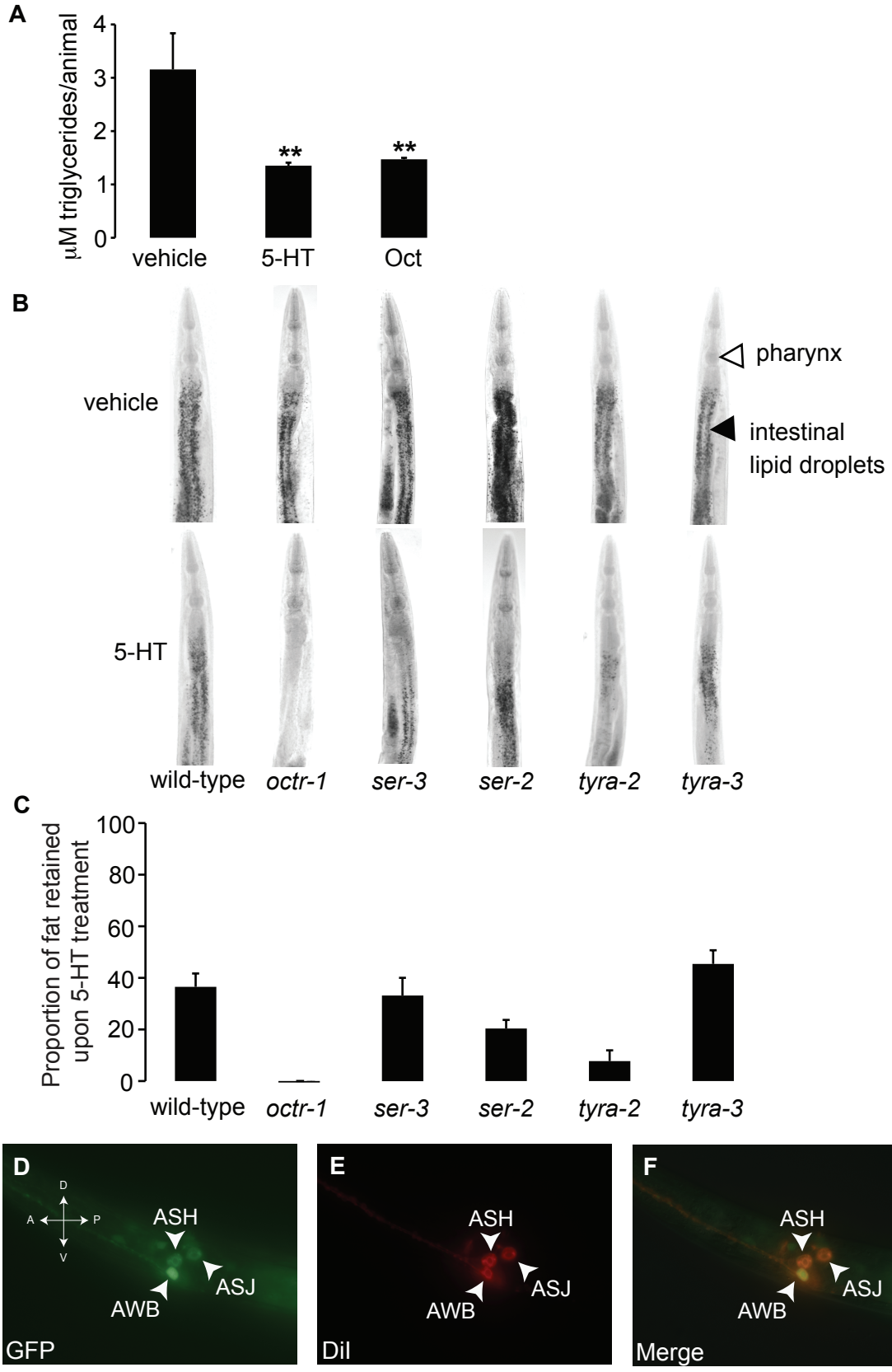
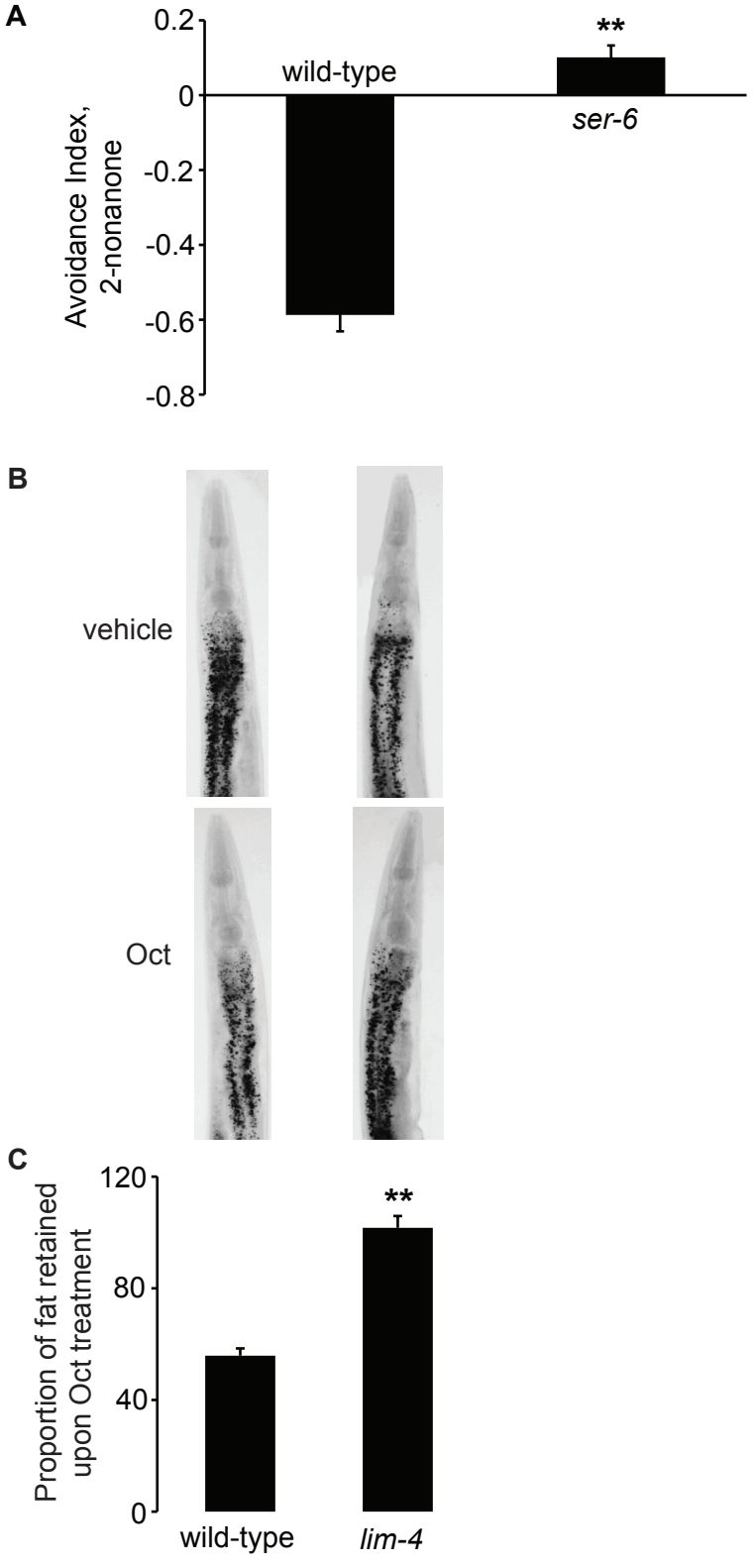
Running title:

A 5-HT neuroendocrine circuit for body fat control

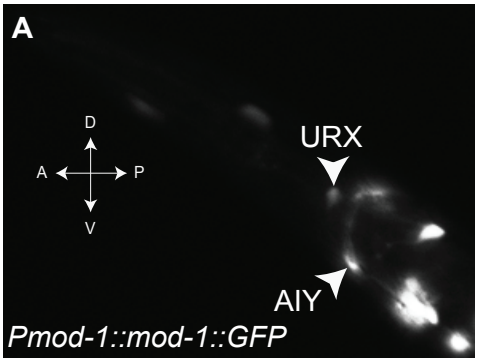
Supplemental Figure 1



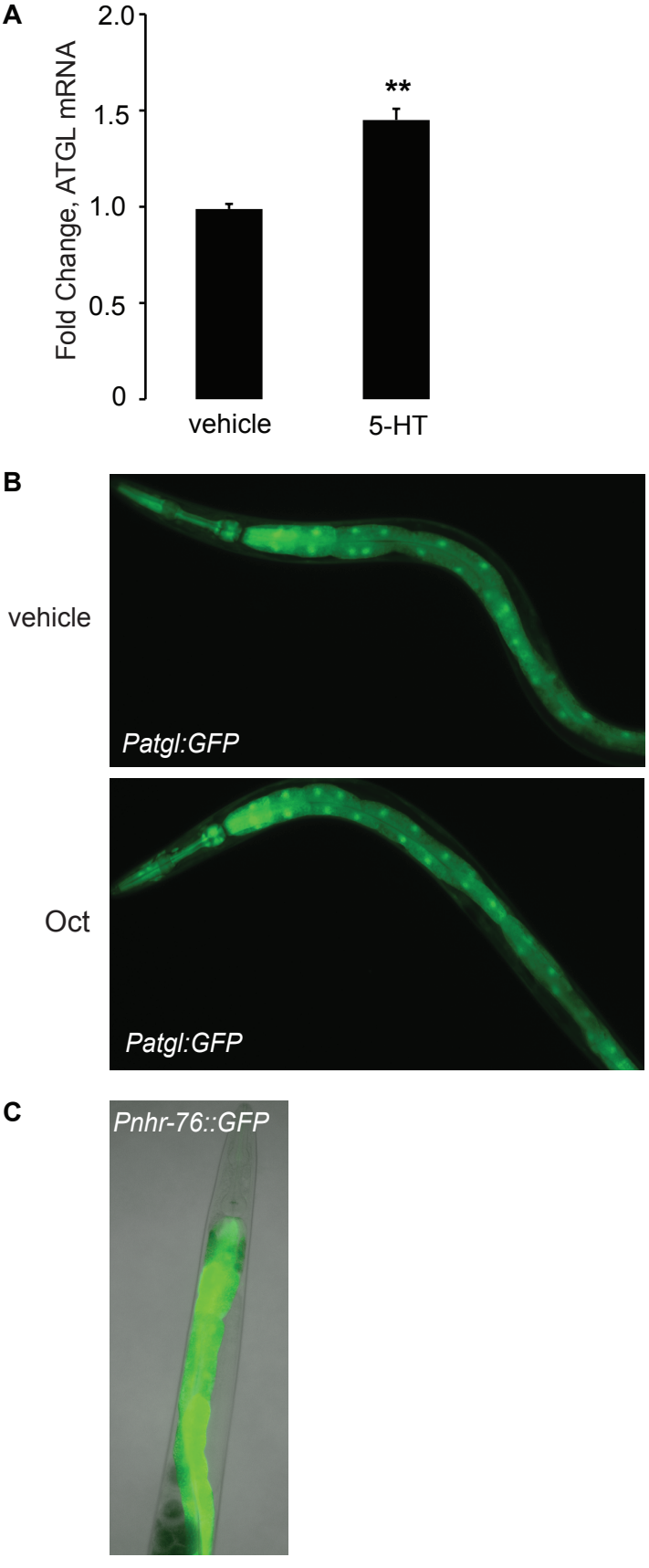
Supplemental Figure 2



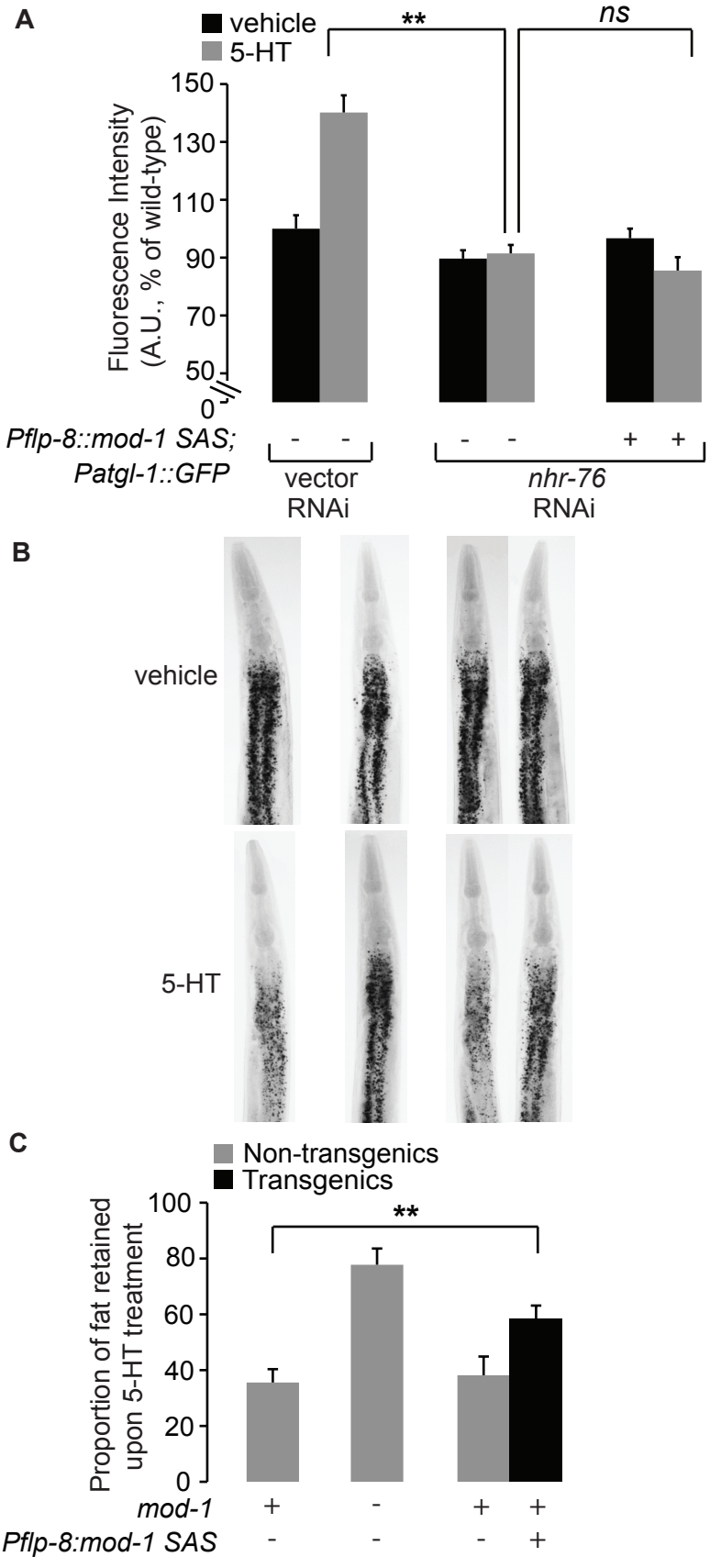
Supplemental Figure 3



Supplemental Figure 4



Supplemental Figure 5



SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. The SER-6 octopaminergic GPCR is required for 5-HT-mediated fat loss, related to Figure 1. A. Biochemical extraction and quantitation of triglycerides recapitulates fat loss upon 5-HT and Oct administration seen with Oil Red O staining. **, $p<0.005$. **B.** Representative images of vehicle (top row) or 5-HT (bottom row) animals fixed and stained with Oil Red O. **C.** The proportion of fat retained upon 5-HT treatment relative to vehicle treatment is shown for each genotype (n=10-15). Octopaminergic receptor mutants *octr-1*, *ser-3* and tyraminergic receptor mutants *ser-2*, *tyra-2* and *tyra-3* do not suppress 5-HT-mediated fat loss. Vehicle-treated *octr-1* mutants have decreased body fat compared to wild-type animals, and exogenous 5-HT treatment further decreases body fat. Data are expressed as a percentage of vehicle-treated wild-type animals (n=10-15) $\pm$ S.E.M. **D.** Transgenic animals expressing *ser-6* under the control of the endogenous promoter show GFP expression throughout the cell body of AWB neurons. The faint green staining on the cell membranes of ASH and ASJ neurons represents bleed-through from the DiI stain shown in panel B. **E.** DiI staining is used to identify amphid sensory neurons in *C. elegans*. DiI staining is restricted to the cell surface of neurons and is not taken up in the cell body. In the D-V view, AWB, ASH and ASJ neurons form a recognizable triplet of neurons (www.wormatlas.org), as shown. **F.** Merge of panels D and E shows *ser-6* expression in AWB neurons (yellow) but not in ASH or ASJ neurons.

Supplemental Figure 2. SER-6 is required for AWB-mediated 2-nonanone avoidance and AWB neurons are essential for Oct-mediated fat loss, related to Figure 2. A. Behavioral avoidance of 2-nonanone seen in wild-type animals is abrogated in *ser-6* mutants (n=80-100 within each experiment, error bars indicate $\pm$ S.E.M. of three experiments). **, $p<0.005$. **B.** Representative images of vehicle (top row) or 5-HT (bottom row) animals fixed and stained with Oil Red O. Genotypes are as indicated in the figure. **C.** The proportion of fat retained upon Oct

treatment relative to vehicle treatment is shown ± S.E.M. (n=10-15). *lim-4* mutants which do not have AWB neurons, suppress Oct-induced fat loss. ***, p*<0.005.

Supplemental Figure 3. MOD-1 expression in URX neurons, related to Figure 4. Transgenic animals expressing *mod-1* under the control of the endogenous promoter show GFP expression in the URX and AIY neurons.

Supplemental Figure 4. *atgl-1* control of serotonin-mediated fat loss, related to Figure 6. **A.** qPCR of endogenous *atgl-1* transcripts in vehicle- and 5-HT-treated wild-type adults. 5-HT treatment significantly increases *atgl-1* expression. Data are expressed as averages of quadruplicate determinations across two independent biological replicates ± S.E.M. **, *p*<0.005. **B.** Addition of exogenous Oct (5mM; lower panel) did not change expression of the *pATGL::GFP* reporter relative to vehicle-treated animals (upper panel). **C.** Image of transgenic animal bearing a 7kb *nhr-76* promoter fused to GFP shows expression in the intestine.

Supplemental Figure 5. 5-HT signaling relays an instructive signal to intestinal tissue to control ATGL expression via the nuclear receptor NHR-76, related to Figure 7. A. The fluorescence intensity of *atgl-1* expression in the third and fourth intestinal cells is quantified and expressed relative to control-treated animals, ± S.E.M. (n=20). Black bars, vehicle treatment; gray bars, 5-HT treatment. **, *p*<0.005 and *ns*, not significant. Inactivation of *nhr-76* suppresses 5-HT-mediated induction of *atgl-1* expression. As expected, this suppression is seen in transgenic animals (+) in which *mod-1* has been inactivated in the URX neurons, as well as in non-transgenic controls (-). In *mod-1*-inactivated transgenic animals (+) on *nhr-76* RNAi, there is no further decrease of *atgl-1* expression compared to vector RNAi-treated control animals. **B.** Representative images of vehicle (top row) or 5-HT (bottom row) animals bearing anti-sense mediated inactivation of *mod-1* in the URX neurons, fixed and stained with Oil Red O. **C.** The

proportion of fat retained upon 5-HT treatment animals relative to vehicle-treated animals is shown for each genotype (n=10-15). Relative to non-transgenic controls (gray bars), *mod-1* inactivation in URX neurons (black bars) leads to suppression of serotonin-mediated fat loss. Data are expressed as a percentage of vehicle-treated wild-type animals ± S.E.M. **, $p<0.005$; *ns*, not significant.

Supplemental Table 1. *C. elegans* genes in the 5-HT fat loss pathway, relates to Figures 1,2,5,6. Oil Red O staining intensity is quantified as a measure of body fat. Within a single experiment, 10-15 animals were quantified ± S.E.M. (n=10-15) and all experiments were repeated at least 3 times. The proportion of fat loss is calculated as the percentage of fat retained upon 5-HT treatment, relative to vehicle-treated animals.

Gene Name	Oil Red O Intensity		Proportion Body Fat Retained
	Vehicle	5-HT	
wild-type	100.0 ± 5.7	38.8 ± 3.7	38.8 ± 3.7
tbh-1(n3247)	100.1 ± 4.4	62.6 ± 6.5	65.5 ± 6.5
ser-6(tm2146)	90.9 ± 3.8	69.0 ± 3.7	78.2 ± 4.5
tbh-1(m3247); ser-6(tm2146)	92.9 ± 1.7	69.2 ± 6.5	74.5 ± 7.0
mod-1(ok103)	87.1 ± 4.8	68.2 ± 3.9	78.2 ± 4.5
mod-1(ok103);ser-6(tm2146)	109.9 ± 5.1	109.8 ± 2.6	99.9 ± 2.4
vector RNAi - HT115 bacteria	100.0 ± 3.9	30.1 ± 4.2	30.1 ± 4.2
atgl-1 RNAi	163.0 ± 5.3	132.5 ± 10.3	81.3 ± 6.3
nhr-76 RNAi	154.8 ± 27.5	149.5 ± 15.0	96.6 ± 9.7

Gene Name	Oil Red O Intensity		Proportion Body Fat Retained
	Vehicle	Oct	
wild-type	100 ± 3.1	54.9 ± 3.6	54.9 ± 3.4
mod-1(ok103)	87.8 ± 3.8	69.9 ± 2.1	79.6 ± 2.2
tph-1(mg280)	116.2 ± 4.0	93.5 ± 6.1	80.5 ± 5.3
ser-6(tm2146)	91.2 ± 5.0	73.8 ± 5.1	80.9 ± 5.6
ser-6(tm2146);tph-1(mg280)	116.1 ± 6.1	94.0 ± 4.2	80.9 ± 3.6
lim-4(ky403)	65.7 ± 3.7	66.9 ± 2.8	101.7 ± 4.2
mod-1(ok103);ser-6(tm2146)	110.1 ± 2.9	110.4 ± 2.7	100.4 ± 2.5

Supplemental Table 2. Fat content in transgenic and non-transgenic *ser-6* rescue strains, relates to Figure 1. Oil Red O staining intensity is quantified as a measure of body fat. Within a single experiment, 10-15 animals were quantified ± S.E.M. (n=10-15) and all experiments were repeated at least 3 times. The proportion of fat loss is calculated as the percentage of fat retained upon 5-HT treatment, relative to vehicle-treated animals.

Genetic Background	**Promoter Driving *ser-6*::gfp**	**Transgenic**	**Oil Red O Intensity**	
			Vehicle	**5-HT**
N2 Bristol	none	N/A	100.0 ± 2.5	34.2 ± 3.3
ser-6(tm2146)	none	N/A	89.2 ± 2.9	66.7 ± 2.5
ser-6(tm2146)	*ser-6*	-	90.7 ± 2.9	66.1 ± 2.9
		+	87.7 ± 2.4	32.3 ± 2.5
	unc-7	-	68.5 ± 4.1	49.4 ± 2.4
		+	63.6 ± 3.3	44.6 ± 2.7
	str-1	-	90.6 ± 1.3	63.6 ± 4.2
		+	88.1 ± 4.9	30.0 ± 3.3

Supplemental Table 3. Fat content in transgenic and non-transgenic *mod-1* rescue strains, relates to Figure 4.

Oil Red O staining intensity is quantified as a measure of body fat. Within a single experiment, 10-15 animals were quantified ± S.E.M. (n=10-15) and all experiments were repeated at least 3 times. The proportion of fat loss is calculated as the percentage of fat retained upon 5-HT treatment, relative to vehicle-treated animals.

Genetic Background	**Promoter Driving *mod-1*::gfp**	**Transgenic**	**Oil Red O Intensity**	
			Vehicle	**5-HT**
N2 Bristol	none	N/A	100.0 ± 2.1	35.9 ± 5.3
mod-1(ok103)	none	N/A	83.7 ± 6.2	64.5 ± 6.0
mod-1(ok103)	*mod-1*	-	80.8 ± 3.1	65.2 ± 6.2
		+	74.3 ± 4.4	32.4 ± 4.7
	ttx-3	-	80.7 ± 1.9	65.1 ± 3.4
		+	77.5 ± 2.4	60.9 ± 2.3
	flp-1	-	77.8 ± 3.3	62.4 ± 3.9
		+	77.9 ± 2.0	62.9 ± 6.5
	gcy-36	-	74.9 ± 4.3	64.4 ± 2.0
		+	81.6 ± 4.9	52.1 ± 2.1
	flp-8	-	79.0 ± 3.0	67.8 ± 2.5
		+	73.0 ± 5.4	40.4 ± 3.0

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Triglyceride Extraction and Quantitation

Triglycerides were extracted from wild-type and mutant *C. elegans* as described (Bligh and Dyer, 1959). Extracted lipids were quantified using the Enzychrom™ Triglyceride Assay kit (Bioassay Systems) according to the manufacturer's instructions.

Behavioral Assays

The assay for behavioral avoidance of the volatile repellant 2-nonanone was conducted as described (Troemel et al., 1997).

Real-Time qPCR

12,000 wild-type worms were cultured from L1 larvae to day 1 adulthood and treated with either vehicle or 5-HT as described above. Animals were harvested by sequential washing in M9 buffer and snap frozen in liquid nitrogen. RNA was extracted using the Trizol reagent (Life Technologies) followed by the RNeasy Mini Kit™ (Qiagen). First strand cDNA synthesis was performed using the M-MuLV1 RT Kit™ (NEB). Second strand synthesis was performed using the IQ™ SYBR Green Supermix (Bio-Rad) on the CFX Connect Real-Time System (Bio-Rad). *atgl-1* primers were optimized for target specificity and linear amplification. Primer sequences are available upon request.

SUPPLEMENTAL LITERATURE CITED

Bligh, E.G., and Dyer, W.J. (1959). A rapid method of total lipid extraction and purification. Can J Biochem Physiol *37*, 911-917

Troemel, E.R., Kimmel, B.E., and Bargmann, C.I. (1997). Reprogramming chemotaxis responses: sensory neurons define olfactory preferences in C. elegans. Cell *91*, 161-169