**Cell Metabolism, Volume *7***

## Supplemental Data

## Article

# Serotonin Regulates *C. elegans* Fat and Feeding through Independent Molecular Mechanisms

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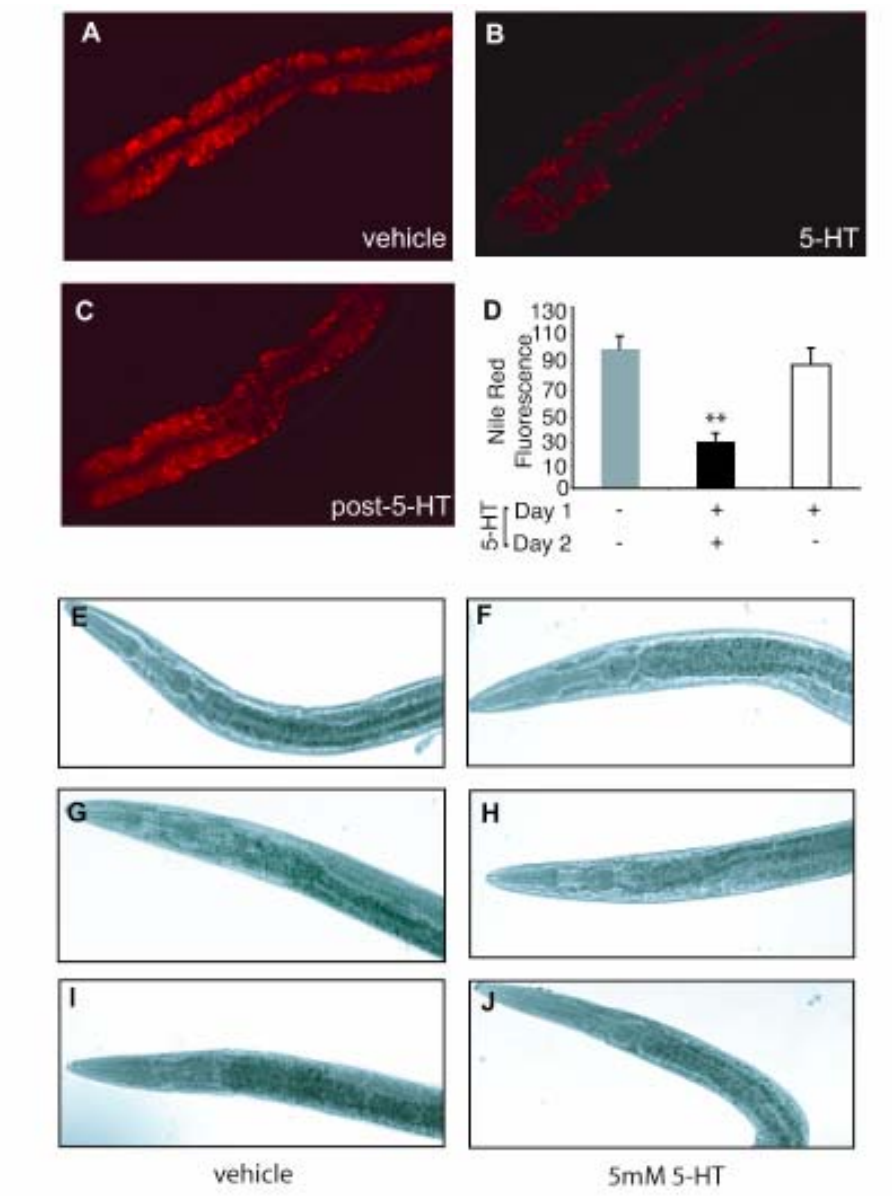


**Figure S1. 5-HT Reduces Fat Content as Gauged by Nile Red and Sudan Black B Staining Methods**

(A and B) Images of Nile Red-stained animals treated with (A) vehicle only, and (B) 5mM 5-HT.

(C) Reduced fat of 5-HT-treated animals was restored within ~ 24 hours once animals were removed from 5-HT-containing plates. In all images, the anterior end of the animals is oriented towards the left.

(D) Quantification of Nile Red fluorescence (n = 8-10 animals per condition, **$p < 0.005$ compared to vehicle-treated animals). The data are expressed as a percentage of vehicle-treated animals on OP50 bacteria.

(E-J) Representative Sudan Black B images of animals treated with (E, G, and I) vehicle, and (F, H, and J) 5-HT, showing fat decreasing effects of 5-HT-treatment.

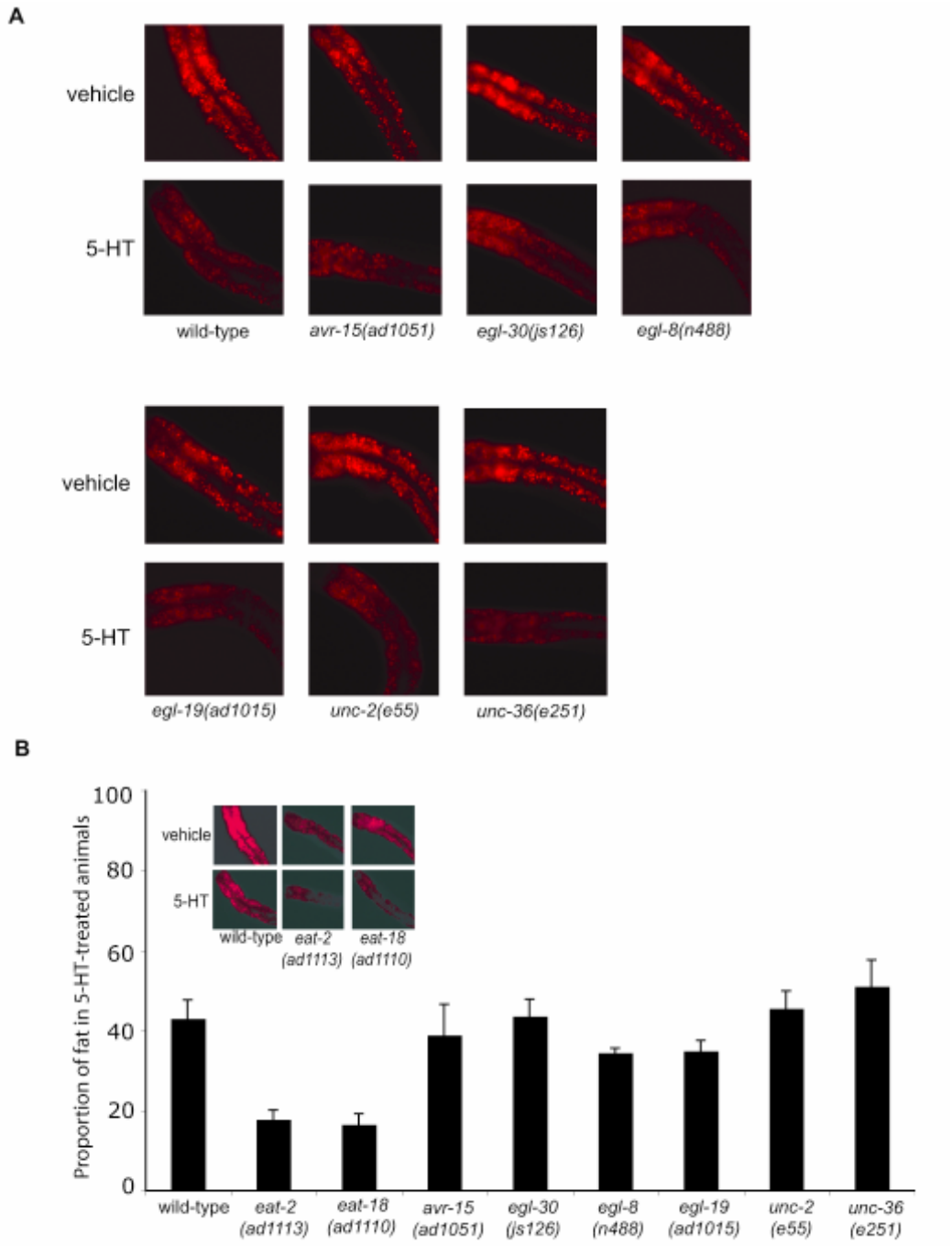


**Figure S2. 5-HT-Induced Fat Reduction Is Independent of Genes Required for Serotonergic Modulation of Feeding, Egg-Laying, and Locomotion**

(A) Representative images of Nile Red-stained animals treated with vehicle (top rows) or 5-HT (bottom rows). The anterior end of the animals is oriented towards the left.

(B) The percent of fat remaining upon 5-HT treatment for each genotype (n = 6-8) relative to vehicle-treated animals. Images in inset are overexposed as *eat-2(ad1113)* and *eat-18(ad1110)* mutants displayed reduced fat levels relative to wild type animals even in the absence of 5-HT treatment.

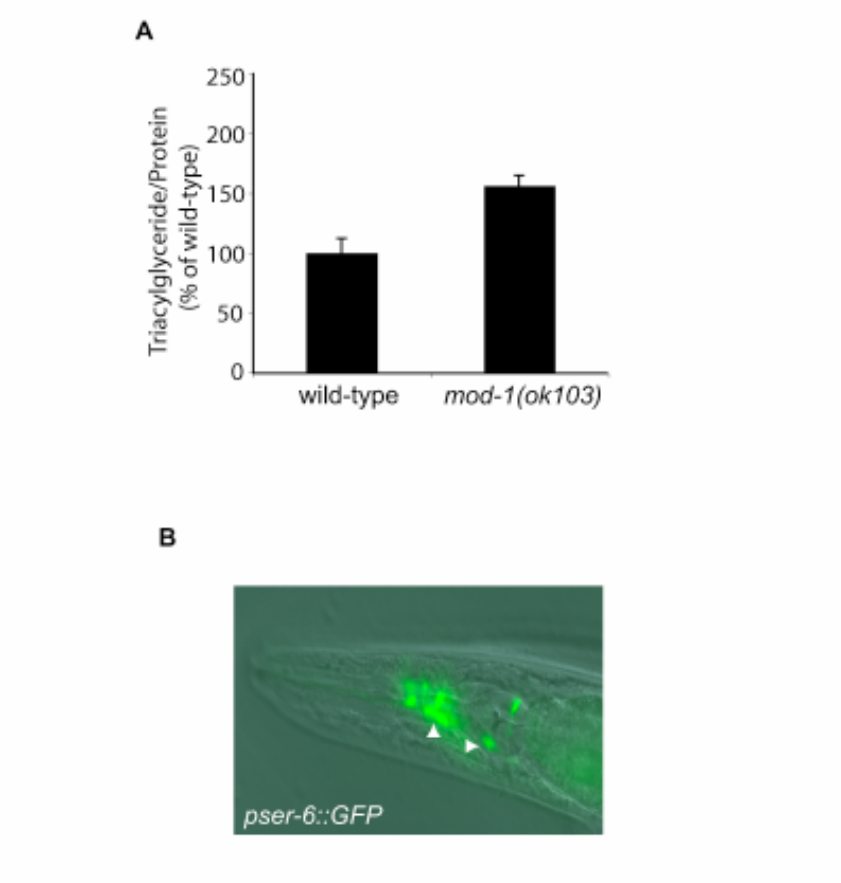


**Figure S3. Biochemical Verification of *mod-1(ok103)* Excess Fat and Expression Pattern of *ser-6***

(A) Values are expressed as a percentage of wild-type.

(B) A GFP reporter fused to the putative *Y54G2A.35/ser-6* promoter was expressed in several head neurons denoted by white arrowheads.

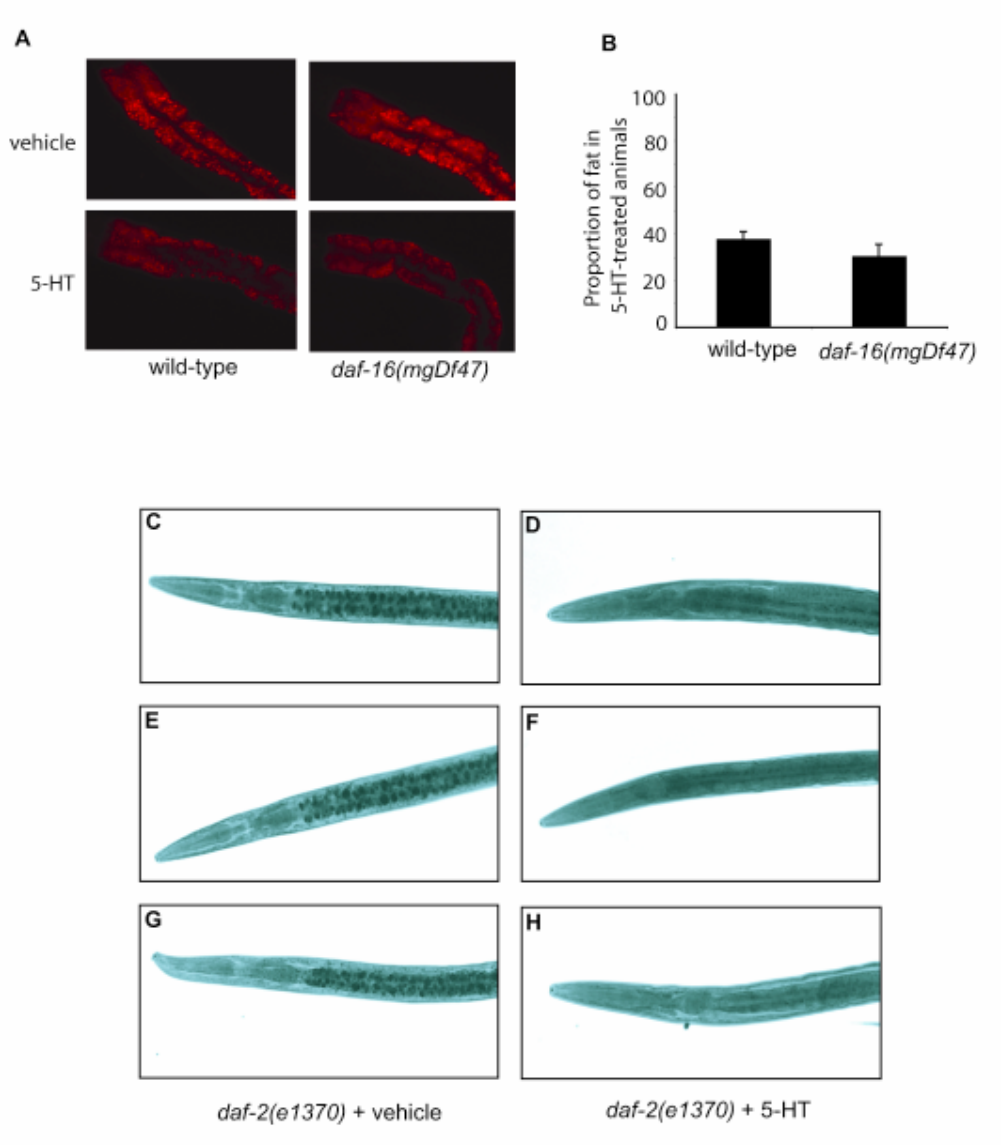


**Figure S4. The Insulin Pathway Is Not Required for Serotonergic Fat Reduction**

(A) *daf-16(mgDf47)* mutation did not alter susceptibility of animals to 5-HT mediated fat reduction.

(B) Quantitation of Nile Red images shown in (A) (n = 8 per genotype).

(C-H) Representative Sudan Black-stained *daf-2(e1370)* animals exposed to either vehicle (C, E, and G) or 5-HT (D, F, and H) showing that, despite excess fat levels, *daf-2(e1370)* animals were susceptible to the fat-reducing effects of exogenous 5-HT.

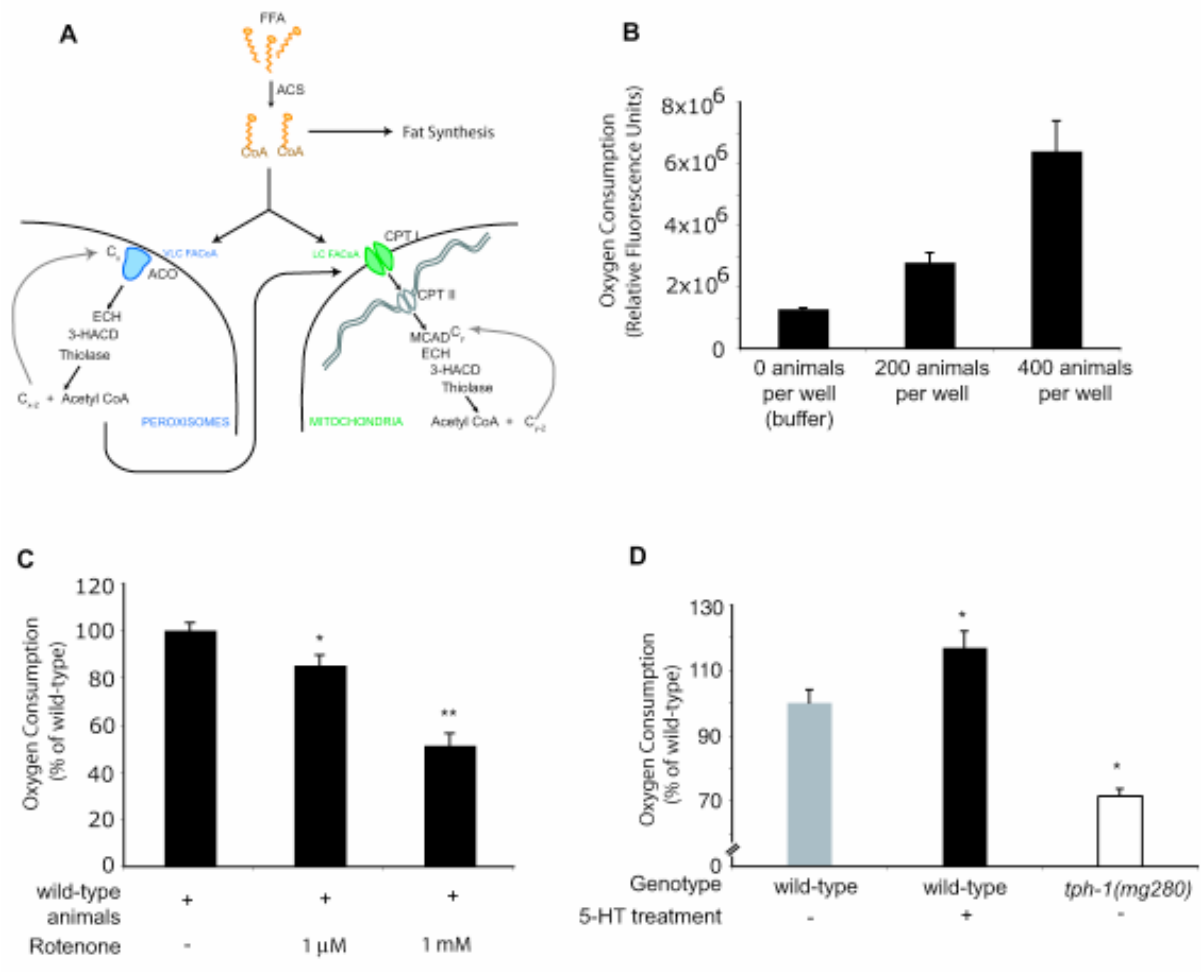


**Figure S5. Pictorial Depiction of Peroxisomal and Mitochondrial β-Oxidation and Validation of the Oxygen Consumption Assay**

(A) Model depicting peroxisomal and mitochondrial β-oxidation. Free fatty acids are activated to their respective CoA derivates by acyl-CoA synthases (ACS). Activated fatty acids are destined for either biosynthetic or degradative pathways. Peroxisomal β-oxidation of very long-chain fatty acids (VLCFA-CoA) is initiated by acyl-CoA oxidases (ACO) whereas long-chain fatty acids (LCFA-CoA) are targeted to the mitochondria, where carnitine-palmitoyl transferases I and II (CPT-1 and CPT-II) catalyze their transfer into the inner lumen of mitochondria. Subsequent breakdown of these mitochondria fatty acyl-CoAs is mediated by mitochondrial acyl-CoA dehydrogenase (MCAD). A trifunctional complex containing enoyl-CoA hydratase (ECH), 3'-OH-acyl-CoA dehydrogenase (3-HACD) and thiolase functions in both organelles resulting in the stepwise breakdown of fatty acyl-CoAs.

(B) Validation of the oxygen consumption assay. We empirically identified assay conditions that produced a linear correlation between oxygen consumption and number of animals tested. In all subsequent tests 200 worms/well were assayed.

(C) Rotenone, an inhibitor of oxidative phosphorylation and mitochondrial electron transport, caused a dose-dependent decrease in oxygen consumption under these assay conditions (*$p < 0.05$; **$p < 0.005$).

(D) Oxygen consumption rate of 5-HT-treated animals was enhanced while that of 5-HT deficient *tph-1(mg280)* animals was reduced (*$p < 0.05$).

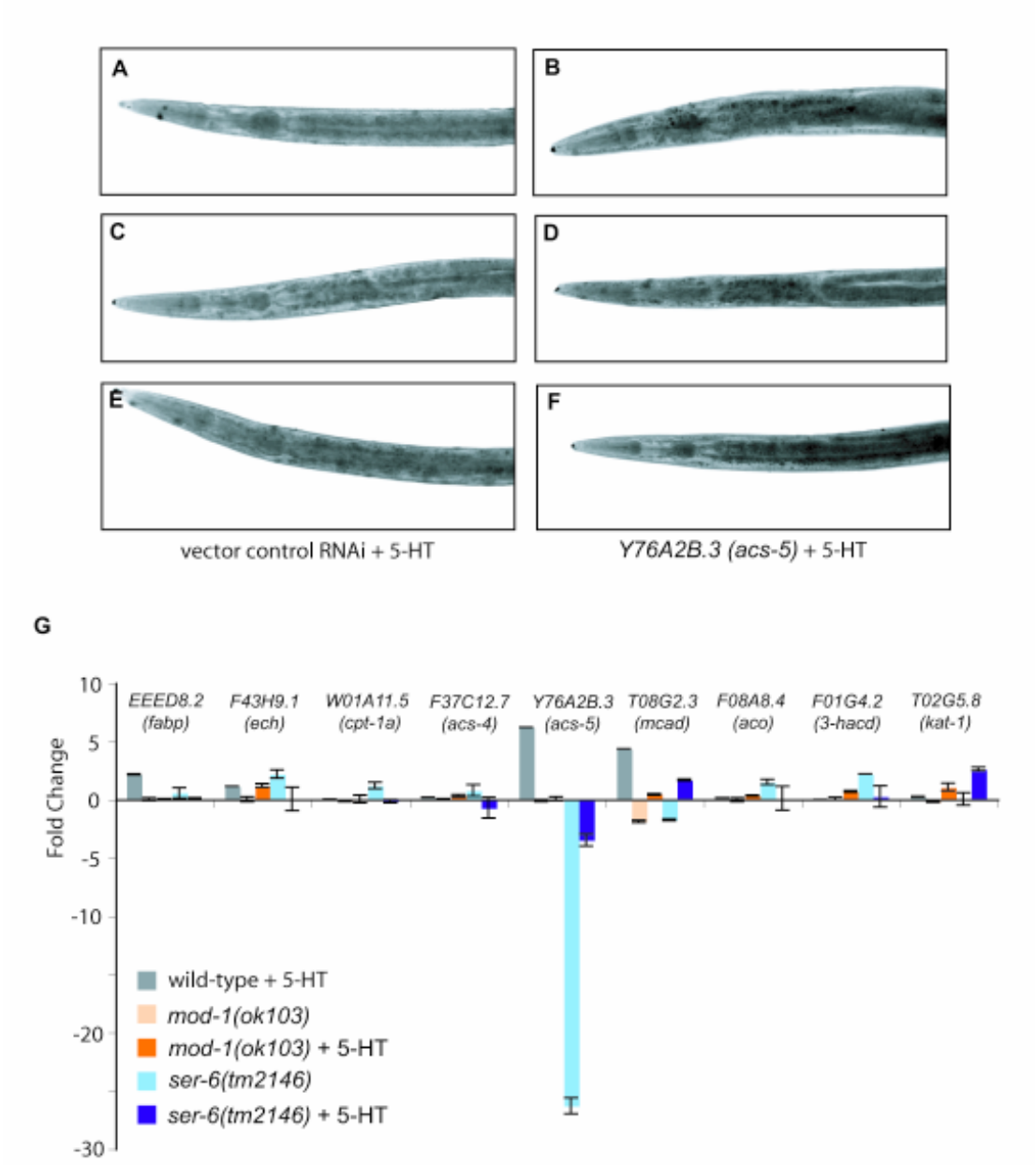


**Figure S6. Verification of 5-HT-Induced Fat Suppression of β-Oxidation Gene *Y76A2B.3* Using Sudan Black and Transcriptional Changes of β-Oxidation Genes in *mod-1(ok103)* and *ser-6(tm2146)* Mutants**

(A-F) Verification of Nile Red results by Sudan Black for *Y76A2B.3(acs-5).* Three representative images showing that, relative to animals on vector RNAi (A, C, and E), *Y76A2B.3(acs-5)* RNAi (B, D, and F) allowed animals to retain more fat upon 5-HT treatment.

(G) qRT-PCR of the nine serotonergic β-oxidation genes for indicated genotypes +/- 5HT treatment. All values are shown as fold changes relative to wild-type animals without 5-HT treatment.

**Table S1. Known and Putative Biogenic Amine Receptors in *C. elegans***

| Gene ID | Gene Name | Dominant Specificity |
|---|---|---|
| F59C12.2 | *ser-1* | Serotonin |
| C02D4.2 | *ser-2* | Octopamine |
| K02F2.6 | *ser-3* | Tyramine |
| Y22D7AR.13 | *ser-4* | Serotonin |
| F16D3.7 | *ser-5* | ND |
| Y54G2A.35 | *ser-6* | ND |
| C09B7.1 | *ser-7* | Serotonin |
| K06C4.6 | *mod-1* | Serotonin |
| F15A8.5 | *dop-1* | Dopamine |
| K09G1.4 | *dop-2* | Dopamine |
| T14E8.3 | *dop-3* | ND |
| C52B11.3 | *dop-4* | ND |
| F10E11.5 | *tyra-2* | Tyramine |
| M03F4.3 | *tyra-3* | ND |
| F14D12.6 | - | ND |
| T02E9.3 | - | ND |
| C24A8.1 | - | ND |

Dominant specificity listed is as determined by cell-based pharmacology studies (references in Chase and Koelle, 2007). ND, not determined.

**Table S2. List of RNAi Clones Used for the 5-HT Metabolic Screen**

| Gene ID | Gene Name |
|---|---|
| Fatty Acid Acylation | |
| F28F8.2/acs-2 | Acyl-CoA Synthetase |
| F41C3.3/acs-11 | Acyl-CoA Synthetase |
| C46F4.2/acs-17 | Acyl-CoA Synthetase |
| F37C12.7 | Acyl-CoA Synthetase |
| F47G6.2 | Acyl-CoA Synthetase |
| Y65B4BL.5 | Acyl-CoA Synthetase |
| R07C3.4 | Acyl-CoA Synthetase |
| R09E10.3 | Acyl-CoA Synthetase |
| Y76A2B.3 | Acyl-CoA Synthetase |
| R09E10.4 | Acyl-CoA Synthetase |
| F46E10.1 | Acyl-CoA Synthetase |
| Fatty Acid β-Oxidation | |
| R07H5.2/cpt-2 | Carnitine Palmitoyl Transferase II |
| Y48G9A.10/cpt-3 | Carnitine Palmitoyl Transferase I |
| F09F3.9/cpt-5 | Carnitine Palmitoyl Transferase I |
| W01A11.5/cpt-6 | Carnitine Palmitoyl Transferase I |
| F41E7.6 | Carnitine Palmitoyl Transferase I |
| T20B3.1 | Carnitine Palmitoyl Transferase I |
| C02B10.1/ivd-1 | Acyl-CoA Dehydrogenase |
| F54D5.7 | Acyl-CoA Dehydrogenase |
| K06A5.6 | Acyl-CoA Dehydrogenase |
| E04F6.5 | Acyl-CoA Dehydrogenase |
| K05F1.3 | Acyl-CoA Dehydrogenase |
| F28A10.6 | Acyl-CoA Dehydrogenase |
| T08G2.3 | Acyl-CoA Dehydrogenase |
| T25G12.5 | Acyl-CoA Dehydrogenase |
| C44B7.8/pmp-1 | Peroxisomal Membrane Transporter |
| C44B7.9/pmp-2 | Peroxisomal Membrane Transporter |
| C48B4.1 | Acyl-CoA Oxidase |
| F25C8.1 | Acyl-CoA Oxidase |
| F59F4.1 | Acyl-CoA Oxidase |
| F08A8.1 | Acyl-CoA Oxidase |
| F08A8.2 | Acyl-CoA Oxidase |
| F08A8.3 | Acyl-CoA Oxidase |
| F08A8.4 | Acyl-CoA Oxidase |
| C29F3.1/ech-1 | Enoyl-CoA Hydratase |
| F38H4.8/ech-2 | Enoyl-CoA Hydratase |
| F43H9.1/ech-3 | Enoyl-CoA Hydratase |
| R06F6.9/ech-4 | Enoyl-CoA Hydratase |
| T05G5.6/ech-6 | Enoyl-CoA Hydratase |
| Y105E8A.4/ech-7 | Enoyl-CoA Hydratase |
| F01G10.2/ech-8 | Enoyl-CoA Hydratase |
| F01G10.3/ech-9 | Enoyl-CoA Hydratase |
| F01G4.2/ard-1 | 3-Hydroxy Acyl-CoA Dehydrogenase |
| R09B5.6/hacd-1 | 3-Hydroxy Acyl-CoA Dehydrogenase |
| B0272.3 | 3-Hydroxy Acyl-CoA Dehydrogenase |
| F54C8.1 | 3-Hydroxy Acyl-CoA Dehydrogenase |
| T02G5.8/kat-1 | Acetoacetyl-CoA Thiolase |
| B0303.3 | 3-ketoacyl-CoA Thiolase |
| Y57A10C.6 | 3-ketoacyl-CoA Thiolase |

| Fatty Acid Desaturation and Elongation | |
|---|---|
| Y67H2A.8/fat-1 | w3 Desaturase |
| W02A2.1/fat-2 | D12 Desaturase |
| W08D2.4/fat-3 | D6 Desaturase |
| T13F2.1/fat-4 | D5 Desaturase |
| W06D12.3/fat-5 | D9 Desaturase |
| VZK822L.1/fat-6 | D9 Desaturase |
| F10D2.9/fat-7 | D9 Desaturase |
| F56H11.4/elo-1 | PUFA elongase |
| F11E6.5/elo-2 | Palmitic acid elongase |
| D2024.3/elo-3 | PUFA elongase |
| C40H1.4/elo-4 | PUFA elongase |
| F41H10.7/elo-5 | PUFA elongase |
| F41H10.8/elo-6 | PUFA elongase |
| F56H11.3/elo-7 | PUFA elongase |
| Y53F4B.2/elo-8 | PUFA elongase |
| F33D4.4 | Desaturase |
| Fatty Acid Binding Proteins | |
| F40F4.3/lbp-1 | Fatty Acid Binding Protein |
| F40F4.2/lbp-2 | Fatty Acid Binding Protein |
| F40F4.4/lbp-3 | Fatty Acid Binding Protein |
| ZK742.5/lbp-4 | Fatty Acid Binding Protein |
| W02D3.7/lbp-5 | Fatty Acid Binding Protein |
| W02D3.5/lbp-6 | Fatty Acid Binding Protein |
| T22G5.2/lbp-7 | Fatty Acid Binding Protein |
| T22G5.6/lbp-8 | Fatty Acid Binding Protein |
| Y40B10A.1/lbp-9 | Fatty Acid Binding Protein |
| EEED8.2 | Fatty Acid Binding Protein |
| EEED8.3 | Fatty Acid Binding Protein |
| F28D1.9 | Fatty Acid Transport Protein |
| Phospholipid Metabolism | |
| F13D12.9 | Phospholipid Biosynthesis |
| C03H5.4 | Phospholipase A2 |
| C07E3.9 | Phospholipase A2 |
| Fatty Acid Synthesis | |
| F32H2.5/fasn-1 | Fatty Acid Synthase |
| W09B6.1/pod-2 | Acetyl-CoA Carboxylase |
| Ketogenesis/Lipid Metabolism | |
| T02G5.7 | Acetoacetyl-CoA Thiolase |
| T02G5.4 | Acetoacetyl-CoA Thiolase |
| F53A2.7 | Acetoacetyl-CoA Thiolase |
| T02G5.8/kat-1 | Acetoacetyl-CoA Thiolase |
| F25B4.6 | HMG-CoA Synthase |
| Y71G12B.10 | HMG-CoA Lyase |
| F10G8.9 | Beta Keto-Acyl Synthetase |
| C25A1.5 | Fatty-Acid Hydroxylase |
| C46C11.1 | Hormone Sensitive Lipase |
| C50D2.7 | Glucokinase |

**Table S3. Nile Red Fluorescence Intensities for Various Epistatic Interactions**

| Strain | RNAi/Bacteria | Nile Red Fluorescence | | Proportion of Fat Retained upon 5-HT Treatment |
|---|---|---|---|---|
| | | Vehicle | 5-HT | |
| Wild-type (N2) | OP50 | 100.0 ± 5.3 | 50.7 ± 3.7 | 50.7 ± 3.7 |
| *mod-1(ok103)* | OP50 | 121.2 ± 4.6 | 81.3 ± 1.9 | 67.1 ± 1.6 |
| *ser-6(tm2146)* | OP50 | 98.2 ± 4.8 | 76.4 ± 4.5 | 77.8 ± 4.6 |
| *ser-6(tm2146);mod-1(ok103)* | OP50 | 124.8 ± 4.8 | 110.9 ± 5.9 | 88.9 ± 4.7 |
| Wild-type (N2) | vector control | 100.0 ± 8.7 | 35.9 ± 5.3 | 35.9 ± 5.3 |
| Wild-type (N2) | *Y76A2B.3(acs-5)* | 112.2 ± 6.1 | 70.3 ± 3.9 | 62.7 ± 3.5 |
| Wild-type (N2) | *F08A8.4(aco)* | 112.2 ± 6.7 | 72.1 ± 6.2 | 64.3 ± 5.5 |
| *mod-1(ok103)* | vector control | 120.0 ± 6.3 | 63.8 ± 3.4 | 53.2 ± 2.8 |
| *mod-1(ok103)* | *Y76A2B.3(acs-5)* | 126.4 ± 6.3 | 96.1 ± 5.1 | 76.1 ± 2.1 |
| *mod-1(ok103)* | *F08A8.4(aco)* | 130.4 ± 6.1 | 100.3 ±10.1 | 76.9 ± 1.8 |
| *ser-6(tm2146)* | vector control | 98.8 ± 7.7 | 71.2 ± 5.9 | 62.7 ± 5.1 |
| *ser-6(tm2146)* | *Y76A2B.3(acs-5)* | 107.2 ± 5.0 | 80.2 ± 3.3 | 65.0 ± 2.3 |
| *ser-6(tm2146)* | *F08A8.4(aco)* | 109.9 ± 5.0 | 80.8 ± 3.1 | 64.0 ± 2.4 |

Vector control and all RNAi constructs were in HT115 bacterial strain, and the reported values are normalized to wild-type on HT115 instead of OP50. All values are means ± SEM (n = 8).

**Table S4. List of Primers Used for qRT-PCR**

| **Gene ID** | **Forward Primer** | **Reverse Primer** |
|---|---|---|
| W01A11.5 | TATGAACCAGCATCAGCTCG | GACGATCGACTCCTTGCCC |
| F08A8.4 | ATATCTGGAGAAGATTCGACC | CAAGTACTTATCGACTGACGG |
| EEED8.2 | TTCTCGAGTTGAGAGATCGG | ATTCTGGCTCTCCGATCCG |
| F37C12.7 | AAACAACTCGAAGAACATGCC | TGCTTATACAGATCATCGAGG |
| Y76A2B.3 | ATTCGAAGCTCCTGGATTGG | CATTATTCTCCATTCTCATCAC |
| F43H9.1 | AGGAAAAGCTGTTGAGGAGG | CTTTATACTTTCAGCGAGCAC |
| T08G2.3 | TCGACAACAAGGTGCGCTC | GAATTTGCGAGGTTCCCTCG |
| F01G4.2 | CAGGACTTATGGATACTCCAC | GGCATGCGGAGTGCTCCG |
| T02G5.8 | GTTAATGAAGCCTTCTCATGTG | CAGCAACTCCGATTTGGCC |