

Supplemental Data

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Neural and Molecular Dissection of a *C. elegans* Sensory Circuit that Regulates Fat and Feeding

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Supplemental Tables

Table S1: List of Strains Used in This Study.

Strain	Pumping rate (% WT)	Egg laying phenotype ^c	Reference
wild type	100 ± 2.8	+	
<i>daf-7(e1372)</i>	81 ± 2.9	+++	Riddle et al., 1981
<i>daf-1(m40)</i>	78 ± 3.4	+++	Riddle et al., 1981
<i>daf-4(m63)</i>	87 ± 2.9	+++	Golden and Riddle, 1984
<i>sma-6(wk7)</i> ^a	100 ± 3.2 ^b	+	Krishna et al., 1999
<i>daf-12(m20)</i> ^a	99 ± 2.6 ^b	+	Riddle et al., 1981
<i>daf-3(mgDf90)</i> ^a	98 ± 2.9 ^b	+	Patterson et al., 1997
<i>tbh1(ok1196)</i> ^a	97 ± 2.8 ^b	+	OMRF ^d
<i>tdc-1(ok914)</i> ^a	95 ± 3.6 ^b	+	OMRF ^d
<i>tyra-2(tm1846)</i> ^a	100 ± 2.8 ^b	+	NBP ^e
<i>ser-2(pk1357)</i> ^a	99 ± 4.3 ^b	+	Tsalik et al. 2003
<i>goa-1(n363)</i> ^a	75 ± 5.9	constitutive	Segalat et al., 1995

<i>dgk-1(sy428)</i> ^a	83 ± 3.6	constitutive	Hajdu-Cronin et al., 1999
<i>eat-4(ky5)</i> ^a	72 ± 7.4	+	Lee et al., 1999
<i>mgl-1(tm1811)</i> ^a	98 ± 5.5 ^b	+	NBP ^e
<i>mgl-2(tm355)</i> ^a	90 ± 7.1 ^b	+	NBP ^e
<i>mgl-3(tm1766)</i> ^a	101 ± 2.8 ^b	+	NBP ^e
<i>glr-1(n2461)</i>	96 ± 4.6 ^b	+	Hart et al., 1995
<i>nmr-1(ak4)</i>	100 ± 3.5 ^b	+	Brockie et al., 2001b
<i>daf-7(e1372); daf-12(m20)</i>	83 ± 4.2	+++	Riddle et al., 1981
<i>daf-7(e1372); daf-3(mgDf90)</i> ^a	98 ± 3.1 ^b	+	This study
<i>daf-1(m40); daf-3(mgDf90)</i> ^a	99 ± 2.8 ^b	+	This study
<i>daf-1(m40); daf-12(m20)</i>	80 ± 3.4	+++	This study
<i>daf-7(e1372); tbh-1(ok1196)</i>	94 ± 3.4 ^b	+++	This study
<i>tdc-1(ok914); daf-7(e1372)</i>	95 ± 3.6 ^b	+++	This study
<i>daf-1(m40); tbh-1(ok1196)</i>	89 ± 2.8 ^b	+++	This study
<i>tdc-1(ok914); daf-1(m40)</i>	91 ± 3.0 ^b	+++	This study
<i>tdc-1(ok914); daf-7(e1372); daf-12(m20)</i>	95 ± 3.7 ^b	+++	This study
<i>daf-1(m40); daf-12(m20); tbh-1(ok1196)</i>	91 ± 4.2 ^b	+++	This study
<i>tdc-1(ok914); daf-1(m40); daf-12(m20)</i>	92 ± 3.1 ^b	+++	This study
<i>daf-1(m40); tyra-2(tm1846)</i>	74 ± 3.9	+++	This study

<i>daf-1(m40); ser-2(pk1357)</i>	88 ± 2.9 ^b	+++	This study
<i>daf-1(m40); ser-2(pk1357); tyra-2(tm1846)</i>	87 ± 3.0 ^b	+++	This study
<i>egl-30(ft9); daf-7(e1372)</i> ^a	78 ± 5.8	constitutive	This study
<i>daf-7(e1372); dgk-1(sy428)</i> ^a	78 ± 7.4	constitutive	This study
<i>goa-1(n363); daf-7(e1372)</i> ^a	70 ± 3.7	constitutive	This study
<i>daf-7(e1372); eat-4(ky5)</i> ^a	66 ± 3.5	+++	This study
<i>daf-7(e1372); mgl-1(tm1811)</i> ^a	77 ± 3.7	+++	This study
<i>mgl-2(tm355); daf-7(e1372)</i>	80 ± 3.9	+++	This study
<i>daf-7(e1372); mgl-3(tm1766)</i> ^a	79 ± 4.5	+++	This study
<i>daf-7(e1372); mgl-3(tm1766); mgl-1(tm1811)</i> ^a	77 ± 4.8	+++	This study
<i>daf-7(e1372); cat-2(e1112)</i>	N/D	+++	This study
<i>daf-7(e1372); egl-3(gk238)</i>	N/D	+++	This study

^a Genotypes that display reduced fat levels when compared to *daf-7(e1372)* or *daf-1(m40)*. Statistical significance (p<0.001) was determined by t-test. Representative images of select genotypes are shown in Figures 1, 4, and 5.

^b Genotypes that display increased feeding rate when compared to *daf-7(e1372)* or *daf-1(m40)*. Statistical significance (p<0.001) was determined by ANOVA with Bonferroni post-test.

^c Young, well-fed wild type animals retained ~10 eggs on average. This phenotype is denoted as (+). Mutants such as *daf-1(m40)* retained ~24 eggs, denoted as (+++).

Phenotypic examples are shown and quantitated in Figure S1. Egg laying constitutive animals displayed an empty uterus phenotype.

^d OMRF: Oklahoma Medical Research Foundation, The *C. elegans* Gene Knock-out Consortium (<http://celeganskoconsortium.omrf.org/>).

^e NBP: National Bioresources Project (<http://www.grs.nig.ac.jp/c.elegans/index.jsp>).

Table S2: Expression Patterns of Interneuron-specific Promoters.

Promoter driving <i>daf-1::gfp</i>	Cells expressing <i>daf-1::gfp</i>	Rescue of <i>daf-1(m40)</i> phenotypes ^a
<i>glr-2</i>	AVA, AVD, AVE, PVC, RMD, AIA, AIB, AVG, RIG, RIA, M1, RIR	None
<i>unc-47</i>	19 D ventral cord neurons, RMEs, AVL, RIS, DVB	None
<i>unc-17</i>	IL2, URA, URB, SAA, SAB, SIA, SIB, SMB, SMD, RMD, AIA, M1, M2, M5, VA, VB, VC, DA, DB, SDQ, HSN, ALN, PLN	None
<i>glr-5</i>	AVA, AVB, AVD, AVE, PVC, <u>RIM</u> , RIC, RMD, SMD, SIB, RME, AVK, RMG, SABVL, SABVR, SABD, RIF, VC, LUA, PVQ, URB, URY, URA, DVA, AIB, HSN	Full
<i>glr-4</i>	AVA, RMD, SMD, SAA, SIB, RIB, <u>RIM</u> , AVH, FLP, RMG, DVA, AUA, PVD, URY, URA, SAB, RIF, DB, PVU	Full
<i>glr-1</i>	AVA, AVB, AVD, AVE, PVC, AIB, RMD, <u>RIM</u> , SMA, AVG, PVQ, URY	Full
<i>ggr-1</i>	AIB, PVR, PVQ, AVH, SMDV	None
<i>flp-1</i>	AVK	None
<i>dop-1</i>	RIS, AVM, ALM, ALN, PLN, PVQ	None
<i>nmr-2</i>	AVA, AVD, AVE, <u>RIM</u> , AVG, PVC	Full
<i>tdc-1</i>	<u>RIM</u> and RIC, UV1	Full
<i>tbh-1</i>	RIC	Partial

daf-1::gfp expression was targeted to indicated cells by each of the listed promoters. Cell names are listed. See www.wormbase.org for additional information.

^a Larval dauer formation, adult excess fat accumulation, reduced pumping rate, and egg retention were monitored. These phenotypes were fully rescued by all promoters that targeted *daf-1::gfp* to RIM interneurons (underlined).

Table S3: List of Promoters Used to Reconstitute *daf-3* in *daf-1(m40);daf-3(mgDf90)*.

	Genetic background	Promoter driving <i>daf-3::gfp</i>	Cells expressing <i>daf-3::gfp</i>	% Dauer (n)^a	% WT pumping rate^b
Control	<i>N2</i> (WT)	None		0 (200)	100 ± 3.4
	<i>daf-1(m40)</i> ^c	None		98 (278)	81 ± 4.8 p<0.001
	<i>daf-3(mgDf90)</i>	None		0 (234)	98 ± 2.9
	<i>daf-1(m40); daf-3(mgDf90)</i>	None		0 (187)	97 ± 3.0
	<i>daf-1(m40); daf-3(mgDf90)</i> ^c	<i>daf-1</i>	many (>80) neurons	40 (181)	83 ± 4.0 p<0.001
Sensory Neurons	<i>daf-1(m40); daf-3(mgDf90)</i>	<i>osm-6</i>	56 ciliated neurons	0 (125)	97 ± 4.6
Pharyngeal Neurons	<i>daf-1(m40); daf-3(mgDf90)</i>	<i>glr-7</i>	9 pharyngeal neurons	0 (98)	95 ± 5.7
Specific Inter-neurons	<i>daf-1(m40); daf-3(mgDf90)</i> ^c	<i>tdc-1</i>	4	18 (186)	84.5 p<0.001
		<i>tbh-1</i>	2	0 (139)	92 ± 5.7

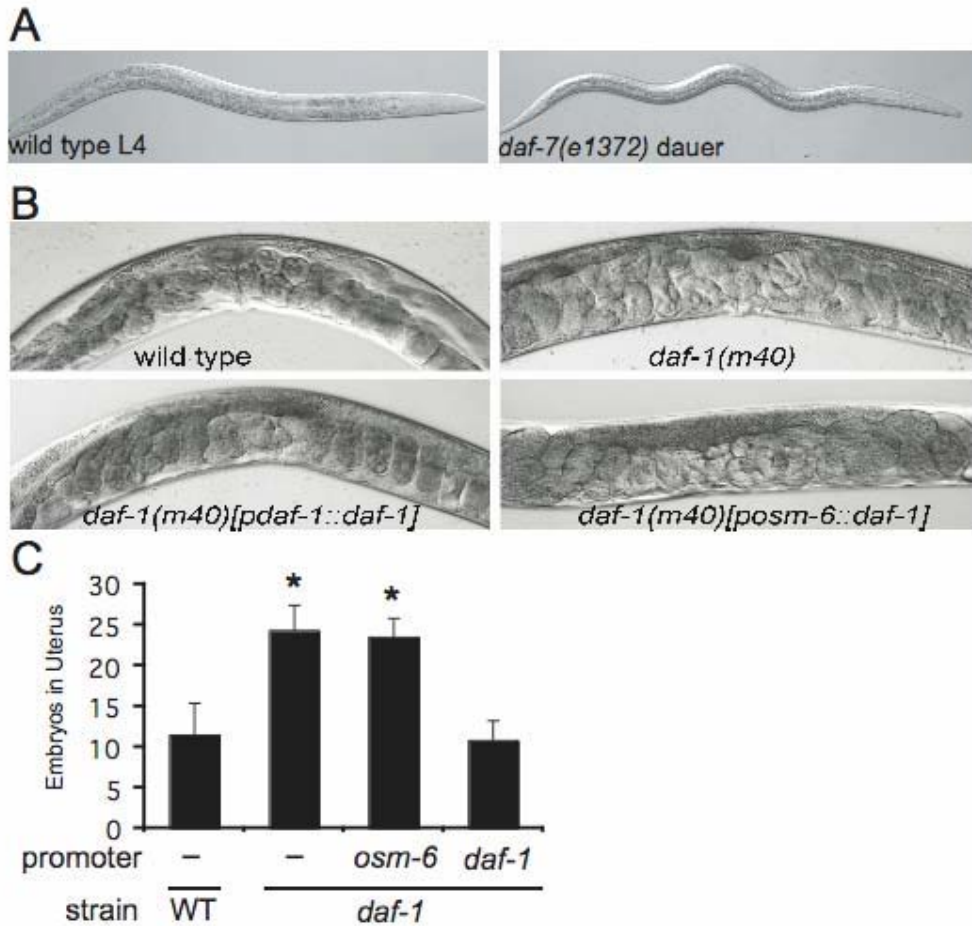
^a % animals that entered dauer when placed on food plates as L1s at 25°C.

^b Average pumping rate of well-fed, young gravid adults. P-values relative to wild type (N2) pumping rate were determined by ANOVA with Bonferroni post-test. P-values <0.001 were considered significant.

^c Genotypes that displayed excess fat levels relative to wild type. Representative images and quantitations are shown in Figure 3.

Supplemental Figures

Figure S1: Examples of Dauer and Egg Retention Phenotypes.

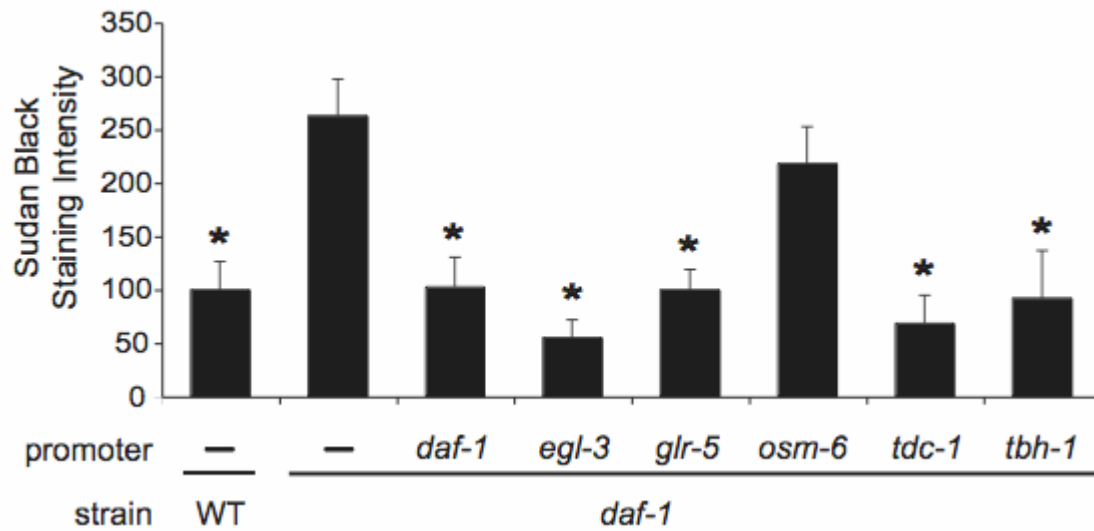


(A) Example of wild-type L4 and *daf-7(e1372)* dauer animals. Dauers were identified by their characteristic thin morphology and constricted pharynxes and resistance to 1% SDS.

(B) Examples of egg retention.

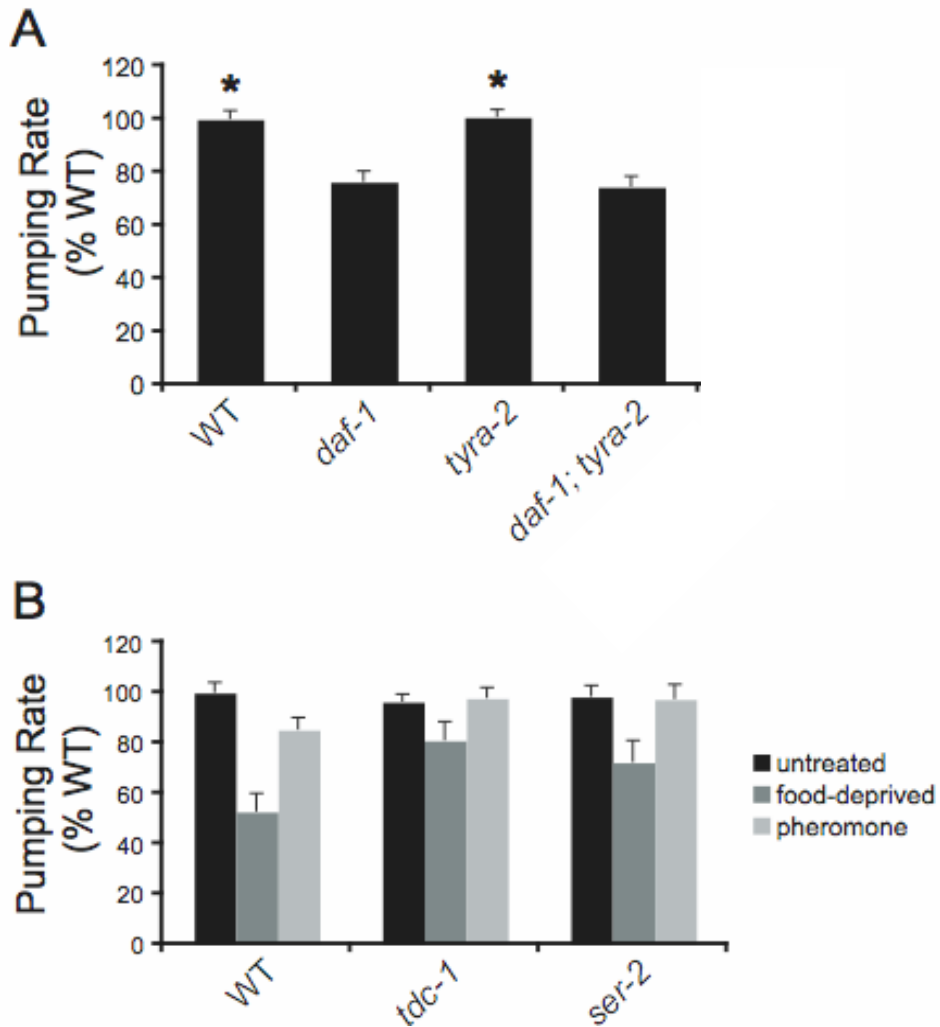
(C) Quantitation of egg retention phenotype for select genotypes. Asterisks indicate statistically significant change ($p < 0.001$) from *daf-1(m40)* as determined by ANOVA with Bonferroni post-test. Standard deviation bars are shown.

Figure S2: Sudan Black B Staining Intensity in Transgenic Lines.



Quantitations of relative amounts of Sudan Black B staining intensities for strains shown in Figure 2C. Asterisks indicate statistical significance ($p < 0.001$) as determined by t-test when comparing transgenic animals and co-stained *daf-1(m40)* non-transgenic animals. Standard error bars are shown.

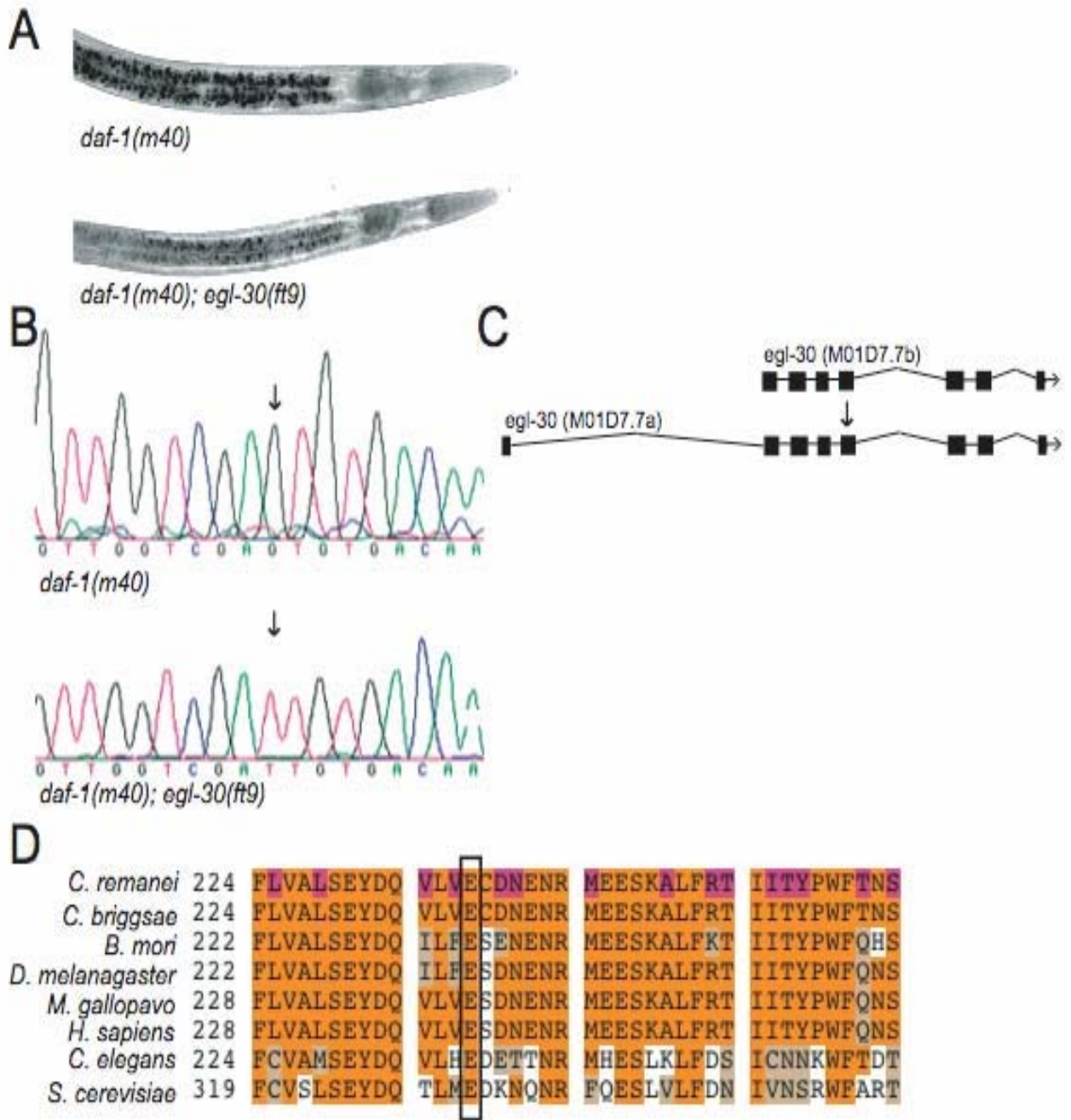
Figure S3: The Tyraminergetic GPCR *ser-2* but not *tyra-2* Acts Downstream of TGF- β Signaling to Regulate Pharyngeal Pumping.



(A) An inactivating mutation in tyraminergetic GPCR *tyra-2* did not rescue the reduced pumping of *daf-1(m40)*. Asterisks indicate statistically significant ($p < 0.001$) change from *daf-1(m40)* as determined by ANOVA. Standard deviation bars are shown.

(B) *tdc-1(ok914)* and *ser-2(pk1357)* animals did not respond to pheromone and showed a smaller reduction in feeding relative to wild type when food deprived.

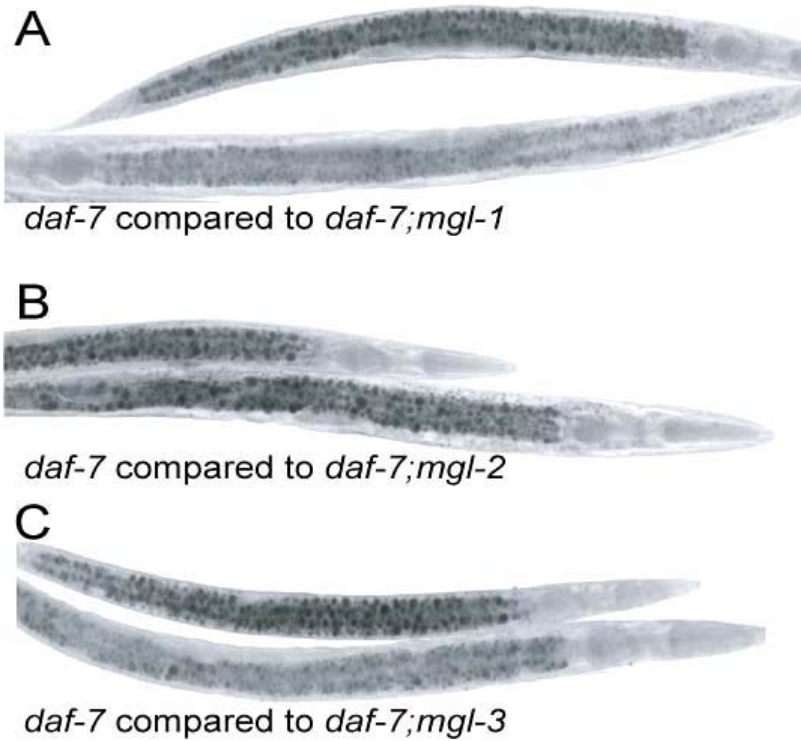
Figure S4: *egl-30(ft9)* Suppresses *daf-1(m40)* Excess Fat.



(A) *egl-30(ft9)* suppressed the increased fat storage of *daf-1(m40)* animals.

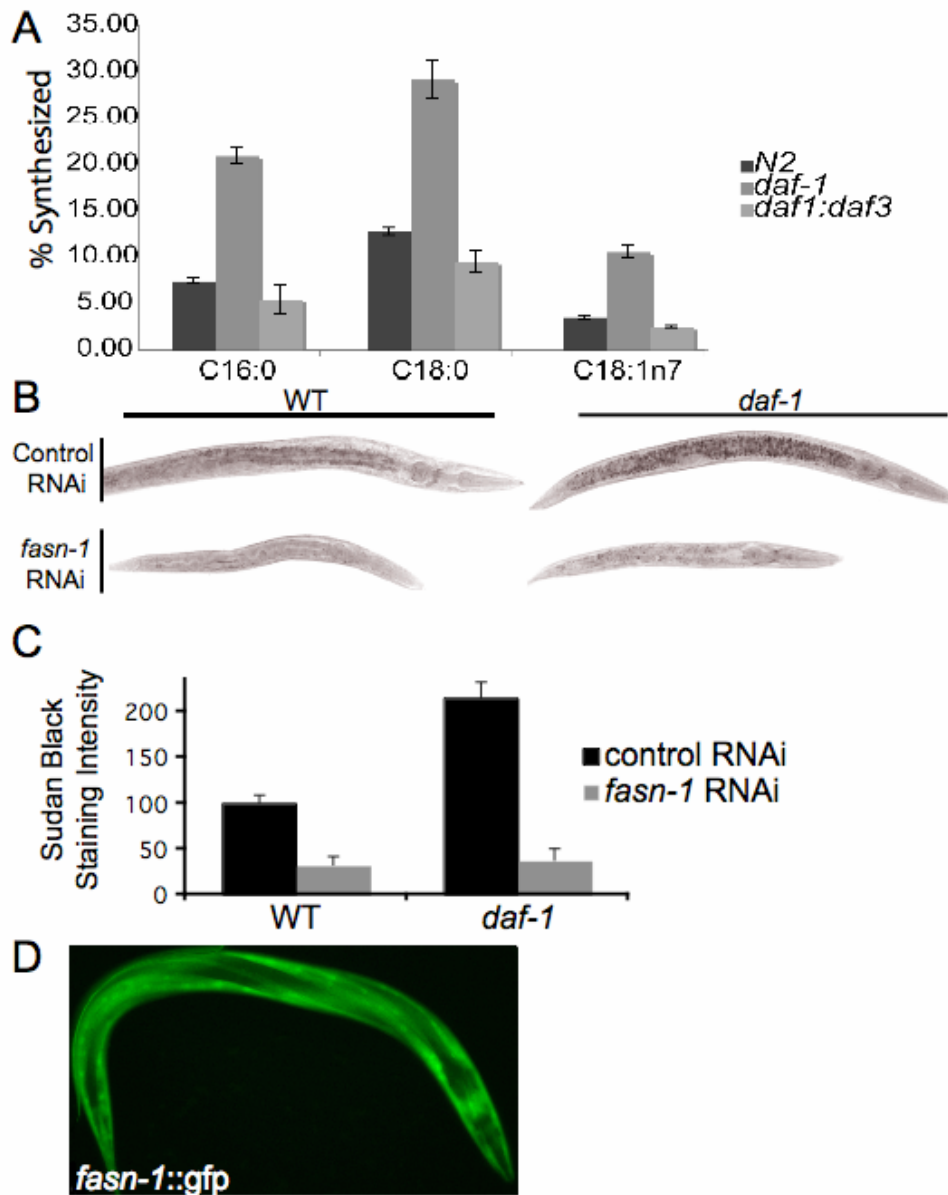
(B-D) *egl-30(ft9)* results in a G to T substitution in the 5th exon of *egl-30a*. This results in a glutamic acid to an aspartic acid change in a highly conserved residue within a highly conserved domain of *egl-30/G_qα*.

Figure S5. Loss of Function Mutations in Metabotropic Glutamate Receptors *mgl-1*, and *mgl-3* but not *mgl-2* Reduce Excess Fat of *daf-7(-)*.



In each experiment, *daf-7(e1372)* and test strain were co-stained in the same tube to minimize variation. Representative images are shown. (A) top: *daf-7(e1372)*; bottom: *daf-7(e1372);mgl-1(tm1811)*, (B) top: *daf-7(e1372)*; bottom: *daf-7(e1372);mgl-2(tm355)*, (C) top: *daf-7(e1372)*; bottom: *daf-7(e1372);mgl-3(tm1766)*.

Figure S6: Fat Synthesis is Upregulated in *daf-1(m40)* Animals.



(A) *de novo* fat synthesis was upregulated in *daf-1(m40)* and was dependent on *daf-3*.

(B-C) RNAi of *fasn-1* resulted in developmental arrest and fat reduction in both wild type and *daf-1(m40)* animals. Standard error bars are shown.

(D) A 6kb *promoter::gfp* for *fasn-1* showed expression in the skin-like epidermis, a key peripheral fat metabolic tissue in *C. elegans*.