

## Supplementary Materials for

### **Octopamine connects nutrient cues to lipid metabolism upon nutrient deprivation**

Jun Tao, Yi-Cheng Ma, Zhong-Shan Yang, Cheng-Gang Zou, Ke-Qin Zhang

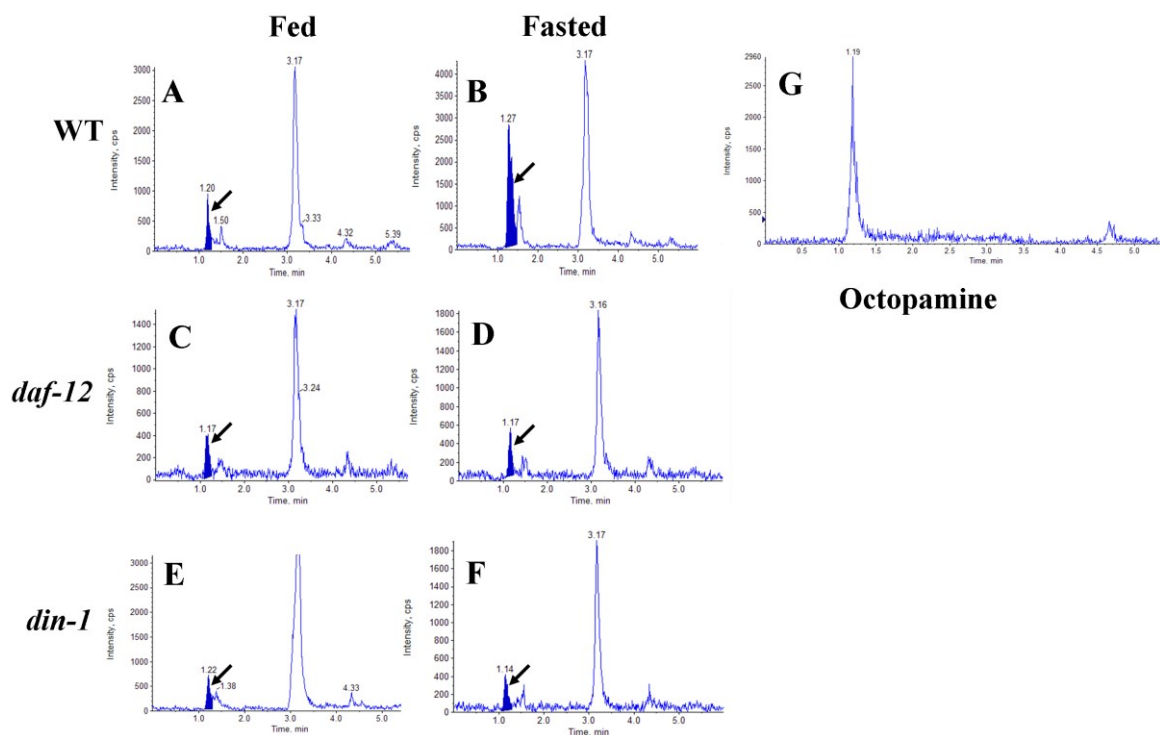
Published 6 May 2016, *Sci. Adv.* **2**, e1501372 (2016)

DOI: 10.1126/sciadv.1501372

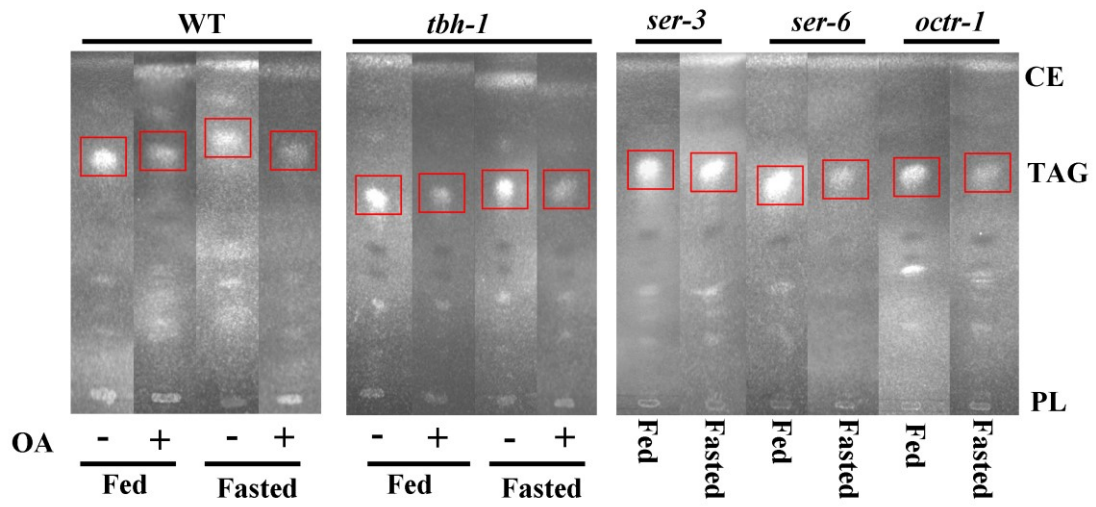
#### **The PDF file includes:**

- fig. S1. LC-MS/MS detection of octopamine contents in worms.
- fig. S2. TLC analysis of total lipids extracted from worms.
- fig. S3. Overexpression of *tbh-1* enhances lipid hydrolysis in well-fed wild-type worms.
- fig. S4. Starvation does not alter the expression of *atgl-1*.
- fig. S5. A mutation in *tbh-1* (*n3247*) does not affect the expression of *fil-1* or *fil-2* in starved worms.
- fig. S6. Knockdown of *lips-6* by RNAi leads to reduced *lips-6* expression.
- fig. S7. Overexpression of *lips-6* in the intestine promotes lipid hydrolysis in well-fed WT worms.
- fig. S8. Octopamine fails to restore lipid hydrolysis and expression of *lips-6* in *ser-3* (*ad1774*) mutants during starvation.
- fig. S9. SER-3 in the neurons and muscle is not involved in lipid hydrolysis during starvation.
- fig. S10. SER-3 is involved in resistance to starvation.
- table S1. ChIP-qPCR analysis of the *tbh-1* promoter.

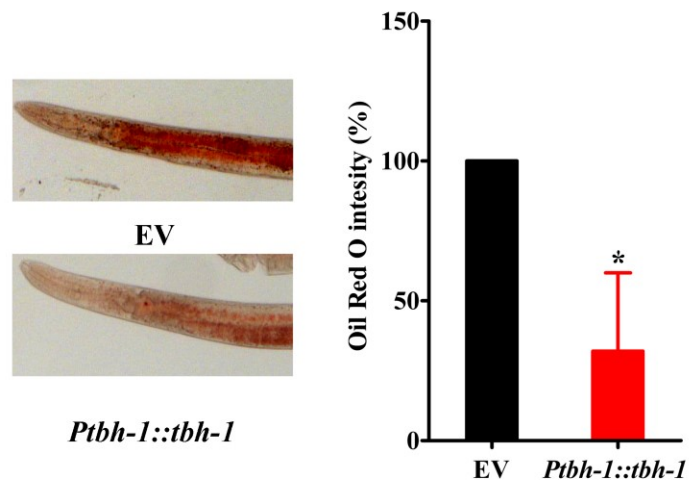
## Supplementary Figures



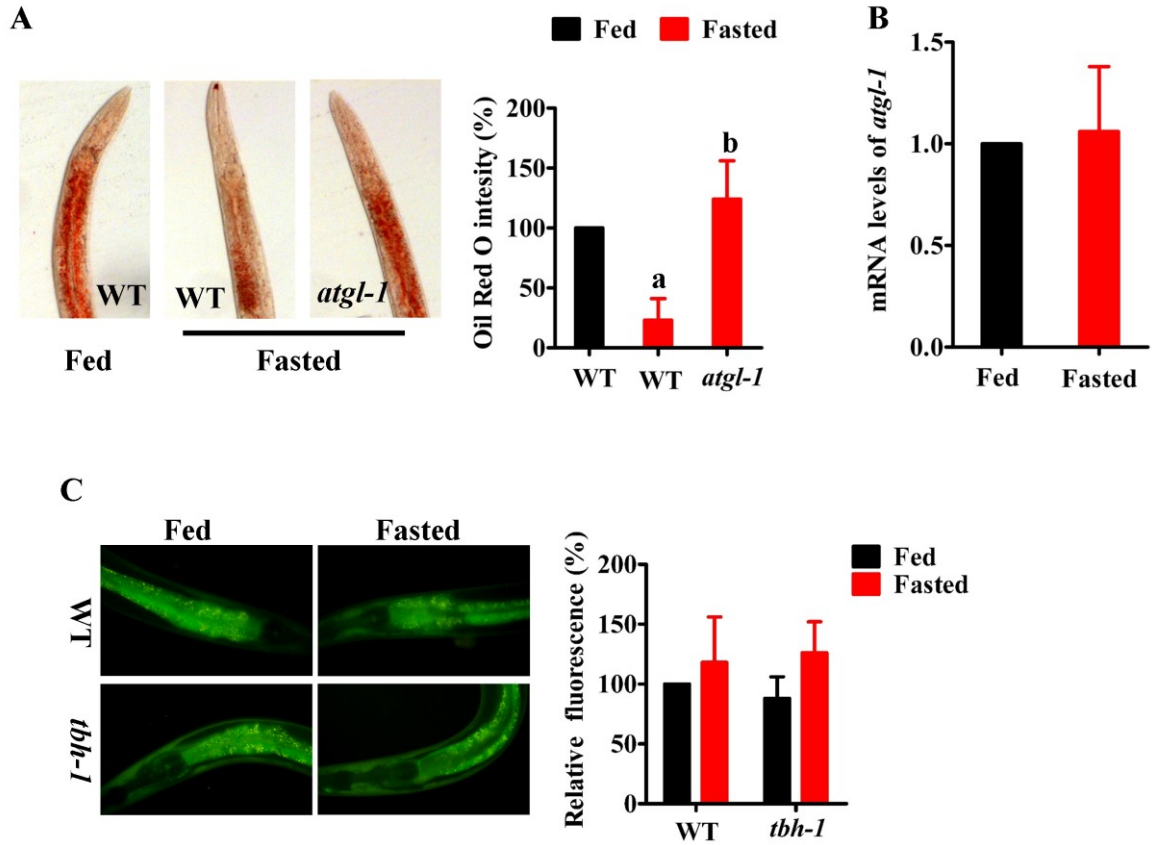
**fig. S1. LC-MS/MS detection of octopamine contents in worms.** Representative chromatograms of (A) well-fed wild-type (WT) worms, (B) starved WT worms, (C) well-fed *daf-12(rh61rh411)* mutants, (D) starved *daf-12(rh61rh411)* mutants, (E) well-fed *din-1(dh127)* worms, (F) starved *din-1(dh127)* worms, and (G) octopamine standard. cps, counts per second. Black arrows indicate the octopamine-specific peaks.



**fig. S2. TLC analysis of total lipids extracted from worms.** WT, wild-type worms; *tbh-1*, *tbh-1(n3247)*; *ser-3*, *ser-3(ad1774)*; *ser-6*, *ser-6(tm2146)*; *octr-1*, *octr-1(ok371)* mutants. CE, cholesterol ester; TAG, triacylglycerol; PL, phospholipids.



**fig. S3. Overexpression of *tbh-1* enhances lipid hydrolysis in well-fed wild-type worms.** The right panel represents relative Oil Red O intensity. EV, empty vector.  
\* $P < 0.05$ .



**fig. S4. Starvation does not alter the expression of *atgl-1*.** (A) The mutation in *atgl-1(tm3116)* led to lipid accumulation in starved worms. The right panel represents relative Oil Red O intensity. <sup>a</sup> $P < 0.05$  versus well-fed wild-type (WT) worms; <sup>b</sup> $P < 0.05$  versus starved WT worms. (B) mRNA levels of *atgl-1*. (C) Fluorescence microscopy of *Patgl-1::atgl-1::GFP*. The right panel shows quantification of GFP levels.

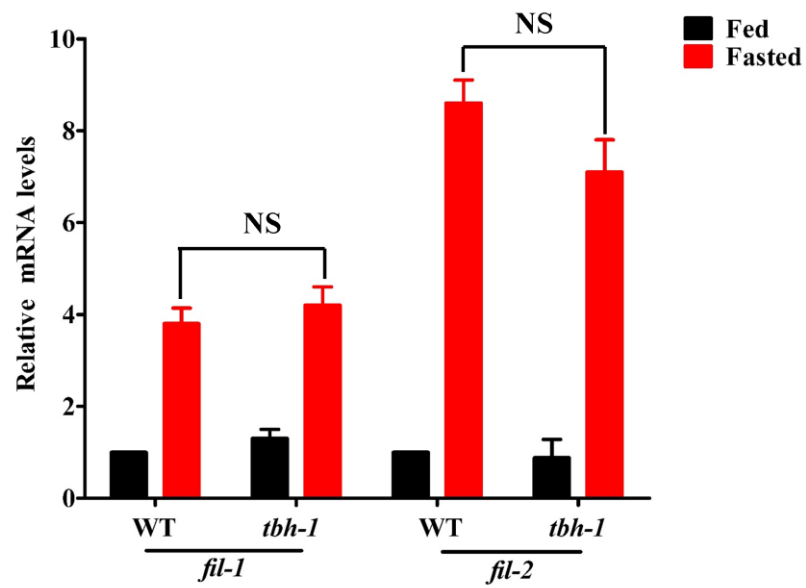


fig. S5. A mutation in *tbh-1(n3247)* does not affect the expression of *fil-1* or *fil-2*

in starved worms. WT, wild-type worms; NS, not significant.

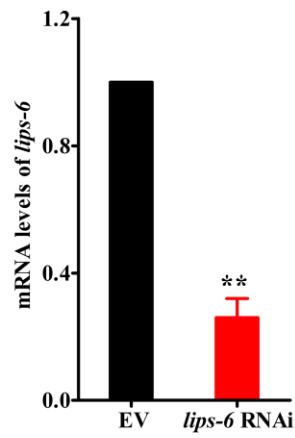
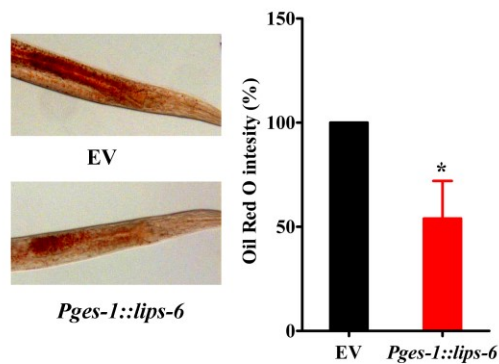
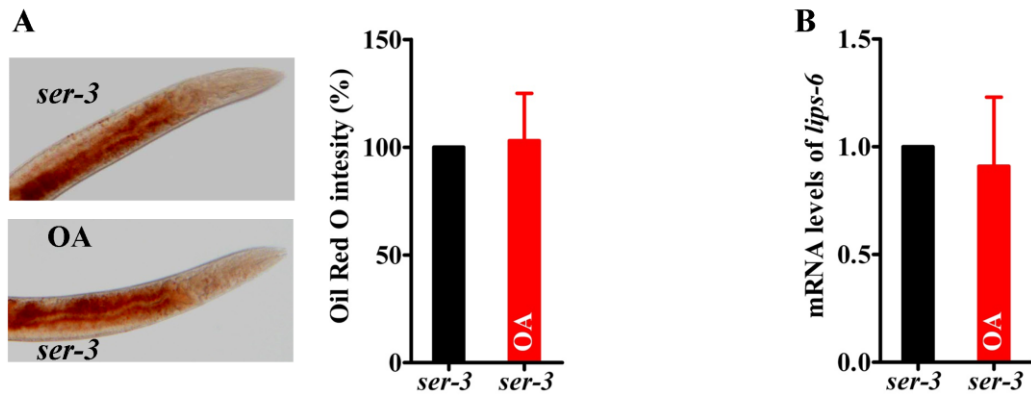


fig.

**S6. Knockdown of *lips-6* by RNAi leads to reduced *lips-6* expression.** EV, empty vector. \*\* $P < 0.01$ .

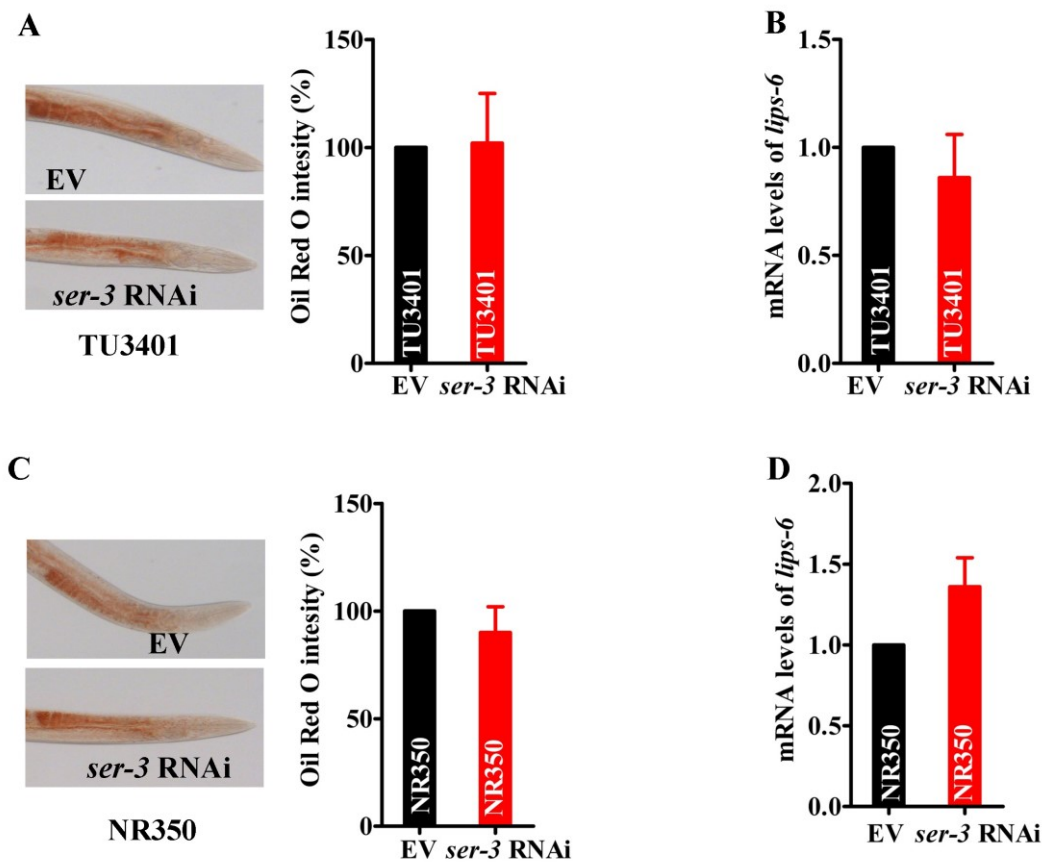


**fig. S7. Overexpression of *lips-6* in the intestine promotes lipid hydrolysis in well-fed WT worms.** The right panel represents relative Oil Red O intensity. EV, empty vector. \* $P < 0.05$ .

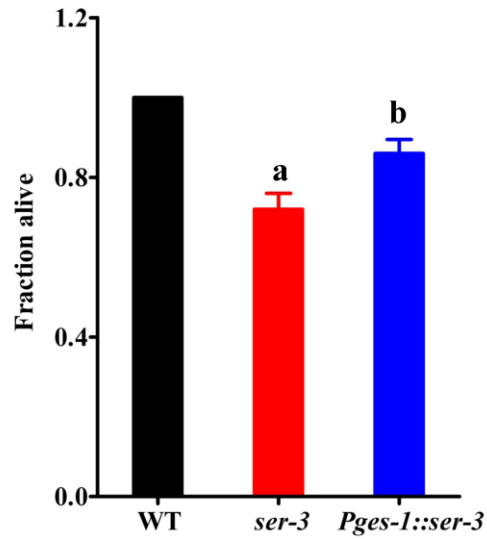


**fig. S8. Octopamine fails to restore lipid hydrolysis and expression of *lips-6* in *ser-3(ad1774)* mutants during starvation.** (A) Oil Red O staining. The right panel represents relative Oil Red O intensity. (B) mRNA levels of *lips-6*.





**fig. S9. SER-3 in the neurons and muscle is not involved in lipid hydrolysis during starvation.** (A and B) Neuronal-specific RNAi of *ser-3* (TU3401) did not alter lipid hydrolysis (A) and expression of *lips-6* (B). The right panel represents relative Oil Red O intensity. (C and D) Muscular-specific RNAi of *ser-3* (NR350) did not alter lipid hydrolysis (C) and expression of *lips-6* (D). The right panel represents relative Oil Red O intensity. EV, empty vector.



**fig. S10. SER-3 is involved in resistance to starvation.** A mutation in *ser-3(ad1774)* led to reduced rate of survival after three days of starvation. However, expression of *ser-3* in the intestine significantly suppressed the sensitivity of *ser-3(ad1774)* mutants to starvation. <sup>a</sup> $P < 0.05$  versus wild-type (WT) worms; <sup>b</sup> $P < 0.05$  versus *ser-3(ad1774)* worms.

**table S1. CHIP-qPCR analysis of the *tbh-1* promoter.**

<b>Distances to ATG</b>		<b>Items</b>	<b>Mean ct</b>	<b><math>\Delta</math>ct</b>	<b><math>\Delta\Delta</math>ct</b>	<b><math>2^{-\Delta\Delta</math>ct}</b>
<b>-4139</b>	1	Fed	21.62	11.42	0	1
		Input	10.2			
		Fasted	21.24	9.54	-1.88	3.68
		Input	11.7			
	2	Fed	20.96	10.53	0	1
		Input	10.43			
		Fasted	21.75	8.98	-1.55	2.93
		Input	12.77			
	3	Fed	22.08	11.05	0	1
		Input	11.03			
		Fasted	21.21	9.23	-1.82	3.53
		Input	11.98			
<b>-3193</b>	1	Fed	21.98	10.44	0	1
		Input	11.54			
		Fasted	20.51	9.18	-1.26	2.39
		Input	11.33			
	2	Fed	22.23	10.44	0	1
		Input	11.79			
		Fasted	21.29	9.44	-1	2
		Input	11.85			
	3	Fed	21.59	10.64	0	1
		Input	10.95			
		Fasted	20.95	9.34	-1.3	2.46
		Input	11.61			
<b>-2711</b>	1	Fed	20.31	10.29	0	1
		Input	10.02			
		Fasted	18.84	8.45	-1.84	3.58
		Input	10.39			
	2	Fed	20.65	10.43	0	1
		Input	10.22			
		Fasted	19	8.71	-1.72	3.29
		Input	10.29			
	3	Fed	20.51	10.06	0	1
		Input	10.45			
		Fasted	17.89	7.9	-2.16	4.47
		Input	9.99			