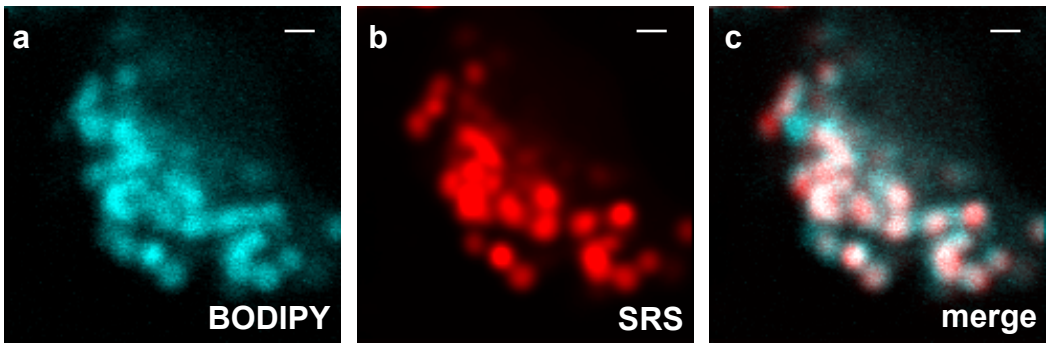
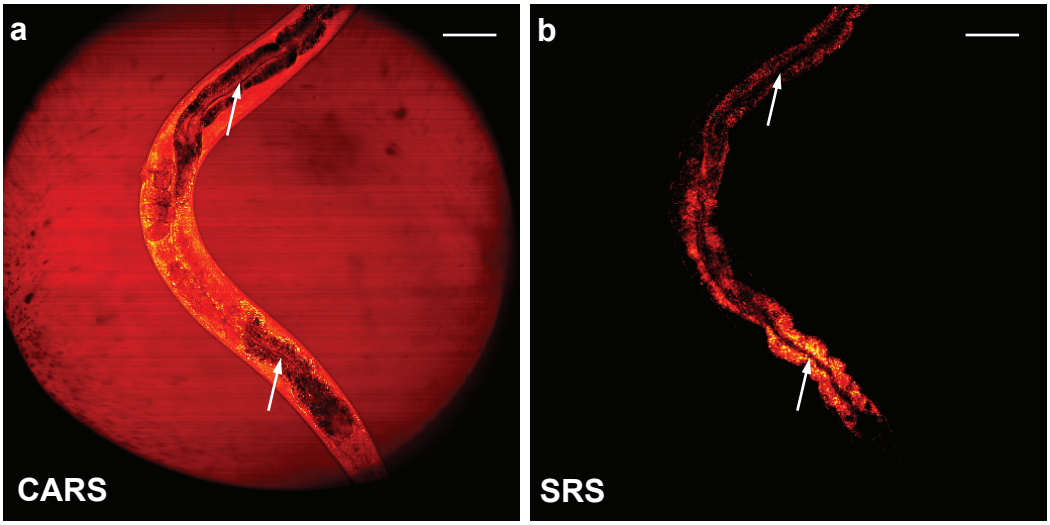


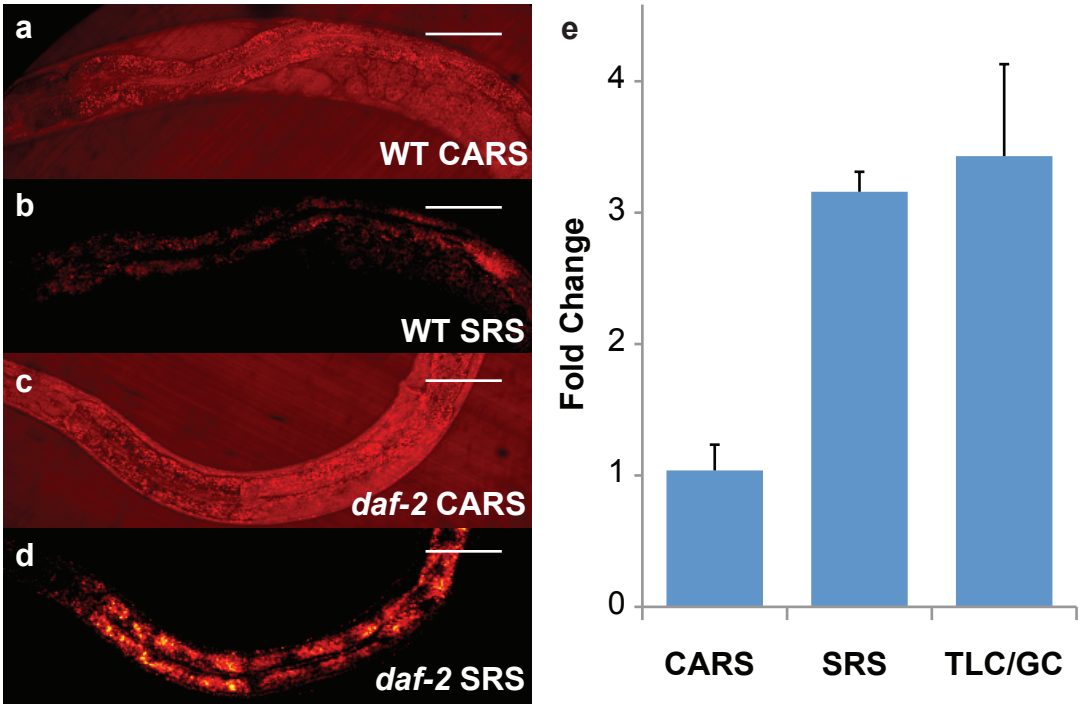
Supplementary Figure 1. Co-localization between BODIPY and SRS signals in lipid droplets in mammalian 293 cells.

a) BODIPY staining reveals lipid droplets in living cells.
b) SRS visualization of lipid storage in lipid droplets.
c) Overlap between BODIPY and SRS signals.
Scale bar, 1μm in all figures



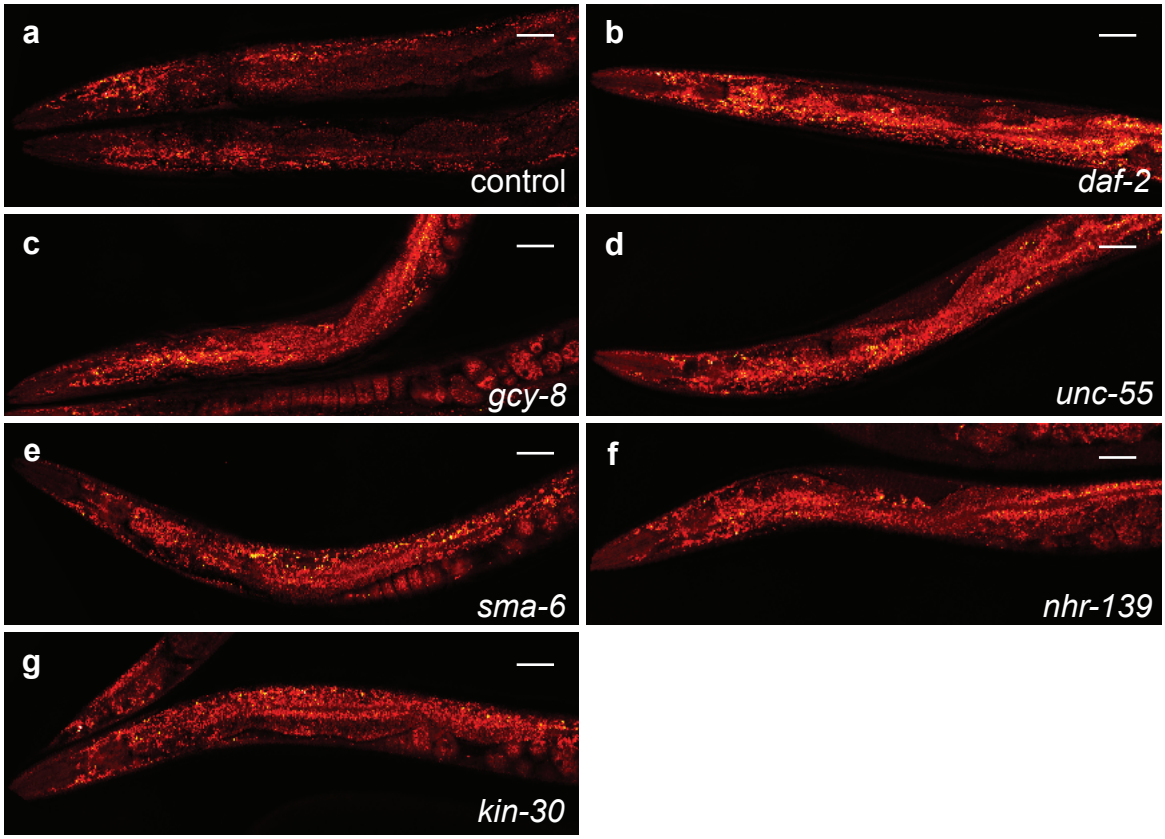
Supplementary Figure 2. Image artifact of CARS due to lipid unrelated structure.

a) CARS signals are significantly affected by the shape of imaging samples. As pointed by arrows, the dark regions low in CARS signals are artifact caused by the intestine structure.
b) SRS imaging of the same animal displays a much more homogeneous pattern along the gut.
Scale bar, 100μm in both figures



Supplementary Figure 3. Quantification of fat storage in the *daf-2(e1370)* mutants based on CARS microscopy.

a) CARS imaging of fat storage in a wild type adult.
b) SRS imaging of fat storage in the same animal in (a).
c) CARS imaging of fat storage in the *daf-2(e1370)* mutant.
d) SRS imaging of the same *daf-2* mutant in (c).
e) CARS microscopy based quantification failed to detect more than 3-fold induction of fat storage in the *daf-2* mutant.
Scale bar, 100μm in all figures



Supplementary Figure 4. New genetic regulators of fat storage identified using label-free SRS microscopy through RNAi screening.

a) Control animals fed with empty vector containing bacteria show normal fat content levels.
b-g) New genes were identified whose inactivation signficantly increases fat storage. $p<0.0001$
Scale bar, 100μm in all figures.

Supplementary Table 1. Summary of newly identified genes responsible for fat storage.

Gene	Brief Description	Expression Pattern	Human	SRS Increase ± STD	p value
daf-2	Insulin/IGF-1 receptor	Neuron, Muscle, Intestine, etc	Yes	0.56 ± 0.16	<0.0001
gcy-28	Natriuretic peptide receptor	Neuron, Muscle, Intestine, etc	Yes	0.34 ± 0.17	<0.0001
B0252.1	FGF receptor	Unknown	Yes	0.54 ± 0.15	<0.0001
sma-6	Type I TGF-beta receptors	Hypodermis, Intestine, Muscle, etc	Yes	0.41 ± 0.03	<0.0001
R09D1.12	Protein kinase	Unknown	Yes	0.46 ± 0.12	<0.0001
kin-30	Tyrosine Kinase	Unknown	Yes	0.26 ± 0.06	<0.0001
unc-55	COUP transcription factor	Neuron, Muscle	Yes	0.37 ± 0.13	<0.0001
nhr-123	nuclear hormone receptor	Neuron, Intestine, Pharynx	Yes	0.46 ± 0.16	<0.0001
nhr-139	nuclear hormone receptor	Unknown	Yes	0.37 ± 0.10	<0.0001
nhr-74	nuclear hormone receptor	Seam cells	Yes	0.21 ± 0.10	0.001

“Expression Pattern” column indicates the tissue where each gene is expressing normally. The information is collected from the wormbase www.wormbase.org.
“Human” column indicates whether each gene has a conserved homolog in human.
All the experiments were performed twice independently.