

Supporting Information

Hou et al. 10.1073/pnas.1318262111

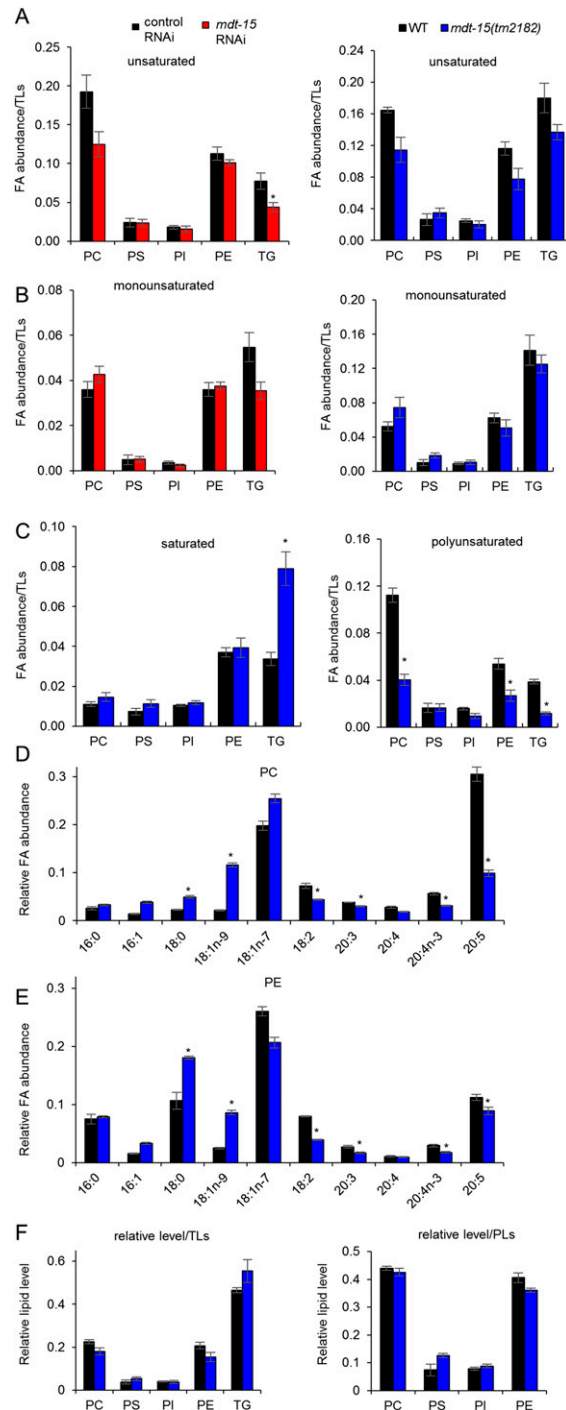


Fig. S1. *mdt-15* is required to maintain normal membrane lipid unsaturation. (A) Bars indicate the average abundance of fatty acids (FAs) relative to total extracted lipids. "Unsaturated" represents the average abundance of phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidylethanolamine (PE), and triglyceride (TG) with C16:1, C18:1n-7, C18:1n-9, C18:2, C20:3, C20:4, C20:4n-3, or C20:5 fatty acyl tails. (B) Bars represent the average abundance of PC, PS, PI, PE, and TG with monounsaturated FAs (C16:1, C18:1n-9, and/or C18:1n-7) relative to total lipids. (C) "Saturated" indicates the average abundance of PC, PS, PI, PE, and TG with C16:0 and/or C18:0 acyl tails; "polyunsaturated" represents lipids with C18:2, C20:3, C20:4, C20:4n-3, and/or C20:5 fatty acyl tails. (D) Bars represent the relative abundance of individual FAs in PC. (E) Bars represent the relative abundance of individual FAs in PE. (F) Bars represent the relative levels of individual lipid species relative to total lipids (TLs) (Left) and relative to total phospholipid (PLs) (Right). In all panels, lipids were isolated from L4-stage *control(RNAi)*, *mdt-15(RNAi)*, WT, or *mdt-15(tm2182)* worms. Error bars represent SEM; $n = 3$; * $P < 0.05$ (two-tailed unequal variance Student *t* test). For *P* values, see Tables S1–S6.

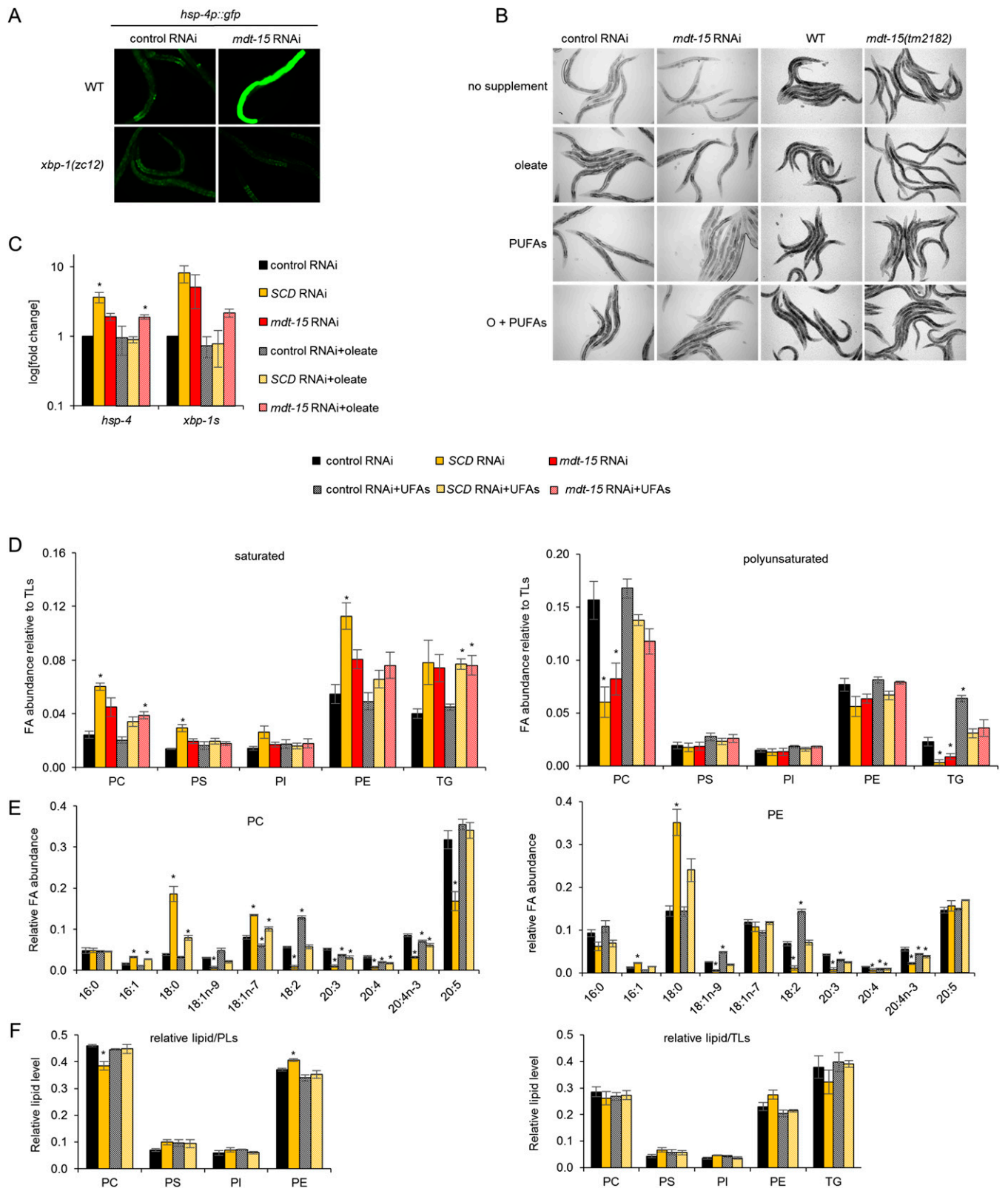


Fig. S2. *mdt-15*-mediated lipid unsaturation is required for maintaining endoplasmic reticulum (ER) homeostasis. (A) Micrographs show *hsp-4p::GFP* expression in WT and *xbp-1(zc12)* worms grown on control or *mdt-15* RNAi. (B) Micrographs illustrate the morphological phenotypes of control(RNAi), *mdt-15* (RNAi), WT, and *mdt-15(tm2182)* worms on plates without lipid supplements (no supplement), with 300 μ M C18:1n-9 (oleate), with 150 μ M C18:2 and 150 μ M C20:5 (PUFAs), or with 150 μ M C18:1n-9 and 300 μ M PUFAs (O+PUFAs). (C) Bar graphs represent the average fold change of *hsp-4* and spliced *xbp-1* (*xbp-1s*) mRNA levels in control(RNAi), SCD(RNAi), and *mdt-15*(RNAi) worms grown in the presence or absence of 300 μ M C18:1n-9 supplement ($n = 3$). P values (vs. control unsupplemented): *hsp-4*: $P = 0.048, 0.059, 0.96, 0.40,$ and 0.022 , respectively; *xbp-1s*: $P = 0.087, 0.25, 0.42, 0.66,$ and 0.060 , respectively. (D) Bars indicate

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the average abundance of saturated and polyunsaturated fatty acyl chains in PC, PS, PI, PE, and TG, relative to total lipids. "Saturated" refers to lipids containing C16:0 and/or C18:0 fatty acyl tails; "polyunsaturated" represents lipids containing C18:2, C20:3, C20:4, C20:4n-3, and/or C20:5 fatty acyl tails. (E) Bar graphs represent the relative abundance of individual FAs in PC (Left) or PE (Right). (F) Bar graphs indicate the relative levels of different lipids relative to total PLs (Left) or TLs (Right). For D, E, and F, lipids were extracted from L4-stage *control(RNAi)*, *SCD(RNAi)*, and *mdt-15(RNAi)* worms grown in the absence or presence of dietary unsaturated FAs (UFAs; C18:1n-9, C18:2 and C20:5 FAs). $n = 3$; error bars represent SEM; $*P < 0.05$ (two-tailed unequal variance Student t test). For P values, see Tables S1–S3 and S7–S9.

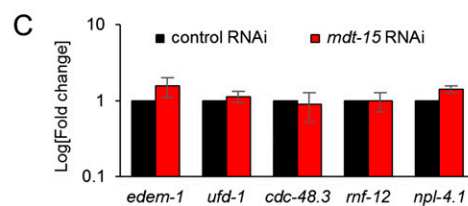
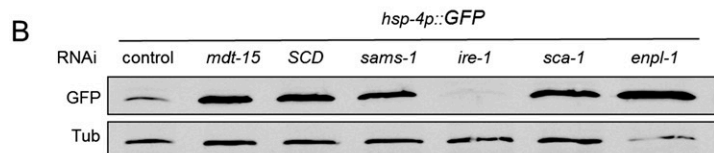
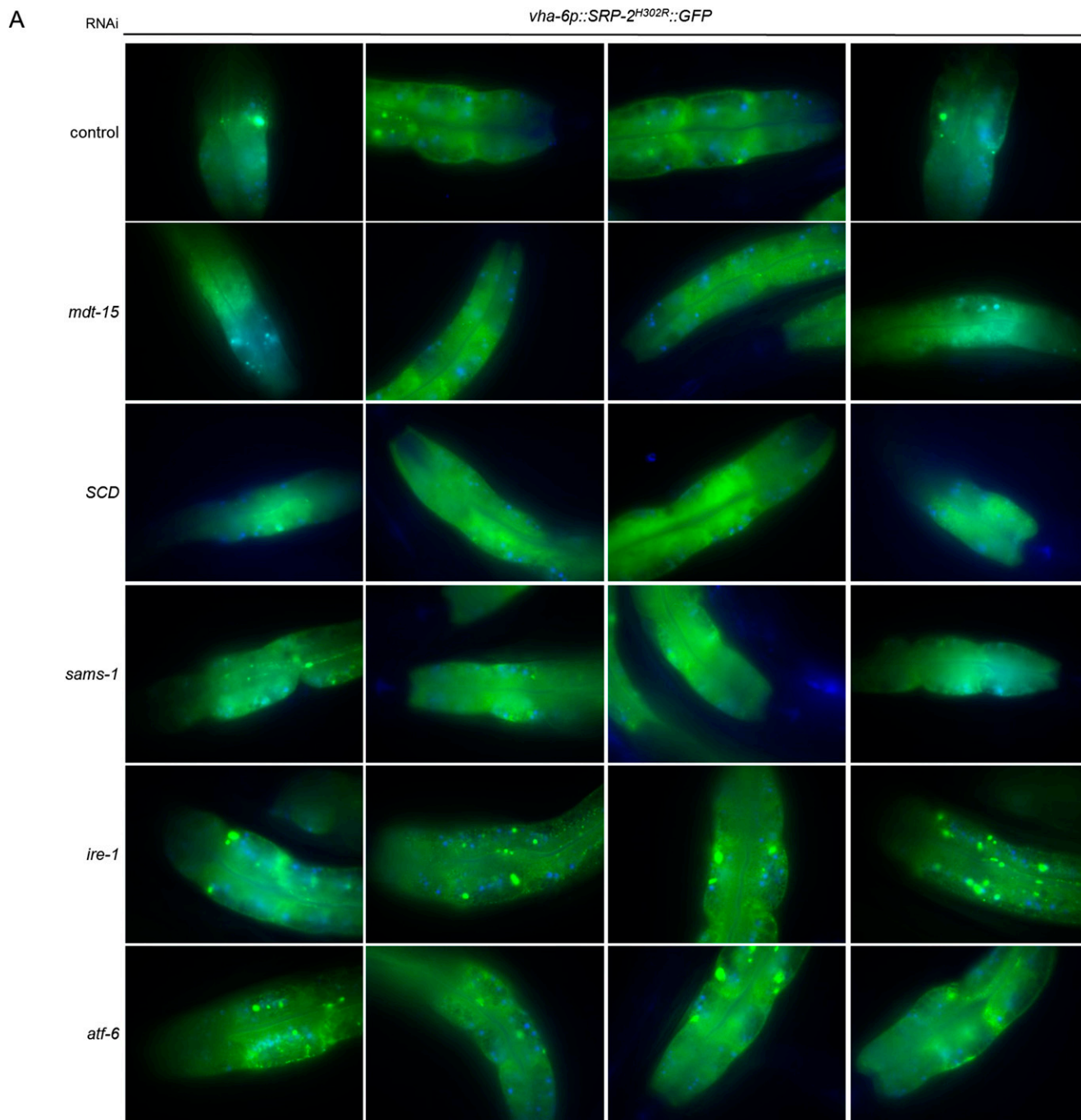


Fig. S3. *mdt-15* depletion or defects in phospholipid (PL) biosynthesis do not enhance misfolded protein aggregation. (A) Micrographs show replicates of SRP-2^{H302R}::GFP aggregates in *control*, *mdt-15*⁻, *SCD*⁻, *sams-1*⁻, *ire-1*⁻, and *atf-6*⁻ depleted worms. Green: GFP; blue: Texas Red (= autofluorescence). The first two pairs of anterior intestinal cells were captured for each condition. (B) Immunoblots show the protein levels of *hsp-4*-promoter driven GFP and endogenous tubulin in *control(RNAi)*, *mdt-15(RNAi)*, *SCD(RNAi)*, *ire-1(RNAi)*, *sca-1(RNAi)*, and *enpl-1(RNAi)* worms. One of three independent experiments is shown. (C) Bar graphs represent the relative fold change of the mRNA levels of five ER-associated protein degradation genes in *control(RNAi)* and *mdt-15(RNAi)* worms. *n* = 3; error bars represent SEM; *P* values vs. *control(RNAi)* (as determined by two-tailed unequal variance Student *t* test): *edem-1*, 0.72; *ufd-1*, 0.75; *cdc-48.3*, 0.25; *mf-12*, 0.56; *npl-4.1*, 0.50.

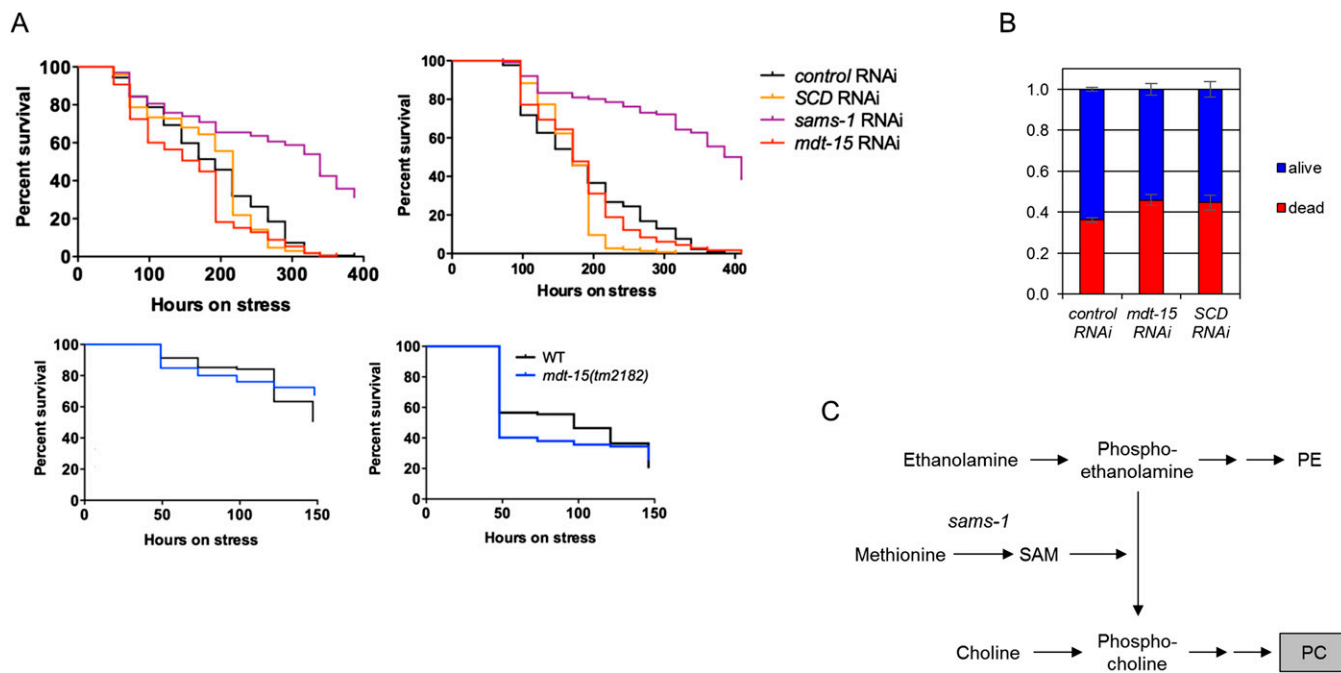


Fig. 54. Defects in membrane PL biosynthesis activate the ER unfolded protein response but do not cause hypersensitivity to exogenous proteostatic stress. (A) The upper two panels represent replicates #2 and #3 of the population survival experiments shown in Fig. 6A. Depicted are survival curves of *control(RNAi)*, *mdt-15(RNAi)*, *SCD(RNAi)*, and *sams-1(RNAi)* worms on tunicamycin; *sams-1* worms are long-lived, as previously reported (1). The lower panels represent additional replicates of WT and *mdt-15(tm2182)* survival on tunicamycin. (B) Bar graphs represent the fractions of live and dead *control(RNAi)*, *mdt-15(RNAi)*, and *SCD(RNAi)* larvae after 48 h on 10 µg/mL tunicamycin (starting from larval stage L1). $n = 3$; error bars represent SEM. (C) The diagram depicts the pathways of PC biosynthesis in *C. elegans* (2).

- Hansen M, Hsu A-L, Dillin A, Kenyon C (2005) New genes tied to endocrine, metabolic, and dietary regulation of lifespan from a *Caenorhabditis elegans* genomic RNAi screen. *PLoS Genet* 1(1):119–128.
- Walker AK, et al. (2011) A conserved SREBP-1/phosphatidylcholine feedback circuit regulates lipogenesis in metazoans. *Cell* 147(4):840–852.

Table S1. P values representing the significance of the change in relative FA abundance in lipid profiles of unsupplemented and supplemented *control(RNAi)* and *mdt-15(RNAi)* worms

| Relative FA abundance | PC | PE | Cardiolipin |
|-------------------------|-------|-------|-------------|
| 16:0 | | | |
| Control RNAi+UFAs | 0.82 | 0.42 | 0.75 |
| <i>mdt-15</i> RNAi | 0.20 | 0.60 | 0.75 |
| <i>mdt-15</i> RNAi+UFAs | 0.04 | 0.33 | 0.97 |
| 16:1 | | | |
| Control RNAi+UFAs | 0.27 | 0.05 | n/d |
| <i>mdt-15</i> RNAi | 0.004 | 0.01 | 0.20 |
| <i>mdt-15</i> RNAi+UFAs | 0.32 | 0.03 | 0.42 |
| 18:0 | | | |
| Control RNAi+UFAs | 0.15 | 0.94 | 0.59 |
| <i>mdt-15</i> RNAi | 0.05 | 0.02 | 0.74 |
| <i>mdt-15</i> RNAi+UFAs | 0.02 | 0.10 | 0.99 |
| 18:1n-9 | | | |
| Control RNAi+UFAs | 0.07 | 0.01 | 0.57 |
| <i>mdt-15</i> RNAi | 0.04 | 0.10 | 0.88 |
| <i>mdt-15</i> RNAi+UFAs | 0.03 | 0.001 | 0.27 |
| 18:1n-7 | | | |
| Control RNAi+UFAs | 0.03 | 0.02 | 0.11 |
| <i>mdt-15</i> RNAi | 0.54 | 0.00 | 0.32 |
| <i>mdt-15</i> RNAi+UFAs | 0.004 | 0.001 | 0.09 |
| 18:2 | | | |
| Control RNAi+UFAs | 0.002 | 0.001 | 0.06 |
| <i>mdt-15</i> RNAi | 0.02 | 0.01 | 0.93 |
| <i>mdt-15</i> RNAi+UFAs | 0.22 | 0.06 | 0.27 |
| 20:3 | | | |
| Control RNAi+UFAs | 0.01 | 0.02 | 0.60 |
| <i>mdt-15</i> RNAi | 0.01 | 0.02 | 0.56 |
| <i>mdt-15</i> RNAi+UFAs | 0.03 | 0.11 | 0.96 |
| 20:4 | | | |
| Control RNAi+UFAs | 0.02 | 0.10 | 0.42 |
| <i>mdt-15</i> RNAi | 0.20 | 0.22 | 0.42 |
| <i>mdt-15</i> RNAi+UFAs | 0.18 | 0.74 | 0.42 |
| 20:4n-3 | | | |
| Control RNAi+UFAs | 0.04 | 0.09 | 0.32 |
| <i>mdt-15</i> RNAi | 0.005 | 0.01 | 0.57 |
| <i>mdt-15</i> RNAi+UFAs | 0.003 | 0.02 | 0.85 |
| 20:5 | | | |
| Control RNAi+UFAs | 0.25 | 0.77 | 0.77 |
| <i>mdt-15</i> RNAi | 0.03 | 0.69 | 0.80 |
| <i>mdt-15</i> RNAi+UFAs | 0.22 | 0.63 | 0.93 |

The P values are for the comparison of unsupplemented *control(RNAi)* worms with unsaturated-FA-supplemented *control (RNAi)*, unsupplemented *mdt-15(RNAi)*, or unsaturated-FA-supplemented *mdt-15(RNAi)* worms, respectively. $n = 3$; statistical analysis by two-tailed Student t test. n/d, FA species not detected in this sample; UFAs, unsaturated fatty acids.

Table S2. P values representing the significance of the change in FA abundance per Tls in lipid profiles of unsupplemented and supplemented *control(RNAi)* and *mdt-15(RNAi)* worms

| FA abundance/Tls | PC | PS | PI | PE | TG | Tls |
|-------------------------|------|------|------|------|-------|------|
| SAT | | | | | | |
| Control RNAi+UFAs | 0.34 | 0.47 | 0.41 | 0.61 | 0.36 | 0.97 |
| <i>mdt-15</i> RNAi | 0.08 | 0.08 | 0.27 | 0.06 | 0.06 | 0.07 |
| <i>mdt-15</i> RNAi+UFAs | 0.02 | 0.05 | 0.38 | 0.16 | 0.02 | 0.06 |
| PUFA | | | | | | |
| Control RNAi+UFAs | 0.62 | 0.17 | 0.18 | 0.56 | 0.002 | 0.08 |
| <i>mdt-15</i> RNAi | 0.03 | 0.91 | 0.74 | 0.16 | 0.05 | 0.06 |
| <i>mdt-15</i> RNAi+UFAs | 0.15 | 0.26 | 0.18 | 0.77 | 0.24 | 0.71 |
| UFA | | | | | | |
| Control RNAi+UFAs | 0.82 | 0.24 | 0.19 | 0.84 | 0.02 | 0.22 |
| <i>mdt-15</i> RNAi | 0.07 | 0.96 | 0.61 | 0.31 | 0.07 | 0.09 |
| <i>mdt-15</i> RNAi+UFAs | 0.16 | 0.44 | 0.37 | 0.61 | 0.77 | 0.06 |
| MUFA | | | | | | |
| Control RNAi+UFAs | 0.36 | 0.41 | 0.43 | 0.20 | 0.14 | 0.08 |
| <i>mdt-15</i> RNAi | 0.26 | 0.95 | 0.27 | 0.69 | 0.08 | 0.06 |
| <i>mdt-15</i> RNAi+UFAs | 0.24 | 0.99 | 0.32 | 0.09 | 0.57 | 0.40 |

The P values are for the comparison of unsupplemented *control(RNAi)* worms with unsaturated-FA-supplemented *control(RNAi)*, unsupplemented *mdt-15(RNAi)*, or unsaturated-FA-supplemented *mdt-15(RNAi)* worms, respectively. $n = 3$; statistical analysis by two-tailed Student *t* test. MUFA, monounsaturated fatty acid; SAT, saturated fatty acid; PUFA, polyunsaturated fatty acid.

Table S3. P values representing the significance of the change in relative lipid levels of unsupplemented and supplemented *control(RNAi)* and *mdt-15(RNAi)* worms

| Relative lipid levels | PC | PS | PI | PE | Cardiolipin | TG |
|-------------------------|------|------|------|------|-------------|------|
| % Tls | | | | | | |
| Control RNAi+UFAs | 0.52 | 0.27 | 0.21 | 0.26 | 0.69 | 0.75 |
| <i>mdt-15</i> RNAi | 0.30 | 0.39 | 0.97 | 0.19 | 0.85 | 0.92 |
| <i>mdt-15</i> RNAi+UFAs | 0.27 | 0.32 | 0.31 | 0.42 | 0.84 | 0.99 |
| % PLs | | | | | | |
| Control RNAi+UFAs | 0.05 | 0.16 | 0.28 | 0.10 | 0.46 | |
| <i>mdt-15</i> RNAi | 0.02 | 0.18 | 0.82 | 0.13 | 0.83 | |
| <i>mdt-15</i> RNAi+UFAs | 0.09 | 0.15 | 0.46 | 0.32 | 0.73 | |

The P values are for the comparison of unsupplemented *control(RNAi)* worms with unsaturated-FA-supplemented *control(RNAi)*, unsupplemented *mdt-15(RNAi)*, or unsaturated-FA-supplemented *mdt-15(RNAi)* worms, respectively. $n = 3$; statistical analysis by two-tailed Student *t* test.

Table S4. P values representing the significance of the change in relative FA abundance in lipid profiles between WT and *mdt-15(tm2182)* worms

| Relative FA abundance | PC | PS | PI | PE | TG |
|-----------------------|-------|-------|------|--------|-------|
| 16:0 | 0.31 | 0.55 | 0.35 | 0.73 | 0.001 |
| 16:1 | 0.05 | 0.005 | 0.51 | 0.06 | 0.66 |
| 18:0 | 0.04 | 0.54 | 0.11 | 0.03 | 0.01 |
| 18:1n-9 | 0.001 | 0.00 | 0.01 | 0.0002 | 0.03 |
| 18:1n-7 | 0.22 | 0.98 | 0.71 | 0.17 | 0.26 |
| 18:2 | 0.02 | 0.16 | 0.32 | 0.0003 | 0.001 |
| 20:3 | 0.02 | 0.08 | 0.60 | 0.05 | 0.02 |
| 20:4 | 0.06 | 0.51 | 0.93 | 0.66 | 0.11 |
| 20:4n-3 | 0.01 | 0.17 | 0.27 | 0.03 | 0.01 |
| 20:5 | 0.002 | 0.06 | 0.01 | 0.04 | 0.003 |

The P values are for the comparison of WT worms with *mdt-15(tm2182)* mutant worms. $n = 3$; statistical analysis by two-tailed Student *t* test.

Table S5. *P* values representing the significance of the change in relative FA abundance per total lipids in lipid profiles between WT and *mdt-15(tm2182)* worms

| FA abundance/TLs | PC | PS | PI | PE | TG | TLs |
|------------------|------|------|------|------|-------|-------|
| SAT | 0.24 | 0.19 | 0.33 | 0.71 | 0.02 | 0.03 |
| PUFA | 0.00 | 0.98 | 0.08 | 0.02 | 0.001 | 0.002 |
| UFA | 0.08 | 0.45 | 0.42 | 0.09 | 0.13 | 0.06 |
| MUFA | 0.20 | 0.17 | 0.68 | 0.36 | 0.49 | 0.96 |

The *P* values are for the comparison of WT worms with *mdt-15(tm2182)* mutant worms. *n* = 3, statistical analysis by two-tailed Student *t* test.

Table S6. *P* values representing the significance of the change in relative lipid levels between WT and *mdt-15(tm2182)* worms

| Relative lipid levels | PC | PS | PI | PE | TG |
|-----------------------|------|------|------|------|------|
| % TLs | 0.10 | 0.27 | 0.71 | 0.12 | 0.24 |
| % PLs | 0.39 | 0.12 | 0.37 | 0.10 | |

The *P* values are for the comparison of WT worms with *mdt-15(tm2182)* mutant worms. *n* = 3; statistical analysis by two-tailed Student *t* test.

Table S7. P values representing the significance of the change in relative FA abundance in lipid profiles of unsupplemented and supplemented *control(RNAi)* and *SCD(RNAi)* worms

| Relative FA abundance | PC | PE | Cardiolipin |
|-----------------------|-------|-------|-------------|
| 16:0 | | | |
| Control RNAi+UFAs | 0.82 | 0.42 | 0.75 |
| SCD RNAi | 0.93 | 0.08 | 0.51 |
| SCD RNAi+UFAs | 0.80 | 0.10 | 0.48 |
| 16:1 | | | |
| Control RNAi+UFAs | 0.27 | 0.05 | |
| SCD RNAi | 0.01 | 0.01 | 0.42 |
| SCD RNAi+UFAs | 0.03 | 0.33 | 0.42 |
| 8:0 | | | |
| Control RNAi+UFAs | 0.15 | 0.94 | 0.59 |
| SCD RNAi | 0.001 | 0.01 | 0.30 |
| SCD RNAi+UFAs | 0.01 | 0.05 | 0.69 |
| 18:1n-9 | | | |
| Control RNAi+UFAs | 0.07 | 0.01 | 0.57 |
| SCD RNAi | 0.01 | 0.01 | 0.64 |
| SCD RNAi+UFAs | 0.09 | 0.04 | 0.44 |
| 18:1n-7 | | | |
| Control RNAi+UFAs | 0.03 | 0.02 | 0.11 |
| SCD RNAi | 0.00 | 0.41 | 0.75 |
| SCD RNAi+UFAs | 0.05 | 0.71 | 0.45 |
| 18:2 | | | |
| Control RNAi+UFAs | 0.002 | 0.001 | 0.06 |
| SCD RNAi | 0.002 | 0.001 | 0.30 |
| SCD RNAi+UFAs | 0.89 | 0.76 | 0.86 |
| 20:3 | | | |
| Control RNAi+UFAs | 0.01 | 0.02 | 0.60 |
| SCD RNAi | 0.001 | 0.004 | 0.12 |
| SCD RNAi+UFAs | 0.02 | 0.004 | 0.77 |
| 20:4 | | | |
| Control RNAi+UFAs | 0.02 | 0.10 | 0.42 |
| SCD RNAi | 0.00 | 0.07 | 0.42 |
| SCD RNAi+UFAs | 0.01 | 0.10 | 0.42 |
| 20:4n-3 | | | |
| Control RNAi+UFAs | 0.04 | 0.09 | 0.32 |
| SCD RNAi | 0.00 | 0.01 | 0.35 |
| SCD RNAi+UFAs | 0.01 | 0.04 | 0.26 |
| 20:5 | | | |
| Control RNAi+UFAs | 0.25 | 0.77 | 0.77 |
| SCD RNAi | 0.01 | 0.50 | 0.76 |
| SCD RNAi+UFAs | 0.48 | 0.03 | 0.75 |

The P values are for the comparison of unsupplemented *control(RNAi)* worms with unsaturated-FA-supplemented *control(RNAi)* worms, unsupplemented *SCD(RNAi)* worms, or unsaturated-FA-supplemented *SCD(RNAi)* worms, respectively. n = 3; statistical analysis by two-tailed Student t test. SCD, stearyl-CoA-desaturase.

Table S8. P values representing the significance of the change in FA abundance per total lipids in lipid profiles of unsupplemented and supplemented *control(RNAi)* and *SCD(RNAi)* worms

| FA abundance/TLs | PC | PS | PI | PE | TG |
|----------------------|-------|------|------|------|-------|
| SAT | | | | | |
| Control RNAi+UFAs | 0.34 | 0.47 | 0.41 | 0.61 | 0.36 |
| <i>SCD</i> RNAi | 0.001 | 0.01 | 0.11 | 0.01 | 0.14 |
| <i>SCD</i> RNAi+UFAs | 0.10 | 0.06 | 0.50 | 0.31 | 0.002 |
| PUFA | | | | | |
| Control RNAi+UFAs | 0.62 | 0.17 | 0.18 | 0.56 | 0.002 |
| <i>SCD</i> RNAi | 0.01 | 0.74 | 0.69 | 0.15 | 0.02 |
| <i>SCD</i> RNAi+UFAs | 0.40 | 0.41 | 0.68 | 0.25 | 0.27 |

The P values are for the comparison of unsupplemented *control(RNAi)* worms with unsaturated-FA-supplemented *control (RNAi)* worms, unsupplemented *SCD(RNAi)* worms, or unsaturated-FA-supplemented *SCD(RNAi)* worms, respectively. n = 3; statistical analysis by two-tailed Student t test.

Table S9. P values representing the significance of the change in relative lipid levels in lipid profiles of unsupplemented and supplemented *control(RNAi)* and *SCD(RNAi)* worms

| Relative lipid levels | PC | PS | PI | PE | CL | TG |
|-----------------------|------|------|------|------|------|------|
| % TLs | | | | | | |
| Control RNAi+UFAs | 0.52 | 0.27 | 0.21 | 0.26 | 0.69 | 0.75 |
| <i>SCD</i> RNAi | 0.49 | 0.08 | 0.06 | 0.12 | 0.88 | 0.42 |
| <i>SCD</i> RNAi+UFAs | 0.64 | 0.27 | 0.89 | 0.40 | 0.81 | 0.80 |
| % PLs | | | | | | |
| Control RNAi+UFAs | 0.05 | 0.16 | 0.28 | 0.10 | 0.46 | |
| <i>SCD</i> RNAi | 0.04 | 0.07 | 0.37 | 0.01 | 0.93 | |
| <i>SCD</i> RNAi+UFAs | 0.56 | 0.23 | 0.91 | 0.35 | 0.64 | |

The P values are for the comparison of unsupplemented *control(RNAi)* worms with unsaturated-FA-supplemented *control(RNAi)* worms, unsupplemented *SCD(RNAi)* worms, or unsaturated-FA-supplemented *SCD(RNAi)* worms, respectively. n = 3; statistical analysis by two-tailed Student t test.

Table S10. Primer sequences used for quantitative PCR

| Gene | Forward | Reverse |
|--------------------|------------------------------|---|
| <i>hsp-4</i> | cgagcgagctattgagtggc | ccgagtaaagtttggagacg |
| <i>xbp-1</i> total | ccgatccacctccatcaac | accgtctgctcctcctcaatg |
| <i>xbp-1s</i> | tgctttggaatcagcagtg | accgtctgctcctcctcaatg (same as for <i>xbp-1</i> total) |
| <i>srp-7</i> | aatgtctccagtacttcggttaatg | aattccgagcgattgaagag |
| Y41C4A.11 | gccatggatTTTTgactgctt | cgtggatTTTTcggagacc |
| <i>act-1</i> | gctggacgtgatcttactgattacc | gtagcagagcttctccttgatg |
| <i>ubc-2</i> | agggagggtgtcttcttctcctcac | cggatttggatcacagagcagc |
| <i>tba-1</i> | gtacactccactgatctctgctgacaag | ctctgtacaagggcaaacagccatg |
| <i>edem-1</i> | ttaacaaggacagcaagaaagtg | cgtggcaatggtttgagag |
| <i>ufd-1</i> | gctccagtggcgaatgat | aacgaaggaatgaaacacgac |
| <i>cdc-48.3</i> | ctgttggatgggaaaaggaa | tccaataaaaagaatcagaagaatgaat |
| <i>rnf-12</i> | aatgaagttcaggaaggaatgc | ttttcgggtgctcctctgc |
| <i>npl-4.1</i> | aatggaggaagcggcaat | tccacagttccacacagctc |