SUPPLEMENTAL FIGURES AND FIGURE LEGENDS

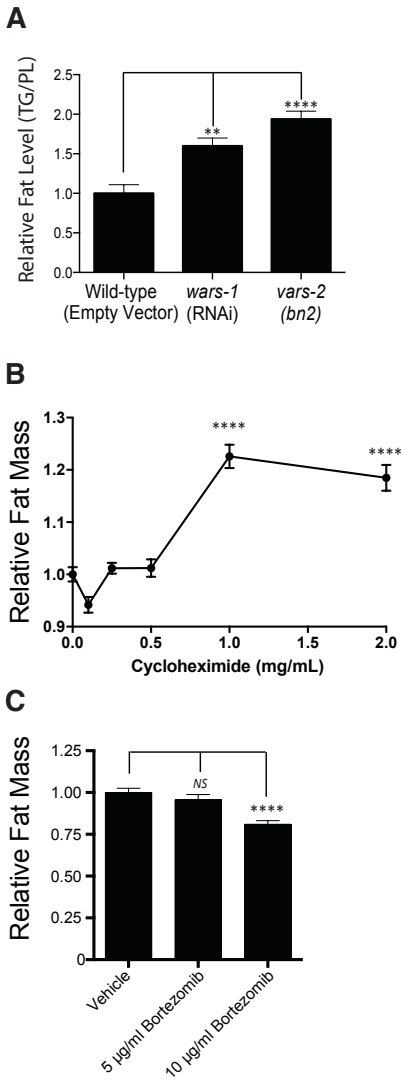

Figure S1. Loss of Function of Protein Translation and UPS Inhibition Leads to Increased Fat Mass in *C. elegans.* Relevant to Figure 1

(A) Biochemical lipid analysis of wild-type animals treated with empty vector and *wars-1* RNAi, or *vars-2* (*bn2ts*) temperature-sensitive mutant animals treated with empty vector RNAi (grown at the non permissive temperature 25°C). Solid phase chromatography was used to separate lipid species and GC/MS was used to quantify total fatty acids in triglyceride and phospholipid lipid fractions. Fat mass is represented as the ratio of triglyceride/phospholipid. n = 3 biological replicates. (B) Dose response curve of wild-type animals grown in the presence of the indicated doses of cycloheximide. L4 animals were transferred to seeded NGM plates containing varying concentration of cycloheximide or vehicle control. Plates were prepared by spreading the appropriate concentration of cycloheximide (from a stock solution of 50 mg/ml in ethanol) onto NGM and then seeding with concentrated HT115. Animals were incubated on cycloheximide plates for 24 hours after which they were harvested for detection of Nile red fluorescent fat levels. n = 4 biological replicates. (C) Bortezomib treatment for 20 hours initiated at the L2 stage of development leads to a dose-dependent reduction in fat mass assessed by fixative staining with Nile red. A similar reduction was achieved for lower levels of the drug for L1 to adult treatment (not shown). N = 26 for vehicle, 48 for 5 µg/mL dose and 42 for 10 µg/mL dose. Bars represent mean ± SEM. **,P < 0.01, ****,P < 0.0001, *NS* not significant by 1-way ANOVA.

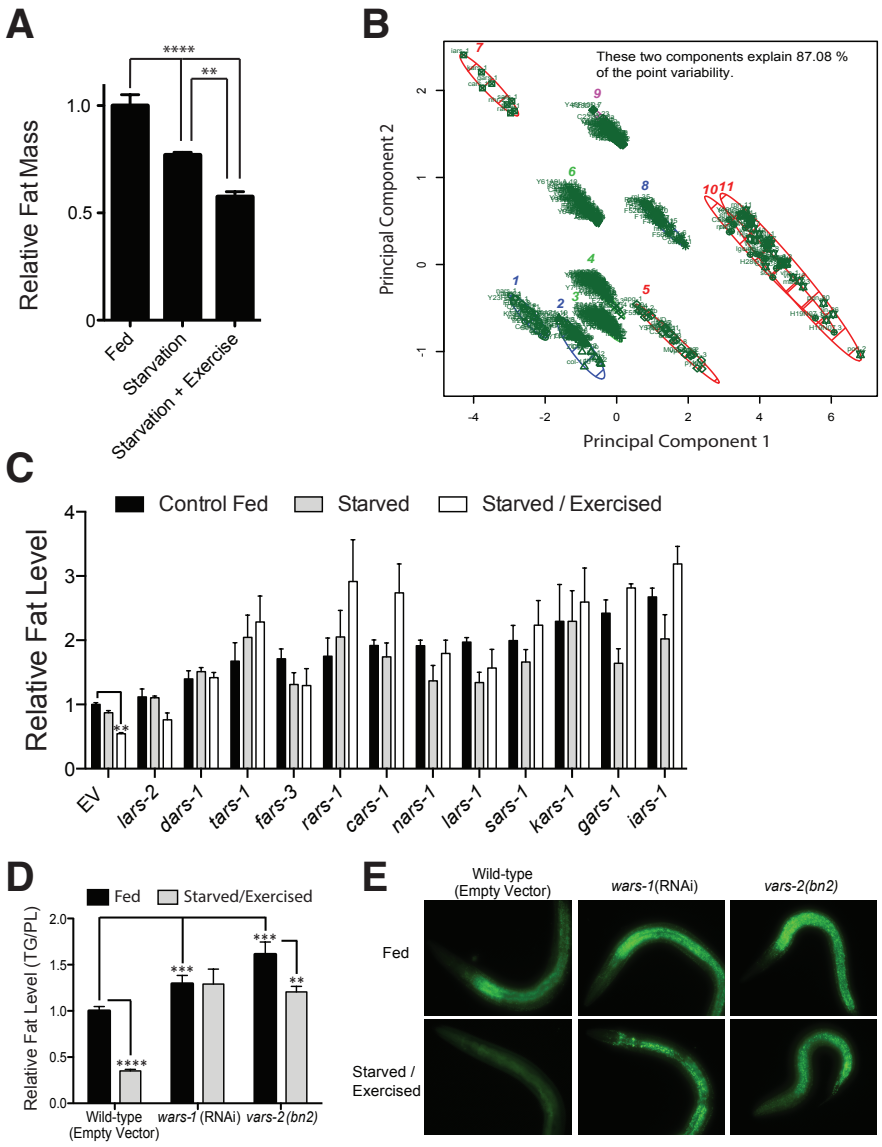

Figure S2. Analysis of Starvation Regulatory Pathways. Relevant to Figure 2

(A) Fixative Nile red staining of stage matched wild-type animals under fed conditions or after 16 hours of starvation in the presence of 100 mM BDM (a paralytic to prevent thrashing, *Starvation*) or without paralytic (*Starvation + Exercise*). **,P < 0.01, ****, P < 0.0001 by one-way ANOVA. n = 6 biological replicates. (B) Principal component analysis of *k*-means clustered fat mass data for 475 fat regulatory genes in the fed state, starved, or starved and exercised. Fat mass was analyzed for wild-type animals after conducting RNAi to the 475 lipid regulatory genes and exposing animals to a 16 hour starvation with and without BDM. Data were expressed as a 3-dimensional vector, log transformed, and clustered by *k*-means clustering. Least squares analysis was used to identify 11 clusters as the grouping of choice. See also Figure 2B for heatmap of clustered data and numerical data in Table S4. (C) Relative fat mass by fixative Nile red staining of stage matched wild-type animals grown on empty vector RNAi (EV) or ARS RNAi in the fed state or after 16 hours of starvation with and without BDM (*Starved* and *Starved / Exercised*, respectively). **, P < 0.01 by two-way ANOVA. n = 4 biological replicates. (D) Solid phase chromatography and GC/MS lipid biochemistry of wild type animals treated with vector RNAi or *wars-1* RNAi, and *vars-2*(*bn2ts*) conditional mutant treated with vector RNAi, under fed

and starved/exercised conditions. Animals were collected at the L4 stage (fed) and fasted for 20 hours (starved/exercised) for biochemical determination of triacylglycerol / phospholipid (TG/PL) ratio. **, P < 0.01, ***, P < 0.001, ****, P < 0.0001, by two-way ANOVA. n = *3* biological replicates. (E) Fixative Nile red staining of wild type animals treated with vector RNAi or *wars-1* RNAi, and *vars-2*(*bn2ts*) conditional mutant treated with vector RNAi, under fed and starved conditions. Worms are stage matched. Bars represent mean ± SEM.

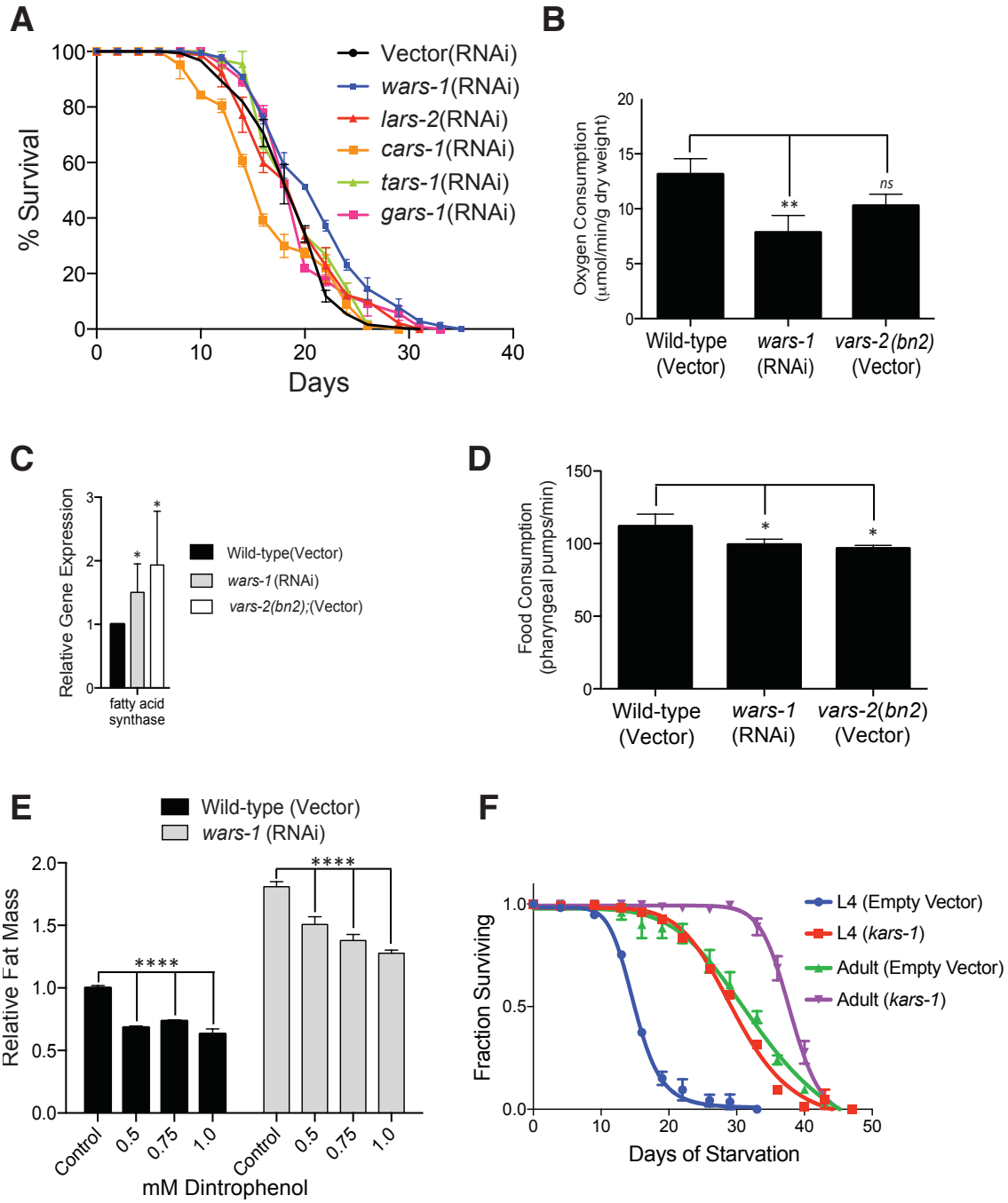

Figure S3. Metabolic Shifts in ARS Gene Inactivations. Relevant to Figure 3
(A) Lifespans of continuously fed wild-type animals treated with empty vector or *wars-1, lars-2, cars-1, tars-1, or gars-1* RNAi from the L1 stage. Lifespan of only *wars-1* deficient animals is

significantly different from wild-type by log rank test (see Table S6). (B) Oxygen consumption of wild-type animals treated with empty vector RNAi, *wars-1* RNAi, or *vars-2* (*bn2ts*) mutants treated with empty vector RNAi. Animals were washed from plates with food and oxygen consumption immediately queried in minimal media with a Clark electrode as indicated in the experimental methods. Bars represent mean ± SEM. n = 6 biological replicates. **, P < 0.01, *ns*, not significant, by 1-way ANOVA. (C) Expression of fatty acid synthase mRNA in stage matched animals fed *wars-1* RNAi, or *vars-2 (bn2ts)* mutants relative to L4 wild-type animals. Bars represent mean ± 95% CI. n = 4 biological replicates. *, P < 0.05 by one-way ANOVA. (D) Pharyngeal pumping rates of ARS deficient animals compared to control animals. Wild-type animals treated with empty vector RNAi or *wars-1* RNAi, or *vars-2*(*bn2ts*) animals treated with empty vector RNAi were grown to late L4 stage and the number of pharyngeal contractions per minute was calculated while fed on lawns of the indicated *E. coli* food source. Bars represent mean ± SEM. n = 4 biological replicates of 10-12 animals per replicate. *, P < 0.05 by 1-way ANOVA. (E) Fat levels of wild-type animals treated with empty vector or *wars-1* RNAi cultured on plates containing the indicated concentration of dinitrophenol or vehicle control. Animals were treated from L1 stage and harvested as adults for analysis of fixative-based Nile red fluorescent fat levels. **** *P* < 0.0001 by 2-way ANOVA. Bars represent mean ± SEM. *n* = 4 biological replicates. (F) Mean starvation survival is extended in wild-type animals treated with *kars-1* versus empty vector RNAi whether starved from the L4 stage or 1 day later as young adults. A significant increase is seen in overall starvation survival in adults versus L4, possibly owing to greater fat mass stores accumulated in adults. Bars represent mean ± SEM of *n* = 3 biological replicates.

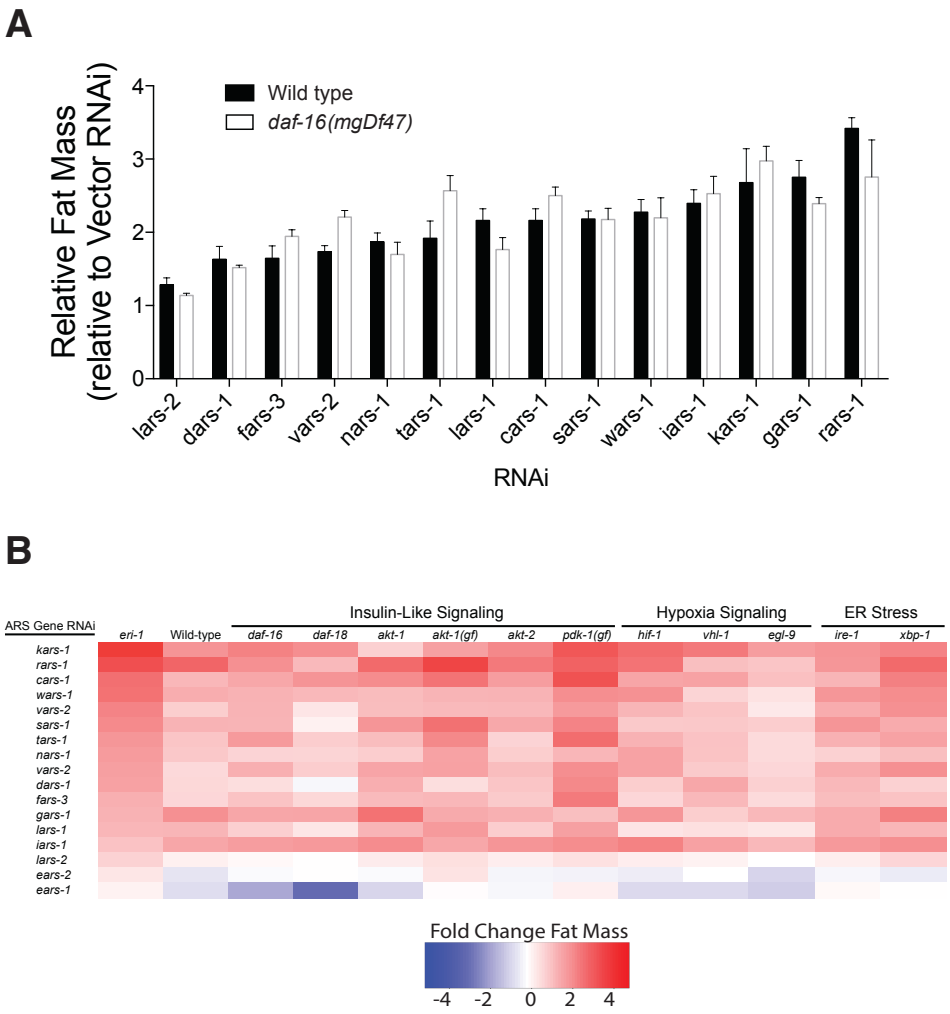

Figure S4. ARS Deficient Starvation Defense Pathway is Independent of *daf-16*/*FoxO*, Hypoxia and ER Stress Signaling. Relevant to Figure 4

(A) Fat levels of wild-type and *daf-16*(*mgDf47*) young adult animals grown on empty vector or various cytoplasmic ARS gene RNAi from the L1 stage and analyzed for fixative-based Nile red fluorescent fat levels. Bars represent mean ± SEM. n = 5 biological replicates. Comparisons are not significant for any RNAi in wild type versus *daf-16* by two-way ANOVA. (B) Heatmap of fat mass by fixative Nile red staining relative to wild type on empty vector RNAi for ARS gene RNAi in the indicated mutants in insulin-like signaling, hypoxia signaling, and ER stress pathways. See also Table S7 for numerical data.

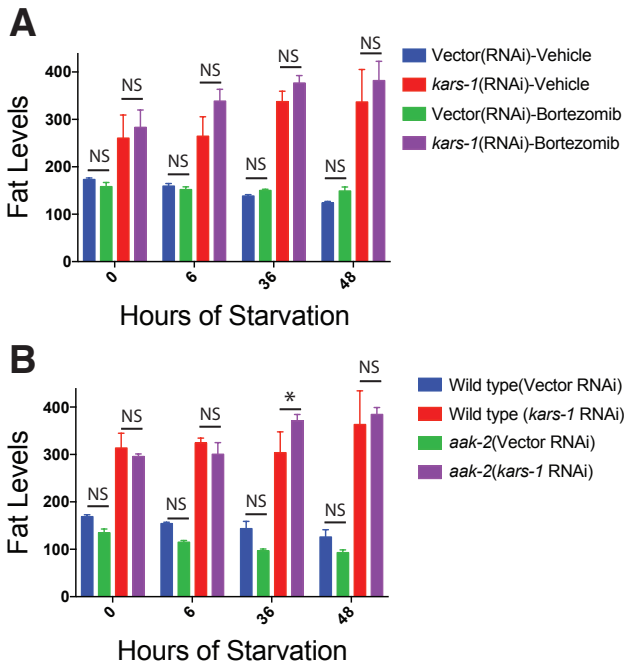

Figure S5. Acute proteasomal inhibition or genetic inactivation of AMPK does not affect baseline fat mass or rate of fat mass loss in starvation. Relevant to Figure 6
(A) Fat levels of wild-type, young adult animals show the predicted doubling of fat mass upon treatment with *kars-1* RNAi, and while vector treated animals lose ~30 percent of fat mass over 48 hours, *kars-1* RNAi allows for conservation of fat mass with starvation. Bortezomib does not significantly affect the rate of fat mass loss in either vector control or *kars-1* RNAi treated animals. Animals were maintained on food until the young adult stage (with 50 µg/ml FUDR from the mid L4 to young adult stage to prevent bagging upon starvation), and pre-treated with 5 µg/ml bortezomib or DMSO vehicle for 6 hours prior to starvation. At the onset of starvation, animals were washed free of food, and 5 µg/ml bortezomib or vehicle was also included in the starvation media Bars represent mean ± SD. n = 3 biological replicates. NS, not significant, by two-way ANOVA. (B) Wild type and *aak-2* mutants show identical patterns of fat mass loss when treated with empty vector or *kars-1* RNAi and subjected to starvation as in (A). A nonsignificant trend towards decreased fat mass was seen in *aak-2* vector control RNAi treated animals, but this decrease was not evident in *kars-1* RNAi treated animals. This suggests that the mechanisms by which *aak-2* suppresses starvation survival is not by reducing fed fat stores or by accelerating the rate of fat mass loss. Bars represent mean ± SEM. n = 3 biological replicates. *, $P < 0.01$ and NS, not significant, by two-way ANOVA.

SUPPLEMENTAL TABLES AND TABLE LEGENDS

Supplemental Table S1. Effect of RNAi Against Each of 514 Fat Regulatory Gene RNAi Targeting 475 Unique Genes on Fat Levels in *eri-1* Animals. Relevant to Figure 1
Supplied as an Excel file.

Supplemental Table S2. GSEA Analysis with Categories Enriched Among High Fat Inducing RNAi Using KEGG Annotation. Relevant to Figure 1.

CATEGORY NAME	NUMBER OF GENES	ES	NES	NOM p-val	FDR q-val	FWER p-val	RANK AT MAX	LEADING EDGE
AMINOACYL-TRNA BIOSYNTHESIS	10	0.81327	2.7386	0	0	0	33	tags=70%, list=7%, signal=74%
PYRIMIDINE METABOLISM	13	0.61471	2.4287	0	0	0	126	tags=77%, list=27%, signal=102%
PURINE METABOLISM	12	0.60137	2.0738	0.01818	0.0080	0.02	126	tags=75%, list=27%, signal=99%
RNA POLYMERASE	9	0.60357	1.8085	0.03174	0.0234	0.07	126	tags=78%, list=27%, signal=104%
VALINE, LEUCINE AND ISOLEUCINE BIOSYNTH	4	0.78970	1.7730	0	0.0252	0.1	33	tags=75%, list=7%, signal=80%
FOLATE BIOSYNTHESIS	4	0.63369	1.4724	0.125	0.1031	0.39	86	tags=75%, list=18%, signal=91%
STARCH AND SUCROSE METABOLISM	4	0.63369	1.4444	0.07547	0.1040	0.43	86	tags=75%, list=18%, signal=91%
CITRATE CYCLE (TCA CYCLE)	3	0.51048	1.0391	0.37288	0.4581	0.97	13	tags=33%, list=3%, signal=34%
BASAL TRANSCRIPTION FACTORS	4	0.33545	0.7290	0.84615	0.8265	1	316	tags=100%, list=67%, signal=296%

Supplemental Table S3. GSEA Analysis with Categories Enriched Among Low Fat Inducing RNAi Using KEGG Annotation. Relevant to Figure 1

CATEGORY NAME	NUMBER OF GENES	ES	NES	NOM p-val	FDR q-val	FWER p-val	RANK AT MAX	LEADING EDGE
PROTEASOME	8	-0.8779	-2.8703	0.000	0.000	0	66	tags=100%, list=14%, signal=114%
OXIDATIVE PHOSPHORYLATION	13	-0.7286	-2.6877	0.000	0.000	0	70	tags=77%, list=15%, signal=88%
CELL COMMUNICATION	3	-0.8189	-1.7834	0.015	0.017	0.07	14	tags=67%, list=3%, signal=68%
RIBOSOME	8	-0.4454	-1.4689	0.076	0.083	0.39	268	tags=100%, list=56%, signal=226%
LYSINE DEGRADATION	3	-0.5196	-1.0415	0.469	0.377	0.93	17	tags=33%, list=4%, signal=34%

Supplemental Table S4. Effect of RNAi Against Each of 475 Fat Regulatory Genes on Fat Mass Under Fed, Starved and Starved/Exercised Conditions and on Starvation Survival. Relevant to Figure 2
Supplied as an Excel file.

Supplemental Table S5. Tabular Starvation Survival Data for Experimental Replicates, Non-Linear Regression Analysis. Relevant to Figures 3, 5, and 6
Supplied as an Excel file.

Supplemental Table S6. Wild-Type vs. ARS Log-Rank Analysis of Lifespan Under Fed Conditions. Relevant to Figure 3. See also Figure S3.

RNAi	No. of subjects	Mean (days)	SEM	95% C.I.	25 %	50 %	75 %	90 %	100 %	95% Median C.I.	Bonferroni-Corrected P-value	
Empty Vector	211	19.01	0.3	18.43 ~ 19.60	16	20	22	24	31	18 ~ 18		
cars-1	94	18.02	0.66	16.72 ~ 19.32	14	16	24	26	31	16 ~ 16	1.000	WT vs. *cars-1*
wars-1	191	21.48	0.4	20.70 ~ 22.27	18	22	24	29	35	20 ~ 20	8.20E-07	WT vs. *wars-1*
lars-2	154	19.58	0.43	18.74 ~ 20.43	16	20	24	29	31	18 ~ 18	0.442	WT vs. *lars-2*
tars-1	134	20.15	0.39	19.39 ~ 20.92	16	20	24	26	31	18 ~ 18	0.152	WT vs. *tars-1*
gars-1	127	19.83	0.44	18.96 ~ 20.69	18	20	22	26	33	18 ~ 18	1.000	WT vs. *gars-1*

Supplemental Table S7. Fat Levels of Wild-Type and *daf-16*, *akt-1*, *akt-2*, *hif-1*, *ire-1*, and *xbp-1* Mutant Animals Treated with ARS RNAi. Relevant to Figure 4.
Supplied as an Excel file.