Kang et al., https://doi.org/10.1083/jcb.201511073

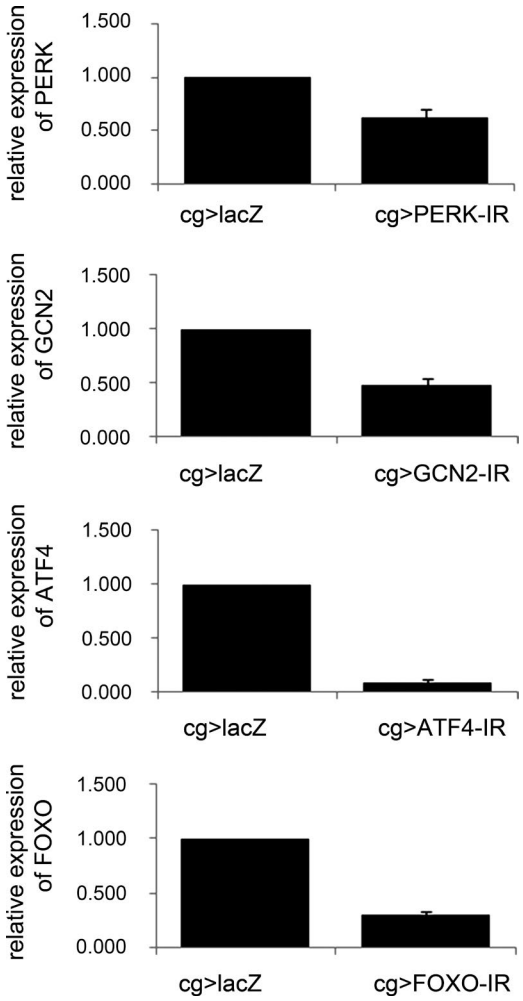

Figure S1. **Knockdown efficiency of the RNAi lines in the developing fat body.** Shown are quantitative RT-PCR results against the indicated genes from dissected third-instar larval fat bodies. The indicated RNAi lines were expressed in that tissue with the *cg-Gal4* driver. As controls, RNAi against *lacZ* was used (left bars in all panels). The error bars indicate SE.

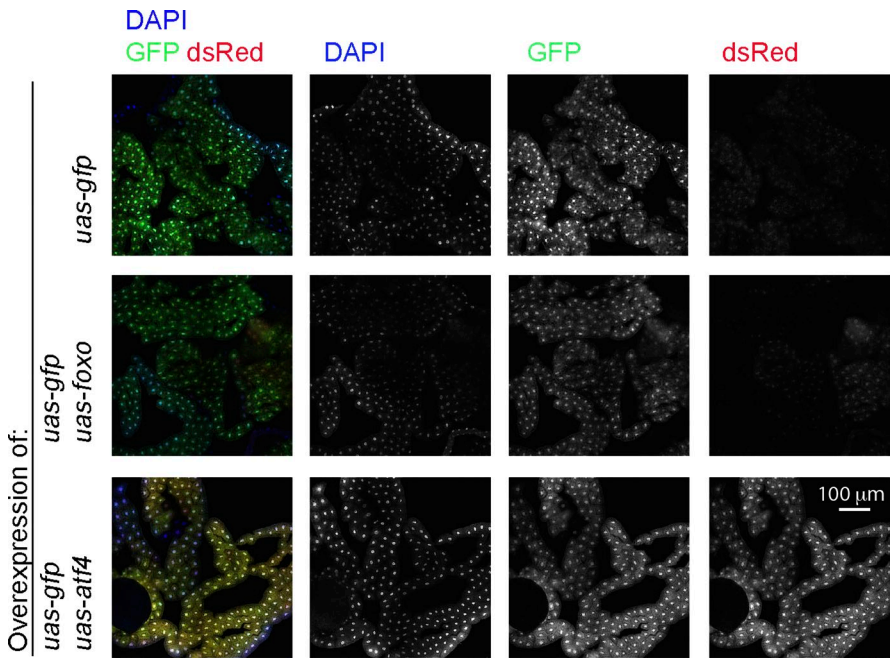

Figure S2. **Overexpression of *atf4*, but not *foxo*, induces 4E-BP intron reporter.** Third-instar larval fat body tissues that overexpress *gfp* only (top) or together with *foxo* (middle) and *atf4* (bottom) are shown. Merged images of GFP (green) and dsRed (red) are shown in left. Individual channels are shown on right. The confocal microscope gain was adjusted to have control tissues show minimal dsRed signal (top right). Under these settings, *atf4* overexpression enhanced *4E-BP intron dsRed* signal (bottom right). *Foxo* overexpression did not enhance the dsRed signal (middle). A scale bar that applies to all panels is shown in the bottom right.

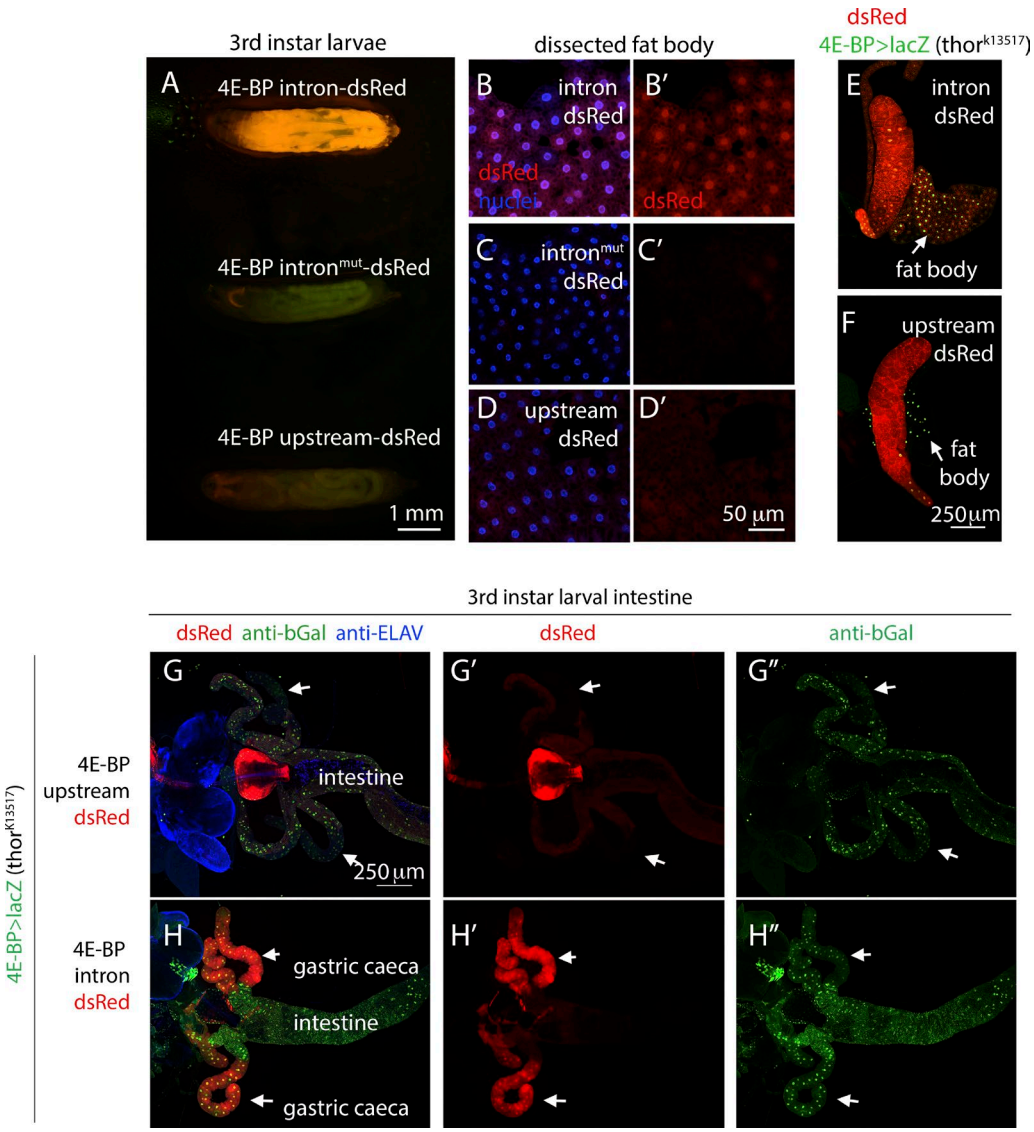

Figure S3. **Comparison of the expression patterns between *4E-BP-lacZ, 4E-BP upstream dsRed,* and *4E-BP intron dsRed*.** In all panels, dsRed is shown in red. (A) Third-instar larvae with the *4E-BP intron dsRed* reporter (top), *4E-BP upstream reporter* (middle), and *4E-BP intron^{mut} dsRed* reporter with ATF4 binding sites impaired (bottom). (B–D) Dissected third-instar fat bodies colabeled with dsRed and the nuclear marker TO-PRO-3 (green). (B′–D′) dsRed single channels of images in B–D. (E and F) Comparison of the dsRed reporters with *4E-BP-lacZ* expression (green) in the salivary glands (left) and the associated fat body (right of salivary glands; indicated by arrows). *4E-BP intron dsRed* is expressed together with *4E-BP-lacZ* in the fat body (E), but the *4E-BP upstream dsRed* expression is not (F; note that the fat body appears green, because of the absence of dsRed). (G and H) Third-instar larval intestines. Anterior is to the left. The larval brain, which is in the anterior side of the intestine, is marked with anti-ELAV (blue). *4E-BP-lacZ* expression is detected with anti–β-galactosidase antibody (green). Unlike the second-instar larval stage (Fig. 4 B), there is significant reporter expression even without exogenous stress. (G) *4E-BP upstream dsRed* reporter expression. Strongest expression is found in the proventriculus. Weak signal is detected in the intestine. (H) *4E-BP intron dsRed* expression. The most prominent signal comes from the gastric caeca (arrows).

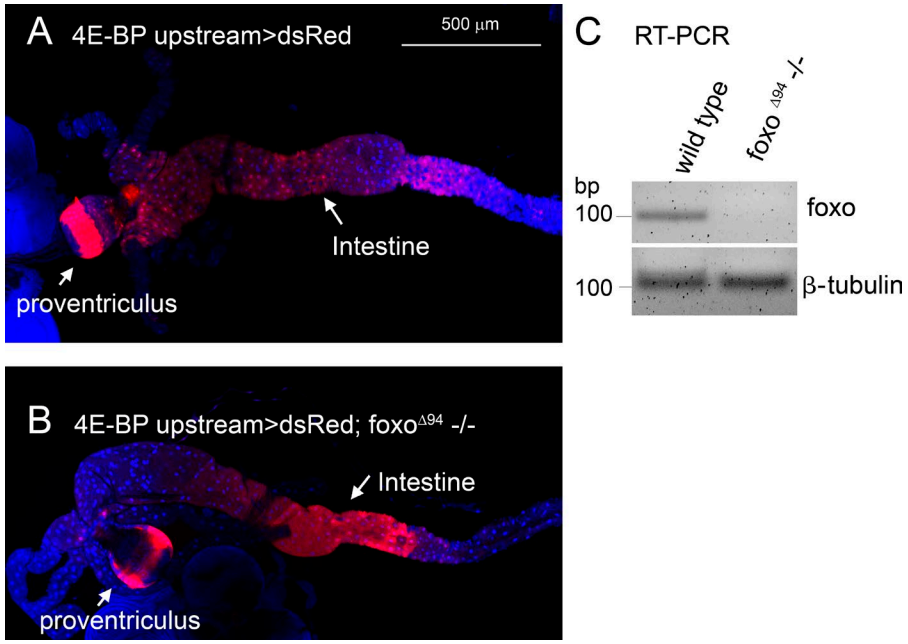

Figure S4. ***4E-BP upstream dsRed*reporter in the *foxo* mutant background.** dsRed in (red) and nuclei detected by TO-PRO-3 in (blue). The detector gains for obtaining these images were higher than those conditions in Fig. S3. *4E-BP upstream dsRed* signals are prominently detected in the proventriculus and the anterior intestine, in both wild-type (A) and *foxo*$^{\Delta 94\,-/-}$ background (B). (C) RT-PCR from adult flies confirm that *foxo*$^{\Delta 94\,-/-}$ flies lack *foxo* transcripts. The bottom gel shows loading control with RT-PCR against β-tubulin.

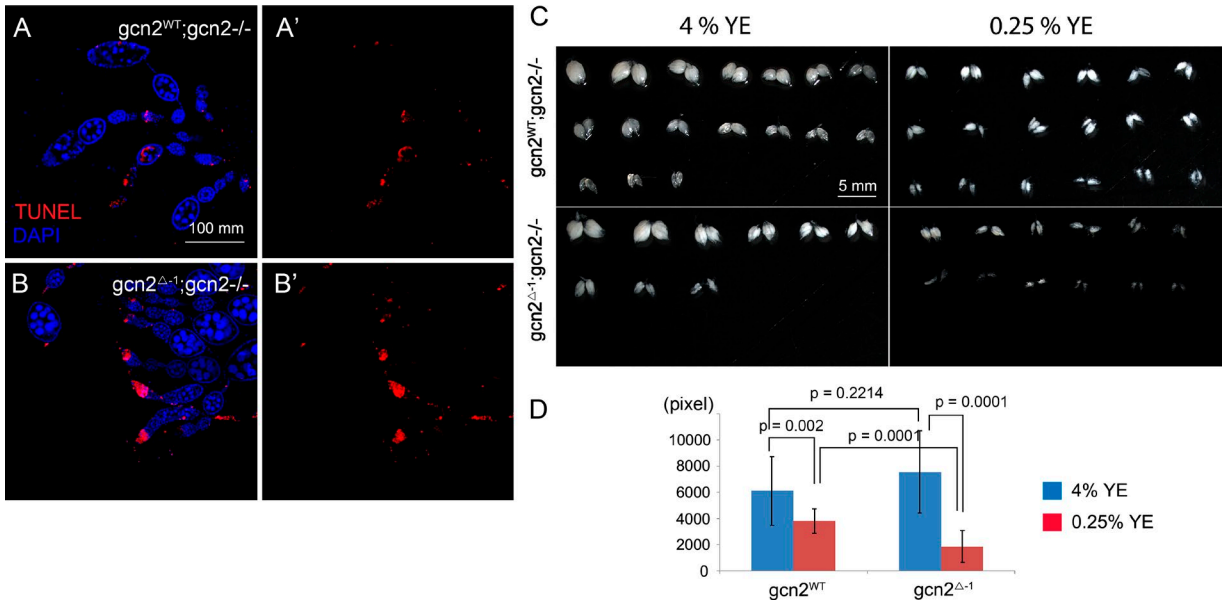

Figure S5. ***Drosophila* ovaries in *gcn2*$^{-/-}$ flies.** (A and B) Ovaries are stained with DAPI (blue) and also assayed for apoptosis through TUNEL labeling (red). The control wild type, *gcn2*$^{wt\ rescue}$; *gcn2*$^{12kb\ -/-}$ (A and A′), and the mutant, *gcn2*$^{\Delta\ rescue}$; *gcn2*$^{12kb\ -/-}$ (B and B′), are shown. A′ and B′ are TUNEL single channels of the images shown in A and B. (C) Dissected ovary sizes in *gcn2* wild-type control (top) and *gcn2* mutants (bottom). Ovaries of flies reared with 4% YE are shown on the left, and those reared with reduced YE food (0.25% YE) are shown on the right. (D) Quantification of the ovary size shown in C indicates that the *gcn2* mutant ovaries decrease in size more than the wild-type control when reared in food with reduced yeast content. The error bars indicate SE.

Provided online are three supplemental Excel tables.

Table S1. Summary of changes in lifespan and their statistical significance in adult flies reared with or without dietary restriction. YE indicates the percentage of yeast extract in food. LS indicates lifespan. n indicates the number of flies analyzed. (Top) Note that *gcn2*$^{wt\ rescue}$; *gcn2*$^{-/-}$ (wild-type equivalent) shows mean and median lifespan extension of 10 and 12 days when reared in low-yeast food, and such effect is significantly reduced in the *gcn2*^{mutant}; *gcn2*$^{-/-}$ (*gcn2* mutant) backgrounds. We analyzed these data using two different methods, low-rank tests and Cox proportional hazard analysis, which both show statistically significant reduction of lifespan in *gcn2* mutant flies reared in 0.25% YE food.

Table S2. Relative fold change of newly synthesized peptides in control versus *gcn2* mutant flies. Full list of proteins that change relative expression after AHA labeling and purification in *gcn2* mutants.

Table S3. GO enrichment by DAVID functional annotation tool.