Supplemental data

Inventory of supplemental information

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Supplemental Figures and Legends

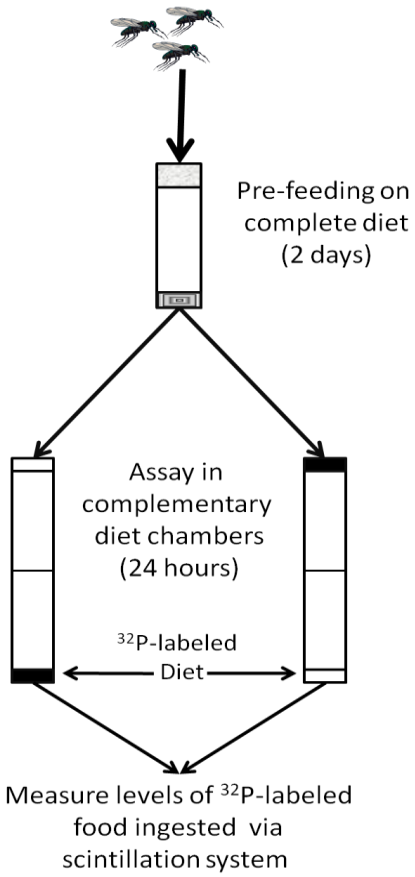

Figure S1. A schematic of the 'nutrient preference' assay, related to Figure 1. Flies were kept on complete diets for 2 days. Flies were separated into 2 populations and transferred to an assay chamber. In this assay chamber they were given a choice between sugar or yeast containing diets; only one of which was radiolabeled. One population was used to measure sucrose consumption and the other population, yeast consumption. Following 24 hours on the radiolabeled diet, yeast or sucrose incorporation was measured by incorporation of radioactivity. Grey food represents the complete diet, white food represents a sugar deficient diet containing 4% yeast extract (4% Y), and black food represents a yeast deficient diet containing 5% sucrose (5% S).

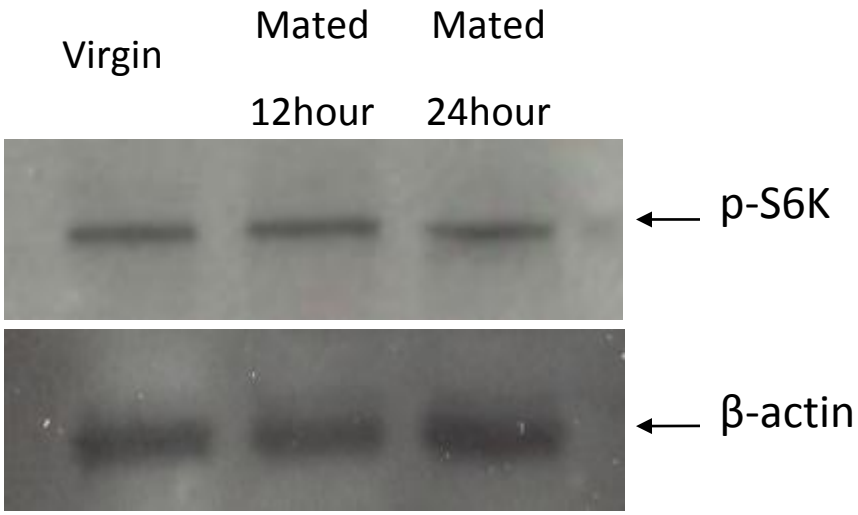

Figure S2. Phosphorylated S6 kinase levels in virgin and mated *D. melanogaster*, related to Figure 2. (A) S6K phosphorylation in the heads of 25 virgin and mated female W1118 adult flies was measured using an antibody against phosphorylated form of S6K. An antibody against β-actin was used as a loading control. Measurements of mated flies were taken 12 or 24 hours after mating.

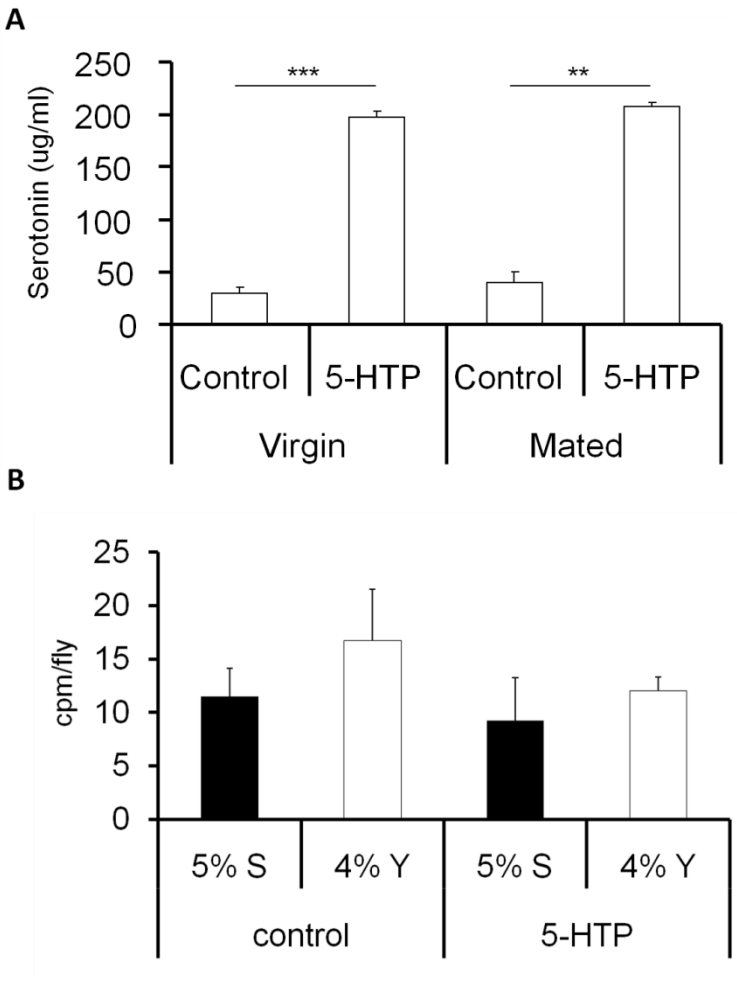

Figure S3. The effects of feeding the serotonin precursor, 5-HTP, to *D. melanogaster,* related to Figure 3*.* A) Effects of feeding 5-HTP on serotonin levels were measured in female *Drosophila*. The flies were fed 3 mg/mL 5-HTP for two days prior to being homogenized and assayed for serotonin levels. 25 heads of virgin and mated females were assayed in triplicate. (B) A nutrient preference assay in male flies fed 5-HTP versus controls. The experiment was similar to that described in Figure 3B except that male flies were used instead of virgin flies. Yeast (white bars) and sucrose (black bars) ingestion was measured as described in Figure 1. Flies were fed 3 mg/mL 5-HTP for two days prior to and during the assay feeding period. Four sets of 15 to 20 whole body flies were analyzed following 5-HTP treatment. **=$p<0.01$, ***=$p<0.001$

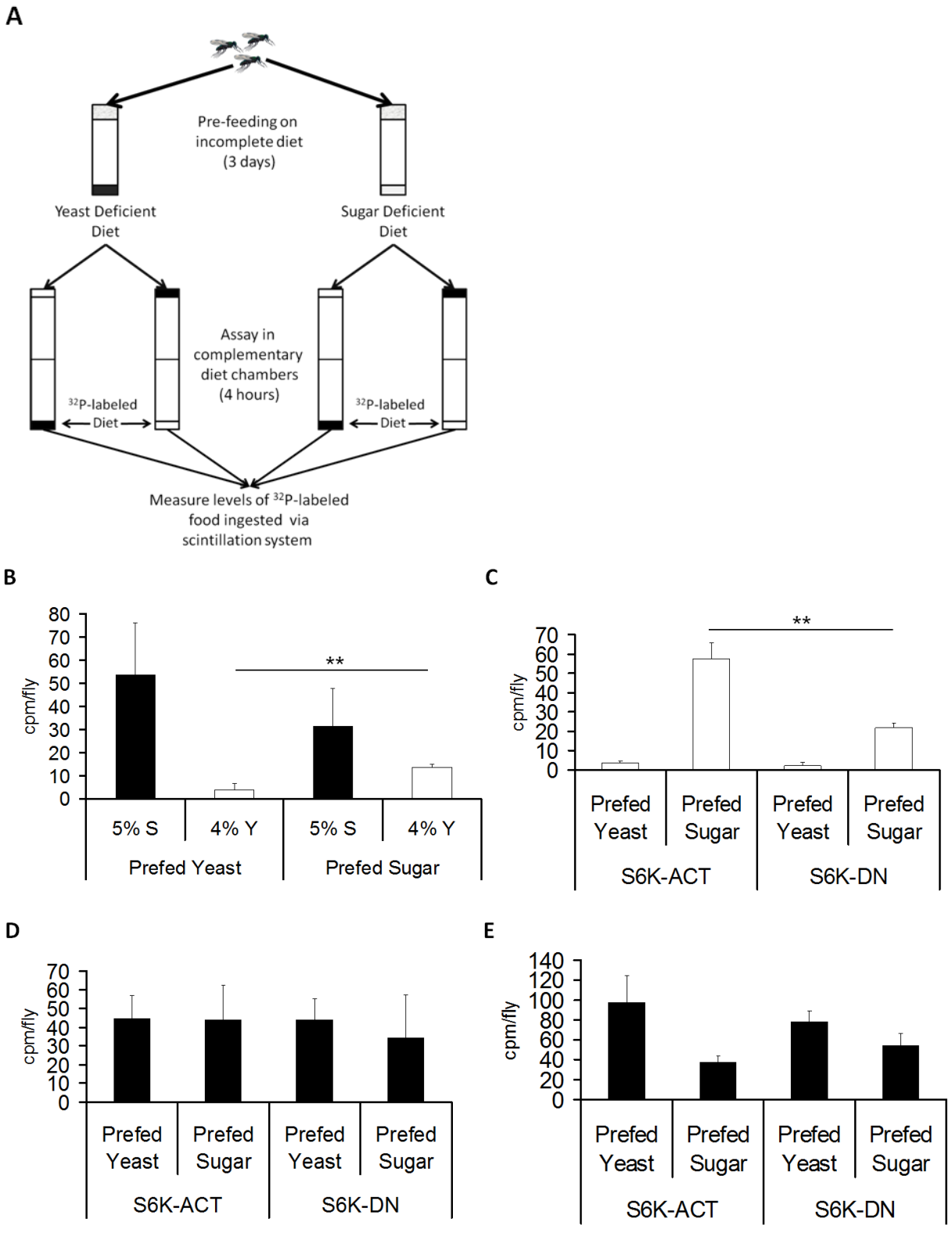
A
Pre-feeding on incomplete diet (3 days)
Yeast Deficient Diet
Sugar Deficient Diet
Assay in complementary diet chambers (4 hours)
32P-labeled Diet
32P-labeled Diet
Measure levels of 32P-labeled food ingested via scintillation system
B
cpm/fly
80
70
60
50
40
30
20
10
0
**
5% S
4% Y
5% S
4% Y
Prefed Yeast
Prefed Sugar
C
cpm/fly
70
60
50
40
30
20
10
0
**
Prefed Yeast
Prefed Sugar
Prefed Yeast
Prefed Sugar
S6K-ACT
S6K-DN
D
cpm/fly
70
60
50
40
30
20
10
0
Prefed Yeast
Prefed Sugar
Prefed Yeast
Prefed Sugar
S6K-ACT
S6K-DN
E
cpm/fly
140
120
100
80
60
40
20
0
Prefed Yeast
Prefed Sugar
Prefed Yeast
Prefed Sugar
S6K-ACT
S6K-DN

Figure S4. The role of S6K in maintaining nutrient balance in *D. melanogaster*, related to Figure 4. (A) A schematic of the 'nutrient balance' assay. Two separate populations of flies were kept on diets deficient in either sucrose or yeast. Each population was then sub divided into 2 populations to measure its sucrose or yeast consumption similar to that described in Figures S1 and 1. Following 4 hours on the radiolabeled diet, yeast or sucrose incorporation was measured by incorporation of radiolabeled tracer. White food represents a sugar deficient diet containing 4% yeast extract (4% Y), and black food represents a yeast deficient diet containing 5% sucrose (5% S). (B & C) Nutrient balancing in male and female flies. (B) The experiment was conducted similarly to that shown in Figure 4A except that male flies were used. 'Nutrient balance' assay in *w1118* males following 3 days pre-feeding on incomplete diets. Sugar consumption (black bars) and yeast consumption (white bars) was measured following pre-feeding on a yeast deficient containing sugar (Prefed sugar) or a sucrose deficient diet containing yeast (Prefed yeast). Four sets of 15 to 20 whole body flies were analyzed. (C) Similar to that described in Figure 4B except that nutrient balance was measured in male flies instead of female flies. A 'nutrient balance' assay showing yeast consumption (white bars) in male flies with altered neuronal levels of S6K activation following pre-feeding on incomplete diets. Nutrient intake of male flies overexpressing either dominant negative (S6K-DN) or constitutively active (S6K-ACT) forms of S6 kinase (S6K) in the neurons driven by the pan neuronal *appl*-gal4 enhancer trap was measured following pre-feeding incomplete diets. The following genotypes were tested *appl-GAL4/+; UAS-S6K*^{STDETE}/+; +/+ (S6K-ACT) and *appl-GAL4/+;* UAS-S6K^{KQ}/+; +/+ (S6K-DN). 4 sets of 15 to 20 flies were analyzed. (D) This is similar to that described in Figure 4B except that sugar consumption is shown here instead of yeast consumption. (E) This is similar to that described in Figure S4C except that sugar consumption is shown here instead of yeast consumption. **=p<0.01

Supplemental Experimental Procedures

Fly stocks and crosses

Strains overexpressing different forms of S6K; the constitutively active UAS-S6K^{STDETE} (S6K-ACT), wild type UAS-S6K^{wt} (S6K-WT) and dominant negative UAS-S6K^{KQ} (S6K-DN) were obtained from Dr. Stewart [1]. The pan neuronal *Appl-GAL4* [2] and *W1118* lines were obtained from the Bloomington Stock Center. The mated females in each experiment were allowed to mate for a minimum of 24 hours prior to assaying, unless otherwise noted.

Cell culture

S2 *Drosophila* cells were grown and maintained in Schneider's Drosophila medium (Invitrogen) with 10% fetal bovine serum as described previously [3].

Western blotting

Fly heads were homogenized using a mortar and pestle homogenizer in extraction buffer (120 mM NaCl, 50 mM tris, 20 mM NaF, 1 mM benzamidine, 1 mM EDTA, 6 mM EGTA, 15 mM Ppi, 1% NP-40). S2 cells were homogenized by sonication in extraction buffer. 20 µg of homogenate was loaded and separated by SDS-PAGE gel and transferred to a PVDF membrane. Membranes were blocked with 5% milk in 1xPBST for 30 minutes at room temperature. Membranes were incubated with dS6Kp-T398 primary antibody (#9209 Cell Signaling) at 1:200 in 5% BSA in 1x PBST overnight. Membranes were washed and incubated in peroxidase-labeled goat anti-rabbit secondary antibody (1:2000). Membranes were visualized using ECL.

Serotonin assay

25 Adult fly heads were homogenized and serotonin levels were measured using the manufacturer's protocol (ELISA serotonin kit, Stanbio). Test flies were fed 3 mg/mL 5-HTP for 48 hours (prior to assay and during the assay feeding period). Serotonin measurements were undertaken in freshly isolated heads as freezing was found to alter serotonin levels (data not shown).

Supplemental References

1. Barcelo, H., and Stewart, M.J. (2002). Altering Drosophila S6 kinase activity is consistent with a role for S6 kinase in growth. Genesis *34*, 83-85.
2. Luo, L.Q., Martin-Morris, L.E., and White, K. (1990). Identification, secretion, and neural expression of APPL, a Drosophila protein similar to human amyloid protein precursor. J Neurosci *10*, 3849-3861.
3. Zid, B.M., Rogers, A.N., Katewa, S.D., Vargas, M.A., Kolipinski, M.C., Lu, T.A., Benzer, S., and Kapahi, P. (2009). 4E-BP extends lifespan upon dietary restriction by enhancing mitochondrial activity in Drosophila. Cell *139*, 149-160.