Extended data

Protein-responsive gut hormone Tachykinin directs food choice and impacts lifespan in *Drosophila*

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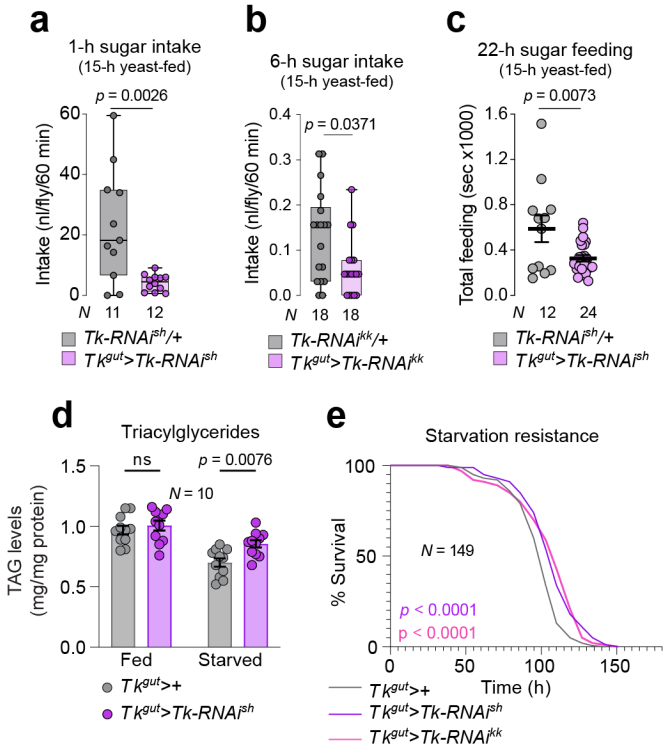
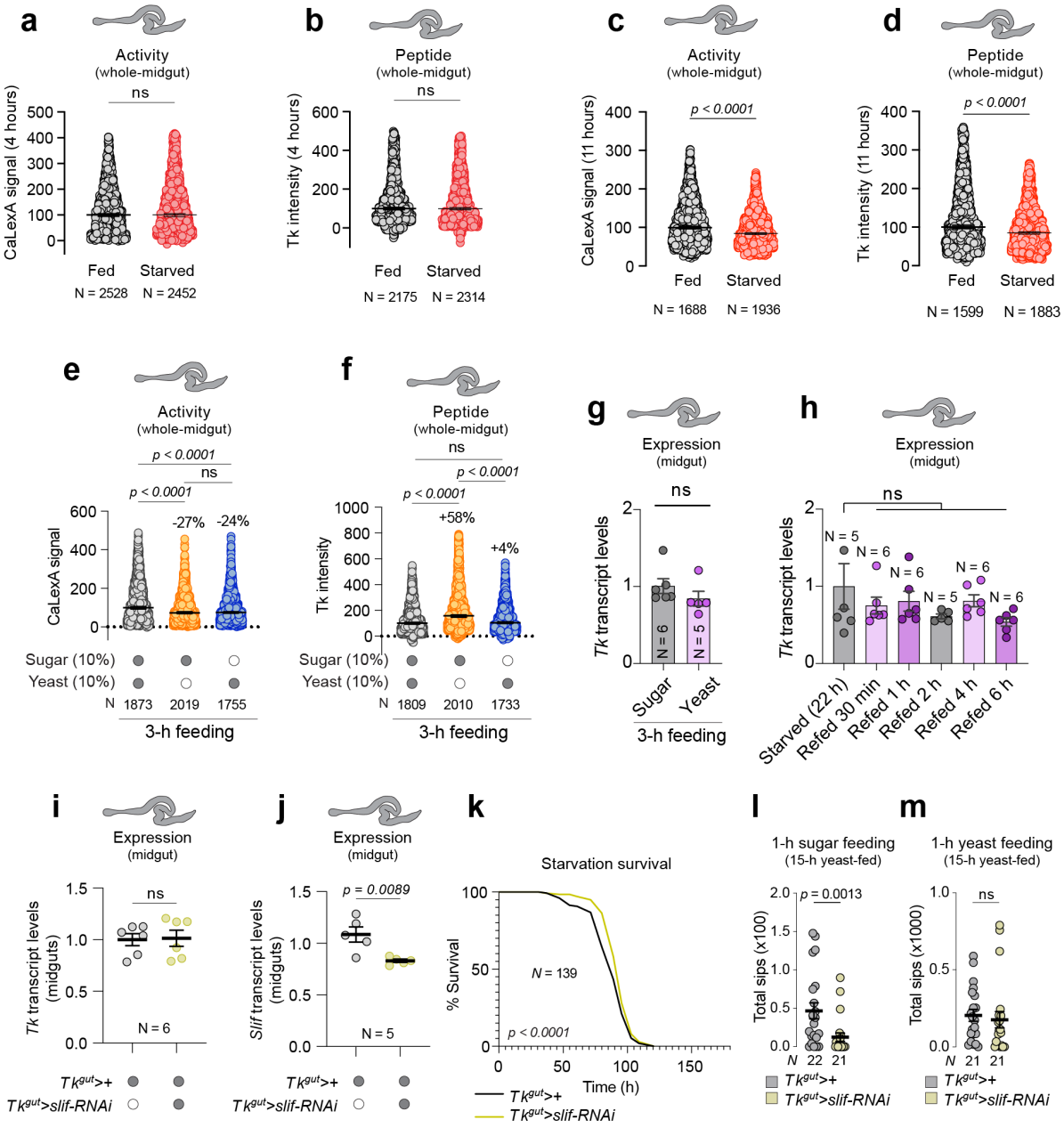
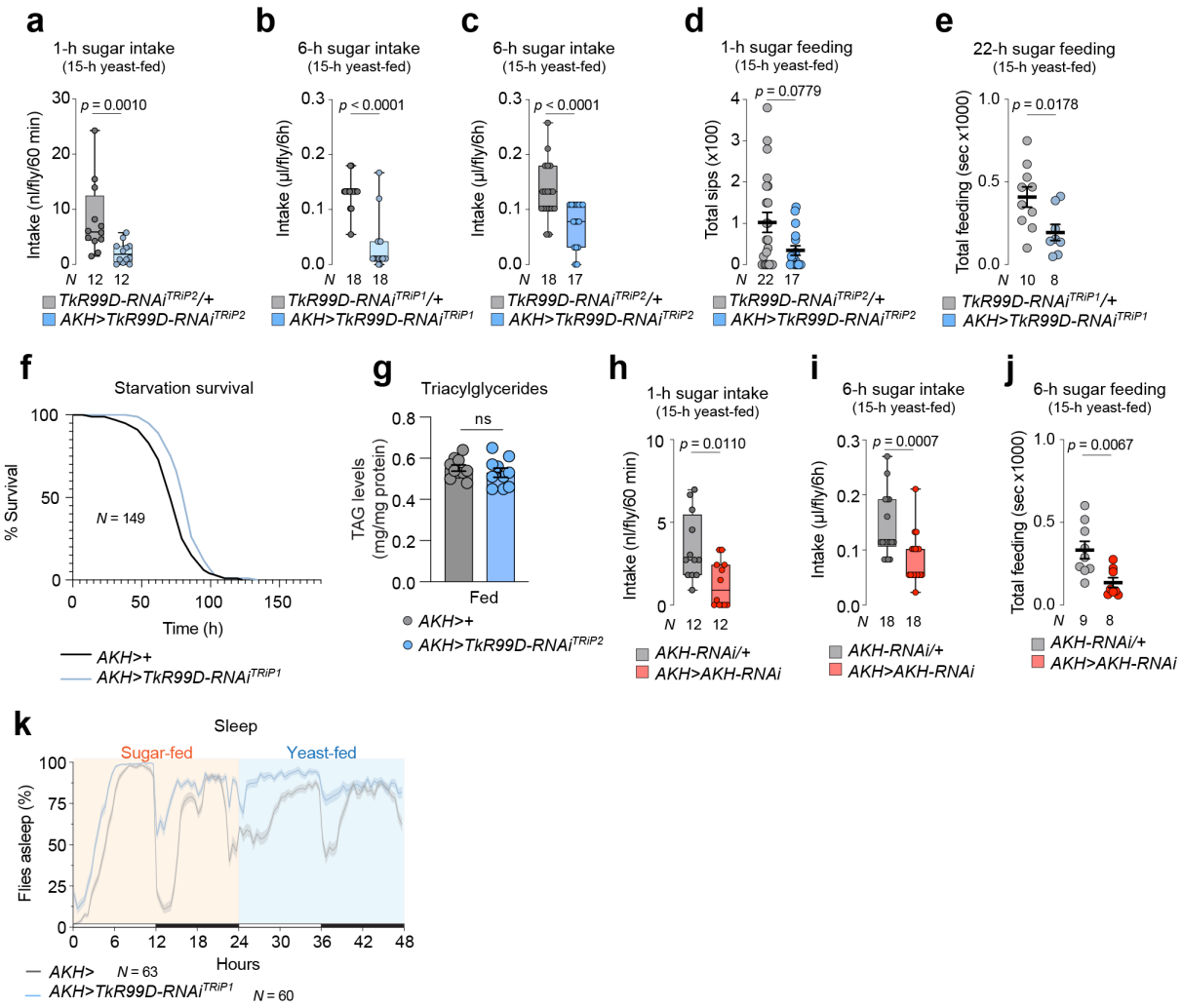


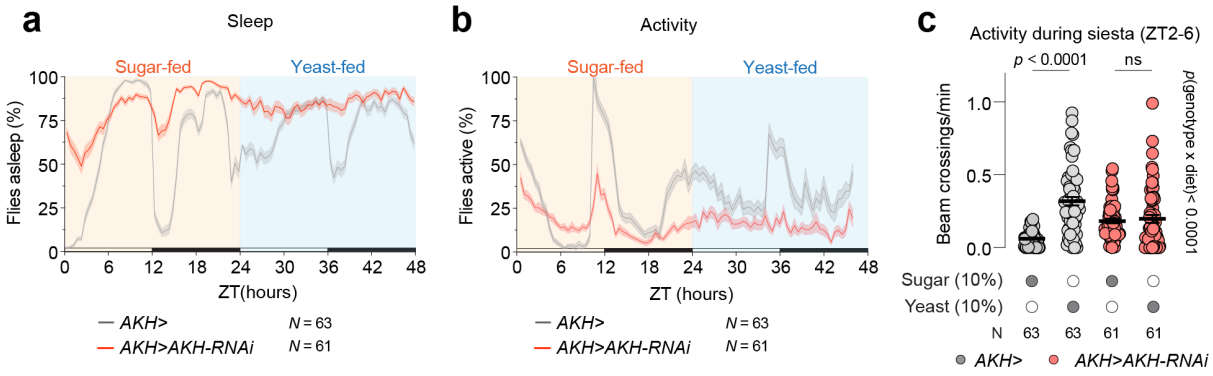
Extended Data Fig. 1. Effects of gut-specific Tk knockdown on feeding behavior and metabolic homeostasis in adult mated females. Comparing controls (*Tk*^{gut}>) and gut-specific *Tk* knockdowns (*Tk*^{gut}>*Tk-RNAi*). **a**, Sugar intake measured over 1 hour in flies fed on a yeast diet for 15 hours. Student's t-test. **b**, Six-hour sugar consumption following a 15-hour yeast pre-feeding. Mann-Whitney U test. **c**, Total feeding duration time on sugar over 22 hours after a 15-hour yeast diet, measured using the FLIC system, showing feeding behavior rather than volume intake. Student's t-test. **d**, Whole-body triacylglyceride (TAG) levels measured under fed and starved conditions, (24 hours) indicating lipid-storage capacity. ANOVA followed by Dunnett's multiple-comparisons test. **e**, Starvation-survival curves with the survival percentage plotted over time (hours). Log-rank (Mantel-Cox) test. Sample sizes (N) and *p* values are indicated in each plot. Data are presented as mean ± SEM. ns, non-significant (p>0.05).



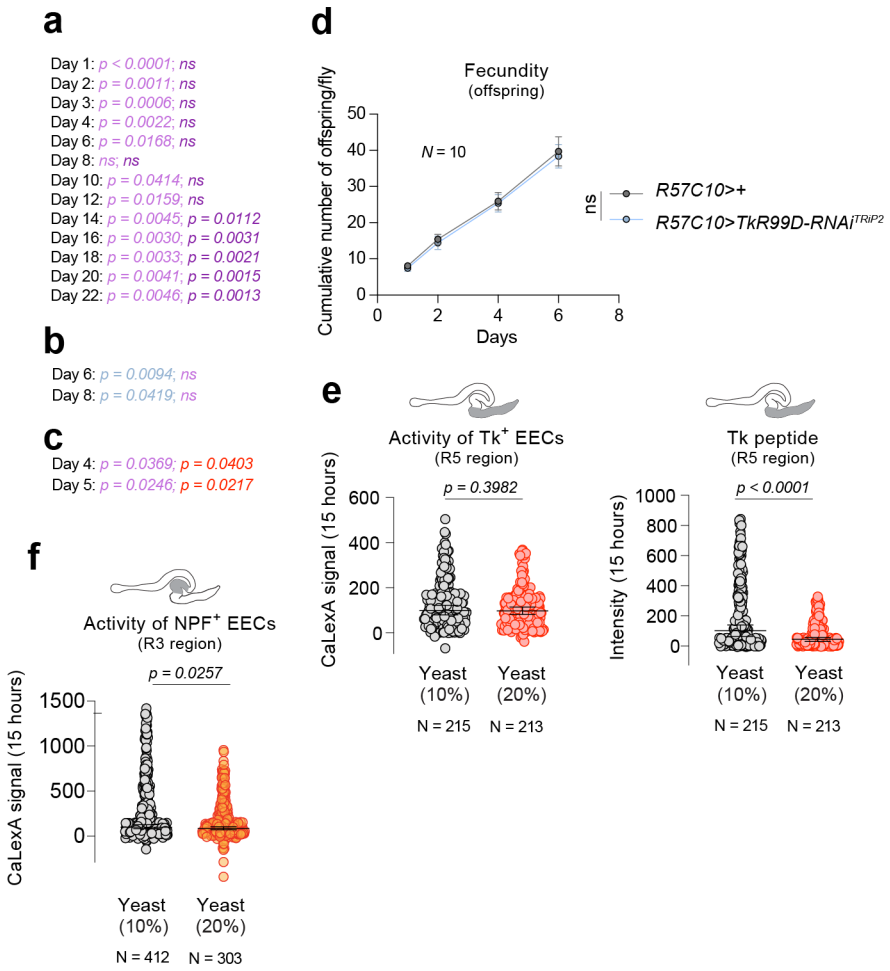
Extended Data Fig. 2. Assessment of mated female midgut Tk$^+$ EEC activity, Tk peptide levels, and transcript expression under different nutritional conditions. **a-d**, Assessment of calcium-signaling activity measured by CaLexA in Tk$^+$ EECs (**a,c**) and Tk peptide intensity (**b,d**) in the midguts of mated females fed standard adult-optimized diet containing 9% sugar and 8% yeast or starved on 1% agarose. Mann-Whitney U test. **e-f**, Effects of a 3-hour period of refeeding on 10% sugar, 10% yeast, or both combined, following 25 hours of food deprivation on calcium signaling, using the CaLexA system (**e**) and Tk intensity (**f**) in Tk$^+$ EECs. Kruskal-Wallis Dunn test. **g-h**, Midgut *Tk* transcript levels in mated females refed with 10% sugar or 10% yeast for 3 hours following a 25-hour period of food deprivation (**g**) and those starved for 22 hours and refed with food containing both sugar and yeast for a range of time periods before dissection (**h**). Student's t-test (**g**) and one-way ANOVA with Dunnett's test (**h**). **i-j**, Analysis of midgut *Tk* and *slif* transcript levels in flies expressing gut-specific *slif* RNAi in Tk$^+$ EECs. Student's t-tests. **k**, Starvation-survival curves for flies expressing *slif* knockdown in Tk$^+$ EECs versus controls. Log-rank (Mantel-Cox) test. **l-m**, FlyPAD feeding assays of total sips of sugar (**l**) and yeast (**m**) over a one-hour test for flies previously fed on a 15-hour yeast diet. Mann-Whitney U test. Sample sizes (N) and *p* values are indicated in each plot. Data are presented as mean ± SEM. ns, non-significant (p>0.05).



Extended Data Fig. 3. Nutrient-intake regulation, metabolic consequences, and sleep effects of TkR99D deficiency in the APCs and loss of AKH signaling in mated females. a,h, Short-term (1-hour) sugar intake following a 15-hour yeast pre-feeding period for *UAS-TkR9D-RNAi* alone. Mann-Whitney U test. **b,c,i**, Longer-term (6-hour) sugar intake following a 15-hour yeast pre-feeding period. Mann-Whitney U tests. (**d**) FlyPAD feeding assays of total sips of sugar over a one-hour test for flies previously fed for 15 hours on yeast diet. Mann-Whitney U tests. **e,j**, FLIC-based sugar-feeding assays over a 22-hour period following a 15-hour yeast pre-feeding period. Mann-Whitney U test. **f**, Starvation-survival curves. Log-rank (Mantel-Cox) test. **g**, Whole-body triacylglyceride levels. Student's t-tests. **k**, Sleep patterns for sugar-fed and yeast-fed controls and *TkR99D-RNAi* flies over 48 hours, suggesting behavioral changes linked to metabolic state. The shaded area represents the standard error of the mean (SEM). Sample sizes (N) and *p* values are indicated in each plot. Data are presented as mean ± SEM. ns, non-significant ($p>0.05$).



Extended Data Fig. 4. Dietary effects on sleep and activity patterns is influenced by AKH signaling females. **a-c** Sleep (**a**) and activity (**b**) profile graphs depicting the percentage of flies asleep or active over time for both control groups and flies with knockdown of *AKH* in the APCs on sugar and yeast diets. The shaded area represents the standard error of the mean (SEM). **c**, Quantitative analysis of activity during midday siesta, spanning Zeitgeber Time (ZT) 2 to 6 hours, in flies with *AKH* knockdown in the APCs, maintained on sugar and yeast diets. Kruskal-Wallis Dunn test and two-way ANOVA to examine interactions. Sample sizes (N) and *p* values are indicated in each plot. Data are presented as mean ± SEM. ns, non-significant ($p>0.05$).



Extended Data Fig. 5. Offspring number in females flies with pan-neuronal *TkR99D* knockdown and significance *p* vales related to Fig. 6a-c. a, *p* values for Figure 6a. **b**, *p* values for Figure 6b. **c**, *p* values for Figure 6c. **d**, Analysis of fecundity in mated females expressing pan-neuronal *TkR99D* knockdown. The graph indicates the cumulative number of offspring per fly over a period of 6 days for control flies (*R57C10>*) and flies with neuronal *TkR99D* knockdown (*R57C10>TkR99D-RNAi*). Two-way ANOVA with Dunnett's multiple-comparisons test. **e**, Quantification of calcium-dependent activity, as indicated by the GFP:tdTomato ratio (driven by *Tk-GAL4*) , in posterior midgut (R5 region) Tk^+ EECs, along with the intensity of anti-Tk staining, in flies fed on diets containing 10% or 20% yeast and 10% sugar. Significance was determined with Mann-Whitney U tests. **f**, Quantification of calcium-dependent activity, as indicated by the GFP:tdTomato ratio (driven by *NPF-Gal4*), in the middle midgut (R3 region) of NPF^+ EECs of flies subjected to the same 10%- and 20%- yeast diets. Significance was determined with Mann-Whitney U tests. Sample sizes (N) and *p* values are indicated. Data are presented as mean ± SEM. ns, non-significant ($p>0.05$).