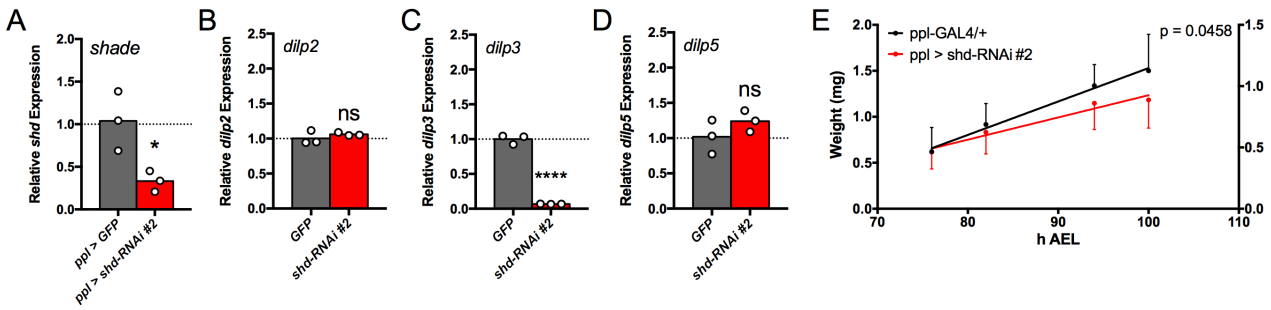
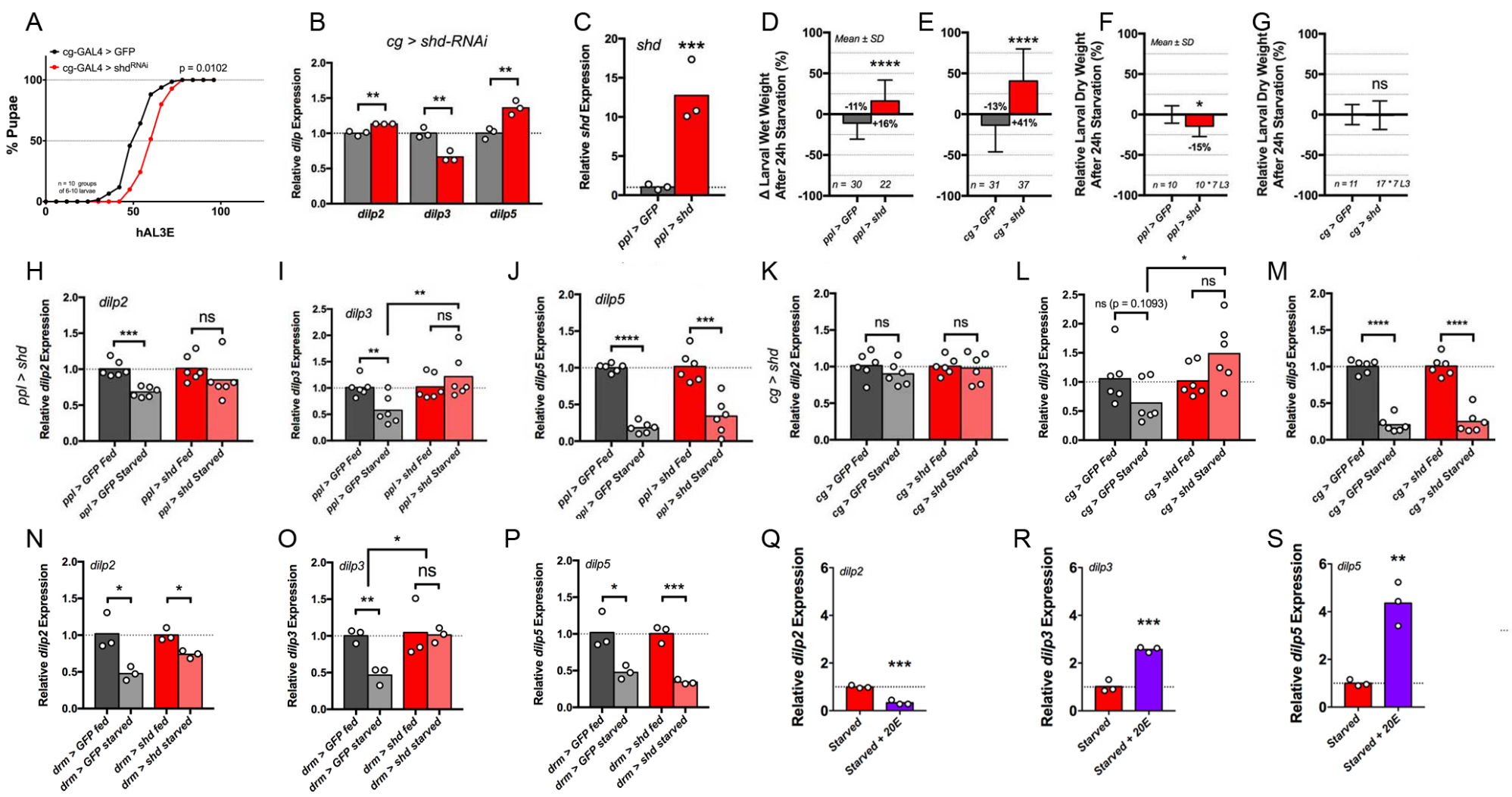


Figure S1. Knockdown of shade in the fat body with a second RNAi line reduces dilp3 expression and results in a reduced growth rate. (A) A second RNAi line directed against shade using ppl-GAL4 significantly reduced detectable levels of shade in whole pupae (when shade is most highly expressed) compared to synchronously timed ppl-GAL4>GFP control pupae. (B-D) Knockdown of shade in the fat body with this second RNAi line did not alter expression of dilp2 or dilp5, but did significantly reduce detectable dilp3 levels (One-way ANOVA, Tukey's test of multiple comparisons, $p < 0.05$). (E) Growth rates of ppl-GAL4>shade-RNAi larvae were significantly reduced when driving a second RNAi line compared to ppl-GAL4/+ larvae in an identical genetic background (Linear regression, ANCOVA, $p < 0.05$).



Development: doi:10.1242/dev.165654: Supplementary information

Figure S2. Overexpressing shade in starved larvae results in wet weight gain and rescues starvation-dependent reductions of *dilp3.* (A) Knockdown of shade in the fat body resulted in significantly delayed maturation onset by about 6h ($p < 0.05$, one-tailed, unpaired T-Test, n = 10 groups of per group 6 cg-gal4/+ larvae and 10 cg-GAL4>shd-RNAi larvae, respectively). Time to pupation was determined as in Figure 1F. All average values shown are mean +/- SD. (B) Knockdown of *shade* in the fat body using *cg-GAL4* results in *dilp3* expression reduction, and an increase in *dilp2* and *dilp5* expression. (C) Overexpressing *shade* using *ppl-GAL4* results in significantly increased *shade* transcript levels in whole pupae compared to overexpression of a GFP. (D-E) Control animals overexpressing GFP in the fat body lose weight during starvation. Animals overexpressing *shade* gain weight (T-test, $p < 0.05$). (F-G) Dry weights of animals starved for 24h show no significant differences in weight between control (fat body overexpression of GFP) and experimental conditions (overexpression of shad in the fat body). Animals were collected at 72h AEL as previously described then starved for 24h on 1% non-nutritive agar plates, re-collected and weighed. The difference in weight in the 24h period is shown as percent change. For dry weight assays, larvae were collected in a dry, perforated eppendorf tube and desiccated at 50°C for 8h. Seven larvae were pooled to determine dry weights. Weights are plotted as relative change in dry weight of *ppl-GAL4 > shade* or *cg-GAL4 > shade* larvae compared to *ppl-GAL4 > GFP* or *cg-GAL4 > GFP* larvae, respectively. (H-M) Overexpression of *shade* in the fat body - using *ppl-GAL4* or *cg-GAL4* - resulted in rescue of starvation-dependent reductions in *dilp3* expression of L3 larvae (ANOVA, $p < 0.05$). No significant changes in *dilp2* or *dilp5* result during *shade* overexpression (ANOVA, $p > 0.05$). Larvae were collected and starved as described above, then collected for RNA extraction from whole L3 larvae and qPCR determination of *dilp2, dilp3* and *dilp5*. (N-P) Overexpression of *shade* in the gut - using *drm-GAL4* - resulted in rescue of starvation-dependent reductions in *dilp3* expression of L3 larvae (ANOVA, $p < 0.05$). No significant changes in *dilp2* or *dilp5* result during *shade* overexpression (ANOVA, $p > 0.05$). (Q-S) Feeding starved animals 1mM 20E resulted in a significant decrease of *dilp2* and a significant increase of *dilp3* and *dilp5* expression (ANOVA, $p < 0.05$). L3 larvae were collected at 72h AEL and placed on filter paper with either 6% EtOH (starved) or 1mM 20E dissolved in 6% EtOH for 24h. Whole larvae were thereafter collected for RNA extraction and qPCR.

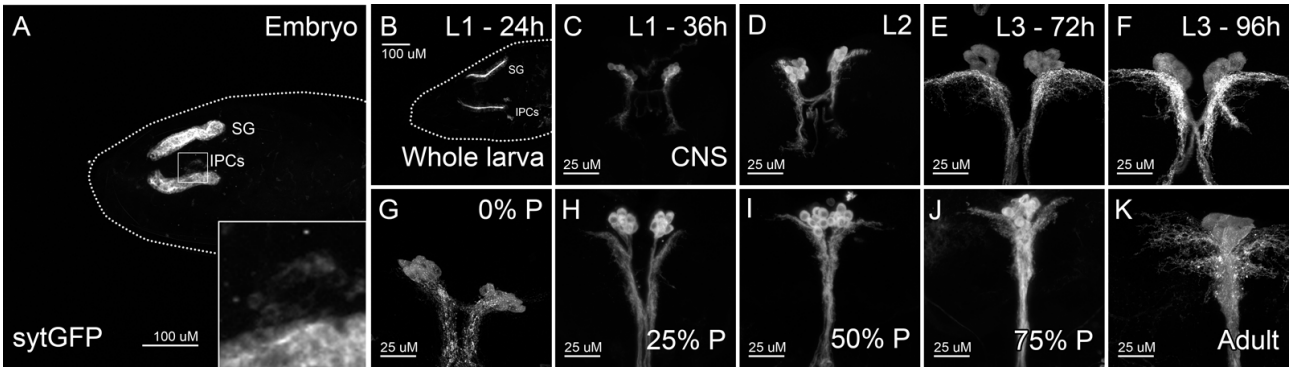


Figure S3. Temporal analysis of the *Dilp2-GAL4^{R}* driver. (A-K) The *Dilp2-GAL4^{R}* driver is active in the salivary glands (SG) and IPCs from late embryonic development throughout development and into adulthood. Note the morphological changes of the IPCs during pupation, where IPC clusters merge centrally, and branching becomes more pronounced thereafter (G-K). IPCs were marked by expression of sytGFP.

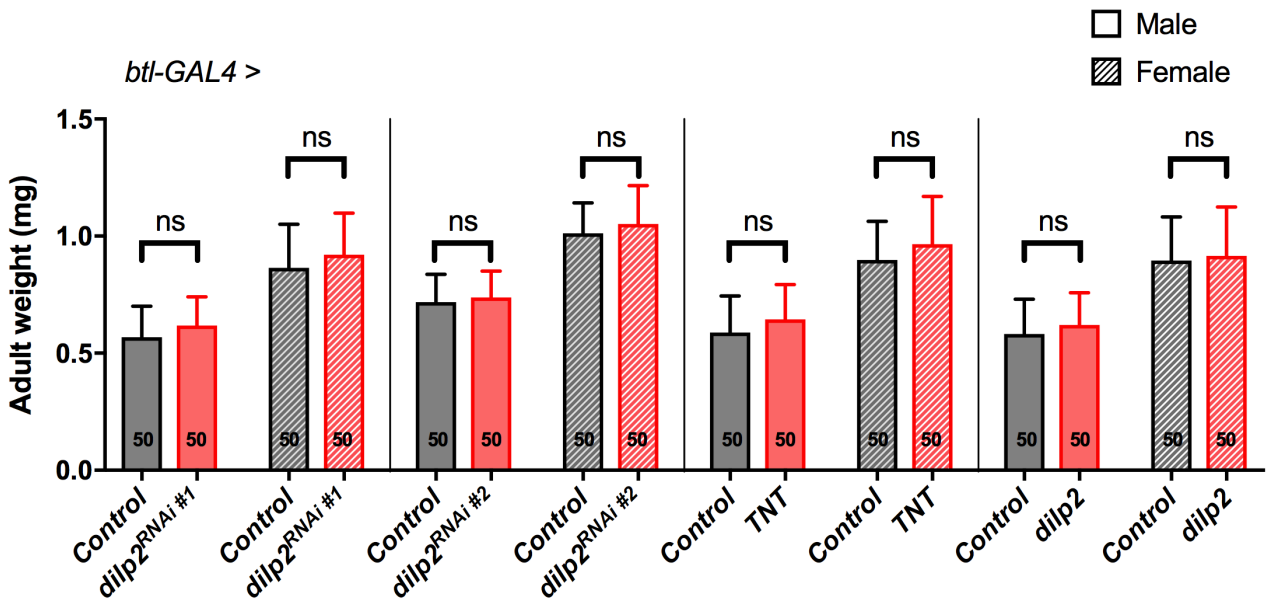


Figure S4. Genetic knockdown or overexpression of *dilp2*, or inhibition of tracheal secretion, do not affect systemic growth. Knockdown of *dilp2* with two different RNAi lines or inhibition of tracheal secretion by overexpression of a tetanus toxin light chain (TNT) did not result in significant weight differences compared to sibling controls ($p>0.05$, ANOVA, n = 50). Similarly, overexpression of *dilp2* using the tracheal *btl-Gal4* did not result in significant weight differences compared to balanced sibling controls ($p>0.05$, unpaired, one-tailed t-test, n = 50). Flies were anesthetized with chloroform and weighed upon reaching sexual maturity. To control for differences in density and nutrition, test genotypes were compared to *CyO, RNAi* or *UAS-TNT* or *UAS-dilp2* control sibling flies.

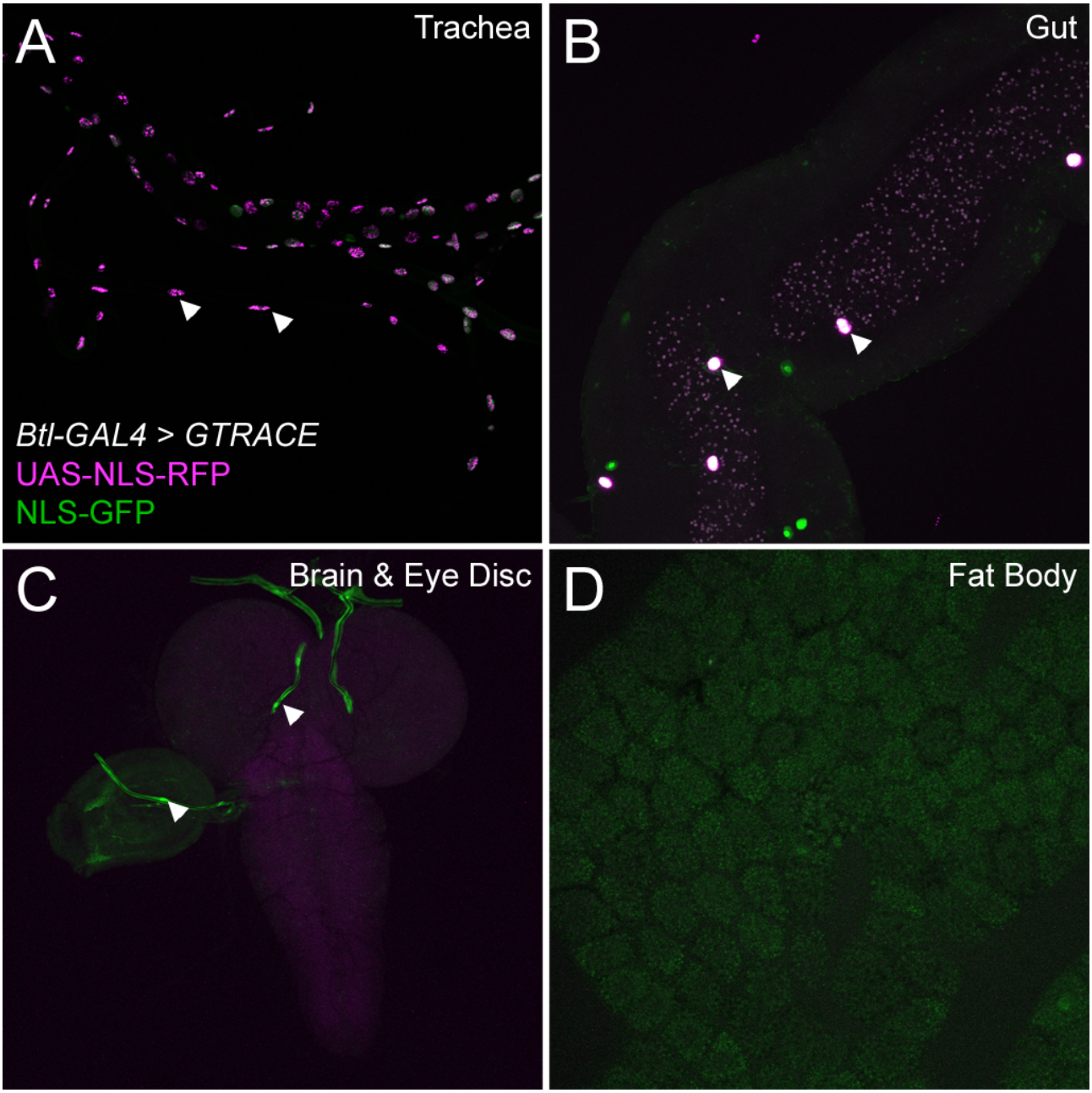


Figure S5. Btl-Gal4 drives expression exclusively in the trachea. When crossed to the G-TRACE reporter line, NLS-RFP and NLS-GFP were only detected in trachea (A) and not in the gut (B), CNS (C) or fat body (D). Wandering *Btl-Gal4 > G-TRACE* larvae were collected and stained for imaging.

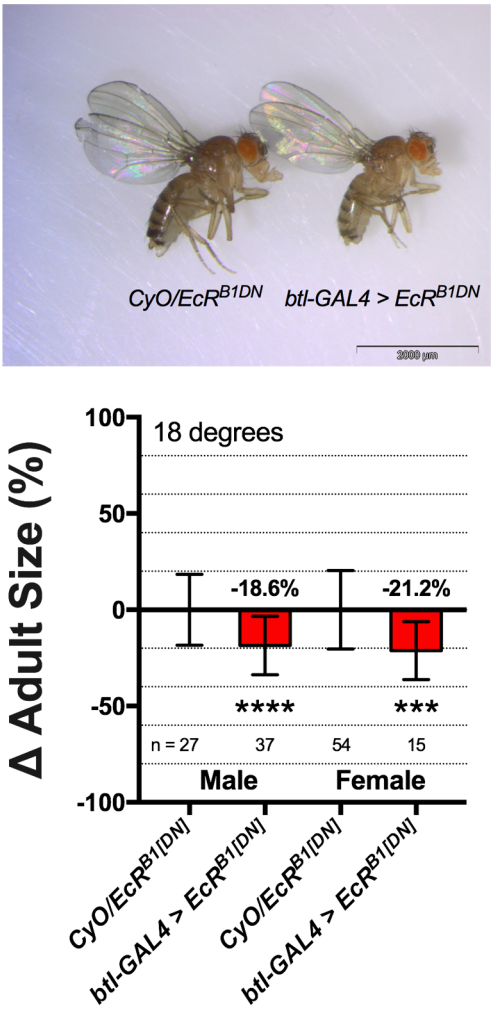


Figure S6. Perturbation of 20E signaling using *btl-GAL4* results in growth defects.

Overexpressing *EcR*$^{B1[DN]}$ with btl-GAL4 at 25°C was lethal, but at 18°C adult flies emerged that were significantly smaller compared to controls (T-test, $p < 0.05$).

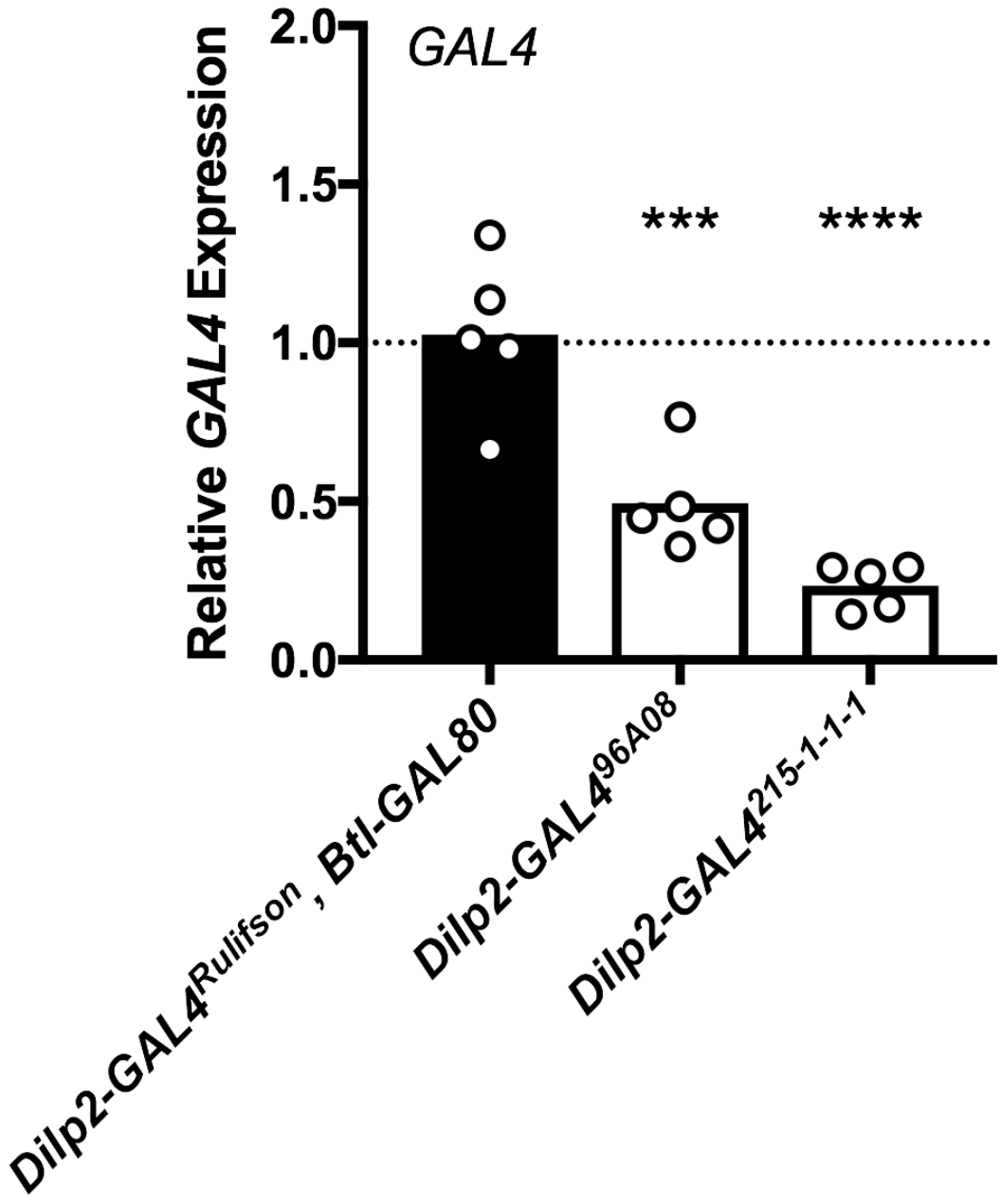


Figure S7. Relative strength of GAL4 expression in different Dilp2-GAL4 driver lines. Dilp2-Gal4[R] is three-fold stronger than Dilp2-Gal4[215-1-1-1] and two-fold stronger than Dilp2-Gal4[96A08]. *GAL4* expression in adult fly heads from *Dilp2-Gal4[R]; btl-Gal80* was significantly higher than other *Dilp2-Gal4s* ($p<0.05$, unpaired, one-tailed T-test). RNA was collected from heads of flies that were density, age and sex-controlled.

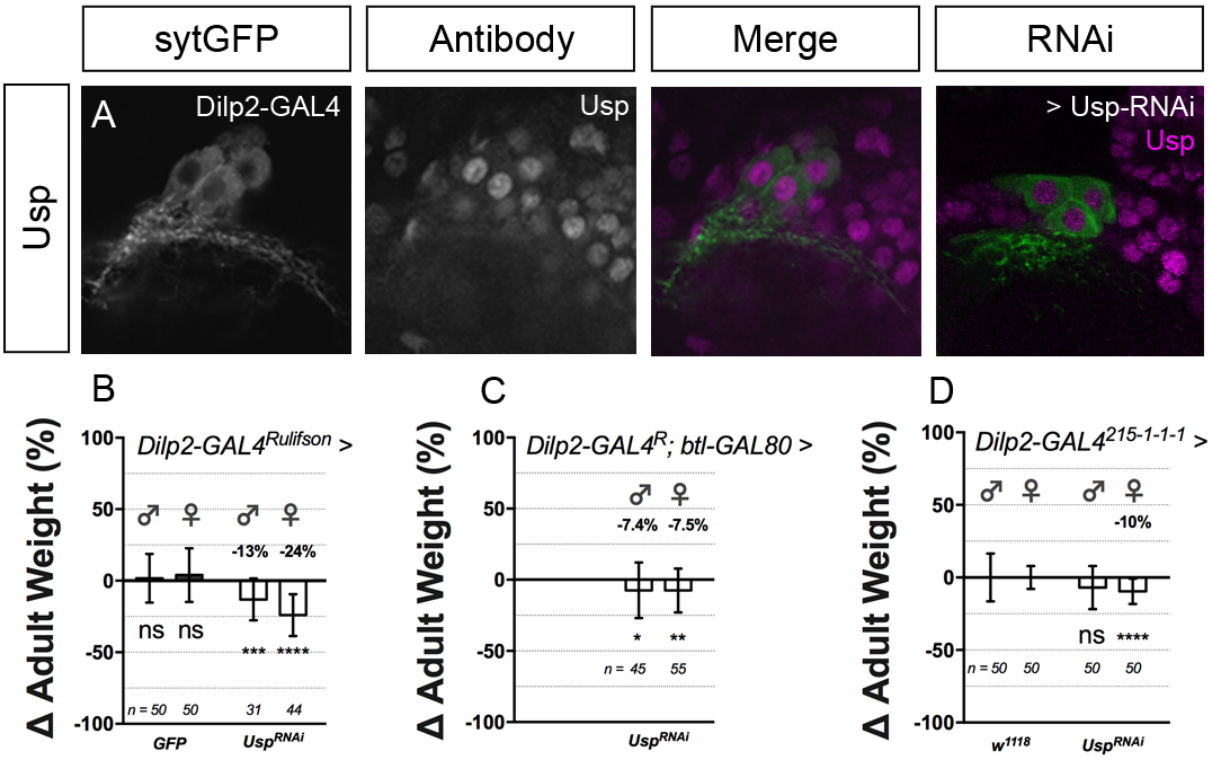


Figure S8. Knockdown of the EcR coreceptor Ultraspiracle (Usp) results in growth defects. (A) Usp expression could be detected and reduced in the IPCs, though knockdown (RNAi) was incomplete. (B) Knockdown of Usp with *Dilp2-GAL4^{R}* results in growth defects in males and females that are less pronounced when the knockdown is controlled by *Dilp2-GAL4^{R}; btl-GAL80* (C). (D) Knockdown with the *Dilp2-GAL4$^{215\text{-}1\text{-}1\text{-}1}$* gives significant growth defects in females. A trend is seen in males, albeit not statistically significant.

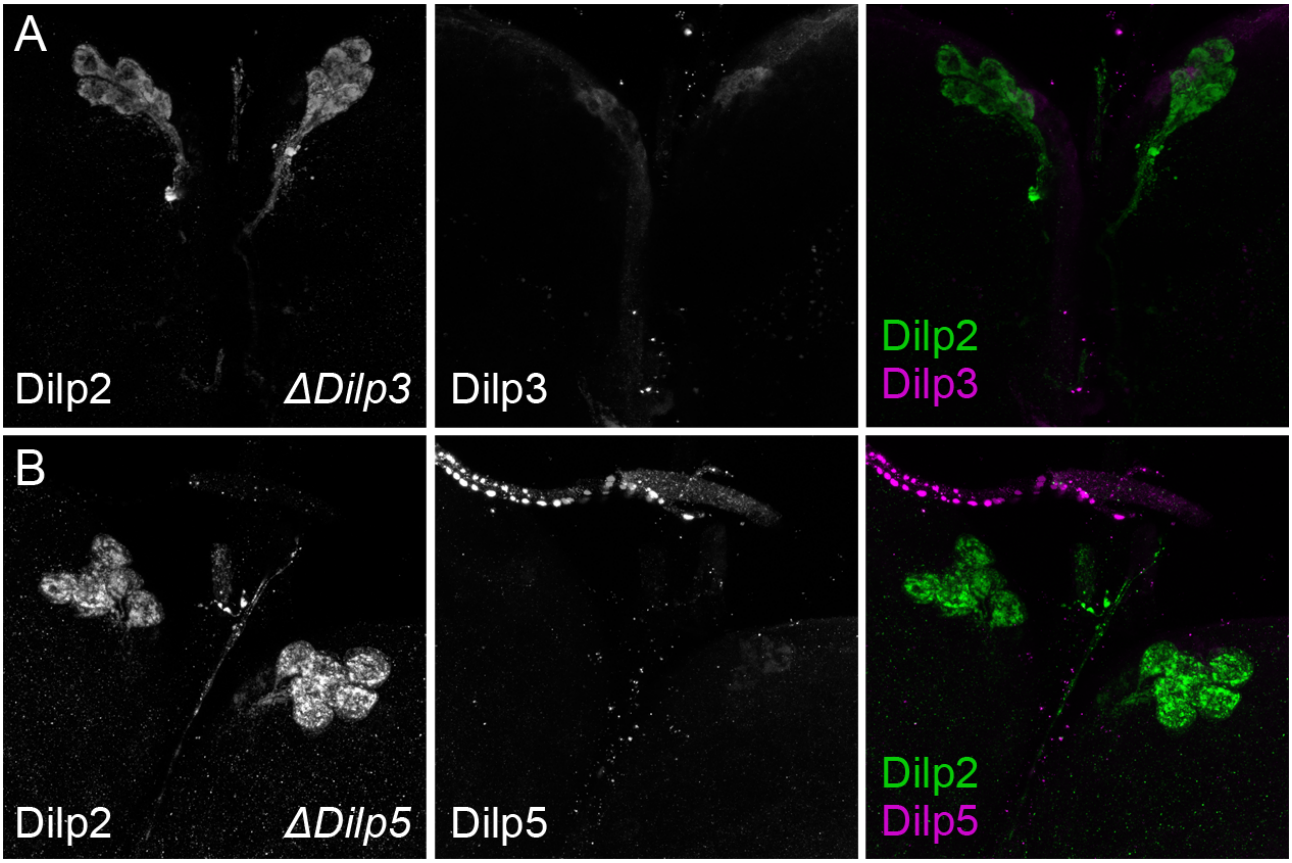


Figure S9: Validation of anti-Dilp3 and anti-Dilp5 antibodies. Neither Dilp3 (A) nor Dilp5 (B) antibody detected signal in *Dilp3* or *Dilp5* deletion mutants, respectively, thereby demonstrating specificity of the antibodies. Dilp2 remained detectable in both. Brains from L3 larvae were dissected and stained with Dilp antibody followed by visualization.

Table S1: Sequences for qPCR primers used in this study

Gene	Forward Primer Sequence (5' > 3')	Reverse Primer Sequence (5'> 3')
dilp2	GCGAGGAGTATAATCCCGTGAT	GGATTGAGGGCGTCCAGAT
dilp3	GCAATGACCAAGAGAACTTTGGA	GCAGGGAACGGTCTTCGA
dilp5	GAGGCACCTTGGGCCTATTC	CATGTGGTGAGATTCGGAGCTA
thor (4e-bp)	CCTGGAGGCACCAAACTTAT	GAAGGGAGTACGCGGAGTT
dInR	AAGCGTGGGAAAATTAAGATGGA	GGCTGTCAACTGCTTCTACTG
Rp49	GCCCAAGGGTATCGACAACA	CTTGCGCTTCTTGGAGGAGA
RpL3	AAGGATGACGCCAGCAAGCCAGTC	TAGCCGACAGCACCGACCACAATC
RpS13	GGGTCTGAAGCCCGACATT	GGCGACGGCCTTCTTGAT
shade	TAATGCAGGGACTGTGGTGC	TCCAACGCTCTGGGGTAAAC