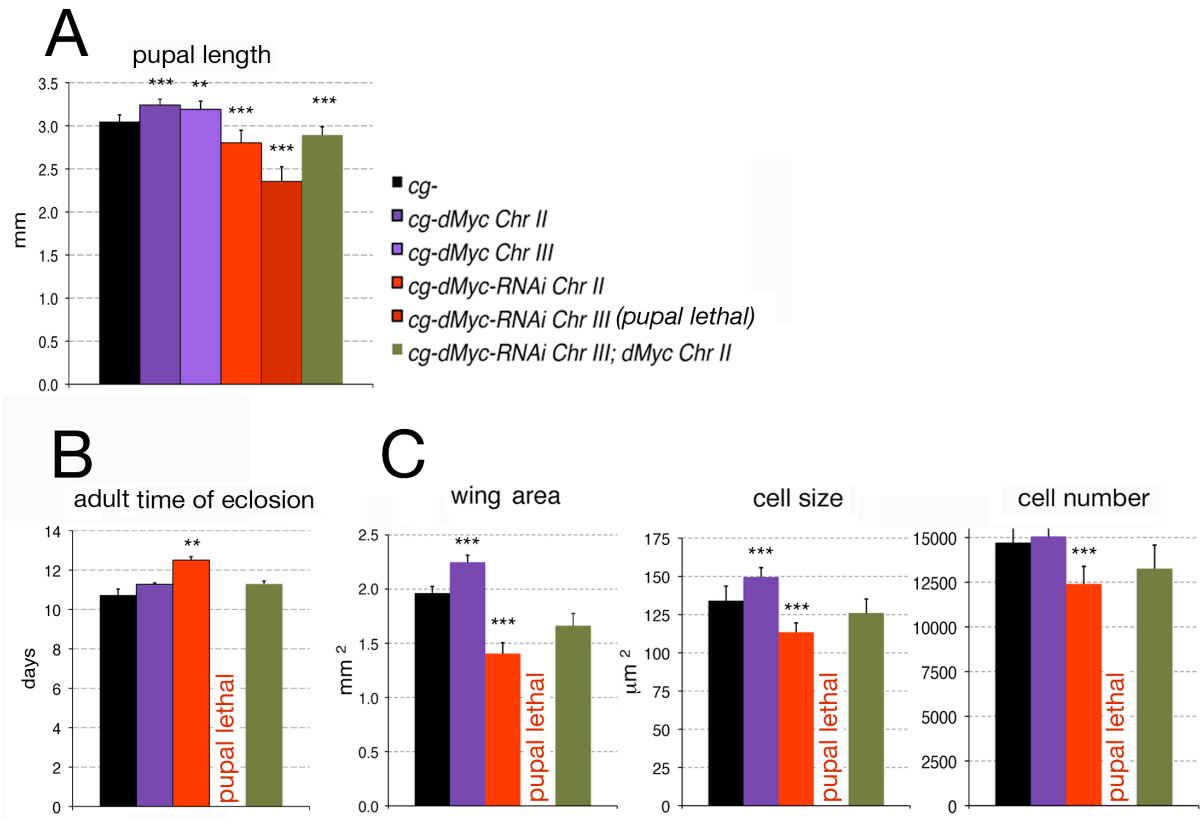
SUPPLEMENTARY MATERIAL

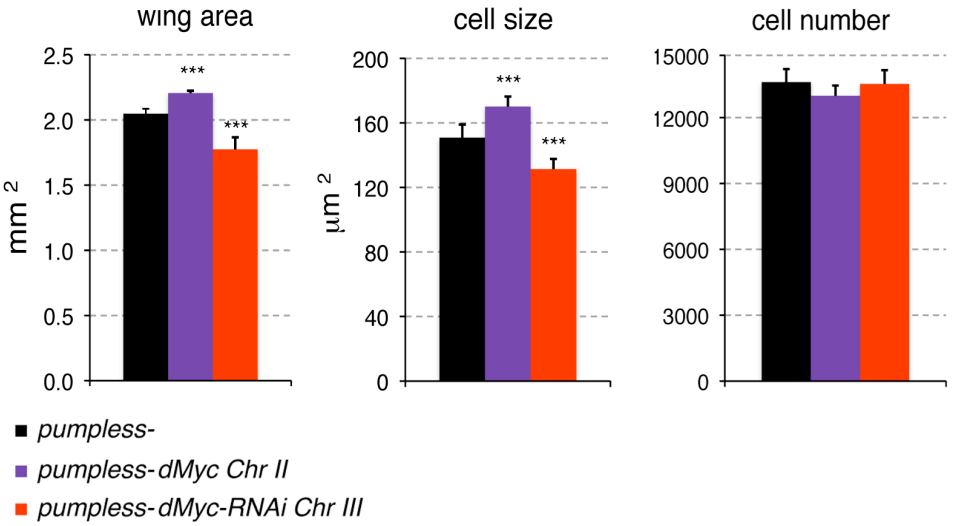
Supplementary Figure 1



Small size and pupa lethality, induced by reduction of dMyc expression in the FB, are rescued by overexpression of dMyc.

(A) Pupal length (mm) of animals expressing different levels of dMyc in the FB using the *cg-Gal4* promoter. Two independent insertions for *UAS-dMyc* and *UAS-dMyc-RNAi* were used. The small size and pupal lethality of *cg-dMyc-RNAi* on Chr III animals was rescued by coexpression of *UAS-dMyc*. ***P*<0.01, ****P*<0.001, *P*-values were calculated from a Student *t*-test. The total number of animals analyzed is reported in Table 1. Error bars represent +/- SD from four independent experiments, at least 10 animals for each genotype were analyzed. (B) Developmental timing of adult eclosion (days). The lethality of *cg-dMyc-RNAi* on Chr III animals was rescued to adulthood by coexpression of dMyc. (C) (C) The size of the rescued *cg-dMyc-RNAi-Chr III; dMyc-Chr II* animals was calculated in the wing of female flies of the indicated genotypes. Error bars represent standard error of the mean (+/- SEM) at least n=15 animals for each genotype were used. **P* <0.05, ***P* <0.01, ****P* <0.001, *P*-values were calculated from Student *t*-test compared to *cg-* control.

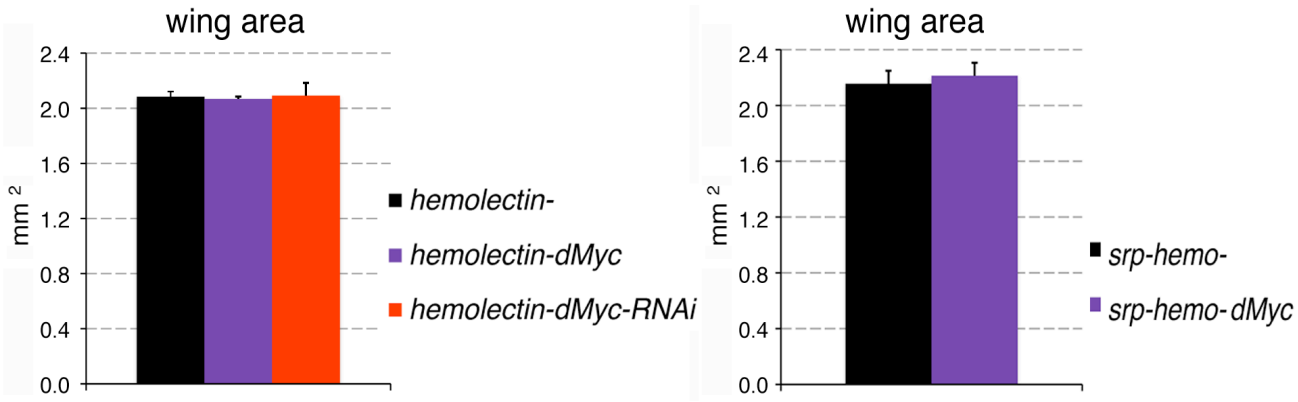
Supplementary Figure 2



Expression of dMyc in the FB using the *pumpless* promoter affects organismal growth.

The UAS/Gal4 system was used to express *UAS-dMyc* or *UAS-dMyc RNAi* in the FB using the *pumpless-Gal4* promoter. Measurement of growth was calculated in the wing of female flies of the indicated genotypes. Error bars represent standard error of the mean (+/- SEM); at least n=15 animals for each genotype were used. ***P <0.001, P-values were calculated from a Student t-test compared to *cg-* control.

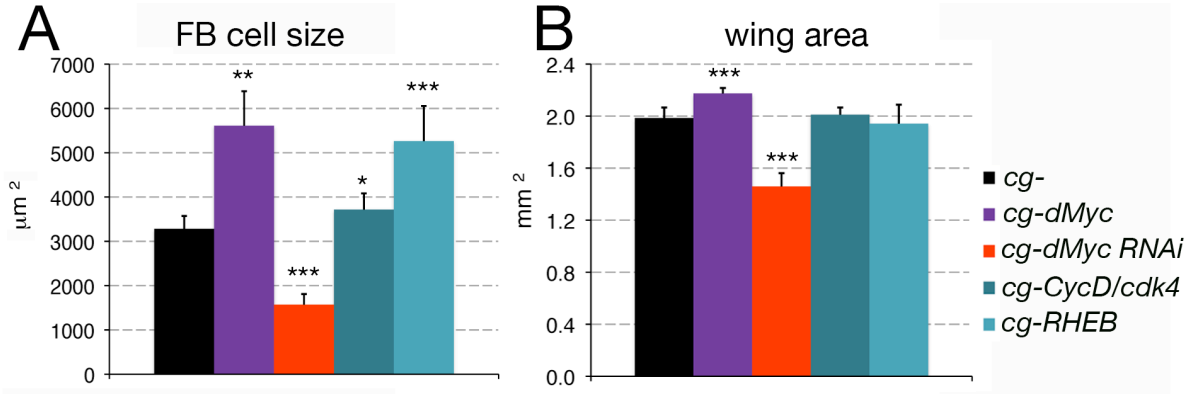
Supplementary Figure 3



Expression of dMyc in the hemocytes using the *hemolectin-Gal4* (A) or the *serpent-hemo-Gal4* (B) promoters, does not affect organismal growth.

The UAS/Gal4 system was used to express *UAS-dMyc* or *UAS-dMyc RNAi* in the hemocytes using *hemolectin-Gal4* or *serpent-hemocytes-Gal4*. Measurement of growth was calculated in the wing of female flies of the indicated genotypes. Error bars represent standard error of the mean (+/- SEM) at least n=15 animals for each genotype were used.

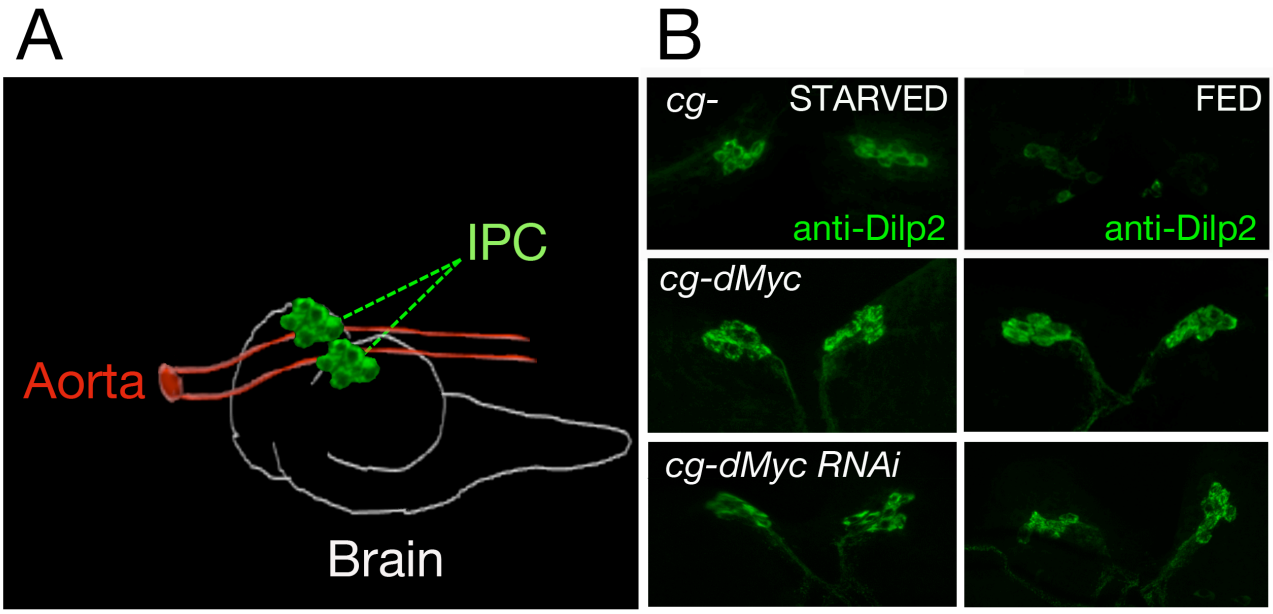
Supplementary Figure 4



Expression of dMyc, CycD/cdk4 and RHEB in the FB affects cell size (A) but only expression of dMyc induces organismal growth (B).

The UAS/Gal4 system was used to express the indicated transgenes in the FB using the *cg-Gal4* promoter. (A) Measurement of the cell size in the FB from third instar larvae of the indicated genotypes, at least n=15 fat bodies were used from each genotype. These experiments show that expression in the FB of the growth inducer genes dMyc, Rheb and CycD/cdk4 significantly increased the mass/size of the fat cells. (B) Measurement of animal growth was calculated in the wing of female flies of the indicated genotypes. **P*<0.05, ***P*<0.01, ****P*<0.001, *P*-values were calculated from a Student *t*-test compared to *cg-* control. Error bars represent standard error of the mean (+/- SEM) at least n=15 animals for each genotype were used.

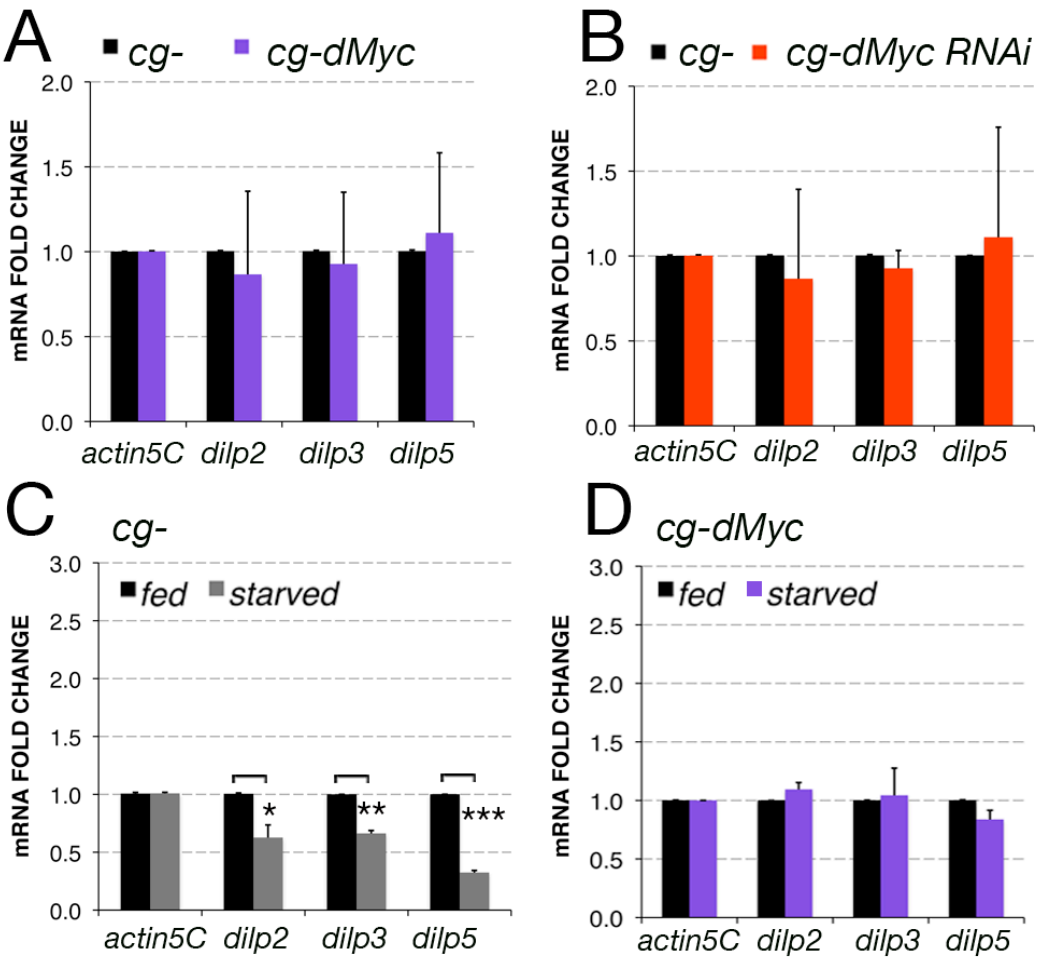
Supplementary Figure 5



DILP2 expression in the Insulin Producing Cells (IPCs).

(A) Schematic representation of *Drosophila* larval brain showing the localization of the IPCs and of the larval aorta or cardiac tube. (B) Representative photographs of DILP2 protein staining in the IPCs in larvae of the indicated genotype in starved or feeding conditions. Immunofluorescence and quantification of DILP2 protein levels was performed using anti-DILP2 antibody and the setting as described in (Geminard et al., 2009).

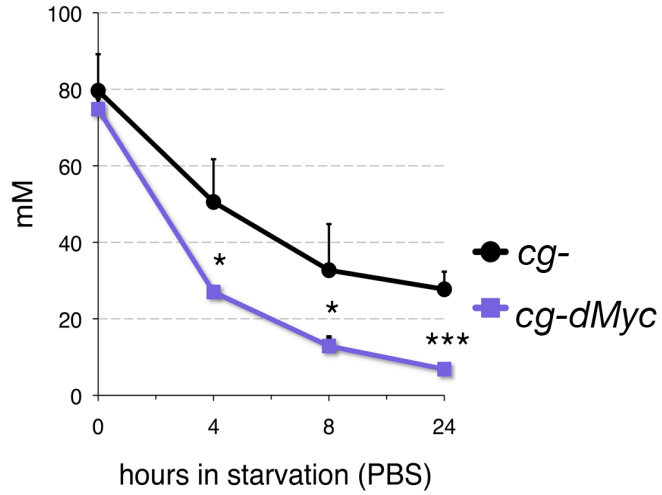
Supplementary Figure 6



***dilp2 dilp3 and dilp5 mRNA* expression in brains from third instar larvae from animals expressing different levels of dMyc in the FB during feeding and starvation.**

Quantitative RT-PCR of *dilps mRNA* expression in brains from *cg*-control and *cg-dMyc* (A) and *cg*-control and *cg-dMyc-RNAi* (B) third instar larvae; *actin5C* was used as the internal control. Quantitative RT-PCR of *dilps mRNAs* expression in brains from fed and starved *cg*-control (C) and *cg-dMyc* (D) third instar larvae; *actin5C* was used as the internal control. *P*-values were calculated from Student *t*-test. Error bars represent +/- SD from three independent experiments.

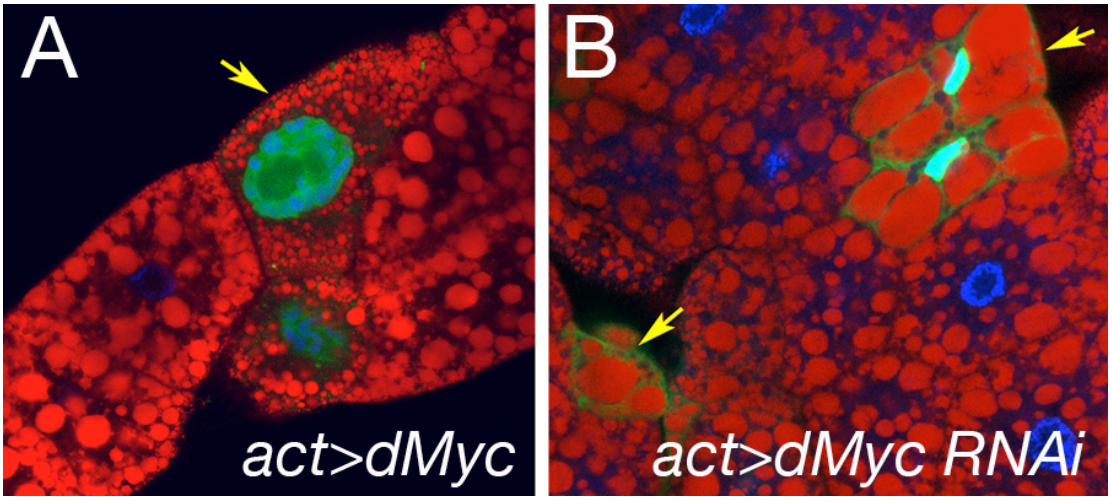
Supplementary Figure 7



Time course of trehalose concentrations in the hemolymph of *cg*-control or *cg-dMyc* larvae at different time points of starvation.

Measurement of trehalose from hemolymph (mM) over time. Error bars represent +/- SD from three independent experiments. 10 larvae were used at each time point per genotype. The amount of sugar decreased over time upon animal starvation. In *cg-dMyc* larvae the level of trehalose was significantly lower in all time points. **P* <0.05, ****P* <0.001, *P*-values were calculated from Student *t*-test compared to *cg-* control.

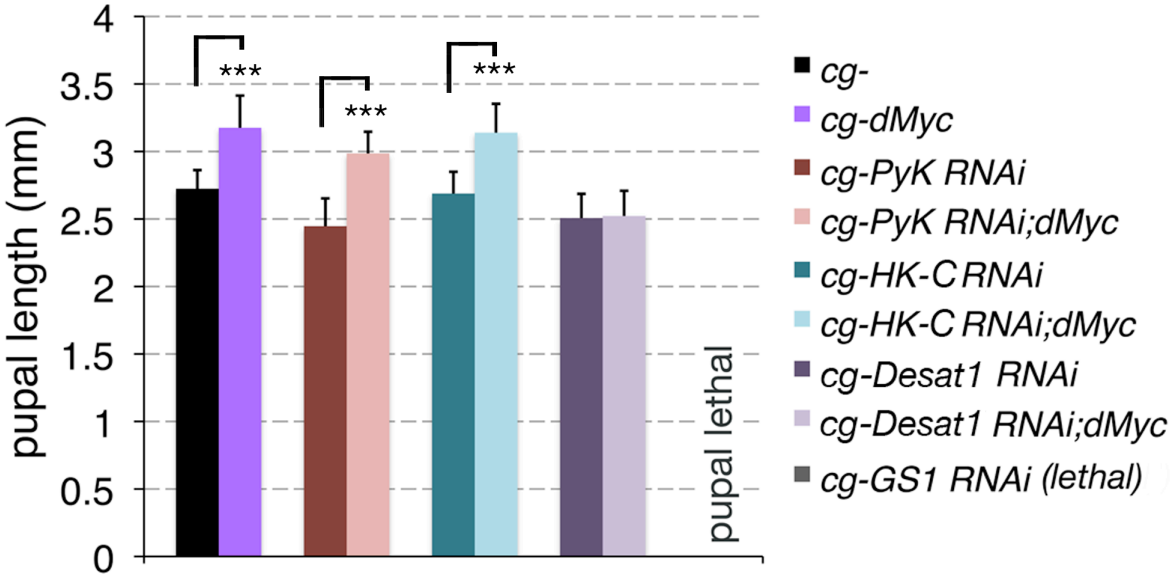
Supplementary Figure 8



Expression of dMyc in FB controls the size of lipid vesicles.

Actin flip-out clones expressing dMyc (A) or dMyc RNAi (B) in the FB. Arrows indicate the clones, which are also marked by coexpression of nGFP. Lipids are stained with Nile Red and cellular membranes with Alexa-488-Phalloidin, nuclei with DAPI.

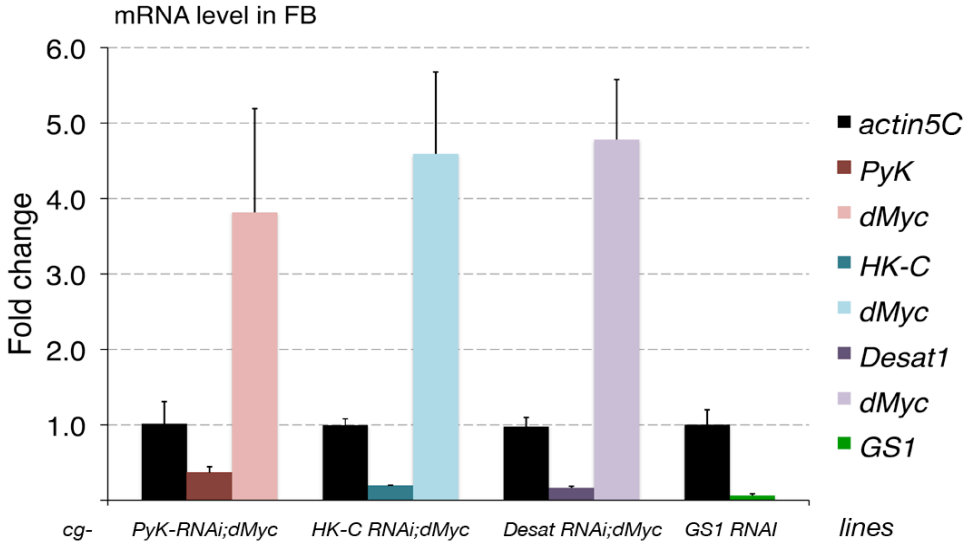
Supplementary Figure 9



Reduction of Desat1 contributes to dMyc regulation of organismal size.

Pupal length was measured from at least (n=20) of each genotype
**P*<0.05, ***P* <0.01, ****P* <0.001 *P*-values were calculated from Student *t*-test. SD from three independent experiments

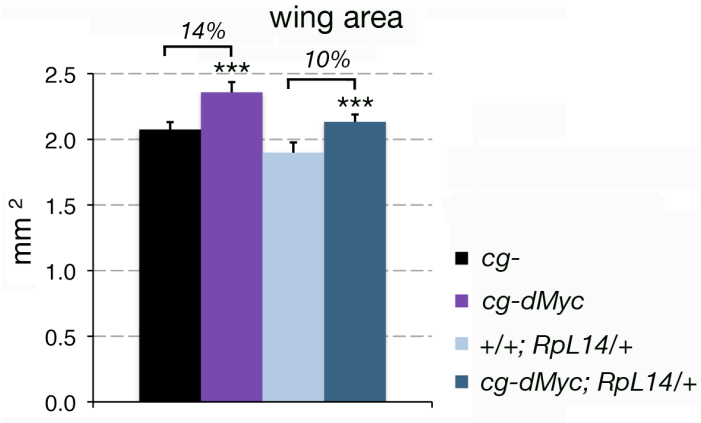
Supplementary Figure 10



Quantitative RT-PCR showing the level of expression for *PyK, HK-C, Desat1* and *dMyc* mRNA in FB from the indicated lines.

The *UAS-GS1RNAi line (Bloomington stock 40836)* showed pupal lethality when used in combination with the *cg-Gal4* driver and was not recombined with *UAS-dMyc* (see also Supplementary Figure 12). *mRNA* levels are expressed in fold change compared to *actin5C* used as a control.

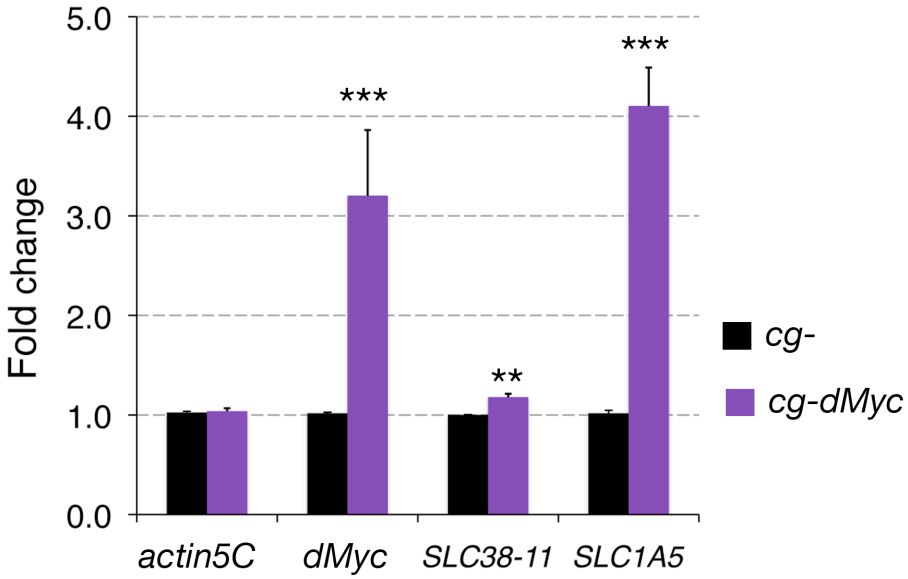
Supplementary Figure 11



Systemic growth induced by dMyc expression in the FB is only partially reduced in *RpL14* heterozygous mutant background.

Growth was measured by calculating the area of the wing from female flies of the indicated genotypes and comparing them to the area of the relative control. No significant difference was found between the size of *cg-* and +/+;*RPL14*/+ animals (Saeboe-Larssen et al., 1997). Error bars represent standard error of the mean (+/- SEM) at least n=15 animals for each genotype were used. ****P* <0.001, P-values were calculated from Student t-test compared to the relative control.

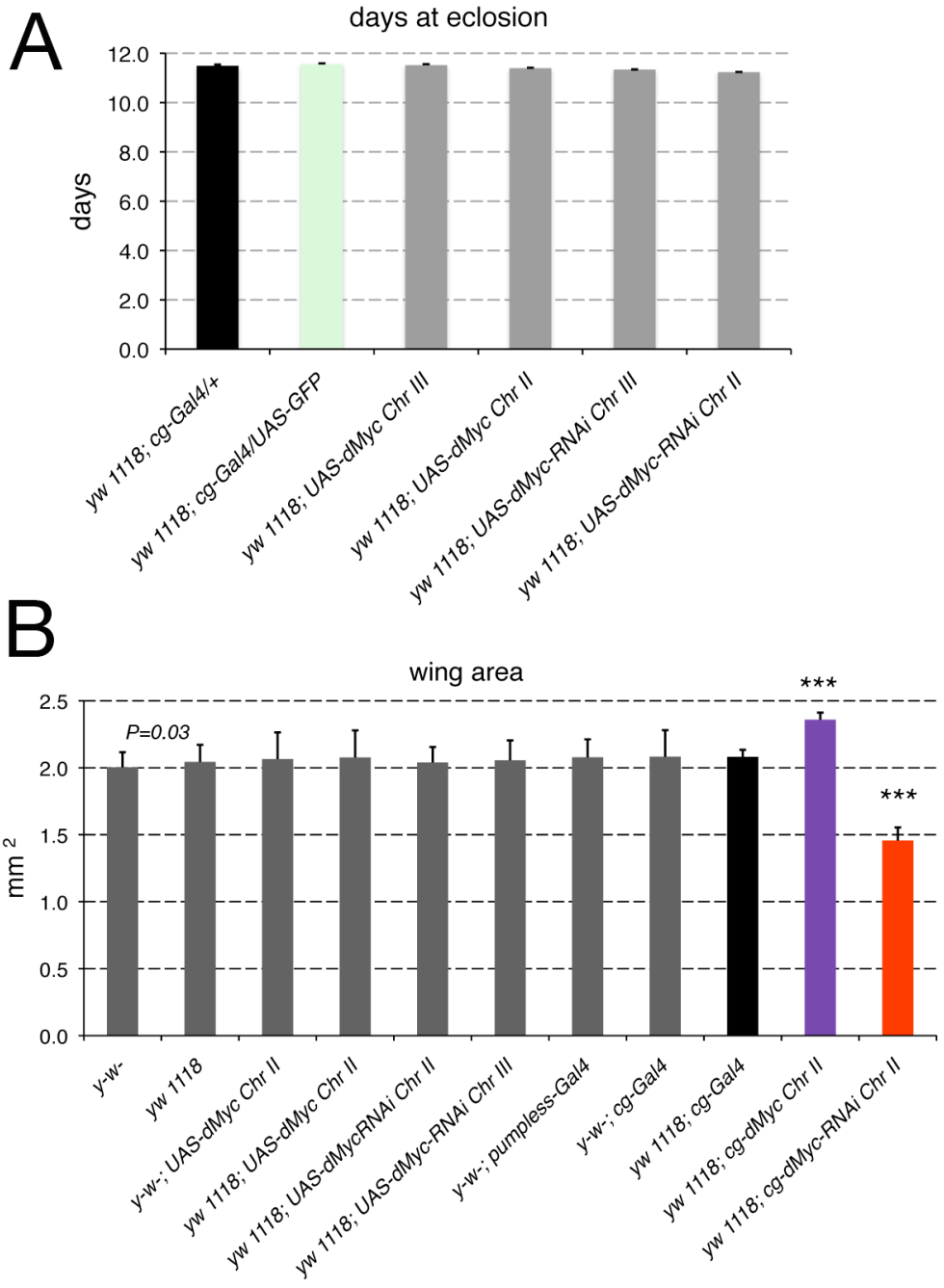
Supplementary Figure 12



Amino acid transporter *SCL38-11* and *SLC1A5 mRNA* expression in FB from third instar larvae expressing dMyc.

Quantitative RT-PCR of *SCL38-11 (CG13743)* and *SLC1A5 (CG*3297) *mRNAs*, *actin5C* was used as the internal control. **P <0.05, **P <0.01, ***P <0.001; P*-values were calculated from a Student *t*-test. Error bars represent +/- SD from three independent experiments.

Supplementary Figure 13



Eclosion time and body size (wing area) of the parental lines used in the growth experiments.

(A) Developmental timing of adult eclosion (days). Error bars represent +/- SD from four independent experiments. At least 10 animals for each genotype were analyzed. (B) Measurement of growth was calculated using the total area of the wing from female flies of the indicated genotypes. Error bars represent standard error of the mean (+/- SEM) at least n=15 animals for each genotype were used. ***P <0.001, P-values were calculated from a Student t-test compared to *cg-* control. All the lines used for our experiments were crossed into yw^{1118} background.

Primers used for quantitative RT-PCRs

actin5C-
F: CACACCAAATCTTACAAAATGTGTGA
R: AATCCGGCCTTGCACATG

Fibrillarin-
F: ATGCGGTACTTGTGTGGATG
R: ATGCGGTACTTGTGTGGATG

CG10798- diminutive (dm or dmyc)
F: CCGACGACCGGCTCTGATAG
R: GGCACGAGGGATTTGTGGGTA

CG2107- Carnitine palmitoyltransferase (CPTI) predicted
F: GAGCCATGATGCAGAGAACA
R: GGTAGCTGGAAGTGTCGCTC

CG3524- Fatty acid synthetase (FAS) predicted
F: GTTGGGAGCGTGGTCTGTAT
R: GGTTTAGGCCAGCGTCAATA

CG11198- Acetyl-CoA carboxylase (ACC) predicted
F: CCGGTAGCTCTGCATCATCT
R: AGACCACCACCAAAGTGTCC

CG9057- Lipid storage droplet 2 (LSP2)
F: CGAATGGGCTTTAACGGCGGC;
R: TCCAGAACGCAGACACACGCA

CG1086- Glucose transporter1 (Glut1)
F: TCTCGGATTCGTCGTCTTCT
R: TGAACGGCAAGAACGTGTAG

CG3001- Hexokinase A (HK-A)
F: GGCACCCAGGGCAACTCCAA
R: GCCTGTGGATGCGTGGACCG

CG8094- Hexokinase C (HK-C)
F: GGCACCCAGGGCAACTCCAA
R: GCCTGTGGATGCGTGGACCG

CG7070- Pyruvate Kinase (PyK)
F: CGAACAGATCGCCGGACGCA
R: TGACAGTAAAGGTGAACTCGCGG

CG17725- Phosphoenolpyruvate carboxykinase (PEPCK)
F: AATCTATGAGGCTCGGGACTGGAC;
R: ATCTTTGGCACCTGACCA

CG8167- Drosophila insulin-like peptide 2 (dilp2)
F: TCTGCAGTGAAAAGCTCAACGA
R: TCGGCACCGGGCATG

CG14167- Drosophila insulin-like peptide 3 (dilp3)
F: CCAGGCCACCATGAAGTTGT
R: TTGAAGTTCACGGGGTCCAA

CG33273- Drosophila insulin-like peptide 5 (dilp5)
F: GAGGCACCTTGGGCCTATTC
R: CATGTGGTGAGATTCGGAGCTAC

CG14049- Drosophila insulin-like peptide 6 (dilp6)
F: TGCGCTTCCCGAAACTG
R: CGGCCTCAGCGATGTG

CG3143- Forkhead box, sub-group O (FOXO)
F: GCAATGTCGAGGAGCTGC
R: GCCCACTGCTGCTGTTGA

CG8846- Thor or 4EBP
F: ACAGCCAACGGTGAAACAC
R: GCGTTGTTTGTATTTCGTTGC

CG5887- Desaturase1 (Desat1)
F: TAAGCAATCGGAAGACCACC
R: AAGATCAGCTTGAGCGGTGT

CG2718- Glutamine synthetase1 (GS1)
F: TGCGTCTGCTGCGTACTGGC
R: CGGCGTTTCCAGGTTGCGGTA

CG42708- Glutaminase (GLS)
F: CGAGACGGGTTCTTCGGCGG
R: CGTGCAGATGCTCAGGCCCC

CG13743- SLC38-11
F: CAGAGCCAGCTTCATTAGCC
R: CGAAATGTGGGTGACCTTCT

CG3297- SLC1A5/minidiscs
F: ATAGGCACTGCATTTGGTCC
R: CTTCGTGAGTCCCAAGGGAG

References:

Geminard, C., Rulifson, E.J., Leopold, P., 2009. Remote control of insulin secretion by fat cells in Drosophila. Cell Metab 10, 199-207.

Saeboe-Larssen, S., Urbanczyk Mohebi, B., Lambertsson, A., 1997. The Drosophila ribosomal protein L14-encoding gene, identified by a novel Minute mutation in a dense cluster of previously undescribed genes in cytogenetic region 66D. Mol Gen Genet 255, 141-151.

APPENDIX I

Analysis of significance

In the experiments showed in 4K, larvae of the followed genotype were used: cg=cg-control (black) cg-myc6=cg-dMyc; Chr II (purple), and cg-137= cg-dMyc-RNAi; Chr II (red).

For three populations of larvae: cg, cg-myc6 and cg-137, denoted henceforth 1, 2 and 3, we are given their initial populations ($n_1 = 444$, $n_2 = 381$ and $n_3 = 418$ individuals respectively) and the number of survivors at the end of each day up to $t = 7$, summarized in the following table:

day	1	2	3
0	444	381	418
1	345	321	319
2	279	274	236
3	232	238	164
4	169	210	90
5	66	113	35
6	12	24	0
7	0	0	0

and displayed in the three curves of the figure. We are asked to estimate the significance of the difference between these three visually distinct curves.

To this end, we introduce the lifespan T_i^k, $i = 1, \ldots, n(k)$ of each individual larva i in population k, rounded to the first day by the end of which it is no longer alive. We compute unbiased estimators in each population for the mean lifespan and its standard deviation:

$$\bar{T}_k = \frac{1}{n_k} \sum_{i=1}^{n_k} T_i^k,$$

$$\sigma_k = \sqrt{\frac{1}{n_k - 1} \sum_{i=1}^{n_k} \left(T_i^k - \bar{T}_k\right)^2}.$$

Then, for each pair of populations, we quantify their difference through the two-sample z-test

$$z_{ab} = \frac{\bar{T}_a - \bar{T}_b}{\sqrt{\frac{(\sigma_a)^2}{n_a} + \frac{(\sigma_2)^2}{n_b}}} \tag{1}$$

with two-tailed p-value

$$p_{ab} = 2\Phi(-|z_{ab}|), \tag{2}$$

where Φ is the normal cumulative distribution function.

The results are:

$$\mu_{1,2,3} = 3.492,\ 4.105,\ 3.029 \quad \text{(in days)},$$

$$\sigma_{1,2,3} = 1.854,\ 1.945, 1.627$$

and

$$p_{12}, p_{13}, p_{23} = 4 \times 10^{-6},\ 9 \times 10^{-5} \text{ and } 0,$$

the last one smaller than machine epsilon. We conclude that the three populations are significantly different.