Supplemental Materials and Methods

DNA Cloning and transgenic flies

dMaf1 RNAi was amplified from cDNA (LD17963) using the following primers: ACAAACAAGTGGCATCACGA and GTTTCACCGGCGTAAAGAGA and cloned into pUAST. A second dMaf1 RNAi fragment, targeting an overlapping, but smaller, region of dMaf1 was amplified using the following primers: ACAAACAAGTGGCATCACGA and CATAACCCACTGCAATGACG, and cloned into pUAST. dMaf1 overexpression construct was amplified from LD17963 with flanking sequences for insertion into the pTHW (5' HA tag) vector from the Drosophila Gateway Vector collection plasmids (T. Murphy, Carnegie Institution of Washington) using the following primers: GGGGACAAGTTTGTACAAAAAAGCAGGCTCCGCGGCCGCCCCCTTCACCATGAAGT TACTTGAAAGTTCACGT and GGGGACCACTTTGTACAAGAAAGCTGG GTCTCAGTACACTTCTTCTTCCATCGAAAAGGG..

Transgenic flies were created by injection of *w118* Drosophila embryos with DNA from P-element-based transformation vectors by Best Gene Inc. (Chino Hills, CA) Lines containing single P-element insertions were made homozygous and crossed to the indicated drivers (*UAS-dMaf1 RNAi, UAS-Maf1*).

Supplemental Figure Legends

Figure S1. Loss of Brf activity has no effects on the levels of Pol I transcription. qRT-PCR of RNA extracted from control (*yw*) and *brf* mutant larvae ($brf^{EY02964}$) showed no change in 5S rRNA and pre-rRNA (Pol I-dependent transcript) levels and a significant decrease in 7SL RNA levels at 48 hr AEL ($p<0.05$, Student's *t*-test). Error bars indicate SEM.

Figure S2. Loss of Brf leads to Cell Autonomous Decreases in Cell Growth in Different Tissues. (A-E) A *brf-RNAi* transgene was ubiquitously expressed using the *da-GAL4* driver (*da>brf RNAi*) and compared to control (*da*>+) larvae. (A) Immunoblotting of larval protein extracts demonstrates a specific reduction in Brf protein levels following expression of the *brf*

RNAi transgene. Levels of β-tubulin were analyzed to ensure equal protein loading. (B) This causes a significant decrease in Pol III-dependent transcript levels, by qRT-PCR ($p<0.05$, Student's *t*-test). Each experiment was performed a minimum of three times, with n=40 per genotype per experiment. Error bars indicate SEM. (C) Reducing Brf levels caused a growth arrest in *Drosophila* larvae. (D, E) The expression of *brf RNAi* in the salivary gland (*ptc-brf RNAi*) significantly reduced gland size when compared to control (*ptc*>+) animals. The salivary gland is indicated by yellow arrows. White, DAPI staining. (F) Cells expressing a *brf RNAi* transgene were created within the *Drosophila* fat body using the *flp-out* system. *Brf RNAi* cells (GFP-positive cells) were smaller than surrounding control (non-GFP) cells. (G) The small cell phenotype of *brf RNAi*-expressing cells was rescued by co-expressing a *UAS-brf* transgene (GFP-positive cells). Scale bar, 100µm. Green, GFP and blue, DAPI staining. In all experiments 120 hr AEL larvae were analyzed.

Figure S3. (A, B) *Brf* mutant clones are rescued in a *Minute* background. Clones (marked by absence of GFP) of either wild-type (A), or *brf* mutant (B) cells were induced in developing wing discs. GFP-marked cells are heterozygous for a dominant *Minute (M/+)* allele of ribosomal protein S3. Genotypes: (A) *ywhsflp*^{122}*/+; +; FRT82B, RpS3, ub-GFP/FRT82B* , (B) *ywhsflp*^{122}*/+; +; FRT82B, RpS3, ub-GFP/FRT 82B, brf*^{EY02964}. (C-E) *Brf* mutant clones are rescued in a *p35* background. The MARCM system was used to generate wing disc clones (marked by GFP) comprising either wild-type cells (C), *brf* mutant cells (D) or *brf* mutant cells expressing a *UAS-p35* transgene (E) Clones were induced 48h AEL and analyzed 120hr AEL. Green, GFP; blue, DAPI staining. Genotypes: *ywhsflp*^{122}*/+; tubGAL*^{4}*, UAS-GFP/+; FRT82B, tubGAL*^{80}*/FRT82B* , (D) *ywhsflp*^{122}*/+; tubGAL*^{4}*, UAS-GFP/+; FRT82B, tubGAL*^{80}*/FRT82B, brf*^{EY02964}, (E) *ywhsflp*^{122}*/+; tubGAL*^{4}*, UAS-GFP/UAS-p35; FRT82B, tubGAL*^{80}*/FRT82B, brf*^{EY02964}. Scale bar, 50 µm.

Figure S4. (A) Fat-body-specific reduction in Brf levels (*cg*>*brf RNAi*) delays larval development when compared to controls (*cg*>+) 122 and 144 hr AEL. (B) Phospho-Akt levels were specifically reduced in whole *brf* homozygous mutant (*brf*^{EY02964}) larvae, when analyzed by immunoblotting. Both total Akt and β-tubulin protein levels remained constant. (C) *InR* mRNA levels were significantly increased ($p<0.05$, Student's *t*-test) in whole *brf* mutant larvae. (D) Peripheral tissues of larvae with Brf silenced in the fat body (*r4*>*brf RNAi*) showed no change in

dilp 2, *3* or *5* mRNA expression levels ($p<0.05$, Student's *t*-test). (E) In *brf* mutant larvae *dilp5* mRNA levels were significantly decreased ($p<0.05$, Student's *t*-test) while *dilp2 and 3* mRNAs were unchanged ($p<0.05$, Student's *t*-test). (F) Decreasing Brf levels specifically in the fat body using the *cg-GAL4* driver significantly increased *dInR* mRNA levels in peripheral tissues of these animals when compared to controls ($p<0.05$, Student's *t*-test). (G) Peripheral tissues of larvae with Brf silenced in the fat body using the *cg-GAL4* driver showed a decrease in *dilp 2* and *5* mRNA expression levels ($p<0.05$, Student's *t*-test) but no change in *dilp3* mRNA expression levels. For qRT-PCR each experiment was performed a minimum of three times, with at least n=32 per genotype per experiment. All qRT-PCR error bars represent SEM. For B-H larvae were analyzed at 96 hr AEL.

Figure S5. (A, B) Fat bodies were dissected from larvae and stained with Nile Red to visualize lipid droplets. (A) DIC and (B) Nile Red images of fat bodies isolated from fed larvae with fat body specific reduction in Brf levels (*cg>brf RNAi*) are shown. Scale bar 100 µm.

Figure S6. Pol III-dependent transcript levels decrease following inhibition of TOR by rapamycin treatment. qRT-PCR of RNA extracted from *Drosophila* S2 cells treated with either DMSO or rapamycin demonstrates a specific decrease in Pol III-dependent transcripts following TOR inhibition. *Nop60B* and *dMyc* mRNA's were measured as positive and negative controls, respectively, for the rapamycin treatment. qRT-PCR data was corrected for *Rp49* mRNA levels. Each experiment was performed a minimum of three times with n=3 per treatment per experiment. Error bars indicate SEM.

Figure S7. (A-F) *brf*, *tsc1* or *tsc1,brf* double mutant clones were induced in the posterior compartment wing imaginal discs in both the presence and absence of *p35* expression. Mutant clones are non-GFP; wild-type sister clones, 2xGFP-positive. Genotypes: panels (A) *en-GAL4, UAS-flp/+; FRT82B, ub-GFP*, (B) *en-GAL4, UAS-flp/+; FRT82B, ub-GFP/FRT82B, tsc1*^{Q87X}, (C) *en-GAL4, UAS-flp/+; FRT82B, ub-GFP/FRT82B, brf*^{EY02964}, (D) *en-GAL4, UAS-flp/+; FRT82B, ub-GFP/ FRT82B, brf*^{EY02964}, *tsc1*^{Q87X}*, (E) en-GAL4, UAS-flp/UAS-p35; FRT82B, ub-GFP/FRT82B, brf*^{EY02964}*, (F) en-GAL4, UAS-flp/UAS-p35; FRT82B, ub-GFP/ FRT82B, brf*^{EY02964}, *tsc1*^{Q87X} . Scale bar, 50 µm.

Figure S8. Brf protein levels do not change in response to starvation. Immunoblot of whole larval extracts prepared from fed, 24 hr and 48 hr starved larvae using antibodies specific to Brf and β-tubulin shows no change in Brf protein levels following starvation.

Figure S9. (A-C) qRT-PCR analyses of RNA extracted from whole larvae following the ubiquitous expression of a *dMaf1 RNAi* transgene (*da*>*dMaf1 RNAi*) compared to control (*da*>+) larvae. In this case, the *dMaf1* RNAi trasngene targets and overlapping but smaller region of dMaf1 than the transgene used in Figure 6. TOR activity was modulated by starving the larvae of dietary protein. tRNA levels were significantly elevated following loss of *dMaf1* (*da*>*dMaf1 RNAi*) compared to controls (*da*>+) when TOR activity was high under normal fed conditions ($p<0.05$, Student's *t*-test). tRNA levels remain elevated in *da*>*dMaf1 RNAi* animals even under starved conditions when control tRNA levels are normally reduced ($p<0.05$, Student's *t*-test). (D, E) Overexpression of a d*Maf1* transgene can restore the elevated tRNA levels of *da*>*dMaf1 RNAi* animals to control (*da*>+) levels. Overexpression of dMaf1 alone had no effect on tRNA levels. (F, G) Ubiquitous expression of a *dMaf1 RNAi* transgene (*da*>*dMaf1 RNAi*) has no effect on levels of the Pol III machinery (Brf, Trf or RPIII128) or levels of pre-rRNA or RP49 mRNA, compared to control (*da*>+) larvae. (H) $tRNA_i^{Met}$ levels are significantly elevated following the ubiquitous expression of either *dMaf1 RNAi* (*da*>*dMaf1 RNAi*) or *tsc1 RNAi* (*da*>*tsc1 RNAi*). $tRNA_i^{Met}$ levels are elevated even further upon expression of both *dMaf1 RNAi* and *tsc1 RNAi* (*da*>*dMaf1 RNAi, tsc1 RNAi*) at 72 hr AEL. For qRT-PCR error bars indicate SEM.

Figure S10, relating to Figure 7. *PPAN* mRNA (a dMyc target gene) was measured as a control for dMyc overexpression under conditions of fed and starved (A) and DMSO and rapamycin treatment (B).

Figure S1

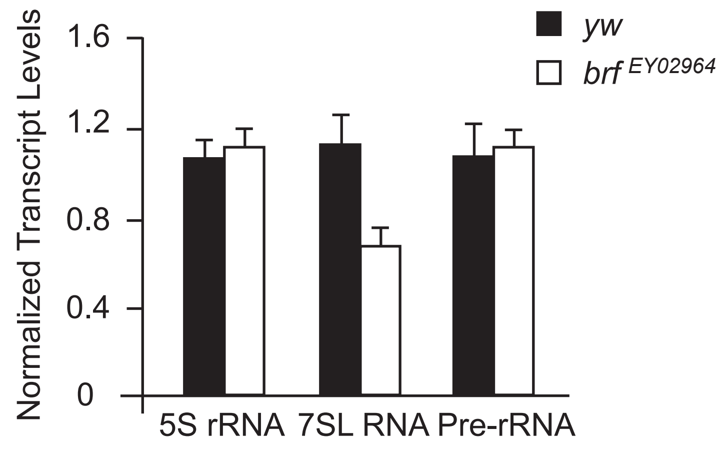
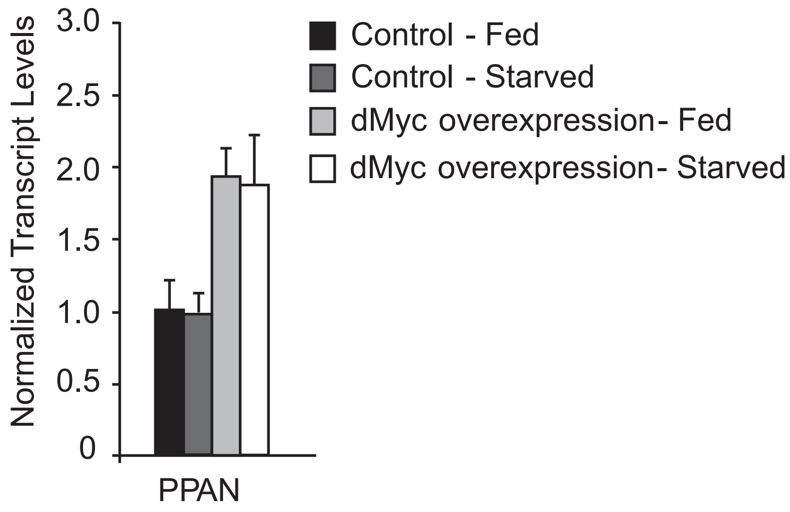


Figure S10



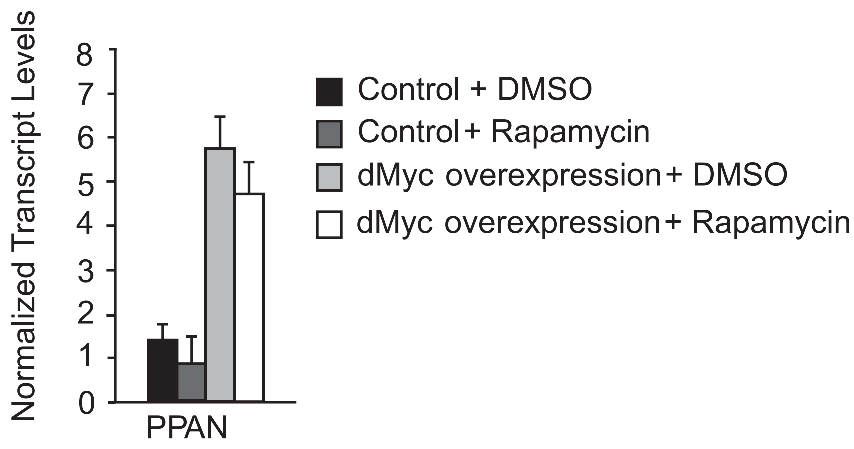
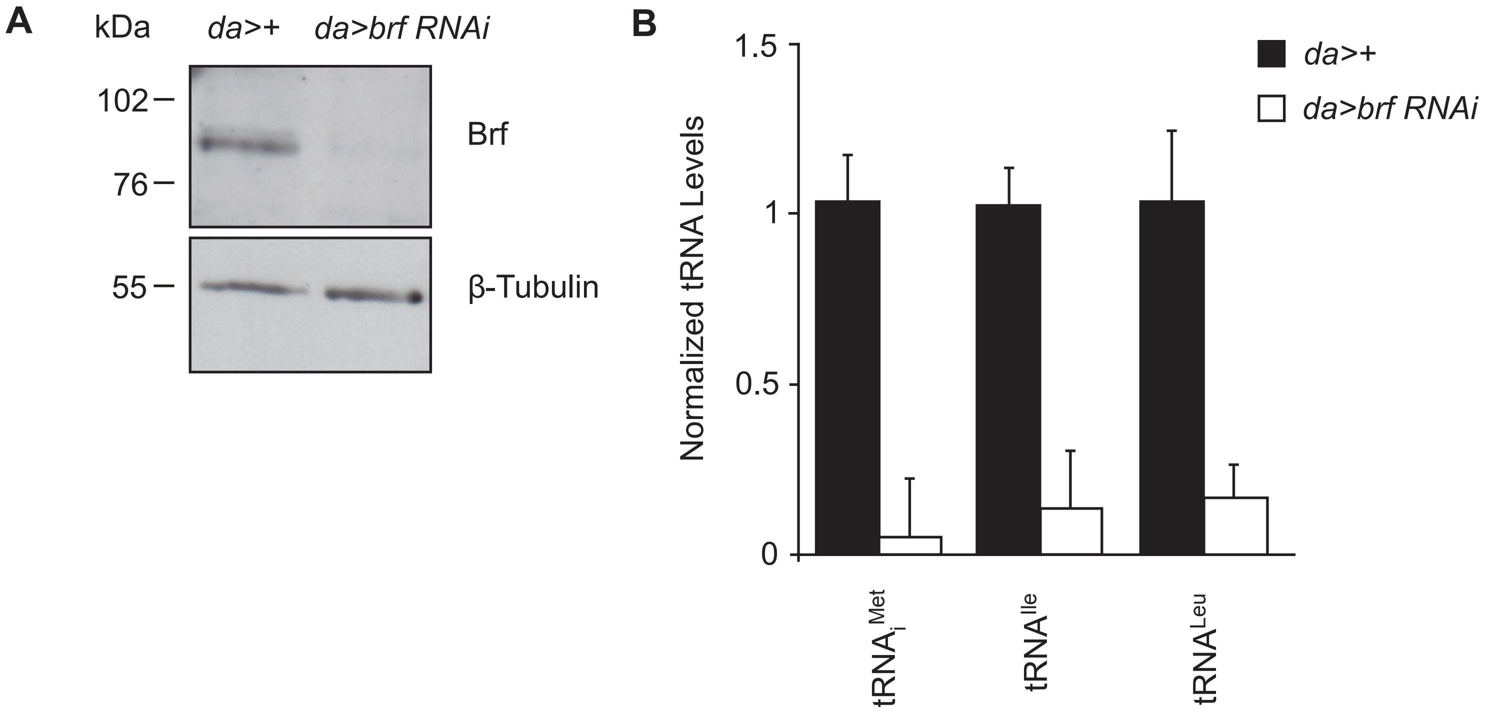
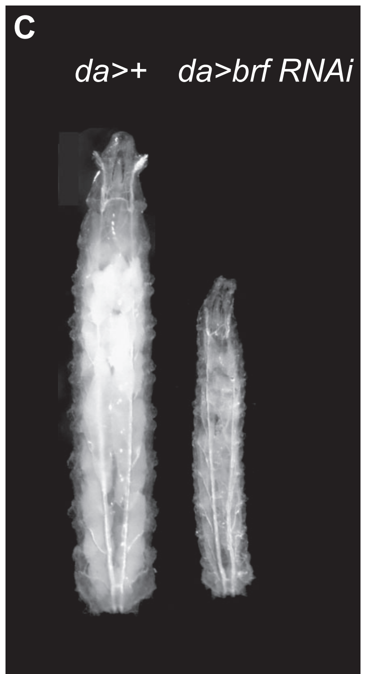
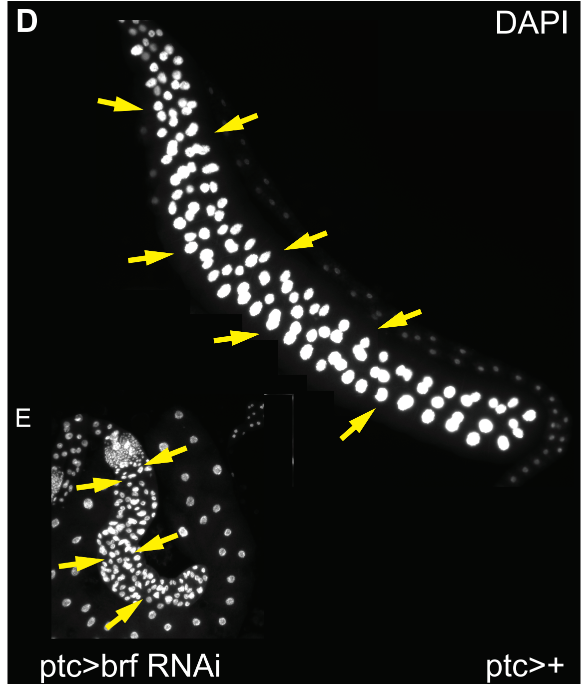
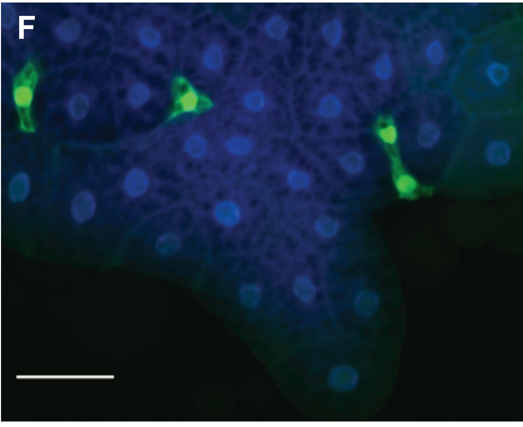


Figure S2









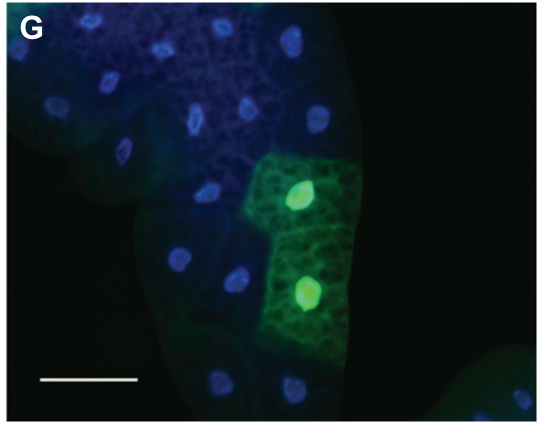
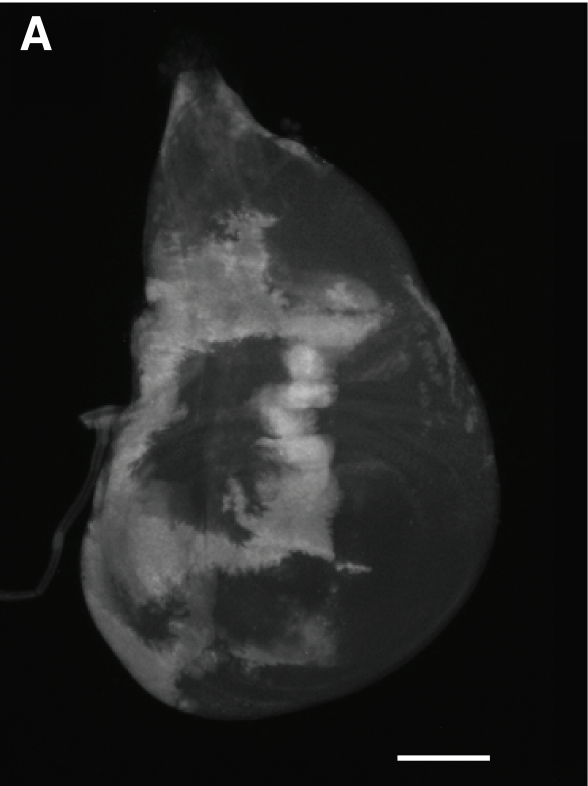
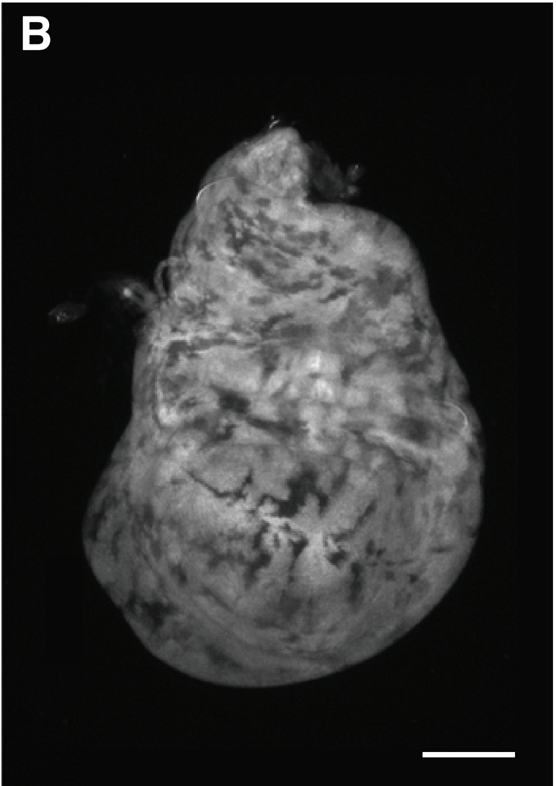


Figure S3



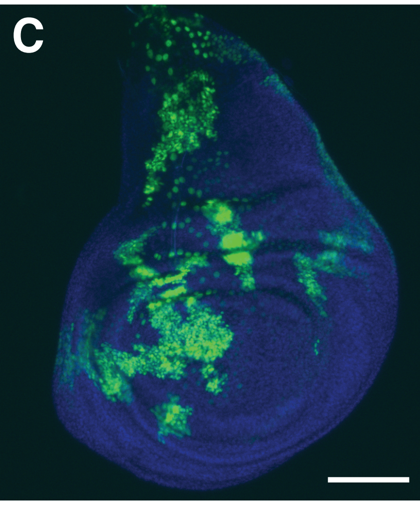


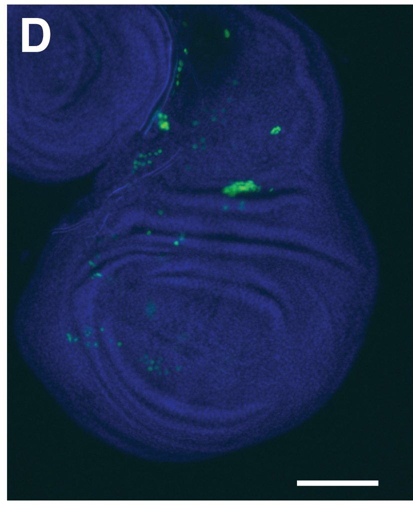
+/+

M/+

brf

M/+ *brf*/+





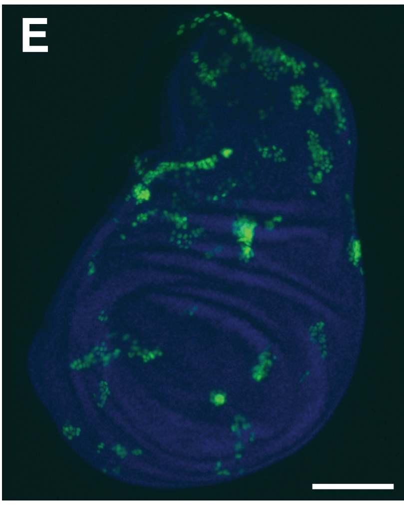
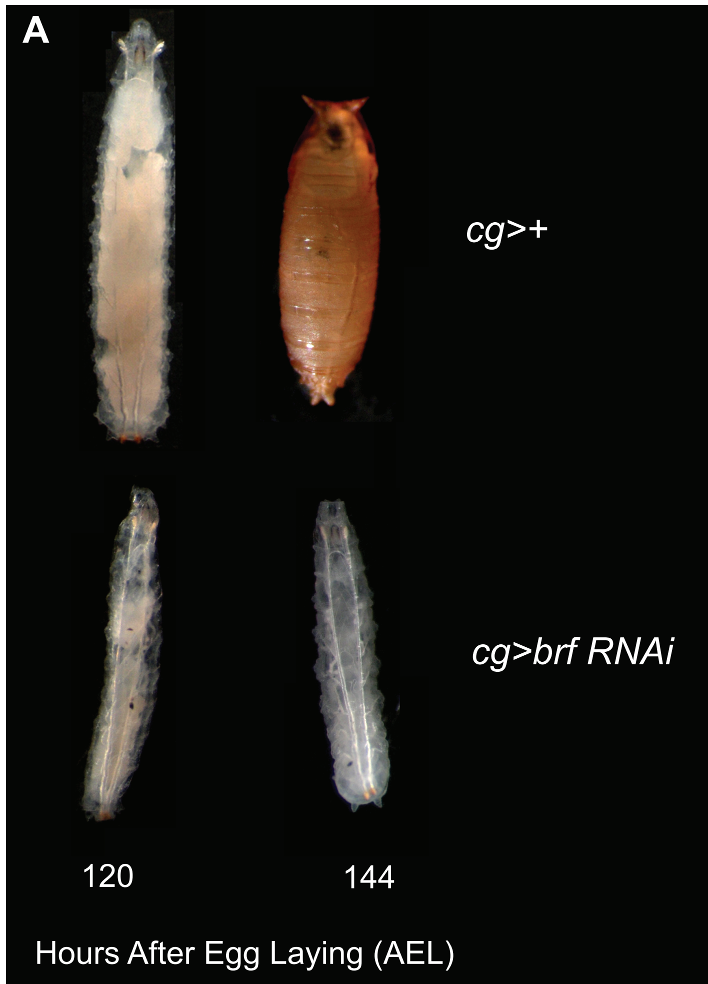
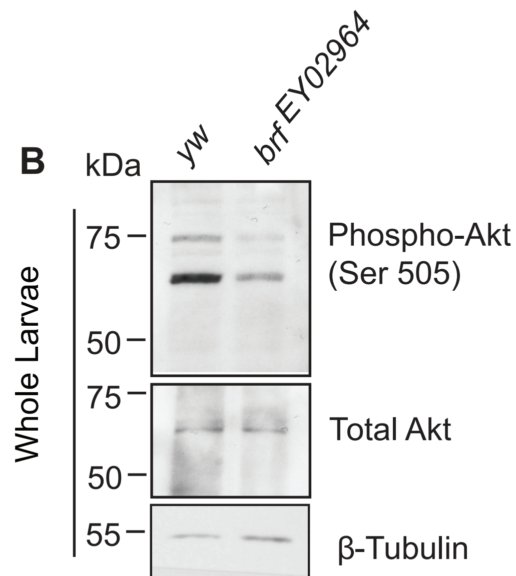
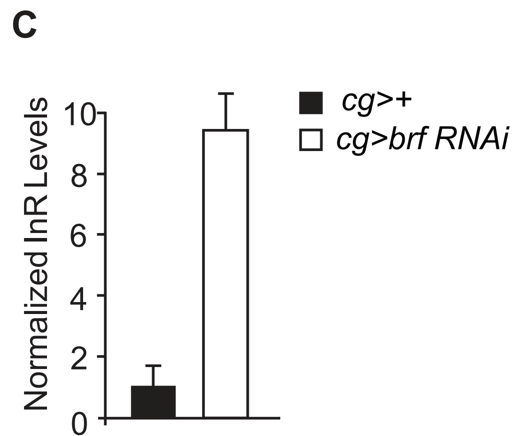
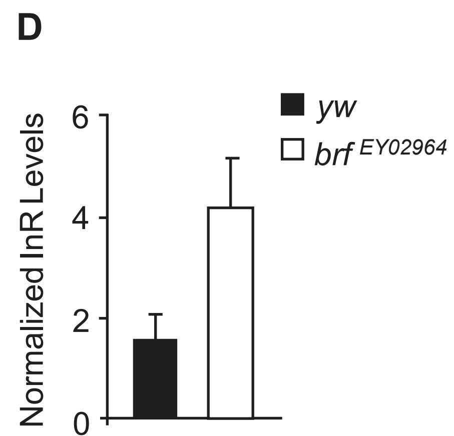


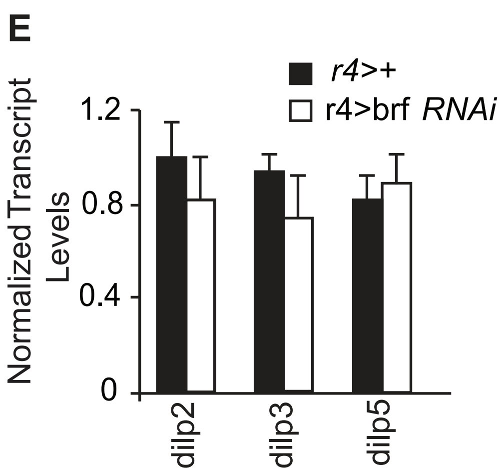
Figure S4

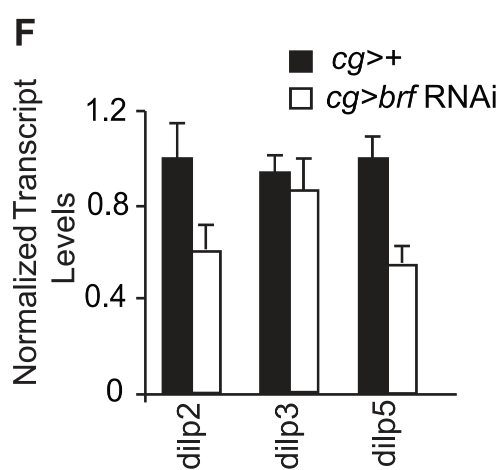












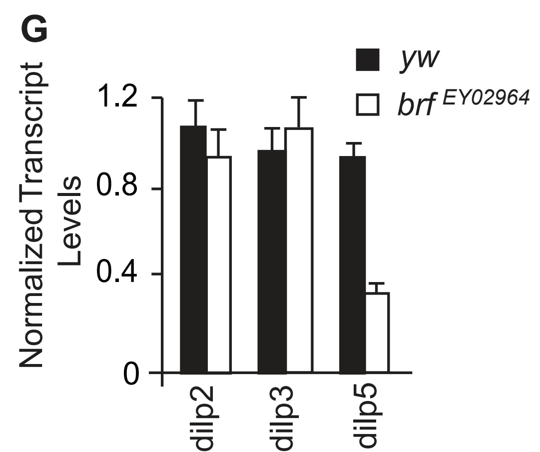


Figure S6

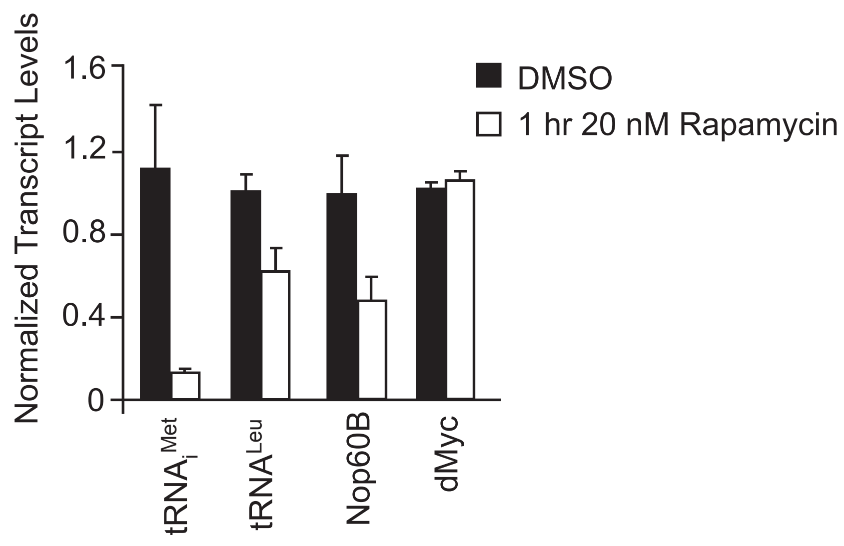


Figure S7

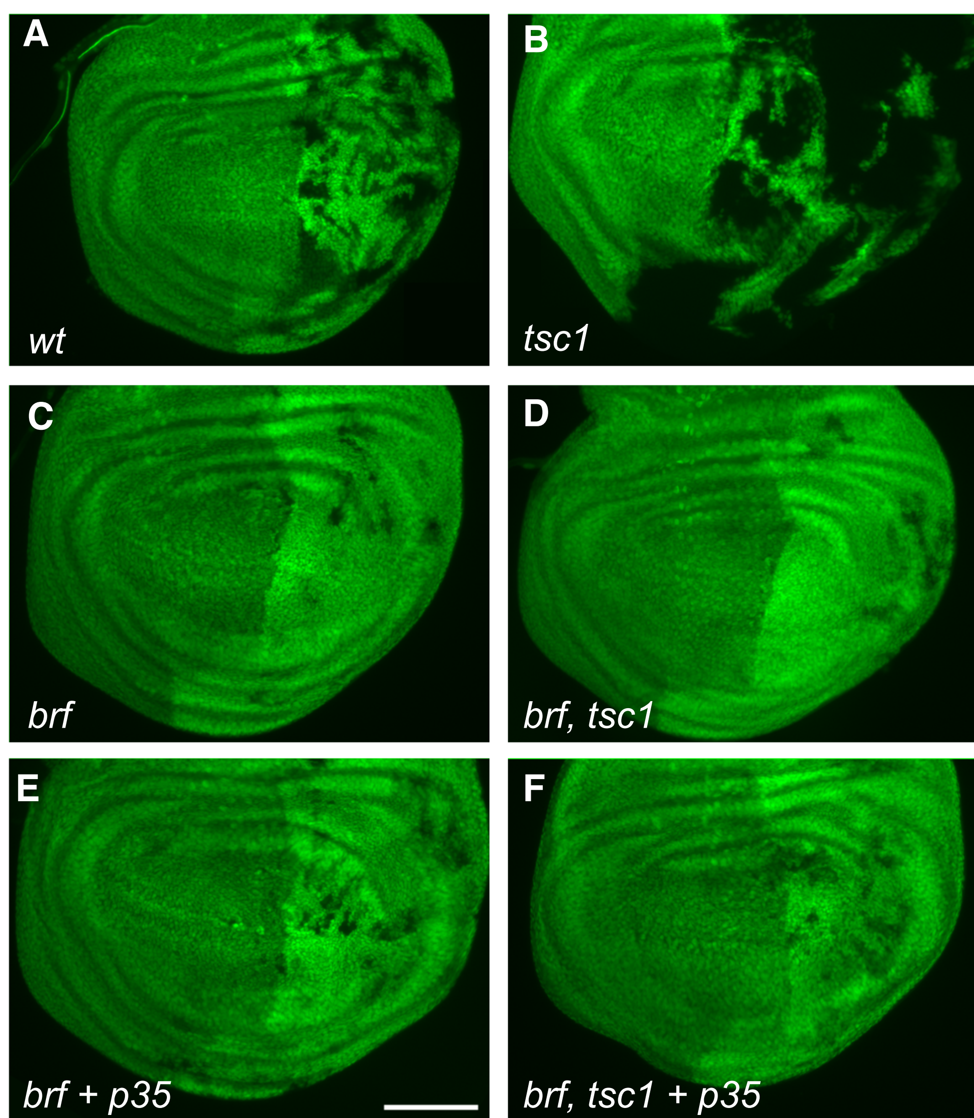


Figure S8

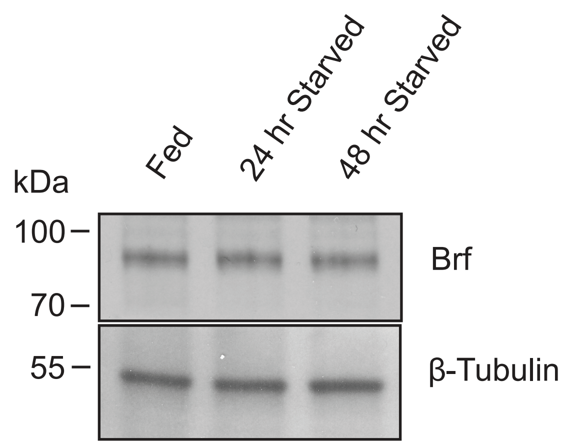


Figure S9