Supplemental Materials and Methods

Drosophila Microarray Analysis

Hybridization was performed by the Functional Genomics Facility at the University of Chicago, and raw data analysis was performed by AI and NLB at the University of Pompeu Fabra.

RNA Isolation, Hybridization, Normalization, and Differential Expression (DE) analysis:

RNA was harvested from biological triplicates of third instar larval eye imaginal discs from animals with the following genotypes:

GMR-Gal4/+  
GMR-Gal4/UAS-miR-11  
GMR-Gal4/+; UAS-dE2F1,UAS-dDP/+  
GMR-Gal4/UAS-miR-11; UAS-dE2F1, UAS-dDP/+  

RNA was extracted from 40-50 eye disc pairs per sample using the TriZol RNA extraction reagent and protocol (Invitrogen). Further purification was performed using RNeasy purification columns according to manufacturer’s protocol (Qiagen). Three replicates for each condition were independently isolated and processed. RNA quality was assessed with the Agilent 2100 Bioanalyzer. In accordance with the Affymetrix protocol (Affymetrix expression manual), all samples were processed and a total of 15ug of fragmented and labeled cRNA were hybridized to the Affymetrix GeneChip arrays (Drosophila genome 385K 2.0). The Chips were then washed and stained using an Affymetrix Fluidics Station 450 and Fluorescence was detected using the Affymetrix GS3000. For each genotype there were three biological replicates, and raw data were background corrected and normalized using the R-Bioconductor (Gentleman et al, 2004) package “affy” (Gautier et al, 2004; Irizarry et al, 2003). The Bioconductor package “arrayQualityMetrics” (Kauffmann et al, 2009) was used to determine the quality and variability of the microarray experiment.
To determine the difference in gene expression relative to wild type (wt), differential expression (DE) analysis was calculated with the Bioconductor package RankProd (Hong et al, 2006). For all comparisons, a gene was considered differentially expressed if it had a p-value ≤ 0.01 irrespective of log2 fold change (Log2FC). To annotate probes with the corresponding Ensembl gene ID, gene symbol and description, we used the Probe annotations to Ensembl gene ID, gene symbol and description, we use the annotation description from Biomart, Ensembl v.55 (Drosophila melanogaster genes; BDGP 5.4) (Hubbard et al, 2007), Flybase (Drysdale, 2008), and affymetrix website (http://www.affymetrix.com/support/technical/annotationfilesmain.affx). Where two or more more probes corresponded to same Ensembl gene, highest absolute value (maximizing) was considered for DE.

Enrichment Analysis (EA):

Functional annotation of genes is based on Gene Ontology (GO) (Gene Ontology Consortium, 2006) (http://www.geneontology.org). EA (overrepresentation) was performed using GiTools (http://www.gitools.org) (Perez-Llamas and Lopez-Bigas 2011) to identify GO categories that might be enriched by the up- or down- regulated genes. To determine the statistical significance (p-value), we used binomial distribution and p-value calculated as:

\[
P(X \geq x) = \sum_{i=0}^{x} \binom{n}{i} p^i (1 - p)^{n-i}
\]

\[
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\]

Where: \( n = \) total no. of genes in the category, \( x = \) number of differently expressed genes in the
category, and \( p \) = frequency of upregulated or downregulated genes

The resulting p-values were adjusted for multiple testing using the Benjamin and Hochberg's method of False Discovery Rate (FDR) (Benjamini and Hochberg, 1995).

References


