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# Supporting Online Material for

## Analysis of *Drosophila* Segmentation Network Identifies a JNK Pathway Factor Overexpressed in Kidney Cancer

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**This PDF file includes:**

## Supplemental figures

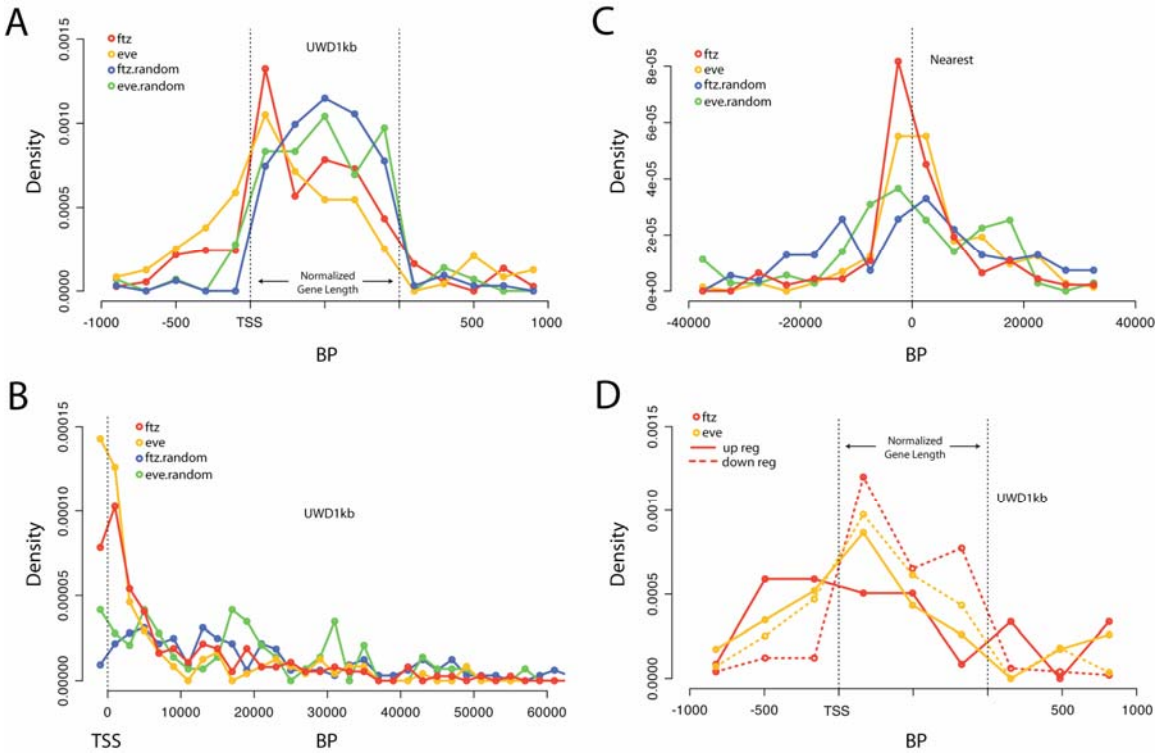


**fig. S1**. Distribution of ChIP-chip probe locations relative to direct target genes. All panels are plotted as histograms, distance plotted along x-axis, density of probes along y-axis. Probe-to-gene data are depicted for Ftz binding probes to differentially expressed genes in *ftz* mutants (red), Eve binding probes to differentially expressed genes in *eve* mutants (orange), permuted probes to differentially expressed genes in *ftz* mutants (blue) and differentially expressed genes in *eve* mutants (green). (A) Distribution of probe locations relative to target genes identified under the UWD1kb* parameter set. Distances are plotted along the x-axis as follows: -1000 to TSS and 0 to +1000 represent real 5' and 3' coordinates, respectively. Coordinates from TSS to 0 represent the normalized position within the length of the gene, scaled for display to 1000bp. Note the enrichment (relative to random probes) of Eve and Ftz binding probe locations surrounding the TSS and the approximately normal distribution of permuted probes. (B) Data from (A) are plotted in absolute distance from the TSS. Again note the increased density of eve and

ftz probes surrounding the TSS. (C) Distribution of probe locations relative to target genes identified under the nearest50kb** parameter set. X-axis depicts absolute distance from the TSS. Again note the enrichment at the TSS and the decreased variance of the experimental distributions relative to permuted probes. (D) Distribution of probe locations relative to up or down regulated direct target genes***. Distances are plotted as in (A). Solid lines represent distributions relative to up regulated genes (mutant > wt), dashed lines represent distributions relative to down regulated genes.

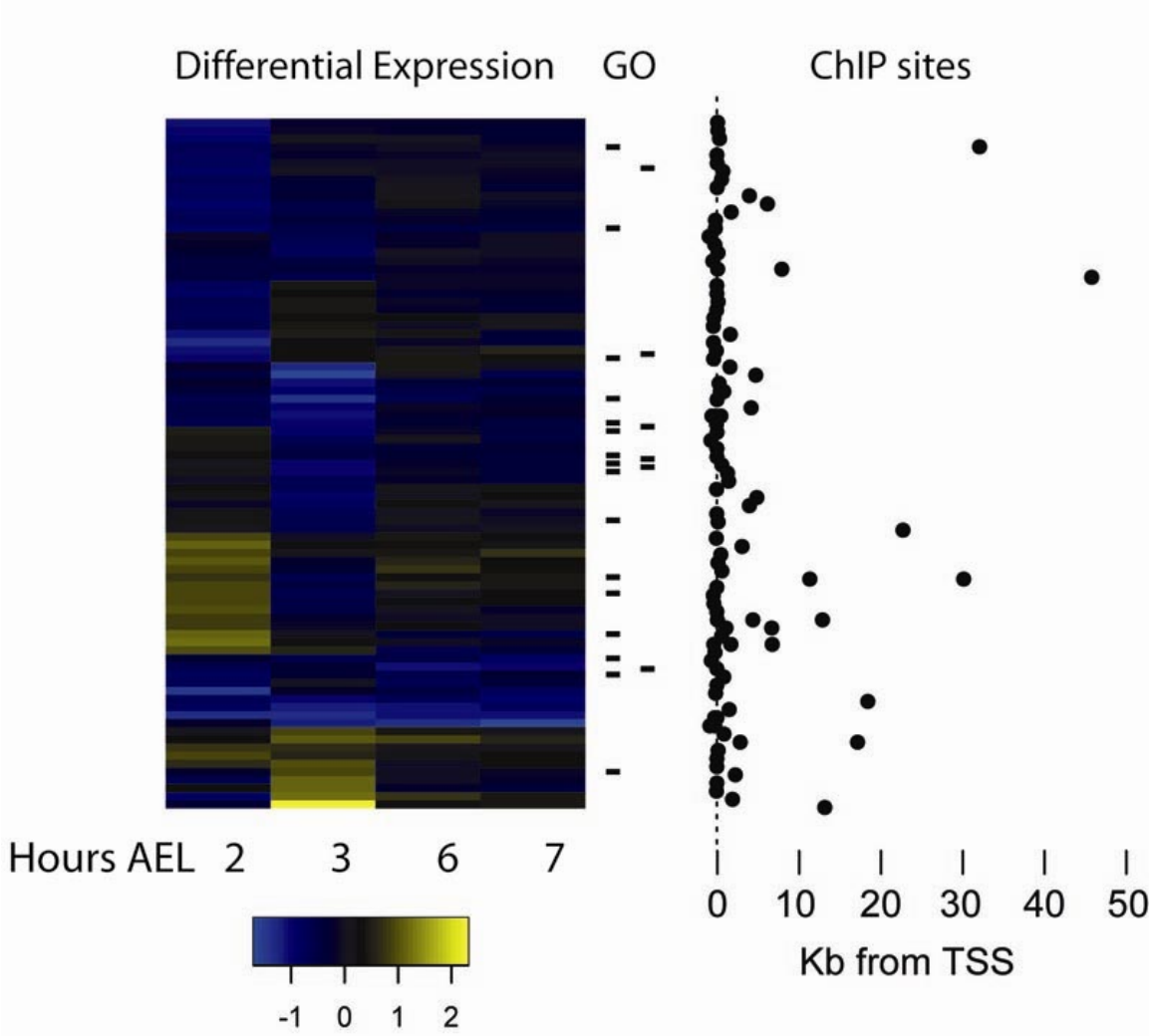


fig. S2. Identification of direct targets of Eve. Eve targets. Heatmap at left depicts $log_2$-fold change in gene expression, mutant vs. wild type, for putative direct targets of Eve. Columns represent time points in hours after egg laying (AEL). Rows depict individual genes, sorted by hierarchical clustering. Genes involved in regulation of development (left) or transcriptional regulation (right) from gene ontology (GO) annotations are marked in the center panel. Locations of binding sites relative to the transcription start site of each gene are represented at right. To confirm the ChIP-chip results, we performed ChIP-qPCR on 22 predicted binding sites near 19 different target genes; 86% of targets were validated by this method (table S7).

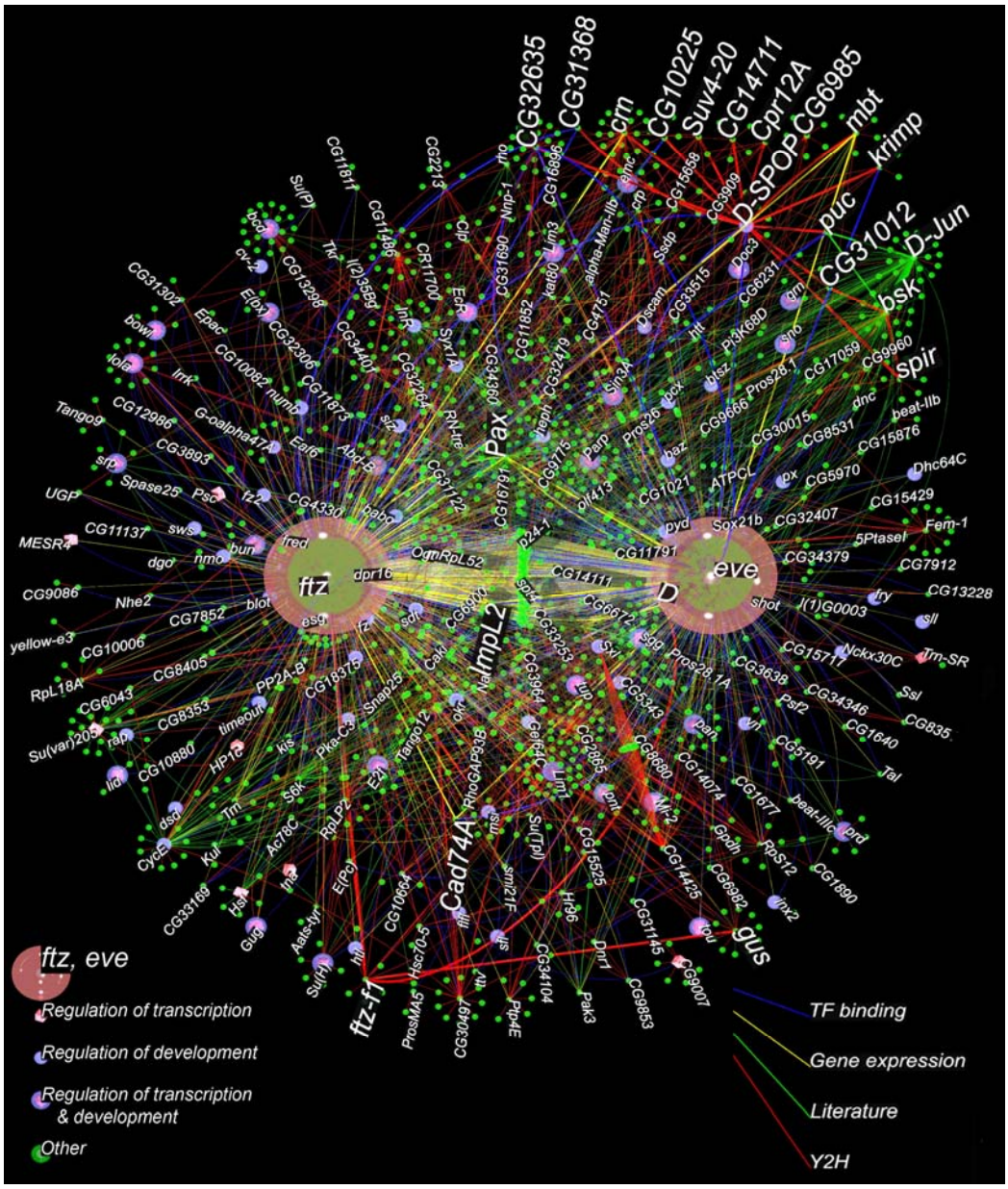


fig. S3. *Drosophila* segmentation network. Full network containing 4084 genes/proteins and 6648 interactions. Genes/proteins, which are direct targets for Ftz and Eve or appear in the subnetworks, are labeled.

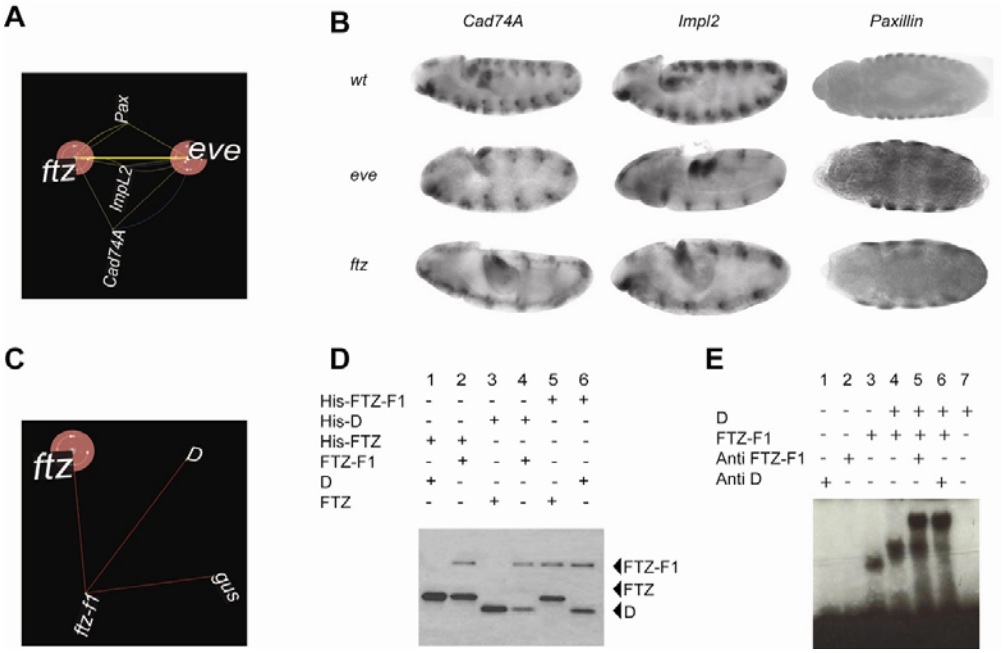


fig. S4. Experimental confirmation of network predictions. (A) Sub-network from Fig 2A shows that cell-adhesion and/or focal-adhesion genes *Cad74a*, *Impl2*, and *Paxillin* are target genes of Eve and Ftz. (B) RNA *in situ* patterns for *Cad74a*, *Impl2*, and *Paxillin* and found defective expression in *eve* and *ftz* mutant embryos. RNA *in situ* localization of selected target genes following the microarray experiments for *ftz* and *eve*. RNA localization for Impl2, Cad74a and Paxillin was performed in wild type, *ftz* and *eve* mutant backgrounds. (C) Sub-network from fig. S3 shows that HMG box transcription factor *Dichaete* interacts with Ftz-F1. (D) *In vitro* interaction between Ftz-F1 and Dichaete in a pull down assay. Ftz-F1 interacts with Dichaete (lane 4 and 6). Ftz-F1 also interacts with Ftz (lane 2 and 5). But Ftz and Dichaete do not interact (lane 1 and 3). (E) Dichaete forms a complex with Ftz-F1 on the *ftz* zebra regulatory element. Dichaete does not bind to the *ftz* regulatory element alone (lane 7). The zebra element was gel shifted after incubation with FTZ-F1 (lane 3). In the presence of Dichaete and Ftz-F1, a supershifted complex formed (lane 4). Antibodies specific either FTZ-F1 (lane 5) or Dichaete (lane 6) further shifted the complex. Fig S4D and fig. S4E indicate that the effect of D on segmentation is likely through a direct physical interaction between D and Ftz-F1 on the *ftz* zebra stripe enhancer.

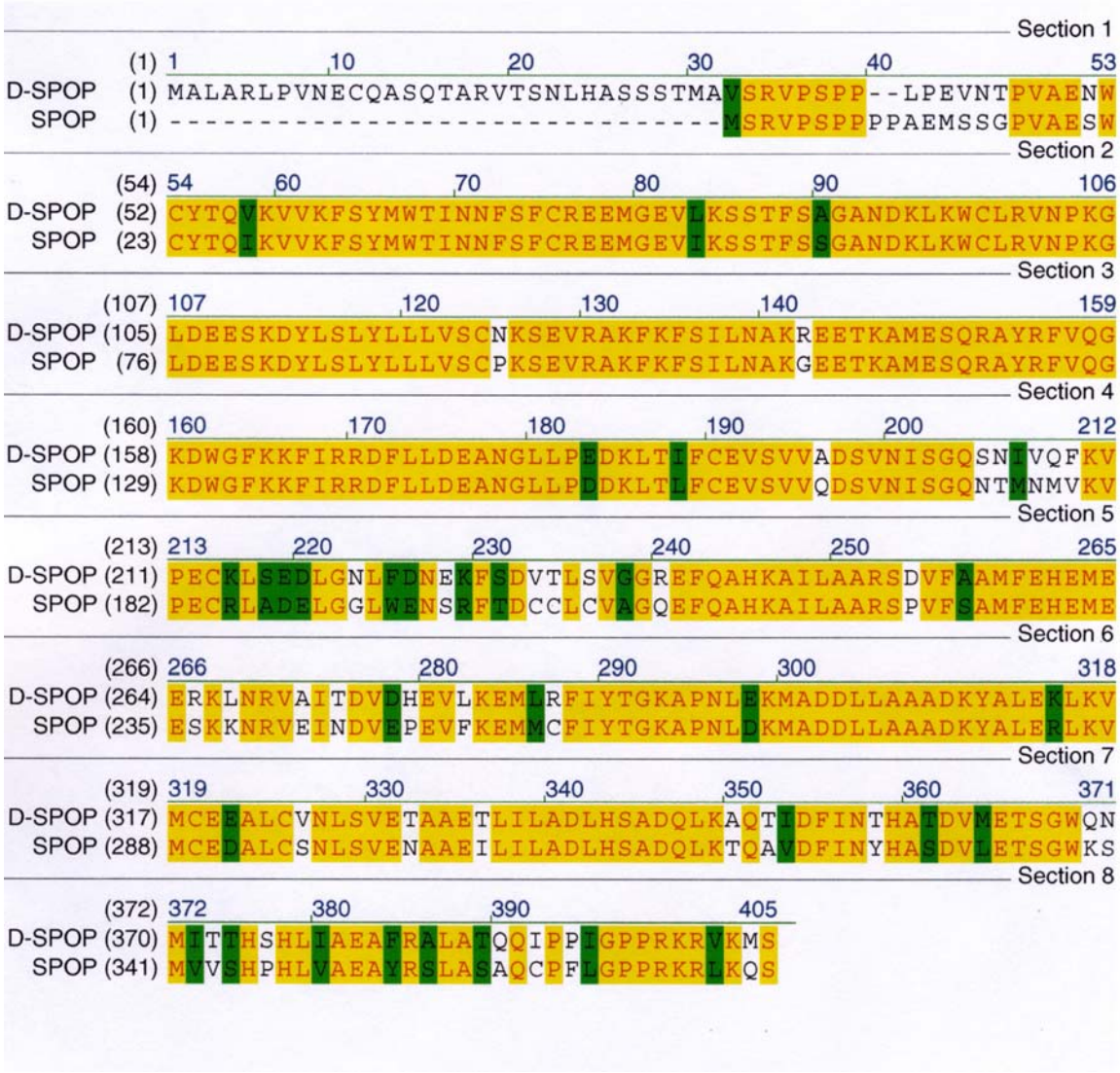


**fig. S5.** Protein alignment for D-SPOP (isoform c) and SPOP. Protein sequence of *Drosophila*-SPOP is 79% identical or 90% conserved to human SPOP.

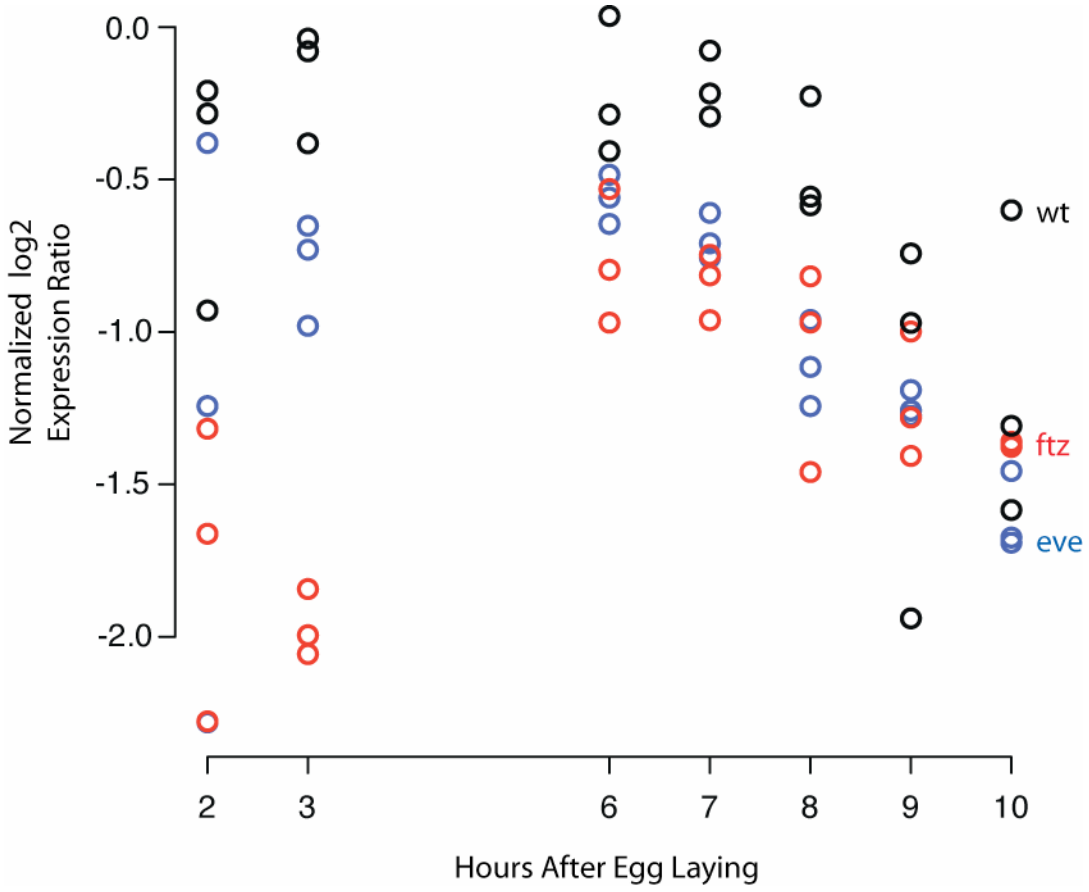


**fig. S6**. D-SPOP expression changes during embryo development in wild type (WT), *ftz* mutant embryos (ftz) and *eve* mutant embryos (eve). D-SPOP expression level decreased in both *ftz* mutant and *eve* mutant animals compared with wild type.

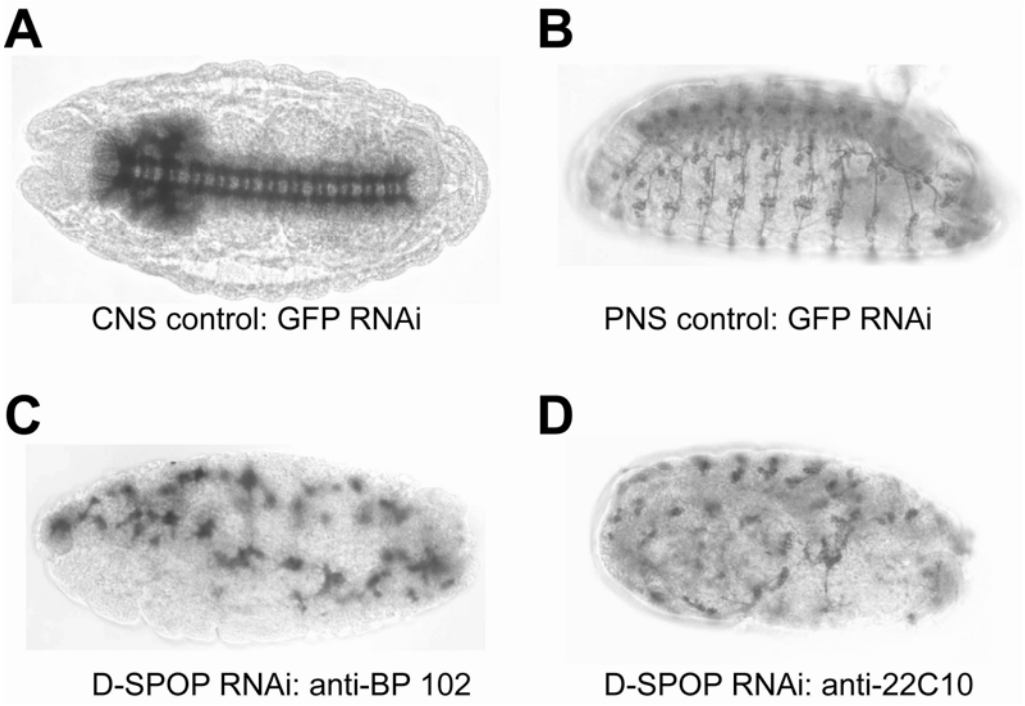


**fig. S7.** SPOP is required for nervous system development. (a) Central nervous system staining by using anti BP102 antibody in RNAi GFP control embryos. (b) Peripheral nervous system staining by using anti 22c10 in GFP RNAi control embryos. (c) Central nervous system staining by using anti BP102 antibody in RNAi D-SPOP embryos. Central nervous system was corrupted. (d) Peripheral nervous system staining by using anti 22c10 in RNAi D-SPOP embryos. Similar results were obtained with P-element insertional mutagenesis alleles of SPOP (data not shown).

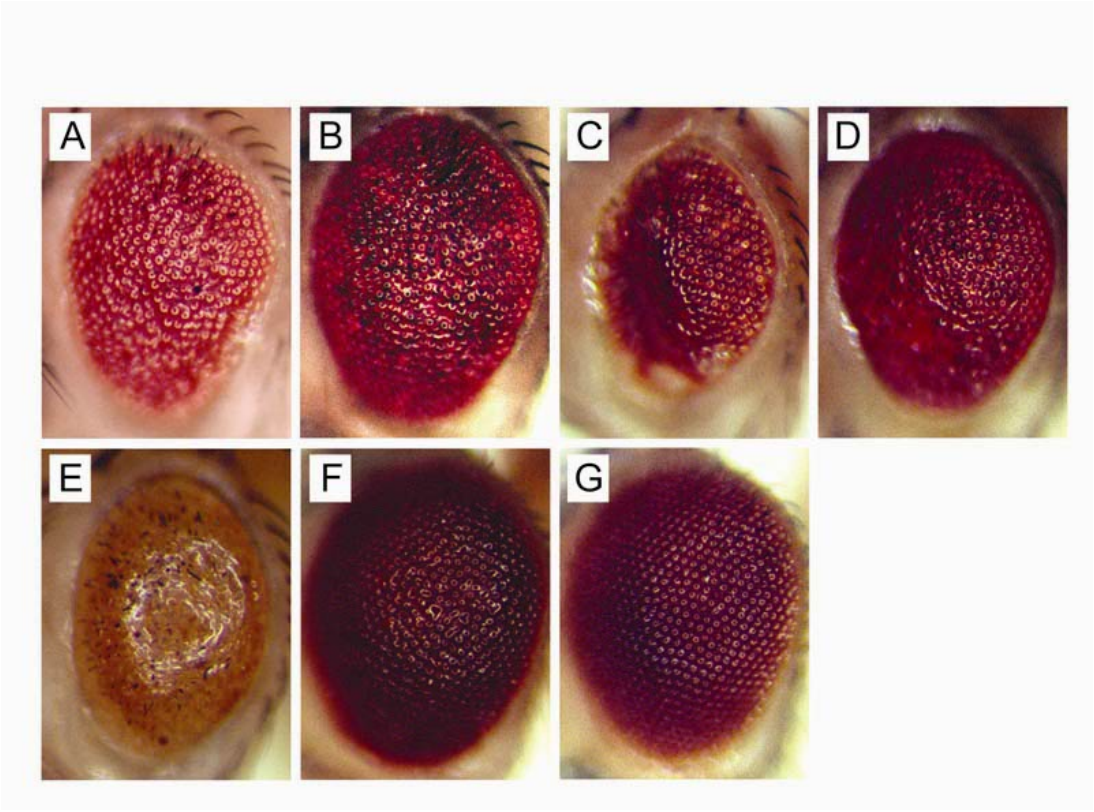


**fig. S8.** Genetic epistasis mapping of *rdx/D-SPOP* in the Eiger/TNF signaling pathway. (A) Ectopic expression of dTAK1 (*sev*-Gal4 *UAS*-dTAK1/+) in developing eyes under the control of *sev* promoter induces apoptosis and generates rough eyes with reduced size. (B) The apoptosis induced by dTAK1 is suppressed by removing one wild-type copy of *D-SPOP* (B, *sev*-Gal4 *UAS*-dTAK1/+; $D\text{-}SPOP^{\Delta 6}$/+). (C) Ectopic expression $Hep^{CA}$ (*sev*-Gal4 *UAS*-$Hep^{CA}$ /+) in developing eyes under the control of *sev* promoter induces apoptosis and generates rough eyes with reduced size. (D) The apoptosis induced by Hep is suppressed by removing one wild-type copy of *D-SPOP* (D, *sev*-Gal4 *UAS*-$Hep^{CA}$ /+; $D\text{-}SPOP^{\Delta 6}$/+). (E) Expression of D-SPOP (*GMR*-Gal4 *UAS-D-SPOP*/+) under the control of GMR promoter produced rough eyes with slightly reduced size. (F) Co-expression of a *bsk* RNAi (*GMR*-Gal4 *UAS-D-SPOP*/*UAS*-bsk-IR) and D-SPOP can recover the reduced eyes induced by D-SPOP. (G) Co-expression of Puc and D-SPOP can recover the reduced eyes induced by D-SPOP (*GMR*-Gal4 *UAS-D-SPOP*/*UAS*-Puc).

## Methods and Materials

### Materials

The D-SPOP cDNA was obtained by RT-PCR. This cDNA encodes isoform C of CG9924 (CG9924-PC; Flybase). HA tag sequence was added to D-SPOP by PCR-amplification. Constructs expressing HA-D-SPOP were generated by placing the coding sequence between the NotI and KpnI sites of pUAST vector. Myc-puc was obtained by the same strategy as HA-SPOP. pMT-HA-Ub were gifts from Jiang J. SPOP cDNA was obtained from RZPD. Using the Topo cloning kit (Invitrogen), SPOP was cloned into pcDNA3.1/V5-His vector. SPOP siRNA and non-target control siRNA were obtained from Dharmacon.

### Drosophila strains

Drosophila strains used include: GMR-*Gal4*, sev-*Gal4*, UAS-*Egr*, UAS-*dTAK1*, UAS-*Hep*$^{CA}$, UAS-*bsk*-IR,,UAS-*Puc*, hib$^{\Delta 6}$, UAS-*D-SPOP*-RNAi, UAS-*D-SPOP*, *ftz*$^{11}$ and *eve*$^{3}$ (Flybase).

### Cell Culture, DNA and SiRNA Transfection,

Drosophila S2 cells were cultured in Schneider's Drosophila Medium with 10% FBS at 25°C. DNA transfections were performed using Effectene Transfection Reagent (QIAGEN), according to the manufacturer's instructions. Cells were lysed 2 days after transfection. Mammalian HEK293 cells were cultured in DMEM medium with 10% FBS at 37 °C in 95% $O_2$-5% $CO_2$. DNA vector transfection was performed using lipofectamine 2000 (Invitrogen). siRNA transfection was performed using DharmaFECT™ 1. Cells were lysed for biochemical analysis 2 days after transfection.

### Microarrays

Null mutant $ftz^{11}$ and $eve^{3}$ alleles available from the Bloomington stock center were balanced over GFP balancers. The desired stages were genotyped and collected for microarrays as previously described (*1*). RNA samples were directly compared to a *Drosophila* reference sample collected throughout embryogenesis from 0-22h as previously described (*2*). Samples were hybridized to a PCR spotted whole-genome *Drosophila* microarray as described in Li and White (*3*).

**Chromatin Immunoprecipitation**

ChIPs were performed on the sequenced strain of Drosophila melanogaster, which is *y;cn bw sp*. Flies were grown at 25°C on a standard medium for amplification. Eggs were laid on plates filled with standard vinegar medium. Embryos were collected in EWB (Embryo Wash Buffer: 0.03% Triton X100, 0.4% NaCl) 2 h after the beginning of egg laying. The ChIP method was an adaptation of a previously published protocol (*4*). Drosophila eggs were fixed by 1.8% formaldehyde in buffer A1 solution (60 mM KCl, 15 mM NaCl, 4 mM MgCl2, 15 mM HEPES (pH 7.6), 0.5% Triton X-100, 0.5 mM DTT, 10 mM sodium butyrate, protease inhibitor cocktail) for 15 min at room temperature. During the fixation, the eggs were homogenized. The reaction was blocked with glycine. After three washes (5 min each, at 4°C in buffer A1), the crushed material was then filtered through Centricon Y-100 columns to recover chromatin. Chromatin was sheared by sonication. Sheared chromatin had an average length of 500 bp. Specific antibodies were added for the IP. For mock control, no antibody was added. The Ftz rabbit polyclonal antibody was a gift from Dr. Henry Krause. Eve rabbit polyclonal antibody was a gift from Dr. Manfred Frasch.

**Analysis of Microarray data**

The developmental time course gene expression data was obtained from wild-type, as well as *ftz* and *eve* mutant flies. Probes not present in all the arrays and those flagged as defective were removed from the data before the normalization. This resulted in 13,229

probes included per array. All data analyses were conducted in Perl and R. Background subtraction was performed with the normexp function (offset=50) in the LIMMA package using median foreground and background intensities (*5*-*7*) . Within-array normalization was performed with print-tip loess, between array normalization was performed by quantile normalization, as implemented in the LIMMA package (*7*). Differentially expressed genes were identified using an empirical Bayes methodology (*8*), with a false discovery rate of 0.001 (*9*), also implemented in the LIMMA package.

**Analysis of Genome wide location data**

High density oligonucleotide tiling arrays used in this study contain 187,023 unique 36-nucleotide probe (or 36-mers) spanning the entire euchromatic genome of Drosophila melanogaster and 4,000 negative control probes. The negative control probes, joined by three randomly selected non-existing 12-mers (with at least one mismatch in Drosophila genome), were designed to estimate the hybridization background due to non-specific binding. Locally weighted linear regression (LOWESS) analysis (*10*) was performed to normalize the ChIP-on-Chip datasets within and across the slides. For each probe, we calculated an intensity P value based on the normalized intensities of all negative control probes which follow an approximately gamma distribution. Intensity dependent Z scores (*11*) were calculated to identify those probes in which ChIP experiments with protein-specific antibodies were significantly greater than mock experiments. All probes with the p-value < 0.01 were considered significant.

We mapped probes to target genes under several scenarios: by mapping probes to all genes whose boundaries they mapped within or within a certain distance from (1, 5, 10, 25, or 50kb); by mapping probes to genes whose transcription start sites were within 1, 5, 10, 25, or 50kb; or by mapping probes to the gene whose transcription start site was nearest, with maximum distances of 1, 5, 10, 25, or 50kb. We also mapped three sets of probes randomly drawn from the same array design to their 'target genes.' For each target gene set, we calculated the enrichment of genes differentially expressed in the two mutant strains. All analyses presented in the text were carried out using the target gene

set identified by mapping probes to all genes they mapped within or within 1kb of, because these criteria produced the greatest enrichment of differentially expressed genes relative to the random probe sets.

**ChIP-qPCR**

The primers for ChIP-qPCR were designed around the binding probes, which were identified from the microarray analysis (for primers see table s9). Amplicon sizes ranged from 100-150bp. After chromatin immunoprecipitation, we performed qPCR reactions on an ABI 7900HT machine, using Qiagen SYBR Green. For each binding site validation, we performed the qPCR from both ChIP and Mock samples with at least three replicates. As a positive control, we used a primer pair from the CG9924 genome region that was validated by our gel shift analysis. For negative controls, we assayed three amplicons from exons of neg-ebony, p53 and actin 5c. Average CT value between ChIP and mock has been calculated.

**Automated literature mining and network modeling**

The literature based interactions that we used in our analysis is a *Drosophila* subset of the GeneWays 6.0 database (*12*). This database is compiled through automated text-mining of nearly 250,000 full-text articles from 78 leading biomedical journals. We remove all non-*Drosophila*-specific interactions. The interaction types extracted by GeneWays system can be classified as physical (such as bind and phosphorylate), logical (like activate and inhibit) and other (i.e contain, homolog, require). Furthermore, we use only those molecular interactions for which all names of the involved genes or proteins are unambiguously mapped to fly genes. To identify genes uniquely, we use the flybase ids of the form FBgnXXXXXX defined by the Flybase Database. We map the gene names using synonym lists provided by the flybase.

We used a multi-stage algorithm for computing our graph layout. We aimed at optimizing multiple criteria: clarity of graph structure, node label readability,

compactness of the graph, and aesthetic appeal. First, using the graph's adjacency matrix, we computed a matrix of lengths of shortest paths connecting every pair of nodes (a distance matrix), assuming that all edges are undirected (*13*). Second, using the distance matrix, we computed a non-metric multidimensional scaling projection of the distance matrix to two-dimensional space (*14*), which served as the initial graph layout. Third, maximizing distances between boundaries of the node labels, we computationally relocated every labeled node that was too close to other labeled nodes. Fourth, keeping coordinates of the labeled nodes immutable, we optimized the rest of the graph. In this optimization, each node was moved to minimize a pseudo-energy function that increased if edge-connected nodes were too close or too far, compared to an optimum distance (an "ideal edge length," an external parameter of the algorithm). The energy function also increased with shortening distance between pairs of nodes that were not connected by a direct edge.

Conceptually, the third and fourth stages of optimization are closely related to "spring" algorithms used in graph layout optimization, e.g. see pp. 303-324 in (*15*). Most of the computation was performed using a home-made script for MatLab 2007b (MathWorks, Inc., http://www.mathworks.com) program. The resulting layout was then used to generate a three-dimensional scene in POV-Ray format; public-domain software POV-Ray (http://www.povray.org/) was used for publication-quality rendering of the final figure.

**Gel shift mobility assay**

Two FTZ-F1 binding sites (CAAGG) in the *ftz* "zebra stripe" enhancer were previously described (*16*). Complementary oligonucleotides that contain one of the binding sites (tctccgtgcctgCAAGGacatttcgccggaggg) were synthesized and resuspended in TEN buffer (TE + 0.1M NaCl). Equal amounts of the oligos were mixed and annealed in 80°C for 10 minutes. They were then cooled to room temperature, precipitated as dsDNA in -20°C with one volume of 5M $NH_3Ac$ and 2 volumes of absolute ethanol, pelleted at 14,000

rpm, washed in 70% ethanol, air dried and resuspended in 50 µl $H_2O$. 10 pmoles of the dsDNA were end labeled with 1µl $\gamma^{32}$P-ATP by 5 units T4 polynucleotide Kinase. The reaction was incubated at 37°C for 1 hour and inactivated at 65°C for 10 minutes. A gel-shift reaction including FTZ-F1 or DICHAETE proteins produced in a TNT T7 Quick Coupled Transcription/Translation system (Promega cat. # L1170) and the transcend tRNA system conjugated to Biotin, end-labeled dsDNA that contains the FTZ-F1 binding sites. This reaction was incubated for 30 minutes at room temperature. Samples were loaded on a pre-run native poly acrylamide gel, and run for 30-40 minutes at 80-100 volts. The gel was then dried on Whatmann paper using BioRad gel dryer at 80°C for 1 hour. The dried gel was then exposed to a Fuji X-ray film for 30-60 minutes until the desired signal was obtained.

**Embryo *in situ* staining**

Embryos were collected at the desired stage, fixed in 4% paraformaldehyde, devitellinized and stored in methanol in -20ºC until use. The riboprobe for RNA *in situ* was *in vitro* transcribed using a template of the target gene cloned in Qiagen's TA cloning vector. *In vitro* transcription was performed using the T7 or SP6 promoter based on the orientation of the cloned insert to produce the antisense sequence. The riboprobe was synthesized using DIG-11-dUTP and hybridized to the embryos overnight (*17*). For protein *in situ* staining, embryos were incubated with EVE antibody (mouse monoclone) and D-SPOP antibody (rabbit polyclone) for 4 hours or overnight. After washes, embryos were incubated with Goat anti mouse HRP secondary antibody and Goat anti rabbit AP secondary antibody. After washes, embryos were stained with DAB and BCIP/NBT. Embryos were mounted with 70% Glycerol and visualized under a compound microscope.

**Immunoblot**

S2 cells and HEk293 cells were lysed with RIPA buffer (50mM Tris-HCl, Ph=7.4; 1% NP-40; 0.25% sodium deoxycholate; 150mM NaCl; 1mM EDTA; 1mM PMSF; 1mM NaF; 1mM $Na_3VO_4$; 1ug/ml aprotinin, leupeptin, pepstatin). Cell lysates were centrifuged at 10,000 g for 10 min at 4°C. Protein concentration of the supernatants was quantified by using a BCA kit (Pierce). After boiling the samples for 3 min, protein was resolved by SDS-Laemmli. Equivalent amounts of total protein were run on Tris-HCl gel. Myc-puc was detected by myc antibody (Invitrogen). Rabbit monoclonal antibody P-c-Jun (ser73) was from Upstate. Monoclonal antibody β-actin was from ABcam. Mono-clone antibody P-JNK was from Cell Signaling. Rabbit poly-clone and mouse mono-clone SPOP antibodies were made from Protein-tech, by using full length SPOP protein as antigen.

**In-vitro pull-down**

Open reading sequences of *ftz*, *dichaete*, *ftz-f1*, *D-SPOP* and *puckered* genes were cloned into p-RSETa cloning vector (Invitrogen Inc.) which includes 6X His. The FTZ, DICHAETE, FTZ-F1, D-SPOP and Puckered proteins were *in vitro* synthesized from this vector using the TNT T7 Quick Coupled Transcription/Translation system (Promega cat. # L1170) and the transcend tRNA system conjugated to Biotin (Promega). Pull-down assays were performed using the Profound PolyHis Pull-Down Protein: Protein interaction kit per the manufacturer instructions (Pierce), and were run on SDS-PAGE and blotted onto PVDF membranes. The proteins were detected using a Streptavidin antibody linked to the Horse-Radish Peroxidase (HRP) enzyme and ECL reagent.

**Immunoprecipitation,**

2 days after transfection, S2 cells were lysed with RIPA buffer. Cell lysates were centrifuged at 10,000 g for 10 min at 4°C. Supernatants were incubated with Anti-HA conjugated agarose beads (Sigma, mouse antibody) for 4 hours at 4°C. The beads were washed and the bound protein complex was eluted in SDS-Laemmli buffer. The protein was detected by immunoblot.

**In Vivo Ubiquitination Assay**

*In vivo* ubiquitination assays were based on the protocol previously described (*18*). Briefly, S2 cells were transfected in 6 well plates with 1 μg UAS-Myc-Puc, and 0.6 μg each of the following constructs: UAS-HA-D-SPOP or pMT-HA-Ub, and Actin-Gal4. 24 hr after transfection, 700 μMCuSO4 was added to the medium for another 24 hr to induce HA-Ub expression. Cells were treated with 50 μM MG132 for 4 hr before harvesting. Cells were first lysed in denaturing buffer (1% SDS/50mMTris [pH 7.5], 0.5mMEDTA/1 mMDTT). After incubation for 5 min at 100ºC, the lysates were diluted 10 times with lysis buffer and then subjected to coimmunoprecipitation with Anti-c-Myc conjugated agarose beads (Sigma, rabbit antibody), followed by immunoblot analysis with myc antibody (Invitrogen).

**Renal cell carcinoma micro-tissue array design**

331 cases of renal cell carcinoma received between 1985 and 1999 were selected from the archives of the Department of Pathology at Yale University School. Both areas of normal kidney tissue and carcinoma were identified in the original stained H&E slides. 0.6 cm tissue cores of each identified area were extracted from the corresponding paraffin-embeded tissue block (in 36 cases normal epithelium was not found) and transferred to two recipient paraffin blocks using the technique described by Knononen et al (19) using a Tissue Microarrayer (Beecher Instriments, Silver Spring, MD). The resulting microarray contained 331 renal carcinoma spots (two-fold redundancy) and 295 non-neoplastic kidney tissue spots (one-fold redundancy). Tissue slides were prepared by cutting 5-μm-thick sections and processed as described previously (*19, 20*). We also constructed a set of TMAs, which contains metastatic tissues from RCC in the Department of Pathology at the University of Chicago. 1.5 cm tissue cores were extracted from the paraffin-embeded tissue block (in 36 cases normal epithelium was not found) and transferred to the recipient paraffin blocks using the technique described by Knononen et al (19) using a Tissue Microarrayer.

**Tissue array Immunhistochemistry**

Multi-tissue slides and renal cell carcinoma tissue microarray slides from the Yale tissue center were stained as described previously (*20*), using the DAKO EnVision TM + System (Dako, Carpinteria, CA). In brief, the slides were deparaffinized by rinsing with xylene, followed by two washes of 100% ethanol and two washes of water. The slides were then boiled in a pressure cooker containing a sodium citrate buffer (pH 6.0) for antigen retrieval. After washing the slides in water, endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide in methanol for 30 minutes at room temperature. After washing with Tris-buffered saline (TBS), the slide was incubated in 0.3% BSA/1x TBS for 30 minutes at room temperature to reduce nonspecific background. Slides were then incubated with 1:100 dilution of SPOP antibody in BSA/TBS buffer at 4°C overnight. The following day, the slides were rinsed three times in 1x TBS buffer with 0.05% Tween 20. Bound antibody was detected by applying horseradish peroxidase-labeled polymer secondary antibody from the DAKO EnVision kit. The slides were washed in Tris-buffered saline-Tween 20 as above, and incubated for 10 min in DAB solution (from Dako). Counterstaining was then performed with hematoxylin, and slides were mounted with Immunomount (Shandon, Pittsburgh, PA). For the multi-tissue array, the staining between 0-1 was considered as weak or negative. For the 331 TMA, 20 tissue spots were missing in the tissue array section used in our analysis. Pathology reports for 23 cases gave the diagnosis as renal cell type, without further specification, and 24 cases were diagnosed as mixed type; these cases were not included in our analysis. Additionally, we did not include 6 tissue samples in which the cells were dead or tissues were damaged. In summary 73 cases from 331 were not included in our analysis.

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**Supplemental tables for "Integrate Drosophila segmentation network leads to identify a biomarker for human kidney cancer"**

## Table S1

## Ftz targets list from gene expression profile analysis

DHR78
IMPL2
FBgn0040010
FBgn0032629
LSPbeta
FBgn0036472
FBgn0031607
FBgn0036350
FBgn0037842
FBgn0036914
FBgn0036263
FBgn0036806
FBgn0035595
FBgn0037548
FBgn0036399
FBgn0038587
FBgn0025455
ADH
FBgn0032202
FBgn0032676
FBgn0011762
USP
FBgn0015621
FBgn0039293
FBgn0011230
FBgn0039633
DIAP2
FBgn0027534
FBgn0010105
FBgn0003031
FBgn0020251
FBgn0013343
FBgn0015269
FBgn0036534
FBgn0036408
FBgn0031419
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FBgn0020369
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FBgn0026262
FBgn0010410
FBgn0003067
FBgn0035404
FBgn0036001

FBgn0036909
FBgn0036002
FBgn0027779
FBgn0035854
FBgn0037282
FBgn0038146
FBgn0032881
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## Table S2

## Eve targets list from expression profile analysis

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## Table S3

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## Table S4

## Eve targets list from ChIP-chip binding sites mapping

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FBgn0030018
FBgn0000557
FBgn0039324
FBgn0011739
FBgn0030400
FBgn0034369
FBgn0033858
FBgn0037635
FBgn0013753
FBgn0004395
FBgn0028875
FBgn0030812
FBgn0038859
FBgn0000256
FBgn0033209
FBgn0030799
FBgn0014467
FBgn0037277
FBgn0031079
FBgn0031303
FBgn0040079
FBgn0037920
FBgn0037306
FBgn0035867
FBgn0039545
FBgn0011260
FBgn0039287
FBgn0038755
FBgn0036372
FBgn0040071
FBgn0036101

FBgn0005672
FBgn0014269
FBgn0032747
FBgn0033989
FBgn0013764
FBgn0037669
FBgn0040961
FBgn0037662
FBgn0000568
FBgn0017457
FBgn0002849
FBgn0015514
FBgn0000667
FBgn0036034
FBgn0032696
FBgn0033745
FBgn0020224
FBgn0016694
FBgn0005659
FBgn0042209
FBgn0025394
FBgn0031236
FBgn0033988
FBgn0029674
FBgn0020240
FBgn0031304
FBgn0025803
FBgn0036484
FBgn0035289
FBgn0029983
FBgn0035576
FBgn0021761
FBgn0031162
FBgn0004647
FBgn0038936
FBgn0030019
FBgn0036634
FBgn0027568
FBgn0030685
FBgn0027581
FBgn0015245
FBgn0029514
FBgn0034216
FBgn0031288
FBgn0026411
FBgn0031706
FBgn0030768
FBgn0040335
FBgn0035074
FBgn0039099
FBgn0039632

FBgn0038671
FBgn0031578
FBgn0036097
FBgn0002863
FBgn0013948
FBgn0037876
FBgn0040912
FBgn0029996
FBgn0024989
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FBgn0024998
FBgn0004924
FBgn0005671
FBgn0035127
FBgn0038008
FBgn0020360
FBgn0036268
FBgn0038452
FBgn0000486
FBgn0015954
FBgn0030865
FBgn0036216
FBgn0015905
FBgn0034227
FBgn0033522
FBgn0033000
FBgn0034897
FBgn0035423
FBgn0003151
FBgn0037443
FBgn0036866
FBgn0040897
FBgn0034299
FBgn0032488
FBgn0038562
FBgn0037767
FBgn0000615
FBgn0040346
FBgn0039054
FBgn0002643
FBgn0029895
FBgn0031195
FBgn0039272
FBgn0014002
FBgn0037154
FBgn0028539
FBgn0029921
FBgn0030613
FBgn0033859
FBgn0035227

FBgn0042204
FBgn0019990
FBgn0035411
FBgn0014009
FBgn0040587
FBgn0029580
FBgn0040107
FBgn0024195
FBgn0039550
FBgn0035460
FBgn0033895
FBgn0001316
FBgn0022342
FBgn0031260
FBgn0032800
FBgn0010398
FBgn0030810
FBgn0037986
FBgn0039410
FBgn0032697
FBgn0037776
FBgn0037240
FBgn0003310
FBgn0039858
FBgn0036697
FBgn0033677
FBgn0015338
FBgn0034877
FBgn0035597
FBgn0031627
FBgn0033551
FBgn0023509
FBgn0019947
FBgn0036373
FBgn0031577
FBgn0033811
FBgn0033867
FBgn0029138
FBgn0001994
FBgn0037506
FBgn0000635
FBgn0036096
FBgn0038904
FBgn0016120
FBgn0039167
FBgn0024432
FBgn0034121
FBgn0015793
FBgn0003114
FBgn0003513
FBgn0040273

FBgn0030650
FBgn0010550
FBgn0030867
FBgn0031287
FBgn0030535
FBgn0039547
FBgn0000634
FBgn0000109
FBgn0032947
FBgn0025832
FBgn0036211
FBgn0038949
FBgn0029861
FBgn0030021
FBgn0040227
FBgn0030649
FBgn0032073
FBgn0039285
FBgn0029948
FBgn0001332
FBgn0030942
FBgn0037586
FBgn0030790
FBgn0039214
FBgn0031044
FBgn0033101
FBgn0030334
FBgn0030866
FBgn0038990
FBgn0030383
FBgn0000028
FBgn0000036
FBgn0027889
FBgn0015904
FBgn0035180
FBgn0024196
FBgn0010602
FBgn0001991
FBgn0038193
FBgn0037015
FBgn0031525
FBgn0036867
FBgn0024251
FBgn0039845
FBgn0037873
FBgn0005612
FBgn0040347
FBgn0039273
FBgn0038931
FBgn0034736
FBgn0000099

FBgn0026597
FBgn0024986
FBgn0034965
FBgn0010482
FBgn0011288
FBgn0027356
FBgn0038418
FBgn0037641
FBgn0027106
FBgn0032380
FBgn0039693
FBgn0030382
FBgn0015396
FBgn0033550
FBgn0038668
FBgn0028489
FBgn0029654
FBgn0034546
FBgn0039692
FBgn0028550
FBgn0026418
FBgn0002023
FBgn0030489
FBgn0038632
FBgn0033698
FBgn0032915
FBgn0033100
FBgn0032166
FBgn0030413
FBgn0000307
FBgn0030349
FBgn0037919
FBgn0024963
FBgn0024321
FBgn0033804
FBgn0025109
FBgn0011766
FBgn0037588
FBgn0032695
FBgn0038948
FBgn0037771
FBgn0003963
FBgn0039741
FBgn0037777
FBgn0031016
FBgn0034966
FBgn0035819
FBgn0036210
FBgn0031261
FBgn0035530
FBgn0011708

FBgn0033357
FBgn0034974
FBgn0033356
FBgn0039350
FBgn0033380
FBgn0039137
FBgn0015778
FBgn0039883
FBgn0033159
FBgn0036698
FBgn0038937
FBgn0034300
FBgn0032710
FBgn0039025
FBgn0032267
FBgn0037874
FBgn0028890
FBgn0031635
FBgn0029891
FBgn0037747
FBgn0001404
FBgn0029896
FBgn0010229
FBgn0029707
FBgn0004652
FBgn0029764
FBgn0031297
FBgn0038591
FBgn0036865
FBgn0038515
FBgn0016078
FBgn0027086
FBgn0002413
FBgn0014028
FBgn0003975
FBgn0030505
FBgn0035623
FBgn0011227
FBgn0024315
FBgn0033210
FBgn0029799
FBgn0031019
FBgn0031047
FBgn0035953
FBgn0035599
FBgn0011283
FBgn0029862
FBgn0037602
FBgn0035818
FBgn0034535
FBgn0031889

FBgn0037642
FBgn0000259
FBgn0035712
FBgn0032431
FBgn0020556
FBgn0003380
FBgn0013973
FBgn0033094
FBgn0032630
FBgn0003425
FBgn0030504
FBgn0014143
FBgn0030381
FBgn0026188
FBgn0037670
FBgn0030731
FBgn0035656
FBgn0033946
FBgn0016917
FBgn0030328
FBgn0034964
FBgn0024991
FBgn0030420
FBgn0024245
FBgn0010300
FBgn0034205
FBgn0033557
FBgn0037198
FBgn0015795
FBgn0030686
FBgn0032234
FBgn0011837
FBgn0004493
FBgn0030179
FBgn0038065
FBgn0036100
FBgn0000588
FBgn0037727
FBgn0039168
FBgn0024326
FBgn0003371
FBgn0023535
FBgn0030350
FBgn0013770
FBgn0039265
FBgn0041092
FBgn0000567
FBgn0037525
FBgn0039412
FBgn0039549
FBgn0030166

FBgn0035894
FBgn0033549
FBgn0004646
FBgn0020445
FBgn0015903
FBgn0034217
FBgn0000179
FBgn0039510
FBgn0038896
FBgn0016754
FBgn0005775
FBgn0037694
FBgn0035995
FBgn0010413
FBgn0004243
FBgn0003512
FBgn0027522
FBgn0038010
FBgn0039035
FBgn0039323
FBgn0028499
FBgn0030888
FBgn0027949
FBgn0032840
FBgn0004359
FBgn0022764
FBgn0038240
FBgn0040286
FBgn0038561
FBgn0035767
FBgn0000242
FBgn0033886
FBgn0003749
FBgn0030522
FBgn0038373
FBgn0031698
FBgn0015756
FBgn0030490
FBgn0030568
FBgn0000570
FBgn0037774
FBgn0031891
FBgn0004055
FBgn0011771
FBgn0037554
FBgn0016685
FBgn0014027
FBgn0034533
FBgn0003964
FBgn0034098
FBgn0004595

FBgn0034878
FBgn0002783
FBgn0039409
FBgn0000504
FBgn0002948
FBgn0039411
FBgn0040533
FBgn0025376
FBgn0010567
FBgn0033182
FBgn0032916
FBgn0004399
FBgn0000119
FBgn0003013
FBgn0015609
FBgn0033471
FBgn0030533
FBgn0038214

## Table S5

## Ftz direct targets

FBgn0036630
FBgn0024150
FBgn0013591
FBgn0003507
FBgn0027660
FBgn0038192
FBgn0020251
FBgn0031759
FBgn0001222
FBgn0015278
FBgn0038264
FBgn0035342
FBgn0032918
FBgn0031398
FBgn0026875
FBgn0036914
FBgn0005410
FBgn0038150
FBgn0035978
FBgn0003177
FBgn0011817
FBgn0020620
FBgn0035692
FBgn0024921
FBgn0038118
FBgn0030024
FBgn0013733
FBgn0016797
FBgn0020245
FBgn0004465
FBgn0010382
FBgn0036103
FBgn0039019
FBgn0001309
FBgn0010247
FBgn0003118
FBgn0030868
FBgn0022069
FBgn0020427
FBgn0023526
FBgn0035340
FBgn0033103
FBgn0035174
FBgn0036826
FBgn0035574
FBgn0038457
FBgn0003175

FBgn0030306
FBgn0000575
FBgn0000546
FBgn0035229
FBgn0015806
FBgn0003274
FBgn0042630
FBgn0035210
FBgn0016697
FBgn0001085
FBgn0031303
FBgn0034240
FBgn0003200
FBgn0027655
FBgn0031236
FBgn0003607
FBgn0033559
FBgn0003656
FBgn0002973
FBgn0029933
FBgn0037443
FBgn0035172
FBgn0032629
FBgn0031684
FBgn0032002
FBgn0034606
FBgn0036398
FBgn0038853
FBgn0029138
FBgn0001994
FBgn0003896
FBgn0032497
FBgn0039688
FBgn0037639
FBgn0039528
FBgn0030942
FBgn0040059
FBgn0037199
FBgn0026616
FBgn0038335
FBgn0033922
FBgn0011288
FBgn0003659
FBgn0000395
FBgn0039266
FBgn0038430
FBgn0011766
FBgn0037771
FBgn0013984
FBgn0010460
FBgn0036350

FBgn0001977
FBgn0033159
FBgn0038816
FBgn0005630
FBgn0037243
FBgn0004837
FBgn0034644
FBgn0001122
FBgn0040295
FBgn0004368
FBgn0036099
FBgn0040884
FBgn0017557
FBgn0030809
FBgn0010409
FBgn0004893
FBgn0035397
FBgn0002284
FBgn0037198
FBgn0037971
FBgn0010389
FBgn0028914
FBgn0003371
FBgn0003715
FBgn0038803
FBgn0033218
FBgn0036461
FBgn0032756
FBgn0029856
FBgn0022764
FBgn0028683
FBgn0039148
FBgn0003048
FBgn0033552
FBgn0005624
FBgn0036856
FBgn0040533
FBgn0001981
FBgn0034071

## Table S6

## Eve direct targets

FBgn0031454
FBgn0038493
FBgn0035116
FBgn0038169
FBgn0034602
FBgn0015300
FBgn0033842
FBgn0036339
FBgn0035650
FBgn0027108
FBgn0030478
FBgn0039732
FBgn0040889
FBgn0013733
FBgn0030452
FBgn0030451
FBgn0031596
FBgn0030064
FBgn0000163
FBgn0040754
FBgn0001138
FBgn0001309
FBgn0035456
FBgn0015621
FBgn0042693
FBgn0028704
FBgn0037632
FBgn0033918
FBgn0039869
FBgn0023526
FBgn0001257
FBgn0038580
FBgn0001220
FBgn0033208
FBgn0031020
FBgn0011666
FBgn0030180
FBgn0038720
FBgn0029941
FBgn0034542
FBgn0037875
FBgn0037295
FBgn0039297
FBgn0036715
FBgn0039095
FBgn0035954

FBgn0023477
FBgn0040709
FBgn0033559
FBgn0038524
FBgn0030341
FBgn0033636
FBgn0019664
FBgn0030534
FBgn0031574
FBgn0032248
FBgn0002989
FBgn0032629
FBgn0039032
FBgn0038620
FBgn0020236
FBgn0029653
FBgn0039263
FBgn0037048
FBgn0035073
FBgn0027524
FBgn0003984
FBgn0037639
FBgn0016081
FBgn0037153
FBgn0038881
FBgn0039014
FBgn0000489
FBgn0030958
FBgn0035569
FBgn0039266
FBgn0003145
FBgn0010349
FBgn0031599
FBgn0033588
FBgn0011300
FBgn0039736
FBgn0031456
FBgn0032833
FBgn0039633
FBgn0013759
FBgn0031448
FBgn0040207
FBgn0028914
FBgn0004066
FBgn0037261
FBgn0032756
FBgn0032348
FBgn0036818
FBgn0000581
FBgn0034738
FBgn0035624

FBgn0001128

## Table S7

## Quantitative real time PCR to validate ChIP-chip results

| gene name | CT(chip)-CT(mock) | Forward primer | Reverse primer |
|---|---|---|---|
| CG7664 (ftz) | 11 | GATTCTGATAAGCCGGCAAGG | GAAAGAAATGACACAATACGAG |
| CG6821 (ftz) | 5.2 | GCGTTAAAAAAGCAAGACCAAAC | GACTAAGACGGGACGATATCC |
| CG5670 (ftz) | 5.5 | ATTGACTGTTTGCTGTCAC | CCGCTCTAGAACTTAATCTAC |
| CG9432 (ftz) | 8.4 | CTCGCGAAAATGTCTCATA | CAGCAATACATCAACATGG |
| CG4533 (ftz) | 8.6 | CTTCCTCGTACCTTTTTAGA | CTGTCTGAGCAGTGACTAAT |
| CG10108 (ftz) | 8.5 | CTACCACTAATGAGCATCC | CGTACCGATCACCTTTATC |
| CG8956 (ftz) | 6 | GCAGCTTGTAGAGCACATA | GACTACTTCAAGGCCATTC |
| CG5594 (ftz) | 4.2 failed | CTTTACGGTGTCCTAATTG | CCTTCGGTTCTTATTGTTAC |
| CG7763 (ftz) | 10 | TCTCTACGTTTTGGTGATAC | CATTGAGTCGAGAACAATAG |
| cg13109-1 (ftz) | 6.8 | TGCATGTATGTATGTAGCC | GCTTAGCTTCAGATACAGATAC |
| cg13109-2 (ftz) | No signal failed | GTATCTAAAATCCCCCAAC | TAGGTCTATGACCCAGTACAC |
| cg3131 (ftz) | 6.5 | CAGGACTTGTCTTTCCTACT | CTGTTCAAGGATGTTTACC |
| cg4567-1 (ftz) | No signal failed | GGTGTAGAACAGAATCCTCTC | TAACTTGTCCGATAGAAACC |
| cg4567-2 (ftz) | 8.5 | CTTACAGCGCTTACATAACT | ATTGGGGAGAAGTGTAAAAC |
| cg5025 (ftz) | 6 | CGAAGATGTAGAGTTTATCG | GCTACTAACGGTATAAAGCAC |
| cg8400 (ftz) | 8.5 | GCCAGCATTTATGAGATAC | TGCGTACTATTCCTACTGAC |
| cg15867 (ftz) | 6 | CTTCTTGGGAGTTATACTAGG | CAACCTAATCCGTTCTAATC |
| cg10035 (ftz) | 11 | GATTTTCCGACTCTTCCAC | GTGCTTGACATTTCTTAGG |
| cg9924 (ftz) | 6 | CACATACGACATATGACAGC | GAACTGAACTGAGACTGAGAG |
| cg13130-1 (eve) | 6.2 | TATTACCTTACCTTCGACCGG | CCGTGGACGAAGTGAAATCA |
| cg13130-2 (eve) | 9.6 | TGTCGAGCACCGGCTGTAT | ATCGGAGGCGTAGCATACCA |
| cg9924 (eve) | 7 | GCCTTTGAGCGACTTAACTCT | GGTGGCCGTGAAACAATG |
| Sucessful rate: 19/22=86% | | | |
| CG9924 positive control | 5.5 | GCAACGATAGCCAACTAGCCA | CAGTCGAGTGTCAAAGGTCAGC |
| | | | |
| neg-ebony2nd exon | 4 | TATGGGCACATTATCGTACA | CTGTTGGAGCGTTATAAGA |
| p53 exon | 3.5 | CAAATCTACAAGGAGTACCA | CCAAAGTAGCAGTTATTGAG |
| Actin 5c exon | 4.7 | GTTACTCTTTCACCACCAC | GTGATCTCCTTCTGCATAC |
| Average of negtive | 4.1 | | |

To validate ChIP microarry result, we performed quantitative real time PCR. For each binding site, we performed the qPCR from ChIP and Mock (Primers see table s9) with at least three replicates. For positive control, we chose a pair primer from CG9924 genome region which was validated by our gel shift analysis. For negative control, we designed three pair primers from exon of neg-ebony, p53 and actin 5c. Average CT value between ChIP and mock has been calculated. To reduce the false positive rate, we only consider

the difference between ChIP and mock higher than 5 as positive result. In the end, the successful rate is 86%.

## Table S8
## Network analysis for ftz or eve direct targets, which have homolog in human

A network was generated by superimposing individual networks created from the following data types: a protein-protein interaction (PPI) network from Giot et al. (6), ChIP-on-chip FTZ and EVE DNA binding networks (this paper, Fig. 1A), an expression profile network (this paper, Fig. 1A) and a network produced by literature data mining using the GeneWays system described previously by Rzhetsky et al. (5). Geneways includes 368,331 full-text research articles representing 100 high-impact biomedical journals and 8,039,972 article abstracts from the PubMed database. “Connectivity (all)” means the connectivity over all data types. Connectivity of a node is defined as the number of edges that end in the node. Betweenness-centrality (BC) is a measure of "importance" of a network node: high BC nodes tend to participate in many shortest paths connecting other nodes. Betweenness-centrality of a node is defined as the proportion of shortest paths between all possible pairs of nodes that include the node. Clustering means the clustering coefficient of a network node, which is the ratio of the actual number of direct connections between the immediate neighbors of the node to the maximum possible number of such direct arcs between its neighbors.

| Gene | FBID | Connectivity (all) | Clustering | Betweenness-centrality | PPI | Literature | Expression | ChIP-on-chip |
|---|---|---|---|---|---|---|---|---|
| CG9924 | fbgn0086364 | 45 | 0.03252 | 248526.3694 | 39 | 1 | 1 | 4 |
| Lim1 | fbgn0026411 | 47 | 0.018583 | 224192.3961 | 39 | 1 | 1 | 6 |
| sgg | fbgn0003371 | 84 | 0.037736 | 187706.6988 | 1 | 80 | 1 | 2 |
| CG11486 | fbgn0035397 | 42 | 0.007317 | 171102.548 | 40 | 0 | 1 | 1 |
| InR | fbgn0013984 | 51 | 0.033333 | 163586.4229 | 0 | 48 | 2 | 1 |
| CycE | fbgn0010382 | 58 | 0.022222 | 132275.1154 | 16 | 38 | 2 | 2 |
| heph | fbgn0011224 | 45 | 0.043544 | 131056.7794 | 6 | 36 | 1 | 2 |
| esg | fbgn0001981 | 32 | 0.002151 | 120850.6629 | 30 | 0 | 1 | 1 |
| pan | fbgn0085432 | 45 | 0.077652 | 117163.2061 | 2 | 40 | 2 | 1 |
| Fem-1 | fbgn0034542 | 26 | 0.01 | 115103.8195 | 24 | 0 | 1 | 1 |
| Ogt | fbgn0040295 | 29 | 0.015873 | 111677.1782 | 26 | 1 | 1 | 1 |
| CG30497 | fbgn0050497 | 31 | 0.02069 | 111337.8673 | 29 | 0 | 1 | 1 |
| CG31122 | fbgn0051122 | 23 | 0.021053 | 101320.7096 | 18 | 0 | 3 | 2 |
| Sin3A | fbgn0022764 | 28 | 0.053333 | 95548.93566 | 19 | 6 | 1 | 2 |
| Clp | fbgn0015621 | 23 | 0.019048 | 93001.74503 | 20 | 0 | 2 | 1 |
| Lim3 | fbgn0002023 | 28 | 0.061594 | 89385.7989 | 21 | 1 | 2 | 4 |
| CG32264 | fbgn0052264 | 26 | 0.042857 | 86713.22703 | 19 | 0 | 3 | 4 |
| CG9960 | fbgn0031454 | 17 | 0 | 83555.11484 | 15 | 0 | 1 | 1 |
| Trn | fbgn0024921 | 29 | 0.036667 | 81486.00671 | 12 | 14 | 2 | 1 |
| RpS12 | fbgn0014027 | 27 | 0.016667 | 81410.5608 | 24 | 0 | 1 | 2 |
| G-oalpha47A | fbgn0001122 | 21 | 0.026316 | 74146.10238 | 12 | 7 | 1 | 1 |
| crp | fbgn0001994 | 22 | 0.05848 | 73572.06455 | 5 | 14 | 1 | 2 |
| E2f | fbgn0011766 | 27 | 0.065359 | 71661.2263 | 1 | 22 | 2 | 2 |
| pyd | fbgn0003177 | 39 | 0.040936 | 67965.67799 | 4 | 21 | 4 | 10 |
| RpLP2 | fbgn0003274 | 20 | 0.03268 | 66605.46827 | 16 | 2 | 1 | 1 |
| RpL18A | fbgn0010409 | 18 | 0.022059 | 65464.4959 | 15 | 1 | 1 | 1 |
| Su(Tpl) | fbgn0014037 | 18 | 0.080882 | 56558.23862 | 12 | 3 | 1 | 2 |
| EcR | fbgn0000546 | 20 | 0.136364 | 55818.28431 | 1 | 14 | 1 | 4 |
| S6k | fbgn0015806 | 37 | 0.068841 | 54645.5415 | 1 | 34 | 1 | 1 |
| Su(H) | fbgn0004837 | 17 | 0.021978 | 52913.28177 | 12 | 3 | 1 | 1 |
| tup | fbgn0003896 | 15 | 0.043956 | 51267.60991 | 13 | 0 | 1 | 1 |
| ATPCL | fbgn0020236 | 10 | 0.027778 | 49619.77726 | 7 | 1 | 1 | 1 |
| prd | fbgn0003145 | 14 | 0.030303 | 49190.49787 | 3 | 9 | 1 | 1 |
| Mi-2 | fbgn0013591 | 14 | 0.090909 | 48896.97645 | 2 | 9 | 1 | 2 |
| CG10664 | fbgn0032833 | 13 | 0.075758 | 47546.25122 | 10 | 0 | 2 | 1 |
| CG15525 | fbgn0039732 | 16 | 0.095238 | 45105.79424 | 13 | 0 | 2 | 1 |
| nmo | fbgn0011817 | 21 | 0.076923 | 41727.30188 | 4 | 15 | 1 | 1 |
| Gpdh | fbgn0001128 | 9 | 0.035714 | 40969.37178 | 6 | 0 | 2 | 1 |
| Sox21b | fbgn0042630 | 15 | 0 | 37449.52955 | 12 | 0 | 1 | 2 |
| numb | fbgn0002973 | 8 | 0 | 37421.26447 | 6 | 0 | 1 | 1 |
| Caki | fbgn0013759 | 11 | 0.083333 | 37209.53441 | 7 | 0 | 2 | 2 |
| Pka-C3 | fbgn0000489 | 10 | 0.027778 | 30804.88662 | 3 | 4 | 2 | 1 |
| l(1)G0003 | fbgn0027335 | 9 | 0.071429 | 30731.93175 | 6 | 0 | 1 | 2 |
| bowl | fbgn0004893 | 7 | 0.066667 | 27488.96662 | 5 | 0 | 1 | 1 |
| bun | fbgn0010460 | 12 | 0.066667 | 27133.9783 | 9 | 0 | 2 | 1 |
| babo | fbgn0011300 | 13 | 0.018182 | 26476.71867 | 4 | 7 | 1 | 1 |
| Ssdp | fbgn0011481 | 8 | 0.095238 | 25397.10952 | 5 | 0 | 2 | 1 |
| Tango9 | fbgn0037971 | 5 | 0 | 24486 | 3 | 0 | 1 | 1 |
| E(bx) | fbgn0000541 | 12 | 0.095238 | 22857.87345 | 4 | 2 | 3 | 3 |
| Hsc70-5 | fbgn0001220 | 8 | 0.142857 | 22331.74059 | 5 | 0 | 1 | 2 |
| rap | fbgn0003200 | 11 | 0.083333 | 21727.1315 | 1 | 8 | 1 | 1 |
| htl | fbgn0010389 | 8 | 0.095238 | 21425.19784 | 1 | 4 | 2 | 1 |
| pnt | fbgn0003118 | 11 | 0.166667 | 21206.952 | 2 | 6 | 1 | 2 |
| okr | fbgn0002989 | 10 | 0.1 | 19606.93287 | 2 | 5 | 1 | 2 |
| Nat1 | fbgn0031020 | 7 | 0.2 | 18060.16178 | 4 | 0 | 2 | 1 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| CG32479 | fbgn0052479 | 8 | 0.1 | 16511.9526 | 3 | 0 | 3 | 2 |
| Eaf6 | fbgn0035624 | 4 | 0 | 16326 | 2 | 0 | 1 | 1 |
| Nnp-1 | fbgn0022069 | 4 | 0 | 16326 | 2 | 0 | 1 | 1 |
| l(2)35Bg | fbgn0001977 | 4 | 0 | 16326 | 2 | 0 | 1 | 1 |
| shot | fbgn0013733 | 12 | 0.142857 | 16032.73405 | 3 | 2 | 5 | 2 |
| fz2 | fbgn0016797 | 11 | 0.142857 | 15210.25161 | 3 | 5 | 1 | 2 |
| Aats-tyr | fbgn0027080 | 5 | 0 | 14863.40358 | 2 | 1 | 1 | 1 |
| CG5343 | fbgn0032248 | 6 | 0.1 | 14397.29229 | 3 | 0 | 2 | 1 |
| fz | fbgn0001085 | 7 | 0 | 14289.85096 | 1 | 3 | 2 | 1 |
| RN-tre | fbgn0020620 | 6 | 0 | 13821.51918 | 4 | 0 | 1 | 1 |
| CG18375 | fbgn0034606 | 7 | 0.066667 | 12755.72464 | 5 | 0 | 1 | 1 |
| CG31302 | fbgn0051302 | 6 | 0 | 12683.57234 | 3 | 0 | 1 | 2 |
| htt | fbgn0027655 | 12 | 0.035714 | 12309.86619 | 0 | 9 | 2 | 1 |
| btsz | fbgn0053555 | 7 | 0.166667 | 12214.05617 | 2 | 0 | 2 | 3 |
| tna | fbgn0026160 | 6 | 0 | 12000.28859 | 4 | 0 | 1 | 1 |
| Trn-SR | fbgn0031456 | 7 | 0.066667 | 11388.39268 | 5 | 0 | 1 | 1 |
| kat80 | fbgn0040207 | 6 | 0.1 | 11175.46335 | 3 | 0 | 2 | 1 |
| CG17059 | fbgn0040754 | 4 | 0 | 9977.685837 | 2 | 0 | 1 | 1 |
| CG16896 | fbgn0035073 | 4 | 0 | 9892.179688 | 2 | 0 | 1 | 1 |
| UGP | fbgn0035978 | 5 | 0.166667 | 9775.156914 | 3 | 0 | 1 | 1 |
| Pi3K68D | fbgn0015278 | 5 | 0 | 9659.609515 | 0 | 3 | 1 | 1 |
| RhoGAP93B | fbgn0038853 | 5 | 0 | 9539.065036 | 3 | 0 | 1 | 1 |
| Dhc64C | fbgn0010349 | 6 | 0 | 9310.093315 | 0 | 4 | 1 | 1 |
| sfl | fbgn0020251 | 5 | 0.166667 | 8700.013352 | 2 | 0 | 2 | 1 |
| Pros26 | fbgn0002284 | 4 | 0 | 8417.167244 | 2 | 0 | 1 | 1 |
| CG8531 | fbgn0033918 | 4 | 0.333333 | 8164 | 2 | 0 | 1 | 1 |
| CG3909 | fbgn0027524 | 3 | 0 | 8164 | 1 | 0 | 1 | 1 |
| CG1640 | fbgn0030478 | 3 | 0 | 8164 | 1 | 0 | 1 | 1 |
| PP2A-B' | fbgn0042693 | 4 | 0.333333 | 8164 | 1 | 0 | 1 | 2 |
| kis | fbgn0086902 | 10 | 0.333333 | 8164 | 0 | 1 | 3 | 6 |
| CG1677 | fbgn0029941 | 4 | 0.333333 | 8164 | 1 | 0 | 2 | 1 |
| grn | fbgn0001138 | 3 | 0 | 8164 | 0 | 1 | 1 | 1 |
| p24-1 | fbgn0030341 | 3 | 0 | 8164 | 1 | 0 | 1 | 1 |
| lid | fbgn0031759 | 3 | 0 | 8164 | 1 | 0 | 1 | 1 |
| CG13298 | fbgn0035692 | 3 | 0 | 8164 | 1 | 0 | 1 | 1 |
| CG1021 | fbgn0037443 | 4 | 0.333333 | 8164 | 1 | 0 | 1 | 2 |
| ProsMA5 | fbgn0016697 | 4 | 0.333333 | 8164 | 1 | 1 | 1 | 1 |
| CG8680 | fbgn0031684 | 3 | 0 | 8164 | 1 | 0 | 1 | 1 |
| CG9086 | fbgn0030809 | 3 | 0 | 8164 | 1 | 0 | 1 | 1 |
| CG4751 | fbgn0032348 | 5 | 0.166667 | 8010.560642 | 2 | 0 | 2 | 1 |
| CG6043 | fbgn0032497 | 5 | 0.166667 | 4271.524541 | 3 | 0 | 1 | 1 |
| CG8351 | fbgn0037632 | 4 | 0 | 3149.374904 | 2 | 0 | 1 | 1 |
| Su(P) | fbgn0004465 | 4 | 0 | 2665.7708 | 2 | 0 | 1 | 1 |
| Gug | fbgn0010825 | 4 | 0.333333 | 2458.18451 | 2 | 0 | 1 | 1 |
| Pros28.1A | fbgn0017557 | 4 | 0.333333 | 2165.427797 | 1 | 0 | 2 | 1 |
| baz | fbgn0000163 | 8 | 0.4 | 2015.342061 | 0 | 5 | 2 | 1 |
| CG9666 | fbgn0036856 | 4 | 0.333333 | 1594.134269 | 2 | 0 | 1 | 1 |
| Psf2 | fbgn0031599 | 4 | 0.666667 | 1426.81948 | 1 | 0 | 2 | 1 |
| E(Pc) | fbgn0000581 | 4 | 0.666667 | 1426.81948 | 1 | 0 | 2 | 1 |
| dsd | fbgn0039528 | 3 | 0 | 1426.152813 | 1 | 0 | 1 | 1 |
| CG1890 | fbgn0039869 | 4 | 0.333333 | 1147.759982 | 2 | 0 | 1 | 1 |
| siz | fbgn0026179 | 6 | 0.666667 | 1147.426648 | 1 | 0 | 3 | 2 |
| mRpL52 | fbgn0033208 | 3 | 0 | 1144.593315 | 1 | 0 | 1 | 1 |
| tou | fbgn0033636 | 3 | 0 | 1144.429342 | 1 | 0 | 1 | 1 |
| Pros28.1 | fbgn0004066 | 3 | 0 | 1139.609982 | 1 | 0 | 1 | 1 |
| CG17672 | fbgn0083978 | 4 | 0 | 1016.295407 | 4 | 0 | 0 | 0 |
| sno | fbgn0005410 | 4 | 0.333333 | 959.390553 | 0 | 2 | 1 | 1 |
| Dnr1 | fbgn0034738 | 3 | 0 | 282.846108 | 1 | 0 | 1 | 1 |
| CG9853 | fbgn0086605 | 5 | 0.333333 | 282.233911 | 3 | 0 | 1 | 1 |
| Tal | fbgn0023477 | 4 | 0.666667 | 0.666667 | 0 | 2 | 1 | 1 |
| Ac78C | fbgn0024150 | 4 | 1 | 0 | 0 | 0 | 3 | 1 |
| CG31145 | fbgn0051145 | 4 | 1 | 0 | 1 | 0 | 1 | 2 |
| CG5970 | fbgn0033842 | 3 | 1 | 0 | 0 | 0 | 2 | 1 |
| Nckx30C | fbgn0028704 | 2 | 0 | 0 | 0 | 0 | 1 | 1 |
| CG6900 | fbgn0030958 | 2 | 0 | 0 | 0 | 0 | 1 | 1 |
| dnc | fbgn0000479 | 5 | 0 | 0 | 0 | 0 | 1 | 4 |
| CG15429 | fbgn0031596 | 2 | 0 | 0 | 0 | 0 | 1 | 1 |
| flfl | fbgn0024555 | 3 | 1 | 0 | 0 | 0 | 2 | 1 |
| fry | fbgn0016081 | 2 | 0 | 0 | 0 | 0 | 1 | 1 |
| sll | fbgn0038524 | 2 | 0 | 0 | 0 | 0 | 1 | 1 |
| e | fbgn0000527 | 1 | 0 | 0 | 0 | 0 | 1 | 0 |
| CG6672 | fbgn0037875 | 2 | 0 | 0 | 0 | 0 | 1 | 1 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| h | fbgn0001169 | 3 | 0 | 0 | 0 | 0 | 3 | 0 |
| sdt | fbgn0003349 | 11 | 1 | 0 | 0 | 0 | 1 | 10 |
| CG3638 | fbgn0026875 | 3 | 1 | 0 | 0 | 0 | 1 | 2 |
| Dscam | fbgn0033159 | 4 | 1 | 0 | 0 | 1 | 1 | 2 |
| CG3893 | fbgn0036826 | 2 | 0 | 0 | 0 | 0 | 1 | 1 |
| Psc | fbgn0005624 | 2 | 0 | 0 | 0 | 0 | 1 | 1 |
| cv-2 | fbgn0000395 | 2 | 0 | 0 | 0 | 0 | 1 | 1 |
| sws | fbgn0003656 | 2 | 0 | 0 | 0 | 0 | 1 | 1 |
| pcx | fbgn0003048 | 3 | 1 | 0 | 1 | 0 | 1 | 1 |
| CG10082 | fbgn0034644 | 2 | 0 | 0 | 0 | 0 | 1 | 1 |
| CG11137 | fbgn0037199 | 2 | 0 | 0 | 0 | 0 | 1 | 1 |
| ttv | fbgn0020245 | 3 | 1 | 0 | 0 | 0 | 1 | 2 |
| Spase25 | fbgn0030306 | 2 | 0 | 0 | 0 | 0 | 1 | 1 |
| Nhe2 | fbgn0040297 | 4 | 0 | 0 | 0 | 0 | 2 | 2 |
| timeout | fbgn0038118 | 2 | 0 | 0 | 0 | 0 | 1 | 1 |
| CG7852 | fbgn0035229 | 2 | 0 | 0 | 0 | 0 | 1 | 1 |
| CG8405 | fbgn0034071 | 2 | 0 | 0 | 0 | 0 | 1 | 1 |
| CG33523 | fbgn0053523 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| CG18616 | fbgn0028495 | 1 | 0 | 0 | 1 | 0 | 0 | 0 |