Supporting Online Material

Materials and Methods

Generation of a genome-wide dsRNA library

A two-round PCR amplification strategy was used to generate a set of gene-specific DNA templates for *in vitro* transcription and targeted RNAi (Fig. S1). Gene-specific primer design was performed using GenomePRIDE (http://pride.molgen.mpg.de) (*1*). In the first step, 21,306 primer pairs (*2*) (see also Table S7) were used to amplify gene-specific fragments from Drosophila genomic DNA based on the genome annotation version 2.0 (*3*) and additional gene predictions (*2*). The amplified fragments were biased towards 3-prime exons with an average length of 408 bp. In the second amplification step, modified adaptor primers were used to add T7-promotor sequences (5-prime: TAATACGACTCACTATAGG) to both ends of the gene-specific fragments. T7-PCR fragments were used as templates for *in vitro* transcription reactions (T7 polymerase MegaScript kits in bulk, Ambion), followed by DNaseI (Ambion) digestion to remove the template DNA. RNA products were purified using 96-well filter plates (MANU03050, Millipore). Both PCR-amplified DNA and purified dsRNA products were assessed by gel electrophoreses and absorbance measurements of the yield, ultimately resulting in the generation of 19,470 (91%) high quality dsRNA fragments. The dsRNA was diluted to working stock concentrations (average of 0.04 µg/µl) and aliquoted into 384-well plates. A one-time synthesis yielded sufficient reagent for approximately 300 genome-wide RNAi screens in 384-well plates. Protocols and supplemental material can be found at http://drsc.med.harvard.edu/viability.

High-throughput RNAi screens

All screen experiments were performed in white, polystyrene 384-well tissue culture plates (Costar 3704, Corning). Screen plates were pre-loaded with an average concentration of 75nM (0.2 µg) dsRNA in 5 µl of 1mM Tris pH7. Kc_{167} cells (*4*) or $S2R^+$ cells (*5*) at a density of 12,000 cells per well in 10 µl serum-free Schneider cell medium (Gibco BRL) were added to the dsRNA assay plates using a liquid dispensing unit (Multidrop, Labsystems). After a 45 minute incubation time, 20 µl of serum-containing medium (10% fetal bovine serum, JRH Biosciences; penicillin-streptomycin, Sigma) was added to each well. Cells were incubated in sealed assay plates at 24°C for five days to allow for depletion of targeted mRNAs and protein products. We assayed cell number using 30 µl per well of an indirect quantitative Luciferase-based assay for ATP-levels (CellTiter, Promega) then read using an Analyst HT 384-well plate reader (Molecular Dynamics).

Computational analysis of RNAi results

To identify conditions with dsRNA-specific diminished cell growth and viability, the numerical readouts were normalized by mean-centering per 384-well plate. Duplicate screens were averaged separately for each cell-type prior to threshold selection. The complete data set is available from our Website (http://drsc.med.harvard.edu/viability and Table S8). We set a threshold of three standard deviations and above to select with 99.9% confidence the most statistically significant averaged *z*-scores, identifying 438 total results that exceeded the set threshhold in either Kc_{167} or $S2R^+$ cells (Table S1). Nearly identical results were obtained using both cell types screened, with the exception of 5 and 68 phenotypes detected only in Kc_{167} cells or $S2R^+$ cells, respectively (defined as cell-type specific phenotypes where the quotient of z-scores in Kc_{167} and $S2R^+$ exceeds 3). Data analysis and data representation were performed using Matlab (Mathworks). The predicted gene targets of the 438 identified dsRNAs were confirmed by

BLASTN searches (*6*) against the published Drosophila genome sequence and mapped to specific chromosomes (*3*). Additional annotation was provided by Hild *et al.* (*2*) (see also http://sunrise.zmbh.uniheidelberg.de/cgi-bin/gbrowse). The identified genes were searched for associated Gene Ontology, mutant allele and RNAi phenotype annotations in FlyBase (http://flybase.bio.indiana.edu/) (*7*). The predicted protein sequences of genes identified by RNAi phenotypes were searched for conserved protein domains using InterPro (version 7.0) (*8*), and manual inspection was used to classify listed domains into functional groups. The predicted protein sequences without any InterPro domain match were categorized under "No Prediction", although these proteins could encode structurally conserved domains that are not detected by the prediction program. To determine an expected frequency of "DNA-Binding" domain proteins within the genome to address the significance of our RNAi screen results, we counted across the entire Drosophila proteome the same InterPro domains that constituted the "DNA-Binding" category of predicted gene products associated with RNAi phenotypes. To determine whether Drosophila genes have putative orthologs in other species, reciprocal best hits of BLASTP (*6*) were selected for every predicted Drosophila protein sequence (*2*) against the protein predictions from *C. elegans* (release 17.102), *A. gambiae* (release 16.2), *H. sapiens* (release 17.34) and *M. musculus* (release 17.3). Databases were obtained from Ensembl (http://www.ensembl.org;) (*9*). *S. cerevisae* sequences were obtained from MIPS (http://mips.gsf.de). In addition, we determined potential homologs by using BLASTP with a cut-off of E < 1e-6. There is precedence in the literature for use of both best-reciprocal BLASTP that underestimates orthologs and threshold approaches that may overestimate orthologs.

Flow Cytometry

Flow cytometry analysis of cell viability and cell cycle phenotypes was performed in Kc_{167} cells

following treatment with dsRNA against *string* (*stg*), *fizzy* (*fzy*), *E2F*, *diminutive* (*dm*), *RpL18A*, *pAbp*, *D-IAP1* (*th*), *CG11700*, *CG9381*, *CG7552*, *CG15455*, *serpent* (*srp*), *Trithorax-like* (*Trl*), *CG6273* (*Eip74EF*), *HFA13017* (*foxo*), *Abdominal-A* (*abd-A*) and *gfp* target genes. 50,000 cells treated with 1 µg dsRNA in triplicate wells of multiple 96-well tissue culture plates were analyzed at one, three and five days (results at three days shown in Fig. 4, Fig. S5, *10*). Triplicate samples were pooled in 12 x 75 mm culture tubes, spun at 1200 rpm for 5 minutes, washed in PBS, resuspended by vortexing in 250 µl staining solution (PBS, 1% Tween20, 100 µg RNaseA (Sigma), 50 µg propidium iodide), incubated for 2 hours and 250 µl PBS added prior to analysis. Samples were analyzed by flow cytometry (FACSCalibur with CellQuest software), collecting 20,000 total (ungated) events with threshold=10 and FL2 voltage~430 (adjusted for each sample so that 2*N* peak on DNA-Area histogram centered at 200). Live cell subsets were gated within the total forward-side scatter dot plots. Cell cycle analysis was performed on histograms of gated counts per DNA-area (FL2-A) by the Watson (pragmatic) curve-fitting algorithm to determine the distribution of 2*N*, 4*N* and >4*N* cells using FlowJo software (Tree Star).

Epistasis analyses

Epistasis analyses tested caspase-dependent cell death by combined dsRNA treatments in Kc_{167} cells. For pan-caspase inhibition, 0.25 µg of each sample dsRNA (as listed above) was used in combination with 40 µM Z-Val-Ala-DL-Asp(OMe)-fluoromethylketone (ZVAD-fmk, Bachem) (Fig. 4C). Z-VAD-fmk stock in DMSO was added to serum-containing media at working concentration before dispensing into wells. Control wells were given equal volumes of DMSO in media. For combined-RNAi epistasis experiments, cells were treated with 0.25 µg of each sample dsRNA together with either 0.25 µg of *gfp* dsRNA (as a negative control), *Nc* dsRNA (Fig. 4C) or *rpr* dsRNA (*10*), for a total of 0.5 µg dsRNA per well in 384-well tissue culture plates. All

epistatic combinations were performed in triplicate wells in separate plates incubated for one, three and five days then assayed with the CellTiter Luciferase-based assay as described above (results at five days shown, Fig. 4). Results were analyzed as the normalized average Relative Light Units obtained, and expressed as the ratio between the test samples and negative controls for each epistatic combination.

Cell Staining and Fluorescence-based Assays

To determine cell viability, cells plated for RNAi (as described above) and incubated for three days were stained for fluorescence microscopy imaging of Hoechst 33342 to detect DNA in all cells and SYTOX green, a membrane impermeable nucleic acid stain (Molecular Probes), to detect dying cells. To detect cell death by apoptosis, cells plated for RNAi were tested for fluorogenic homogeneous caspase(s) activity (Homogeneous Caspases Assay, Roche) and fragmented dsDNA breaks (In situ Cell Death Detection Kit, Roche). Caspase activity was performed in replicate wells using 384-well tissue culture plates and detected after two or three days RNAi using an Analyst HT 384-well plate reader (Molecular Dynamics). DNA fragmentation was detected by fluorescence microscopy imaging of fluorescein-labelled terminal transferase activity in cells five and seven days after treatment with dsRNA in 8-well chamber slides (VWR) and manually counted for the percentage of co-stained versus total stained nuclei.

Quantitative RT-PCR

For quantitative RT-PCR of *reaper* expression, cells treated with dsRNA in triplicate wells in 6-well tissue culture plates and grown for three days were pooled for total RNA extraction (Trizol). Total RNA was DNase I treated (QIAGEN), quantified (Bioanalyzer, Agilent), reverse transcribed (Superscript III, Invitrogen) and qPCR-amplified (LightCycler, Roche). Samples

were qPCR-amplified in triplicate and normalized for RNA levels based on *rp49* expression.

Supporting Text

Success rate, putative false negatives and false positives

The absence of certain predicted gene results may indicate that the assay conditions limited their detection. The mechanism for generating RNAi phenotypes can generate hypomorphic to null-like conditions for any given gene product, potentially generating more (or less) favorable conditions for assessing any given gene function. Certain proteins involved in signaling and metabolism may not be identified if cell growth in serum-containing media masked requirements for specific growth factors or metabolic functions (see Table S1). In other instances, the duration of RNAi for five days (approximately 4 cell divisions) may be insufficient to uncover long-term cytotoxic effects on the population. In addition, we might have missed phenotypes where the corresponding dsRNA was not successfully synthesized.

Specificity of dsRNA targeting sequences and gene silencing

The RNAi library was designed to avoid homologous gene regions, as limited by the current knowledge of predicted gene models. We have no indication that lack of target specificity was an issue. We recovered only a single member of large related families (although other members were expressed), as discussed in the text for the examples of the transcription factors *CG15455* (AML1) and *serpent* (GATA1). The mechanism for RNAi proceeds through dsRNA templates processed into sequence matches with lengths of 21 nt perfect identity. We assessed whether cross-matches might occur for known highly homologous genes in our phenotypic set through identity of the targeting dsRNA sequence with other off-target loci in the *Drosophila* genome. For a set of genes identified in our screen and predicted to have highly-related Histone-fold

domains, we found that 5/6 of these genes were targeted by highly-specific dsRNA fragment sequences that did not contain any sequence identity cross-match ≥ 21 bp to other genes (aside from the intended target). In one case (1/6), the dsRNA fragment targeting His3.3A (recovered with phenotype of *z*-score 3.1) contained a single isolated stretch of 41 nt with cross-match identity to His3.3B, as one might expect for very closely related genes. A limitation of RNAi screening approaches may be homology constraints in primer design for the generation of dsRNAs trageting very closely related genes. However, the respective His3.3B dsRNA did not contain any cross-match to His3.3A and was not selected with phenotypes in our screen (*z*-score 1.8). With another set of genes identified in our screen and predicted to encode homeobox containing proteins, we found that 5/9 of these genes were also targeted with specific dsRNAs without any cross-match identity ≥ 21 bp to other genes. For 4/9 homeobox genes, cross match lengths of 21-24 nt were found to one or several other genes (e.g., *eyg* dsRNA contained 21 nt match to *CG1319*). However, in all of these cases (4/9), the respective dsRNAs that target the cross-matched genes did not themselves result in detectable phenotypes (e.g., CG1319 dsRNA). As evident from these tests and our overall functional results, cross-match sequence identity to off-target genes does not appear to be a major problem in identifying specific phenotypic results. Although the mechanism leading to transcript destruction by RNAi proceeds through 21 nt dsRNA templates, the use of long dsRNAs (~400 bp) may help avoid non-specific silencing from minor cross-match identities. A long dsRNA is processed into different discrete 21 nt templates, so that any single 21 nt stretch with perfect cross-match identity is either unlikely to occur at all, or becomes quite rare in a large pool of different 21 nt RNAs. In contrast to off-target genes, the targeted transcript would still be effectively targeted for destruction by the perfect match of any and all of the differing 21 nt products of the processed dsRNA. Furthermore, since the RNAi mechanism occurs at the nucleic acid and not amino acid level, it is possible to identify long

regions within most transcript sequences, including untranslated regions, that do not contain multiple 21 nt stretches of identity to other genes.

Comparison with RNAi phenotypes in C. elegans

We examined whether the genes we identified with RNAi cell growth and viability phenotypes may have conserved roles also uncovered in similarly scaled genome-wide RNAi screens in *C. elegans* for developmental phenotypes (*11*). We first determined all the potential orthologs by reciprocal best BLASTP between the fly and worm proteomes, then identified the corresponding *C. elegans* orthologous phenotypes. This analysis showed that 20% of the *C. elegans* orthologs of the entire proteome exhibited developmental phenotypes (890/4345) (*11*), whereas 47% of the *C. elegans* orthologs of the set of 438 genes associated with *Drosophila* RNAi cell phenotypes exhibited developmental phenotypes (85/181). Although the cellular and organismal functional requirements and assay resolution are not directly comparable, this increase in the frequency of conserved orthologous sequences as well as a conserved essential function in cells and in the worm validate that this set likely carries important *in vivo* functions, as well.

See http://drsc.med.harvard.edu/viability for supporting figures, tables and all primer and phenotype information.

Supporting Figure and Table Legends

Figure S1: Experimental approach for genome-wide RNAi screens.

(A) Synthesis of a genome-equivalent set of dsRNA for RNAi studies. Gene-specific templates were made using a two step approach, employing PCR-amplification of genomic DNA and re-amplification to incorporate T7-promoters. 19,470 dsRNA generated by simultaneous T7 *in vitro* transcription (T7 MegaScript, Ambion) were purified and diluted to working concentration before use. (B) High-throughput RNAi screening procedure. One synthesis generated sufficient dsRNA for hundreds of phenotypic screens. The dsRNA was reformatted at working concentration (25-100nM) into 384-well tissue-culture plates amenable for a wide-variety of high-density analyses, allowing a throughput of up to 40,000 individual experiments per day. For any specific study, approx. 10^4 cells are added directly to the pre-aliquoted dsRNA in 384-well plates and alternatively processed according to assay conditions. In this study, cells were bathed with dsRNA in serum-free media, incubated for five days to ensure protein depletion and analyzed by luciferase-based detection using a plate reader for luminescence (Analyst HT, Molecular Devices). Demonstrated alternative approaches (italics) include co-transfection of reporter constructs and assay detection based on fluorescence imaging by automated microscopy (data not shown).

Figure S2: Independent genome-wide RNAi screens show highly reproducible phenotypes.

(A) Scatter plot of quantitative genome-wide RNAi phenotypes. Correlation coefficient of *z*-scores from duplicate screens was 0.86. Red lines demarcate phenotypes classified with a *z*-score of three standard deviations or above selected for further analysis (with the exception of filtered positive controls, shown but not selected). Results were also reproducible for control dsRNA added to every 384-well screen plate, with relative luciferase units in the following ranges: *D*-

IAP1, 0.18±0.14 (positive control for loss of cell viability); *Rho1*, 0.85±0.12 (negative control), *gfp*, 1.00±0.13 (negative control); and no dsRNA, 1.10±0.09. (B) Results from two independent genome-wide RNAi screens in Kc_{167} cells. Each RNAi experiment (a single well) is represented by a shaded box. 19,470 experiments are shown in each panel, with an outline of a 384-well plate depicted in the upper left corner. Results in each plate were mean-centered prior to overall analysis. Grey values indicate zscore, with darker shades representing a below average result. Note that each plate had four control wells containing either *D-IAP1*, *gfp*, *Rho1* or no dsRNA. The *D-IAP1* dsRNA phenotypes are evident by the dark spot in the upper left corner of each paneled plate, indicative of fewer cells and a lower signal. Right panels. Magnification of selected results and the associated numerical *z*-scores. Nearly identical results were obtained using both cell types, with the exception of 5 and 68 phenotypes detected only in Kc_{167} or $S2R^+$ cell types, respectively.

Figure S3: RNAi against ribosomal genes show similar quantitative phenotypes.

(A) Quantitative phenotypes upon dsRNA treatment against *D-IAP*, *gfp* and *wg*. Different shades of green to red represent *z*-scores from -1.0 to 7.0 (see colorbar). Results determined for assays of each duplicate screen in both of two cell types (columns; two results shown for both Kc_{167} cells and $S2R^+$ cells). Quantitative results were normalized by cell type. Note that both *D-IAP** (added control) and *D-IAP* (within the RNAi library) dsRNA yielded equivalent severity of phenotypes (red), whereas *gfp* and *wg* dsRNA had no obvious growth or cell-viability phenotypes (green).

(B) Quantitatively similar RNAi phenotypes for ribosomal proteins. Shown are results for 37 genes annotated as ribosomal subunits (rows; gene name as in FlyBase), of which 34 exhibited quantitatively similar RNAi phenotypes (top panel). Several phenotypes for putative false-negative RNAi experiments are shown in the lower section (green panels).

Figure S4: CG15455 is a homolog of mammalian acute-myeloid leukaemia transcription factor family.

Cell-based RNAi screens identified a role in cell survival for an uncharacterized Drosophila gene, CG15455, encoding an AML homolog. Shown are all predicted *Drosophila* and known human Runt-domain proteins. Of the human proteins, CG15455 shares highest homology to AML1 and AML2, with 73% identity and 87% similarity within the Runt-domain amino acid sequences (BLASTP; for comparison, *Drosophila* Runt protein shows 70% identity and 82% similarity). CG15455 expression was detected throughout developing embryos (data not shown).

Figure S5: Cell cycle profiles associated with CG11700 and CG15455 severe RNAi phenotypes.

Flow cytometry cell cycle histograms of DNA content in *CG11700* and *CG15455* dsRNA-treated cells. Only "viable cells" based on unfragmented DNA content were analyzed (see Fig. 4), using a curve-fitting algorithm to determine the distribution of cells with 2*N*, 4*N* and >4*N* DNA that estimate the percentage of cells undergoing G1/S, G2/M and endoreplication, respectively. At three days, as compared to *gfp* RNAi, *CG11700* and *CG15455* RNAi both resulted in a concominant decrease in the frequency of cells in G1/S stage of the cell cycle with an increase in either G2/M (*CG11700*, from 11% to 26%, $p < 0.00001$) or >4*N* DNA populations (*CG15455,* from 6% to 18%, $p < 0.00001$).

Figure S6: Genes identified with severe cell viability defects exhibit ectopic reaper expression.

Induction of *reaper* expression upon RNAi depletion of specific transcription factors required for cell maintenance. Quantitative PCR experiments detected increased expression levels of the pro-apototic gene, *reaper*, from reverse-transcribed mRNA isolated from cells treated for three days

with indicated dsRNAs. Expression levels are represented as the crosspoint difference (Cx) observed during qPCR of each sample, which reflect approximately eight and more fold-differences in *reaper* levels as compared to cells treated with *gfp* dsRNA (left-most column).

Figure S7: Gene products associated with Drosophila cell viability phenotypes are more conserved in other organisms.

Percent orthologs found for predicted *Drosophila* proteome (light grey) or of the selected 438 *Drosophila* amino acid sequences associated with RNAi cell phenotypes (dark grey) when searched using BLASTP for reciprocal best matches against other predicted genome sequences. In each case, a greater proportion of orthologs (as shown by fold differences) were found for gene products associated with RNAi cell growth and viability phenotypes than between entire proteomes: *S. cerevisae* (Sc) 15% and 33%, *C. elegans* (Ce) 32% and 53%, *A. gambiae* (Ag) 53% and 71%, *M. musculus* (Mm) 39% and 59% and *H. sapiens* (Hs) 40% and 59%. A common set of proteins for genes associated with RNAi phenotypes were conserved across all species (22%; Table S5), particularly genes involved in protein translation (70%, 38/54).

Figure S8: Phenotypic distribution associated with conserved and animal-specific homologs.

The distribution of *Drosophila* RNAi phenotypes associated with genes with conserved homologs as defined by BLASTP with a cut-off of E < 1e-6 (see TableS5). In the set of genes identified by the 438 dsRNA growth and viability phenotypes (3 < *z-score*), 67% had homologs either in yeast, *C. elegans, A. gambiae, M. musculus* or *H. sapiens*. We separated the homologs into two sets depending on whether a match was found in yeast or not ("yeast" versus "animal"), and then determined the distribution of homolog conservation by phenotypic severity. As shown here, genes in the most severe phenotypic class were represented by significantly fewer yeast

homologs and more without a match than in the total set of RNAi phenotypes. Significance was determined by Pearson's Chi-squared test. We also identified orthologs as defined by reciprocal BLASTP and analyzed the associated quantitative *Drosophila* RNAi phenotypes. By this approach, 61% had detected orthologs, with 28% yeast orthologs and 34% animal orthologs (see Table S5). The results were similar as for homologs: genes in the most severe phenotypic class were represented by significantly fewer yeast othologs (7%, $p < 0.00001$) and more without a match (56%, $p < 0.001$) than in the total set of RNAi phenotypes.

Table S1: Genes identified by 438 dsRNA-induced cell growth and viability phenotypes.

Genes were identified from dsRNAs with averaged phenotypic *z*-scores of three or more standard deviations from the mean in cell number assays in either Kc_{167} cells or $S2R^+$ cells. 'Gene' is gene name as listed by Symbol in BDGP (*7*). 'DsRNA ID' is internal reference number for nucleic acid sequence targeted by RNAi, described here by dsRNA length in base pairs. 'Z' refers to *z*-score, listing the average value for the RNAi phenotypes obtained in duplicate screens in each of two cell types (Kc_{167} cells or $S2R^+$ cells). 'Functional Group' represents nine manually-assigned categories for genes identified with functionally-related predicted InterPro protein domain signatures and other additional evidence, including an unassigned group (presented as 'Other' on Figure 3). 'InterPro Evidence' is the protein domain signature number as determined.

Table S2: Chromosomal distribution of genes, dsRNAs and phenotypic hits.

Shown are the numbers by chromosomal location of predicted targeted genes, sequence specific dsRNA targeting fragments and the dsRNAs identified with an RNAi phenotype.

Percentage shown is either that of the entire genome, the entire set of dsRNAs or the entire set of RNAi phenotypes. Chr, chromosome. U, unlinked. Left (L) and Right (R)

arms of chromosomes 2 and 3 are listed separately.

Table S3: Genes identified by RNAi phenotypes with associated Drosophila alleles.

Drosophila alleles are shown as annotated in Flybase (*7*). See table for details.

Table S4: Functional groups classified by InterPro domain prediction.

InterPro results classified into functionally related groups. See also Table S1 for complete list of genes and specific IPR domains assigned within each group. Queries were performed with InterPro 7.0 (*8*).

Table S5: Orthologs and Homologs of cell growth and viability hits.

'Orthologs' are defined as reciprocal best BLASTP hits against the five proteomes in S. *cerevisiae, C. elegans, A. gambiae, M. musculus* and *H. sapiens* (protein databases obtained from ENSEMBL, see also Materials and Methods). *Drosophila* proteins with 'homologs' in other species were defined as having at least one BLASTP hit with E < 1e-6. Total number of matches are those identified from the set of 438 predicted *Drosophila* amino acid queries. Summary panels (grey): percentage is the total distribution of orthologs/homologs found and categorized as either "no match", "yeast" (yeast and animal matches) or "animal" (no yeast match). Result panels (white): percentage is of the 438 sequences identified in the screen that also identified a match in the proteome of the searched organinisms: *S. cerevisiae* (Sc), *C. elegans* (Ce), *A. gambiae* (Ag), *M. musculus* (Mm) or *H. sapiens* (Hs).

Table S6: Human disease homologs of Drosophila genes with RNAi cell growth and

viability phenoytpes.

Predicted genes identified in *Drosophila* RNAi screen were translated and searched by BLASTP for human homologs associated with human disease. Symbol: human gene name.

References

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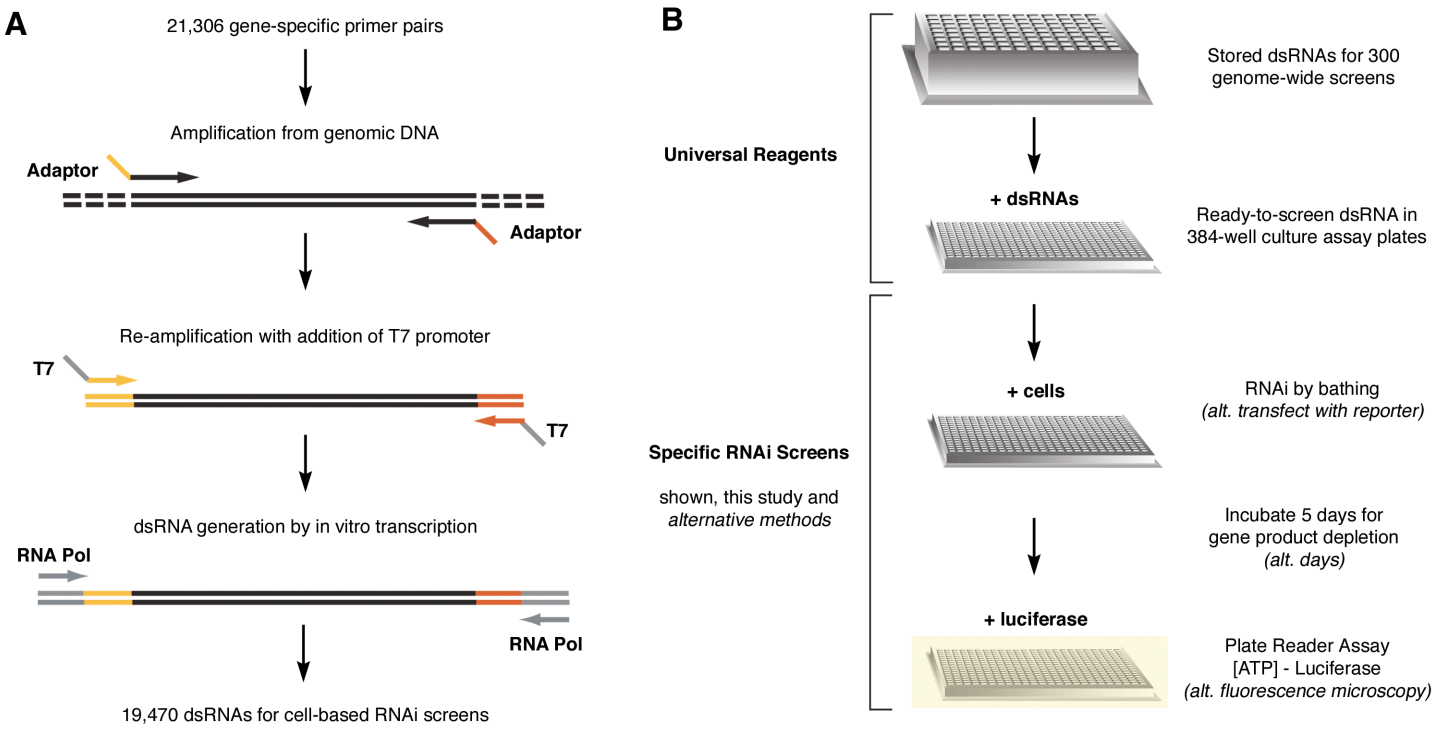
Figure S1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Adaptor
Adaptor
Re-amplification with addition of T7 promoter
T7
T7
dsRNA generation by in vitro transcription
RNA Pol
RNA Pol
19,470 dsRNAs for cell-based RNAi screens
B
Universal Reagents
Stored dsRNAs for 300 genome-wide screens
+ dsRNAs
Ready-to-screen dsRNA in 384-well culture assay plates
Specific RNAi Screens
shown, this study and alternative methods
+ cells
RNAi by bathing
(alt. transfect with reporter)
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+ luciferase
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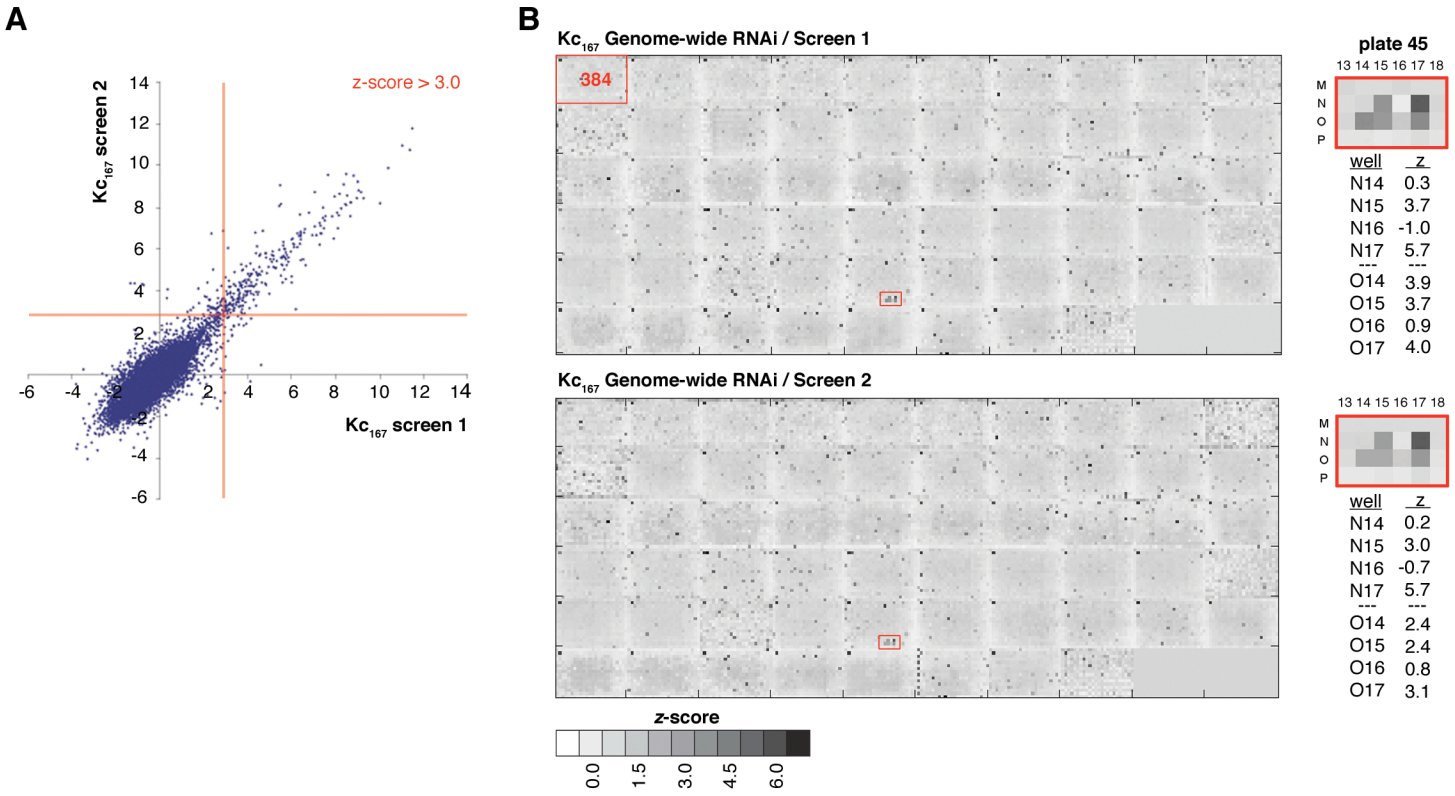
(alt. fluorescence microscopy)

Figure S2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Kc167 screen 1
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O
P
well z
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N15 3.7
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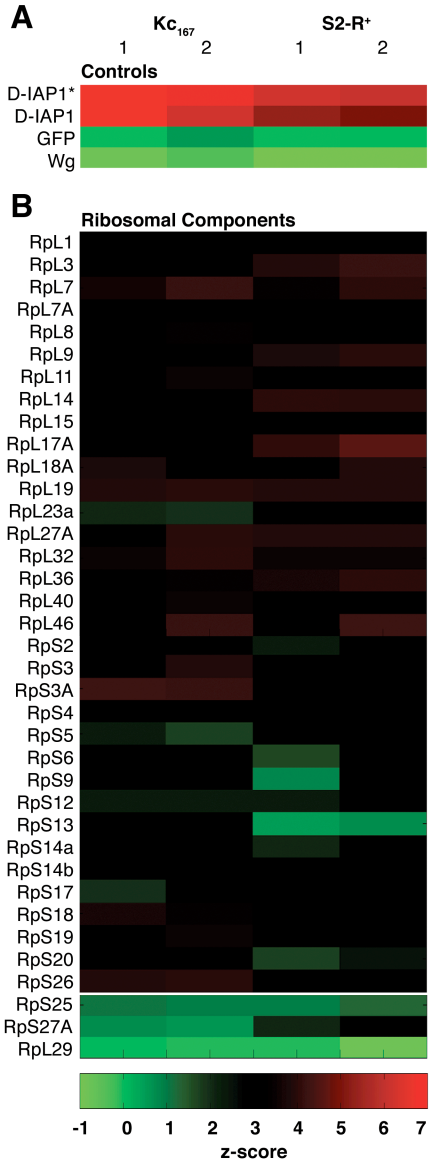
Figure S3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RpL3
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Figure S4

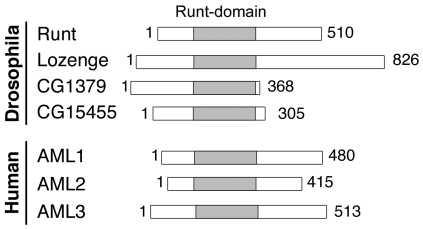
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Figure S5

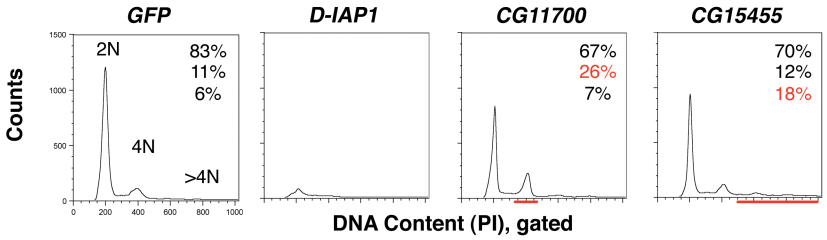
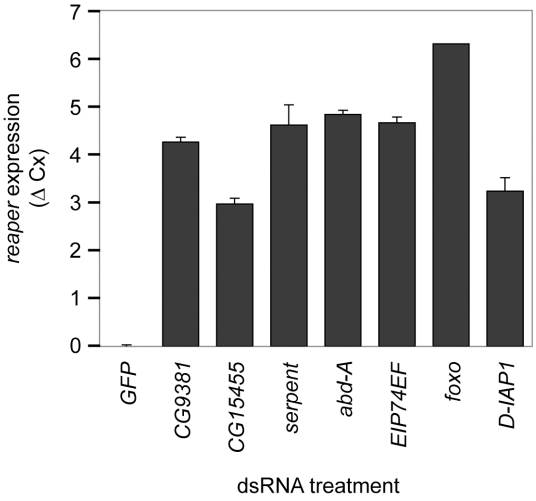

GFP
D-IAP1
CG11700
CG15455
Counts
2N
4N
>4N
83%
11%
6%
67%
26%
7%
70%
12%
18%
1500
1000
500
0
0
200
400
600
800
1000
DNA Content (PI), gated

Figure S6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6
5
4
3
2
1
0
reaper expression
(Δ Cx)
GFP
CG9381
CG15455
serpent
abd-A
EIP74EF
foxo
D-IAP1
dsRNA treatment

Figure S7

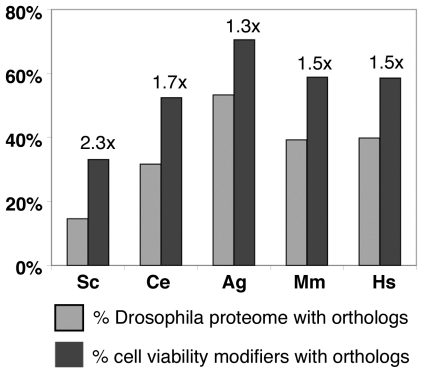
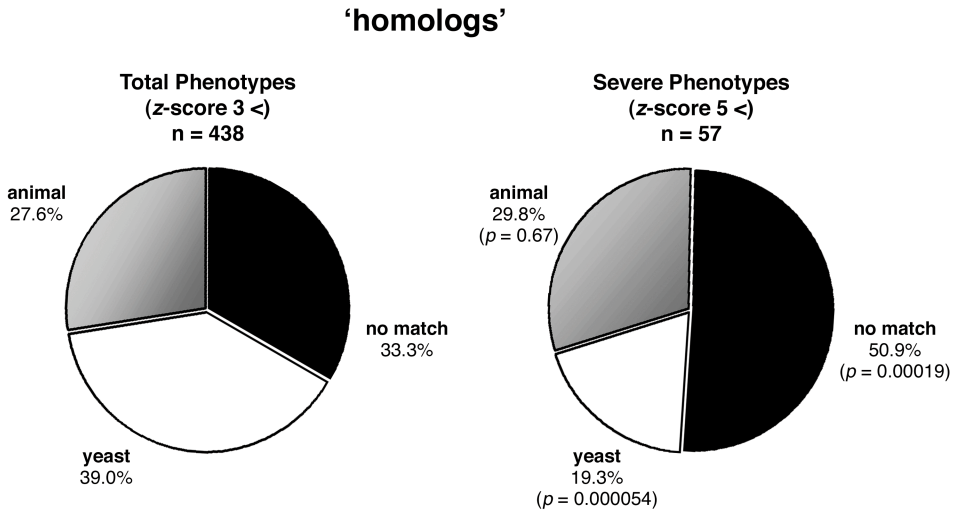

80%
60%
40%
20%
0%
2.3x
1.7x
1.3x
1.5x
1.5x
Sc
Ce
Ag
Mm
Hs
% Drosophila proteome with orthologs
% cell viability modifiers with orthologs

Figure S8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Total Phenotypes
(z-score 3 <)
n = 438
animal
27.6%
no match
33.3%
yeast
39.0%
Severe Phenotypes
(z-score 5 <)
n = 57
animal
29.8%
(p = 0.67)
no match
50.9%
(p = 0.00019)
yeast
19.3%
(p = 0.000054)

Table S1: RNAi Cell Growth and Viability Hits (3 ≤ *z* -score)

Gene name (BDGP R2)	dsRNA ID	dsRNA length	*z* -score [Kc_{167}]	*z* -score [$S2R^+$]	Functional group assignment (based on IPR evidence)	InterPro 7.0 Evidence
CG14023	HFA02353	499	**6.5**	**2.2**		
smt3	HFA03611	269	**3.3**	**1.6**	Proteasome and Ubiquitin	IPR000626; Ubiquitin
CG8222	HFA03080	568	**5.3**	**0.8**	Signaling	IPR000719; Protein kinase; IPR007110; Immunoglobulin-like
RpS13	HFA03419	275	**3.4**	**1.0**	Ribosome and protein synthesis	IPR000589; Ribosomal protein S15
CG13097	HFA02199	516	**3.4**	**3.5**	RNA-binding	IPR007151; Mpp10 protein
CG13098	HFA02200	498	**2.1**	**3.0**		
CycE	HFA03295	512	**2.0**	**3.3**	Cell cycle and Replication	IPR004367; Cyclin, C-terminal
Fs(2)Ket	HFA03328	517	**4.0**	**3.3**	Other	IPR001494; Importin-beta, N-terminal
cad	HFA03502	494	**3.3**	**4.4**	DNA-binding	IPR001356; Homeobox
CG9324	HFA03201	249	**3.5**	**5.1**	Proteasome and Ubiquitin	IPR008012; Proteasome maturation factor UMP1
HmgD	HFA04619	155	**2.0**	**3.2**	DNA-binding	IPR000910; HMG1/2 (high mobility group) box
ken	HFA04696	510	**3.7**	**3.8**	DNA-binding	IPR007087; Zn-finger, C2H2 type; IPR000210; BTB/POZ domain
RpL19	HFA04649	550	**3.9**	**3.9**	Ribosome and protein synthesis	IPR000196; Ribosomal protein L19e
Rpt1	HFA07542	509	**4.5**	**2.9**	Proteasome and Ubiquitin	IPR003959; AAA ATPase, central region
CG1383	HFA06425	518	**3.8**	**3.8**		
CG8055	HFA07061	248	**4.2**	**3.2**	Other	IPR005024; Eukaryotic protein of unknown function DUF279
psq	HFA07668	503	**5.6**	**4.2**	DNA-binding	IPR007889; Helix-turn-helix, Psq
CG7745	HFA07037	510	**1.8**	**3.8**	DNA-binding	IPR006578; MADF
CG10228	HFA05983	506	**4.1**	**3.8**	RNA-binding	IPR006569; Regulation of nuclear pre-mRNA protein
igl	HFA07631	278	**2.6**	**3.0**	Signaling	IPR000048; IQ calmodulin-binding region
CG8092	HFA07076	495	**1.7**	**3.8**		
CG8392	HFA07159	469	**3.1**	**3.2**	Proteasome and Ubiquitin	IPR001353; Multispecific proteasome protease
CG6984	HFA07013	567	**1.6**	**3.3**	Energy and Metabolism	IPR001753; Enoyl-CoA hydratase/isomerase
ProsMA5	HFA07514	493	**5.2**	**4.6**	Proteasome and Ubiquitin	IPR001353; Multispecific proteasome protease
pAbp	HFA07659	490	**3.6**	**3.0**	Ribosome and protein synthesis	IPR002004; Polyadenylate-binding protein/HECT-associated; IPR00050
RpL11	HFA07537	287	**3.2**	**3.0**	Ribosome and protein synthesis	IPR003236; Mitochondrial ribosomal protein L5
CG12031	HFA08235	516	**3.2**	**2.4**		
CG2162	HFA08557	380	**3.4**	**3.1**		
Cdc27	HFA11112	519	**0.6**	**3.0**	Cell cycle and Replication	IPR001440; TPR repeat
CG18656	HFA10335	515	**1.3**	**4.0**		
CG18632	HFA10330	505	**4.1**	**4.0**		
RpS9	HFA11273	212	**3.0**	**1.6**	Ribosome and protein synthesis	IPR001912; Ribosomal protein S4
CG9007	HFA11051	487	**3.1**	**2.4**	Zn-finger	IPR001965; Zn-finger-like, PHD finger; IPR001214; Nuclear protein SET
Trl	HFA11308	517	**5.9**	**4.6**	DNA-binding	IPR007087; Zn-finger, C2H2 type; IPR000210; BTB/POZ domain
Prosbeta2	HFA11257	512	**2.1**	**3.6**	Proteasome and Ubiquitin	IPR001353; Multispecific proteasome protease
th	HFA11404	502	**7.0**	**6.1**	Zn-finger	IPR001841; Zn-finger, RING; IPR001370; Baculovirus inhibitor of apopto
Pros26	HFA11256	516	**2.7**	**3.1**	Proteasome and Ubiquitin	IPR001353; Multispecific proteasome protease
CycT	HFA11124	478	**3.7**	**3.1**	Cell cycle and Replication	IPR006671; Cyclin, N-terminal domain

CG6273	HFA10613	507	**6.1**	**4.7**	DNA-binding	IPR000418; Ets-domain
Rpn1	HFA11274	506	**3.3**	**2.8**	Proteasome and Ubiquitin	IPR002015; Proteasome/cyclosome, regulatory subunit
cno	HFA12374	496	**3.0**	**4.5**	Signaling	IPR001478; PDZ/DHR/GLGF domain; IPR000253; Forkhead-associated
CG12000	HFA12186	481	**3.4**	**3.4**	Proteasome and Ubiquitin	IPR001353; Multispecific proteasome protease
noi	HFA12383	520	**2.1**	**3.5**	Zn-finger	IPR000690; Zn-finger, C2H2 matrin type
CG1475	HFA12265	348	**2.6**	**3.2**	Ribosome and protein synthesis	IPR005822; Ribosomal protein L13
Prosbeta3	HFA16801	514	**2.7**	**3.9**	Proteasome and Ubiquitin	IPR001353; Multispecific proteasome protease
CG14712	HFA14935	510	**1.1**	**3.4**		
CG12207	HFA14477	516	**0.4**	**3.3**	Other	IPR002482; Peptidoglycan-binding LysM
CG5166	HFA15727	434	**7.0**	**4.8**		
CG16941	HFA15166	507	**1.6**	**3.8**	Proteasome and Ubiquitin	IPR000626; Ubiquitin; IPR000061; SWAP/Surp
CG14281	HFA14762	415	**-0.5**	**3.1**	Signaling	IPR002048; Calcium-binding EF-hand
bnl	HFA16913	489	**0.8**	**3.6**	Signaling	IPR002348; Interleukin 1/heparin-binding growth factor
CG12254	HFA14483	510	**4.4**	**5.5**		
cdc2c	HFA16921	185	**0.6**	**3.7**	Cell cycle and Replication	IPR000719; Protein kinase
CG13847	HFA14697	496	**1.6**	**3.4**		
Rpn7	HFA16841	502	**3.9**	**3.2**	Proteasome and Ubiquitin	IPR000717; Proteasome component region PCI
orb	HFA17021	512	**5.6**	**5.1**	RNA-binding	IPR000504; RNA-binding region RNP-1 (RNA recognition motif)
Rpt5	HFA16842	571	**4.2**	**2.6**	Proteasome and Ubiquitin	IPR003593; AAA ATPase
Pros26.4	HFA16799	499	**4.8**	**2.8**	Proteasome and Ubiquitin	IPR003959; AAA ATPase, central region
Rpn2	HFA16839	519	**3.2**	**2.5**	Proteasome and Ubiquitin	IPR002015; Proteasome/cyclosome, regulatory subunit
stg	HFA17071	517	**2.3**	**4.0**	Cell cycle and Replication	IPR001763; Rhodanese-like
CG12054	HFA14467	480	**4.5**	**3.4**	Zn-finger	IPR007087; Zn-finger, C2H2 type
CG11484	HFA17135	496	**1.9**	**3.4**	Proteasome and Ubiquitin	IPR000449; Ubiquitin-associated domain; IPR000504; RNA-binding regi
EG:8D8.6	HFA18583	495	**3.1**	**2.5**	Other	IPR000182; GCN5-related N-acetyltransferase
CG14800	HFA17958	230	**3.3**	**1.6**	DNA-binding	IPR006055; Exonuclease
z	HFA18855	514	**3.3**	**2.6**		
CG12236	HFA17830	520	**4.3**	**5.0**	DNA-binding	IPR007087; Zn-finger, C2H2 type; IPR000210; BTB/POZ domain
CG3918	HFA18331	500	**3.4**	**3.0**		
Bx42	HFA17743	506	**2.6**	**3.7**	DNA-binding	IPR004015; SKIP/SNW domain
BcDNA:GH10646	HFA17735	479	**4.7**	**5.0**	Zn-finger	IPR000315; Zn-finger, B-box; IPR001258; NHL repeat
nej	HFA18801	506	**3.2**	**2.6**	DNA-binding	IPR000433; Zn-finger, ZZ type; IPR001487; Bromodomain; IPR003101;
CG17779	HFA18138	439	**3.2**	**2.7**	Energy and Metabolism	IPR001395; Aldo/keto reductase; IPR005983; KCNAB voltage-gated K+
CG1905	HFA19821	495	**3.3**	**3.1**		
CG12719	HFA19495	439	**4.2**	**3.2**	DNA-binding	IPR001005; Myb DNA-binding domain
CG4453	HFA19904	499	**1.4**	**3.9**	Zn-finger	IPR001876; Zn-finger, Ran-binding
RpP2	HFA00783	254	**-0.2**	**3.8**	Ribosome and protein synthesis	IPR001813; Ribosomal protein 60S
CG2807	HFA00535	480	**4.3**	**6.1**		
aop	HFA00801	508	**3.3**	**6.1**	DNA-binding	IPR000418; Ets-domain
CG15410	HFA00425	518	**3.5**	**4.3**	Energy and Metabolism	IPR003439; ABC transporter; IPR003593; AAA ATPase
CG15415	HFA00430	506	**0.6**	**3.1**		
odd	HFA00832	489	**2.7**	**4.9**	Zn-finger	IPR007087; Zn-finger, C2H2 type
RpL40	HFA00782	129	**2.9**	**3.2**	Ribosome and protein synthesis	IPR000626; Ubiquitin; IPR001975; Ribosomal protein L40e
RpL27A	HFA00781	308	**3.5**	**3.9**	Ribosome and protein synthesis	IPR001196; Ribosomal protein L15
Cf2	HFA00744	517	**2.8**	**4.1**	Zn-finger	IPR007087; Zn-finger, C2H2 type
His3.3A	HFA03343	492	**2.4**	**3.9**	DNA-binding	IPR000164; Histone H3

Rpn11	HFA03422	510	**3.8**	**3.6**	Proteasome and Ubiquitin	IPR000555; Mov34
Lam	HFA03359	512	**1.7**	**3.3**	Other	IPR001664; Intermediate filament protein
eIF-4a	HFA03526	510	**3.6**	**4.2**	Ribosome and protein synthesis	IPR001650; Helicase, C-terminal
CG7105	HFA03009	220	**7.5**	**4.8**		
CG7424	HFA03055	160	**2.1**	**3.3**	Ribosome and protein synthesis	IPR000552; Ribosomal protein L44E
raw	HFA03599	513	**1.3**	**3.6**		
CG13109	HFA02207	497	**2.7**	**3.9**	DNA-binding	IPR001092; Basic helix-loop-helix dimerization domain bHLH
hoip	HFA03546	368	**4.0**	**5.3**	Ribosome and protein synthesis	IPR000231; Ribosomal protein L30e
RpL7	HFA03417	577	**4.0**	**3.8**	Ribosome and protein synthesis	IPR005998; Ribosomal protein L7, eukaryotic form
Pros35	HFA03401	502	**3.1**	**3.6**	Proteasome and Ubiquitin	IPR001353; Multispecific proteasome protease
RpL9	HFA03418	315	**2.6**	**3.9**	Ribosome and protein synthesis	IPR000702; Ribosomal protein L6
CG14917	HFA02401	501	**0.9**	**3.3**	Signaling	IPR001660; Sterile alpha motif SAM
CG17008	HFA02557	489	**3.6**	**3.4**	RNA-binding	IPR000504; RNA-binding region RNP-1 (RNA recognition motif)
CG6043	HFA02922	513	**4.5**	**5.6**		
CG9282	HFA03185	435	**3.2**	**3.3**	Ribosome and protein synthesis	IPR000988; Ribosomal protein L24E
BG:DS07721.3	HFA01970	280	**3.7**	**3.0**		
CycE-x2	HFA03296	329	**1.8**	**5.3**	Cell cycle and Replication	IPR004367; Cyclin, C-terminal
fzy	HFA03534	499	**2.1**	**5.3**	Cell cycle and Replication	IPR000002; Cdc20/Fizzy
CG5953	HFA02914	513	**2.4**	**3.0**	DNA-binding	IPR006578; MADF
CG17331	HFA02603	280	**3.9**	**3.8**	Proteasome and Ubiquitin	IPR001353; Multispecific proteasome protease
RpS26	HFA03420	341	**3.9**	**2.9**	Ribosome and protein synthesis	IPR000892; Ribosomal protein S26E
CG15166	HFA02467	354	**2.9**	**3.2**		
Dox-A2	HFA03318	495	**4.0**	**2.6**	Proteasome and Ubiquitin	IPR000717; Proteasome component region PCI
CG12775	HFA03704	477	**3.8**	**2.7**	Ribosome and protein synthesis	IPR001147; Ribosomal protein L21e
CG17949	HFA03757	193	**5.8**	**3.4**	DNA-binding	IPR000558; Histone H2B
CG10614	HFA04082	284	**4.5**	**2.9**	DNA-binding	IPR001356; Homeobox
CG15665	HFA04256	261	**3.1**	**1.0**	Other	IPR002110; Ankyrin
CG4046	HFA04442	136	**3.1**	**2.7**	Ribosome and protein synthesis	IPR000754; Ribosomal protein S9
CG3751	HFA04414	386	**3.3**	**3.4**	Ribosome and protein synthesis	IPR001976; Ribosomal protein S24e
blw	HFA04675	493	**5.4**	**3.0**	Energy and Metabolism	IPR005294; ATP synthase F1, alpha subunit
RpL17A	HFA04648	194	**3.3**	**4.5**	Ribosome and protein synthesis	IPR000218; Ribosomal protein L14b/L23e
CG3124	HFA04339	499	**1.9**	**3.4**		
CG13550	HFA04191	506	**2.0**	**3.6**		
CG3195	HFA04344	202	**3.9**	**4.8**	Ribosome and protein synthesis	IPR000911; Ribosomal protein L11
RpL46	HFA04651	343	**3.8**	**3.9**	Ribosome and protein synthesis	IPR000077; Ribosomal protein L39e
Mov34	HFA04624	505	**4.7**	**5.3**	Proteasome and Ubiquitin	IPR003639; Mov34, subtype 1
CG3183	HFA04984	508	**3.6**	**1.5**		
CG9469	HFA05013	513	**2.7**	**4.0**		
CG11198	HFA06059	504	**3.3**	**2.8**	Energy and Metabolism	IPR005482; Biotin carboxylase, C-terminal
Hey	HFA07440	506	**2.7**	**4.1**	DNA-binding	IPR001092; Basic helix-loop-helix dimerization domain bHLH
Pabp2	HFA07501	385	**3.4**	**3.9**	RNA-binding	IPR000504; RNA-binding region RNP-1 (RNA recognition motif)
CG13755	HFA06423	154	**6.1**	**3.3**	Other	IPR007110; Immunoglobulin-like; IPR003961; Fibronectin, type III
CG13739	HFA06410	492	**0.9**	**3.6**		
CG12208	HFA06118	500	**1.3**	**3.3**	DNA-binding	IPR001628; Zn-finger, C4-type steroid receptor
CG12912	HFA06227	304	**7.0**	**5.4**		
CG12904-x2	HFA06219	513	**3.8**	**4.5**	Energy and Metabolism	IPR005821; Ion transport protein

CG12897	HFA06212	452	**4.4**	**4.5**		
CG18381-x2	HFA06742	504	**2.3**	**3.1**	DNA-binding	IPR007087; Zn-finger, C2H2 type; IPR000210; BTB/POZ domain
Prosbeta5	HFA07517	505	**2.3**	**3.2**	Proteasome and Ubiquitin	IPR001353; Multispecific proteasome protease
CG17326	HFA06663	568	**3.7**	**3.9**	Zn-finger	IPR007087; Zn-finger, C2H2 type
CG13235	HFA06355	154	**5.3**	**3.6**		
CG13222	HFA06343	485	**4.6**	**4.4**	Other	IPR000618; Insect cuticle protein
CG13165	HFA06292	388	**4.5**	**5.0**		
Su(z)2	HFA07558	510	**4.2**	**4.1**	Zn-finger	IPR001841; Zn-finger, RING
mam	HFA07648	505	**2.9**	**4.5**		
mig	HFA07650	500	**6.0**	**5.1**	Zn-finger	IPR007087; Zn-finger, C2H2 type
CG8171	HFA07088	509	**5.9**	**4.8**		
CG12305-x2	HFA06127	508	**5.3**	**4.5**		
CG8332	HFA07151	207	**4.2**	**2.5**	Ribosome and protein synthesis	IPR002222; Ribosomal protein S19/S15
RpP1	HFA07539	320	**2.5**	**3.5**	Ribosome and protein synthesis	IPR001813; Ribosomal protein 60S
CG15607	HFA06560	483	**3.3**	**5.1**	Zn-finger	IPR007087; Zn-finger, C2H2 type
RpL18A	HFA07538	299	**3.5**	**3.6**	Ribosome and protein synthesis	IPR002670; Ribosomal L18ae protein
CG14494	HFA06465	210	**1.4**	**3.1**		
CG14504	HFA06475	291	**2.9**	**4.5**		
CG9811-x2	HFA07344	286	**4.5**	**4.8**	Signaling	IPR003579; Ras small GTPase, Rab type
RpS18	HFA07540	266	**3.6**	**2.7**	Ribosome and protein synthesis	IPR001892; Ribosomal protein S13
CG18001	HFA07818	207	**1.5**	**3.4**	Ribosome and protein synthesis	IPR002675; Ribosomal L38e protein
CG13798	HFA08333	479	**2.9**	**4.3**		
RpL8	HFA08695	505	**3.2**	**3.4**	Ribosome and protein synthesis	IPR002171; Ribosomal protein L2
CG18334	HFA08532	503	**3.0**	**3.4**	Ribosome and protein synthesis	IPR002171; Ribosomal protein L2
CG12188	HFA08263	448	**0.3**	**3.4**	DNA-binding	IPR003034; DNA-binding SAP; IPR004018; RPEL repeat
CG14952	HFA08412	367	**0.9**	**4.6**		
CG12740	HFA08293	379	**1.7**	**3.1**	Ribosome and protein synthesis	IPR002672; Ribosomal L28e protein
CG14978	HFA08437	499	**-1.1**	**3.6**	Other	IPR004018; RPEL repeat
CG14975	HFA08434	144	**2.9**	**3.3**		
Ubi-p63E	HFA08703	426	**7.7**	**5.9**	Proteasome and Ubiquitin	IPR000626; Ubiquitin
CG14982	HFA08440	499	**1.1**	**4.3**		
scrt	HFA08736	509	**0.8**	**4.4**	Zn-finger	IPR007087; Zn-finger, C2H2 type
CG7468	HFA08593	313	**1.6**	**3.9**	Other	IPR001107; Band 7 protein
CG14821	HFA10192	514	**2.6**	**4.3**		
CG17742	HFA10285	279	**1.5**	**4.1**		
CG14833	HFA10203	430	**4.5**	**5.0**	Other	IPR000008; C2 domain
CG8294	HFA10977	431	**1.3**	**3.9**		
CG13683	HFA10041	165	**6.8**	**4.8**		
CG13675	HFA10034	517	**2.3**	**3.8**	Other	IPR002557; Chitin binding Peritrophin-A
CG13673	HFA10032	287	**0.9**	**3.5**		
CG6694	HFA10696	500	**2.6**	**4.6**	Zn-finger	IPR000571; Zn-finger, C-x8-C-x5-C-x3-H type
RpL14	HFA11269	191	**2.9**	**4.0**	Ribosome and protein synthesis	IPR005824; KOW
CG3982	HFA10394	493	**1.0**	**3.3**		
CG7283	HFA10798	479	**2.3**	**3.7**	Ribosome and protein synthesis	IPR002143; Ribosomal protein L1
CG4328	HFA10410	489	**1.7**	**3.4**	DNA-binding	IPR001356; Homeobox
CG17615	HFA10275	481	**1.0**	**3.4**	DNA-binding	IPR001781; Zn-binding protein, LIM

eyg	HFA11344	497	**3.4**	**4.6**	DNA-binding	IPR001356; Homeobox
CG10682	HFA09778	570	**0.8**	**3.5**	Proteasome and Ubiquitin	IPR000608; Ubiquitin-conjugating enzymes
ara	HFA11322	516	**0.4**	**3.1**	DNA-binding	IPR001356; Homeobox
RpS4	HFA11272	245	**3.1**	**3.2**	Ribosome and protein synthesis	IPR000876; Ribosomal protein S4E
CG17689	HFA10280	479	**1.1**	**3.3**		
CG6419	HFA10634	505	**0.2**	**3.1**	DNA-binding	IPR000910; HMG1/2 (high mobility group) box
ind	HFA11355	502	**1.7**	**5.0**	DNA-binding	IPR000008; C2 domain
CG5151	HFA10487	500	**-0.1**	**4.0**		
Rpn12	HFA11275	502	**3.1**	**4.2**	Proteasome and Ubiquitin	IPR006746; 26S proteasome non-ATPase regulatory subunit Nin1/mts3
CG6064	HFA10578	493	**0.4**	**3.8**		
CG6884	HFA10739	493	**5.1**	**5.6**		
CG6846	HFA10726	425	**2.7**	**4.0**	Ribosome and protein synthesis	IPR005824; KOW
HLH106	HFA11182	493	**4.3**	**2.3**	DNA-binding	IPR001092; Basic helix-loop-helix dimerization domain bHLH
CG15869	HFA11739	412	**3.5**	**3.8**		
CG11451	HFA11663	519	**1.0**	**3.6**		
CG13256	HFA11703	252	**3.4**	**4.6**	Other	IPR002172; Low density lipoprotein-receptor, class A; IPR000859; CUB
CG13257	HFA11704	440	**0.2**	**3.4**		
CG12977	HFA11687	330	**2.0**	**3.6**		
Eip78C	HFA11864	508	**1.0**	**4.3**	DNA-binding	IPR000536; Ligand-binding domain of nuclear hormone receptor
Pros54	HFA11876	487	**1.4**	**3.1**	Proteasome and Ubiquitin	IPR003903; Ubiquitin interacting motif
CG7177	HFA11813	507	**0.5**	**3.3**	Signaling	IPR000719; Protein kinase
msopa	HFA11895	228	**1.7**	**3.5**		
CG14459	HFA11719	431	**3.9**	**5.6**		
Qm	HFA11947	256	**3.2**	**3.6**	Ribosome and protein synthesis	IPR001197; Ribosomal protein L10E
CG9805	HFA12339	518	**0.8**	**3.1**	Ribosome and protein synthesis	IPR000717; Proteasome component region PCI
CG1161	HFA12180	205	**4.3**	**4.3**	Other	IPR008853; TMEM9
CG2099	HFA12302	195	**3.0**	**4.2**	Ribosome and protein synthesis	IPR001780; Ribosomal protein L35Ae
Scr	HFA12604	494	**3.8**	**4.4**	DNA-binding	IPR001356; Homeobox
alphaTub84B	HFA12622	507	**0.6**	**3.4**	Cell cycle and Replication	IPR003008; Tubulin/FtsZ, GTPase
CG10040	HFA12500	506	**1.2**	**5.0**	Zn-finger	IPR007087; Zn-finger, C2H2 type
alphaTub84D	HFA12623	507	**0.4**	**3.2**	Cell cycle and Replication	IPR003008; Tubulin/FtsZ, GTPase
CG11603	HFA14333	290	**5.5**	**5.0**		
CG11745-x2	HFA14371	514	**1.2**	**3.3**	DNA-binding	IPR000637; HMG-I and HMG-Y DNA-binding domain (A+T-hook)
CG9836	HFA16572	375	**1.9**	**4.7**	Energy and Metabolism	IPR002871; Nitrogen-fixing NifU-like, N-terminal
CG16777	HFA15152	504	**1.6**	**3.4**		
CG9381-x2	HFA16484	386	**6.2**	**6.0**	Zn-finger	IPR001841; Zn-finger, RING
karyopherin-alpha3	HFA16976	502	**1.0**	**3.3**	Other	IPR002652; Importin alpha-like protein, beta-binding region;
alphaTub85E	HFA16899	511	**0.3**	**3.4**	Cell cycle and Replication	IPR003008; Tubulin/FtsZ, GTPase
RpL3	HFA16834	262	**2.7**	**4.1**	Ribosome and protein synthesis	IPR000597; Ribosomal protein L3
CG18158	HFA15311	477	**5.8**	**5.5**	DNA-binding	IPR001628; Zn-finger, C4-type steroid receptor; IPR000536; Ligand-binc
Pros25	HFA16798	231	**2.6**	**4.2**	Proteasome and Ubiquitin	IPR001353; Multispecific proteasome protease
CG5844	HFA15890	514	**4.4**	**5.3**	Energy and Metabolism	IPR001753; Enoyl-CoA hydratase/isomerase
Fad	HFA16668	133	**4.6**	**1.1**	Energy and Metabolism	IPR001522; Fatty acid desaturase, type 1
BcDNA:LD41548	HFA14165	172	**0.2**	**3.0**	Energy and Metabolism	IPR000819; Peptidase M17, cytosol aminopeptidase, C-terminal
CG9930	HFA16585	501	**5.2**	**3.6**	DNA-binding	IPR001356; Homeobox
His4r	HFA16703	365	**3.1**	**5.0**	DNA-binding	IPR001951; Histone H4

CG7552	HFA16257	264	**6.6**	**4.1**	Other	IPR001202; WW/Rsp5/WWP domain
CG5143	HFA15724	486	**2.5**	**4.4**	Signaling	IPR001452; SH3 domain; IPR003961; Fibronectin, type III
CG6118	HFA15968	498	**5.4**	**2.7**	Other	IPR000210; BTB/POZ domain
srp	HFA17068	482	**4.8**	**3.1**	DNA-binding	IPR000679; Zn-finger, GATA type
abd-A	HFA16897	363	**6.3**	**5.3**	DNA-binding	IPR001356; Homeobox
CG7305	HFA16225	499	**1.2**	**3.2**		
cpo	HFA16926	502	**0.2**	**3.6**	RNA-binding	IPR000504; RNA-binding region RNP-1 (RNA recognition motif)
CG18435	HFA15327	470	**2.9**	**3.7**	RNA-binding	IPR000504; RNA-binding region RNP-1 (RNA recognition motif)
CG12349	HFA14503	298	**1.8**	**3.2**	RNA-binding	IPR000504; RNA-binding region RNP-1 (RNA recognition motif)
CG7901	HFA16337	373	**4.0**	**3.7**	Signaling	IPR002554; Protein phosphatase 2A, regulatory B subunit (B56 family)
CG14307	HFA14786	518	**4.2**	**3.9**		
CG17836	HFA15282	509	**5.7**	**4.5**	DNA-binding	IPR000637; HMG-I and HMG-Y DNA-binding domain (A+T-hook)
CG5035	HFA15701	475	**3.3**	**3.2**		
CG5466	HFA15808	493	**6.5**	**4.6**		
E2f	HFA16655	492	**0.6**	**3.7**	DNA-binding	IPR003316; Transcription factor E2F/dimerisation partner (TDP)
CG13844	HFA14694	274	**1.5**	**4.0**	Energy and Metabolism	IPR002076; GNS1/SUR4 membrane protein
CG17622	HFA15247	405	**4.0**	**5.3**		
CG7031	HFA16178	508	**3.1**	**5.3**		
RpS3	HFA16838	508	**3.6**	**2.3**	Ribosome and protein synthesis	IPR001351; Ribosomal protein S3, C-terminal
CG13824	HFA14674	216	**5.8**	**5.0**		
CG13820	HFA14670	157	**2.4**	**3.8**		
msi	HFA17003	508	**3.7**	**0.1**	RNA-binding	IPR000504; RNA-binding region RNP-1 (RNA recognition motif)
CG5079	HFA15713	421	**1.2**	**3.3**		
CG4759	HFA15638	389	**2.7**	**3.9**	Ribosome and protein synthesis	IPR001141; Ribosomal protein L27e
CG14554	HFA14897	321	**0.2**	**3.4**		
CG14236	HFA14730	145	**4.7**	**2.5**		
CG5606	HFA15840	501	**-0.2**	**3.0**		
CG12425	HFA14515	398	**2.3**	**4.5**		
CG12852	HFA14558	330	**4.1**	**4.4**		
CG16918-x2	HFA15165	253	**3.6**	**3.3**	Other	IPR001254; Peptidase S1, chymotrypsin family
CG15504	HFA15045	506	**1.7**	**4.4**	DNA-binding	IPR001275; DM DNA-binding; IPR005173; DMRTA motif
ATPsyn-gamma	HFA14094	507	**2.8**	**3.2**	Energy and Metabolism	IPR000131; H+-transporting two-sector ATPase, gamma subunit
CG15507	HFA15048	475	**-1.0**	**3.0**		
CG7808	HFA16318	376	**4.3**	**2.4**	Ribosome and protein synthesis	IPR001047; Ribosomal protein S8E
RpL32	HFA16835	456	**3.8**	**3.6**	Ribosome and protein synthesis	IPR001515; Ribosomal protein L32e
CG1883	HFA15394	319	**4.2**	**2.5**	Ribosome and protein synthesis	IPR000554; Ribosomal protein S7E
wts	HFA17096	504	**4.3**	**5.3**	Signaling	IPR000719; Protein kinase
CG12071	HFA14471	507	**3.1**	**4.9**	Zn-finger	IPR007087; Zn-finger, C2H2 type
CG11522	HFA14323	547	**3.2**	**3.1**	Ribosome and protein synthesis	IPR000915; Ribosomal protein L6E
Med	HFA16737	495	**0.5**	**3.5**	DNA-binding	IPR001132; Dwarfin protein
RpS3A	HFA17168	450	**4.4**	**3.1**	Ribosome and protein synthesis	IPR001593; Ribosomal protein S3Ae
CG9905	HFA17160	518	**0.8**	**3.9**	Proteasome and Ubiquitin	IPR000449; Ubiquitin-associated domain; IPR000504; RNA-binding regi
ATPsyn-beta	HFA17194	508	**5.0**	**2.0**	Energy and Metabolism	IPR004100; H+-transporting two-sector ATPase, alpha/beta subunit, N-te
RpL36	HFA18708	400	**3.4**	**3.9**	Ribosome and protein synthesis	IPR000509; Ribosomal protein L36E
A3-3	HFA17225	495	**0.7**	**3.1**	DNA-binding	IPR004827; Basic-leucine zipper (bZIP) transcription factor
EG:22E5.12	HFA18516	340	**3.7**	**3.4**	Zn-finger	IPR001841; Zn-finger, RING

CG18843	HFA18184	505	**3.7**	**1.7**	RNA-binding	IPR003107; RNA-processing protein, HAT helix; IPR001440; TPR repea
dm	HFA18762	505	**4.6**	**5.0**	DNA-binding	IPR001092; Basic helix-loop-helix dimerization domain bHLH
CG13020	HFA17892	509	**1.8**	**4.1**	Other	IPR007110; Immunoglobulin-like
CG12632	HFA17843	581	**0.9**	**3.2**	DNA-binding	IPR001766; Fork head transcription factor
CG16828	HFA18119	516	**3.3**	**4.2**	Signaling	IPR001806; Ras GTPase superfamily
CG4985	HFA18383	495	**1.6**	**3.2**	Zn-finger	IPR002515; Zn-finger, C2HC type
CG12683	HFA17874	779	**3.2**	**3.8**		
CG15470	HFA18053	469	**5.5**	**4.0**		
CG14447	HFA17940	157	**3.8**	**2.3**	Signaling	IPR001478; PDZ/DHR/GLGF domain
CG11700	HFA17794	456	**7.2**	**5.7**	Proteasome and Ubiquitin	IPR000626; Ubiquitin
Rpt4	HFA18713	307	**3.2**	**2.5**	Proteasome and Ubiquitin	IPR005937; 26S proteasome subunit P45
RpL7A	HFA18709	355	**2.5**	**3.3**	Ribosome and protein synthesis	IPR004038; Ribosomal protein L7Ae/L30e/S12e/Gadd45
CG3075	HFA18272	497	**5.6**	**4.4**	DNA-binding	IPR003958; Transcription factor CBF/NF-Y/archaeal histone; IPR007124
RpS14a	HFA18710	475	**3.2**	**2.0**	Ribosome and protein synthesis	IPR001971; Ribosomal protein S11
RpS14b	HFA18711	475	**3.3**	**2.3**	Ribosome and protein synthesis	IPR001971; Ribosomal protein S11
CG15341	HFA18026	519	**2.4**	**4.2**	Ribosome and protein synthesis	IPR004172; L27 domain
CG15365	HFA18038	504	**3.7**	**4.1**		
CG9725	HFA18442	338	**4.4**	**3.3**	Proteasome and Ubiquitin	IPR000626; Ubiquitin
sbr	HFA20368	515	**1.9**	**3.0**	Other	IPR002075; Nuclear transport factor 2 (NTF2); IPR001611; Leucine-rich
Rpt3	HFA20283	513	**3.5**	**3.0**	Proteasome and Ubiquitin	IPR005937; 26S proteasome subunit P45
CG15223	HFA19661	149	**3.5**	**2.5**		
CG15740	HFA19706	490	**3.7**	**5.1**	Other	IPR004019; YLP motif
CG12720	HFA19496	515	**4.1**	**4.4**		
Smr	HFA20288	494	**4.7**	**4.6**	DNA-binding	IPR001005; Myb DNA-binding domain
CG18646	HFA19816	513	**3.9**	**4.0**	Signaling	IPR000331; Rap/ran-GAP; IPR000210; BTB/POZ domain
CG2033	HFA19831	386	**3.5**	**2.4**	Ribosome and protein synthesis	IPR000630; Ribosomal protein S8
CG15753	HFA19718	509	**4.0**	**4.1**		
CG15759	HFA19724	436	**4.1**	**1.9**	Energy and Metabolism	IPR000863; Sulfotransferase
CG12454	HFA19458	177	**5.3**	**3.8**		
rut	HFA20367	516	**3.3**	**4.0**	Signaling	IPR001054; Guanylate cyclase
CG8198	HFA20092	197	**0.0**	**3.0**	Other	IPR000361; Protein of unknown function, HesB/YadR/YfhF
CycD	HFA20233	509	**0.6**	**3.7**	Cell cycle and Replication	IPR006670; Cyclin
bss	HFA20315	300	**7.2**	**6.3**	DNA-binding	IPR000910; HMG1/2 (high mobility group) box
CG4416	HFA19902	498	**1.0**	**3.3**	Zn-finger	IPR007087; Zn-finger, C2H2 type
RpS19	HFA20281	377	**3.5**	**2.6**	Ribosome and protein synthesis	IPR001266; Ribosomal protein S19e
CG8926	HFA20123	376	**2.1**	**3.2**	Signaling	IPR001849; Pleckstrin-like
B-H2	HFA19335	487	**4.6**	**4.5**	DNA-binding	IPR001356; Homeobox
CG5960	HFA19967	514	**4.2**	**5.2**	Signaling	IPR001936; Ras GTPase-activating protein
CG15063	HFA19637	187	**5.2**	**3.4**		
CG14200	HFA19555	514	**2.7**	**4.2**	Zn-finger	IPR007087; Zn-finger, C2H2 type
CG15455	HFA20526	382	**7.4**	**5.3**	DNA-binding	IPR000040; Acute myeloid leukemia 1 protein (AML 1)/Runt
RpL15	HFA20963	305	**3.1**	**3.2**	Ribosome and protein synthesis	IPR000439; Ribosomal protein L15e
X14215_zmbh_38	HFA21265	367	**5.4**	**5.0**	DNA-binding	IPR000558; Histone H2B
X14215_zmbh_40	HFA21267	403	**4.8**	**5.9**	DNA-binding	IPR000164; Histone H3; IPR007124; Histone-fold/TFIID-TAF/NF-Y; IPR(
	HFA00038	191	**3.8**	**3.6**		
	HFA00256	276	**3.1**	**4.3**		

HFA00976	509	**1.3**	**3.8**		
HFA01162	517	**1.4**	**4.3**	Zn-finger	IPR007087; Zn-finger, C2H2 type
HFA01163	496	**0.4**	**3.2**		
HFA01234	319	**5.2**	**5.9**	RNA-binding	IPR000504; RNA-binding region RNP-1 (RNA recognition motif)
HFA01296	379	**1.4**	**3.8**		
HFA01375	498	**5.3**	**6.9**		
HFA01452	291	**2.3**	**6.1**	Energy and Metabolism	IPR002524; Cation efflux protein
HFA01564	498	**1.0**	**4.0**		
HFA01595	190	**3.8**	**4.7**		
HFA03760	131	**5.1**	**6.1**	DNA-binding	IPR002119; Histone H2A
HFA03850	508	**3.2**	**2.5**		
HFA03998	176	**1.1**	**4.5**		
HFA04017	509	**1.0**	**4.3**		
HFA05108	169	**0.7**	**3.1**		
HFA05209	177	**5.2**	**5.3**		
HFA05234	142	**5.8**	**4.3**		
HFA05259	181	**5.6**	**4.8**		
HFA05360	441	**2.5**	**5.5**		
HFA05468	251	**5.9**	**6.3**		
HFA05553	512	**0.4**	**4.5**		
HFA05623	227	**5.0**	**4.4**		
HFA05689	148	**3.8**	**2.2**		
HFA05695	135	**4.2**	**3.9**		
HFA05715	301	**4.8**	**5.5**		
HFA05769	191	**5.1**	**5.7**		
HFA05792	138	**3.1**	**3.0**		
HFA05818	264	**3.6**	**3.2**		
HFA05853	284	**4.7**	**5.1**		
HFA05871	305	**3.8**	**3.9**		
HFA07932	299	**5.1**	**4.4**		
HFA07938	310	**4.5**	**4.3**		
HFA07992	504	**3.3**	**2.0**	Signaling	IPR001849; Pleckstrin-like
HFA08061	489	**1.7**	**3.2**	Other	IPR004018; RPEL repeat
HFA08063	217	**5.3**	**5.2**		
HFA08080	441	**3.6**	**3.6**		
HFA08748	514	**1.2**	**4.0**		
HFA08770	350	**1.3**	**3.2**		
HFA08772	323	**2.2**	**3.3**		
HFA08800	214	**4.0**	**5.7**		
HFA08841	489	**2.8**	**3.2**	Proteasome and Ubiquitin	IPR003653; SUMO/Sentrin/Ubl1 specific protease
HFA08896	500	**2.8**	**4.1**		
HFA08897	512	**3.6**	**3.7**	DNA-binding	IPR004827; Basic-leucine zipper (bZIP) transcription factor
HFA08919	329	**6.2**	**5.7**		
HFA08991	485	**3.2**	**3.9**		
HFA09005	333	**2.6**	**3.2**		
HFA09009	327	**3.9**	**3.8**		

HFA09047	216	**2.6**	**3.3**		
HFA09067	561	**2.1**	**3.3**		
HFA09069	281	**2.0**	**4.0**		
HFA09076	321	**3.3**	**3.7**		
HFA09077	172	**5.8**	**4.6**		
HFA09206	163	**2.3**	**3.1**		
HFA09230	262	**6.8**	**6.8**		
HFA09263	492	**4.4**	**6.5**	Zn-finger	IPR007087; Zn-finger, C2H2 type
HFA09271	359	**0.8**	**3.3**		
HFA09354	278	**3.2**	**3.7**		
HFA09394	305	**1.8**	**3.6**		
HFA09406	246	**1.7**	**3.1**		
HFA09408	379	**2.4**	**3.9**		
HFA11462	264	**1.6**	**5.2**		
HFA11593	278	**-1.7**	**3.2**	DNA-binding	IPR006578; MADF
HFA12011	239	**-1.4**	**3.2**		
HFA12050	271	**1.1**	**3.1**		
HFA12065	323	**2.3**	**4.2**		
HFA12447	506	**2.9**	**3.7**		
HFA12666	494	**5.4**	**6.4**		
HFA12723	511	**2.3**	**4.5**		
HFA12788	285	**5.6**	**5.7**		
HFA12796	235	**4.6**	**5.6**		
HFA13017	390	**5.5**	**5.3**	DNA-binding	IPR001766; Fork head transcription factor
HFA13053	351	**1.9**	**4.2**		
HFA13139	145	**4.6**	**4.8**		
HFA13145	276	**2.6**	**3.2**		
HFA13174	237	**5.3**	**3.0**		
HFA13190	198	**1.0**	**3.2**		
HFA13271	271	**4.2**	**6.2**		
HFA13286	168	**1.1**	**3.4**		
HFA13298	418	**6.8**	**7.3**		
HFA13530	299	**0.7**	**4.2**		
HFA13640	809	**-0.6**	**4.7**		
HFA13643	486	**0.9**	**4.7**	Other	IPR000313; PWWP domain
HFA13670	350	**0.7**	**4.2**		
HFA13740	265	**2.6**	**4.3**		
HFA13791	161	**1.0**	**4.4**		
HFA17708	449	**2.8**	**5.9**		
HFA17248	199	**4.6**	**6.6**		
HFA17379	366	**1.9**	**4.7**		
HFA17394	247	**5.7**	**5.9**		
HFA17506	290	**5.2**	**5.4**		
HFA17532	246	**3.3**	**4.7**		
HFA17609	242	**3.2**	**2.6**		
HFA17612	319	**5.6**	**5.9**		

	HFA18953	181	**5.4**	**5.1**		
	HFA18963	276	**3.5**	**4.8**		
	HFA18999	248	**5.6**	**5.4**		
	HFA19001	370	**6.3**	**6.3**		
	HFA19029	348	**5.1**	**6.3**		
	HFA19214	415	**3.0**	**5.8**	Energy and Metabolism	IPR005821; Ion transport protein
	HFA19236	178	**1.8**	**3.5**		
	HFA19267	339	**1.2**	**3.5**		
	HFA20451	414	**0.4**	**4.8**		
so	HFA07693	402	**1.9**	**3.9**	DNA-binding	IPR001356; Homeobox
CG8179	HFA07091	309	**4.6**	**5.0**		
CG13483	HFA10025	517	**0.7**	**3.5**	DNA-binding	IPR000910; HMG1/2 (high mobility group) box
BG:DS07721.3	HFA01970	280	**3.1**	**4.5**		
CG13260	HFA02245	508	**0.4**	**3.5**		
CG15157	HFA02458	503	**5.1**	**5.7**		
bs	HFA04676	511	**3.8**	**5.0**	DNA-binding	IPR002100; Transcription factor, MADS-box
CG14975	HFA08434	144	**5.0**	**3.9**		
Ubi-p63E	HFA08703	426	**5.9**	**6.9**	Proteasome and Ubiquitin	IPR000626; Ubiquitin
CG8615	HFA11016	579	**3.2**	**4.1**	Ribosome and protein synthesis	IPR000039; Ribosomal protein L18e
CG14155	HFA10157	481	**3.4**	**5.4**	RNA-binding	IPR000504; RNA-binding region RNP-1 (RNA recognition motif)
CG16918-x2	HFA15165	253	**2.4**	**3.7**	Other	IPR001254; Peptidase S1, chymotrypsin family
RpL32	HFA16835	456	**3.5**	**3.7**	Ribosome and protein synthesis	IPR001515; Ribosomal protein L32e
RpL22	HFA18707	481	**3.8**	**4.3**	Ribosome and protein synthesis	IPR002671; Ribosomal L22e protein
CG15469	HFA18052	392	**4.0**	**4.4**	DNA-binding	IPR007087; Zn-finger, C2H2 type; IPR000637; HMG-I and HMG-Y DNA-
CG15783	HFA18089	514	**0.9**	**4.5**	DNA-binding	IPR003654; Paired-like homeodomain protein, OAR
CG4136	HFA18349	515	**3.2**	**5.8**	DNA-binding	IPR001356; Homeobox
CG9817	HFA18446	472	**3.1**	**4.9**	Zn-finger	IPR007087; Zn-finger, C2H2 type
fru-x2	HFA16952	515	**2.8**	**3.4**	Other	IPR000210; BTB/POZ domain
EG:133E12.2	HFA18495	495	**2.0**	**4.7**	DNA-binding	IPR001628; Zn-finger, C4-type steroid receptor; IPR000536; Ligand-binc
CG4453	HFA19904	499	**2.1**	**3.4**	Zn-finger	IPR001876; Zn-finger, Ran-binding
CG11769	HFA14378	516	**3.4**	**3.2**		
CG15567	HFA15105	260	**3.6**	**4.3**		

Table S2: Distribution of genes, dsRNAs and phenotypic hits

Chr	Predicted Genes Ensembl (v12.3.1)	%	dsRNA	%	Phenotypes	%
X	2292	17%	3512	17%	79	18%
2L	2444	18%	3705	17%	60	14%
2R	2687	20%	3837	18%	79	18%
3L	2612	19%	4061	19%	100	23%
3R	3392	25%	5123	24%	112	26%
4	82	1%	131	1%	4	1%
U			856	4%	4	1%

Table S3: Genes identified by RNAi phenotypes with associated Drosophila alleles

Gene	Phenotype *in vivo**	RNAi in Cultures†	*z* [Kc_{167}]	*z* [$S2R^+$]
A3-3	Semi-lethal		0.7	3.1
abd-A	Lethal		6.3	5.3
aop	Lethal		3.3	6.1
ara	Lethal		0.4	3.1
B-H2	Visible		4.6	4.5
blw	Lethal		5.4	3.0
bnl	Lethal		0.8	3.6
bs	Lethal		3.8	5.0
bss	Viable, Behavioral		7.2	6.3
cad	Lethal		3.3	4.4
Cdc27	Lethal		0.6	3.0
cdc2c	Lethal		0.6	3.7
CG11473	Uncharacterized		4.0	4.4
CG12054	Uncharacterized		4.5	3.4
CG12719	Lethal		4.2	3.2
CG15365	Lethal		3.7	4.1
CG17836	Uncharacterized		5.7	4.5
CG2162	Uncharacterized		3.4	3.1
CG15469	Uncharacterized		4.0	4.4
CG5953	Uncharacterized		2.4	3.0
CG7552	Modifier (cycE and pnr)		6.6	4.1
CG8055	Uncharacterized		4.2	3.2
CG8092	Uncharacterized		1.7	3.8
CG8926	Uncharacterized		2.1	3.2
cno	Lethal		3.0	4.5
cpo	Lethal		0.2	3.6
CycE	Lethal		2.0	3.3
dm	Lethal		4.6	5.0
Dox-A2	Lethal		4.0	2.6
E2f	Lethal		0.6	3.7
eIF-4a	Lethal		3.6	4.2
Eip78C	Viable		1.0	4.3
eyg	Semi-lethal		3.4	4.6
fru	Lethal		2.8	3.4
Fs(2)Ket	Lethal		4.0	3.3
fzy	Lethal		2.1	5.3
EG:133E12.2 (Hr4)	Lethal		2.0	4.7
His3.3A	Uncharacterized		2.4	3.9
HmgD	Lethal (with GAL4 lines)		2.0	3.2
hoip	Lethal		4.0	5.3
ind	Visible, Neurodefective		1.7	5.0
ken	Lethal		3.7	3.8
Lam	Lethal		1.7	3.3
mam	Lethal		2.9	4.5
Med	Visible (Lethal in trans)		0.5	3.5
Mov34	Lethal		4.7	5.3
msi	Lethal		3.7	0.1
nej	Lethal		3.2	2.6
noi	Lethal		2.1	3.5
odd	Lethal		2.7	4.9
orb	Lethal		5.6	5.1
pAbp	Lethal	Cell death increase (1)	3.6	3.0
Pabp2	Uncharacterized		3.4	3.9
Pros25	Uncharacterized		2.6	4.2
Pros26	Lethal	Cell death increase (2)	2.7	3.1

psq	Lethal		5.6	4.2
raw	Lethal		1.3	3.6
CG14975 (Rdh)	Viable		5.0	3.9
RpL11	Lethal		3.2	3.0
RpL14	Lethal		2.9	4.0
RpL18A	Uncharacterized		3.5	3.6
RpL19	Lethal		3.9	3.9
RpL3	Uncharacterized		2.7	4.1
RpL22	Lethal		3.8	4.3
RpL36	Lethal		3.4	3.9
RpL9	Lethal		2.6	3.9
Rpn2	Lethal (in trans)	Cell death increase (2)	3.2	2.5
RpP2	Lethal		-0.2	3.8
RpS13	Lethal		3.4	1.0
RpS26	Lethal		3.9	2.9
RpS3	Visible, Minute		3.6	2.3
RpS3A	Lethal		4.4	3.1
Rpt1	Lethal	Cell death increase (2)	4.5	2.9
rut	Lethal		3.3	4.0
sbr	Lethal	Cell growth decrease (3,4)	1.9	3.0
Scr	Lethal		3.8	4.4
scrt	Visible		0.8	4.4
Smr	Uncharacterized	Cell death increase (1)	4.7	4.6
smt3	Lethal		3.3	1.6
so	Lethal		1.9	3.9
srp	Lethal		4.8	3.1
stg	Lethal		2.3	4.0
Su(z)2	Lethal		4.2	4.1
th	Lethal	Cell death increase (5)	7.0	6.1
Trl	Lethal		5.9	4.6
wts	Lethal		4.3	5.3
z	Visible		3.3	2.6

* most severe phenotype reported in FlyBase

† previously described dsRNA phenotypes in *Drosophila* cell cultures
(1) S2 cells, Ramet et al., 2002, Nature 416(6881)
(2) S2 cells, Wojcik and DeMartino, 2002, J. Biol. Chem. 277(8): 6188--6197
(3) S2 cells, Gatfield et al., 2001, Curr. Biol. 11(21): 1716--1721
(4) SL2 cells, Herold et al., 2001, RNA, N.Y. 7(12): 1768--1780
(5) S2 cells, Igaki et al., 2002, J. Biol. Chem. 277(26): 23103--23106

Source: FlyBase, PubMed

Table S4. Functional groups classified by InterPro prediction.

Functional Group†	N*
Ribosome and protein synthesis	56
DNA-binding	62
Proteasome and Ubiquitylation	34
Zn-finger proteins	25
Signaling factors	18
Energy and Metabolism	16
RNA-binding	12
Cell cycle and DNA Replication	11
All others	26
Predicted proteins with identifiable domain(s)	260
Predicted proteins without identifiable domain	178

Queries performed with InterPro 7.0.

† InterPro results classified into one of functionally related groups.
See Supplementary Information Table 1 for complete list of genes and specific IPR domains assigned within each group.

* Number of proteins identified with InterPro domains found in 438 translated gene sequences.

Table S5: Orthologs and homologs of cell growth and viability hits

RNAi phenotype		'Orthologs' Reciprocal best BLASTP								'Homologs' BLASTP with p < 1e-6							
		171 39.0%	**117** 26.7%	**150** 34.2%	**117** 26.7%	**187** 42.7%	**248** 56.6%	**206** 47.0%	**208** 47.5%	**146** 33.3%	**171** 39.0%	**121** 27.6%	**171** 39.0%	**238** 54.3%	**284** 64.8%	**267** 61.0%	**269** 61.4%
Name	**Fragment ID**	**No match**	**Yeast**	**Animal**	**Sc**	**Ce**	**Ag**	**Mm**	**Hs**	**No match**	**Yeast**	**Animal**	**Sc**	**Ce**	**Ag**	**Mm**	**Hs**
CG4710	HFA00038			x			x					x			x		
HDC00793	HFA00256	x								x							
CG3327	HFA00425	x									x		x	x	x	x	x
CG15415	HFA00430			x			x				x		x	x	x	x	x
CG2807	HFA00535		x		x	x	x	x	x		x		x	x	x	x	x
CG11924	HFA00744	x									x		x	x	x	x	x
CG15442	HFA00781		x		x	x	x	x	x		x		x	x	x	x	x
CG2960	HFA00782		x		x	x	x	x	x		x		x	x	x	x	x
CG4087	HFA00783		x		x	x	x	x	x		x		x	x	x	x	x
CG3166	HFA00801			x			x	x	x			x		x	x	x	x
CG3851	HFA00832			x		x	x				x		x	x	x	x	x
HDC01260	HFA00976	x								x							
CG32830	HFA01162	x								x							
CG32955	HFA01163	x									x		x	x	x	x	x
CG31762	HFA01234			x			x				x		x	x	x	x	x
HDC02272	HFA01296	x								x							
CG32970	HFA01375	x								x							
HDC02637	HFA01452	x										x		x	x	x	x
HDC02899	HFA01564	x								x							
HDC02973	HFA01595	x								x							
BG:DS07721.3	HFA01970	x								x							
BG:DS07721.3	HFA01970	x								x							
CG13097	HFA02199		x		x	x	x	x	x		x		x	x	x	x	x
CG13098	HFA02200			x		x	x	x	x			x		x	x	x	x
CG13109	HFA02207			x			x					x			x		x
CG13260	HFA02245	x								x							
CG14023	HFA02353			x					x	x							
CG31868	HFA02401			x					x			x			x	x	x
CG15157	HFA02458	x								x							
CG15166	HFA02467	x								x							
CG31761	HFA02557			x		x		x	x		x		x	x	x	x	x

CG17331	HFA02603			x			x	x	x	x	x			x		x	x	x	x	x
CG5953	HFA02914	x										x								
CG6043	HFA02922					x			x						x				x	
CG7105	HFA03009	x										x								
CG7424	HFA03055			x			x	x	x	x	x			x		x	x	x	x	x
CG8222	HFA03080					x			x	x	x			x		x	x	x	x	x
CG9282	HFA03185			x			x	x	x	x	x			x		x	x	x	x	x
CG9324	HFA03201					x			x	x	x				x			x	x	x
CG3938	HFA03295					x		x	x	x	x			x		x	x	x	x	x
CG3938	HFA03296					x		x	x	x	x			x		x	x	x	x	x
CG10484	HFA03318			x			x	x	x	x	x			x		x	x	x	x	x
CG2637	HFA03328			x			x	x	x	x	x			x		x	x	x	x	x
CG5825	HFA03343	x												x		x	x	x	x	x
CG6944	HFA03359					x		x	x	x	x			x		x	x	x	x	x
CG4904	HFA03401					x		x	x	x	x			x		x	x	x	x	x
CG4897	HFA03417			x			x	x	x	x	x			x		x	x	x	x	x
CG6141	HFA03418			x			x	x	x	x	x			x		x	x	x	x	x
CG13389	HFA03419			x			x	x	x	x	x			x		x	x	x	x	x
CG10305	HFA03420			x			x	x	x	x	x			x		x	x	x	x	x
CG18174	HFA03422			x			x	x	x	x	x			x		x	x	x	x	x
CG1759	HFA03502					x		x	x	x	x				x		x	x	x	x
CG9075	HFA03526			x			x	x	x	x	x			x		x	x	x	x	x
CG4274	HFA03534					x			x	x	x			x		x	x	x	x	x
CG3949	HFA03546			x			x	x	x	x	x			x		x	x	x	x	x
CG12437	HFA03599					x		x	x						x		x	x		
CG4494	HFA03611			x			x	x		x	x			x		x	x		x	x
CG12775	HFA03704			x			x	x	x	x	x			x		x	x	x	x	x
CG17949	HFA03757			x			x	x	x	x	x			x		x	x	x	x	x
CG31618	HFA03760			x			x	x	x	x	x			x		x	x	x	x	x
CG9480	HFA03850			x			x	x	x	x	x			x		x	x	x	x	x
HDC04273	HFA03998	x										x								
CG30424	HFA04017	x										x								
CG33152	HFA04082					x			x					x		x	x	x	x	x
CG13550	HFA04191	x										x								
CG30387	HFA04256					x		x	x	x	x			x		x	x	x	x	x
CG3124	HFA04339	x										x								
CG3195	HFA04344			x			x	x	x	x	x			x		x	x	x	x	x
CG3751	HFA04414			x			x	x	x	x	x			x		x	x	x	x	x
CG4046	HFA04442			x			x	x	x	x	x			x		x	x	x	x	x
CG17950	HFA04619	x													x			x	x	x
CG3416	HFA04624			x			x	x	x	x	x			x		x	x	x	x	x
CG3661	HFA04648			x			x	x	x	x	x			x		x	x	x	x	x
CG2746	HFA04649			x			x	x	x	x	x			x		x	x	x	x	x
CG3997	HFA04651	x												x		x	x		x	x
CG3612	HFA04675			x			x	x	x	x	x			x		x	x	x	x	x
CG3411	HFA04676			x			x	x	x	x	x			x		x	x	x	x	x
CG5575	HFA04696					x			x					x		x	x	x	x	x

CG3183	HFA04984				x				x	x	x				x			x	x	
CG9469	HFA05013	x										x								
HDC05227	HFA05108	x										x								
HDC05561	HFA05209	x										x								
HDC05643	HFA05234	x										x								
HDC05705	HFA05259	x										x								
HDC06050	HFA05360	x										x								
HDC06312	HFA05468	x										x								
CG30470	HFA05553	x										x								
HDC06794	HFA05623	x										x								
HDC07044	HFA05689	x										x								
HDC07108	HFA05695	x										x								
HDC07108	HFA05715	x										x								
HDC07256	HFA05769	x										x								
HDC07332	HFA05792	x										x								
HDC07396	HFA05818	x										x								
HDC07436	HFA05853	x										x								
HDC07480	HFA05871	x										x								
CG10228	HFA05983			x			x	x	x	x	x			x			x	x	x	x	x
CG11198	HFA06059			x			x	x	x	x	x			x			x	x	x	x	x
CG33183	HFA06118				x			x	x	x	x				x			x	x	x	x
CG30089	HFA06127				x				x						x				x		
CG12897	HFA06212	x										x									
CG12904	HFA06219				x			x	x	x	x				x			x	x	x	x
CG12912	HFA06227	x										x									
CG13165	HFA06292				x				x						x				x		
CG13222	HFA06343	x													x				x		
CG13235	HFA06355	x										x									
CG13739	HFA06410	x										x									
CG33141	HFA06423				x				x	x	x				x			x	x	x	x
CG30497	HFA06425				x			x	x	x	x				x			x	x	x	x
CG14494	HFA06465	x										x									
HDC07114	HFA06475	x										x									
CG30460	HFA06560	x													x				x		x
CG17326	HFA06663				x						x				x				x	x	x
CG12052	HFA06742				x				x						x			x	x	x	x
CG6984	HFA07013				x			x	x	x	x			x			x	x	x	x	x
CG7745	HFA07037	x										x									
CG8055	HFA07061			x			x	x	x	x	x			x			x	x	x	x	x
CG8092	HFA07076				x			x	x	x	x			x			x	x	x	x	x
CG8171	HFA07088				x			x	x	x	x				x			x	x	x	x
CG8179	HFA07091	x										x									
CG8332	HFA07151			x			x	x	x	x	x			x			x	x	x	x	x
CG8392	HFA07159			x			x	x	x	x	x			x			x	x	x	x	x
CG9811	HFA07344				x			x	x					x			x	x	x	x	x
CG11194	HFA07440				x				x	x	x				x				x	x	x
CG2163	HFA07501			x			x	x	x	x	x			x			x	x	x	x	x

CG10938	HFA07514			x		x	x	x	x	x			x		x	x	x	x	x
CG12323	HFA07517			x		x	x	x	x	x			x		x	x	x	x	x
CG7726	HFA07537			x		x	x	x	x	x			x		x	x	x	x	x
CG6510	HFA07538			x		x	x	x	x	x			x		x	x	x	x	x
CG4918	HFA07539			x		x	x	x	x	x			x		x	x	x	x	x
CG8900	HFA07540			x		x	x	x	x	x			x		x	x	x	x	x
CG1341	HFA07542			x		x	x	x	x	x			x		x	x	x	x	x
CG3905	HFA07558				x			x						x			x	x	x
CG18285	HFA07631				x			x						x			x		
CG8118	HFA07648				x			x						x			x		
CG8367	HFA07650				x			x					x		x	x	x	x	x
CG5119	HFA07659			x		x	x	x	x	x			x		x	x	x	x	x
CG2368	HFA07668				x			x						x			x	x	x
CG11121	HFA07693				x		x	x	x	x			x		x	x	x	x	x
CG40278	HFA07818			x		x	x	x	x	x			x		x	x	x	x	x
HDC07858	HFA07932	x									x								
CG32334	HFA07938	x									x								
CG1044	HFA07992				x			x	x	x				x			x	x	x
CG32264	HFA08061				x		x	x	x	x				x		x	x	x	x
HDC08312	HFA08063	x									x								
HDC08349	HFA08080	x									x								
CG12031	HFA08235			x		x	x	x	x	x			x		x	x	x	x	x
CG32296	HFA08263				x			x	x	x				x			x	x	x
CG12740	HFA08293				x		x	x	x	x				x		x	x	x	x
CG32306	HFA08333	x												x			x		
CG14952	HFA08412	x									x								
CG14975	HFA08434	x									x								
CG14975	HFA08434	x									x								
CG32264	HFA08437				x		x	x	x	x				x		x	x	x	x
CG14982	HFA08440				x			x						x			x		
CG1263	HFA08532			x		x	x	x	x	x			x		x	x	x	x	x
CG2162	HFA08557				x		x	x	x	x				x		x	x	x	x
CG32245	HFA08593				x		x	x	x	x				x		x	x	x	x
CG1263	HFA08695			x		x	x	x	x	x			x		x	x	x	x	x
CG11624	HFA08703				x		x		x	x			x		x	x	x	x	x
CG11624	HFA08703				x		x		x	x			x		x	x	x	x	x
CG1130	HFA08736				x			x	x	x			x		x	x	x	x	x
HDC08568	HFA08748	x									x								
HDC08642	HFA08770	x									x								
HDC08645	HFA08772	x									x								
HDC08744	HFA08800	x									x								
CG10107	HFA08841				x			x					x		x	x	x	x	x
HDC08980	HFA08896	x									x								
CG17888	HFA08897				x		x	x	x	x				x		x	x	x	x
HDC09080	HFA08919	x									x								
CG32048	HFA08991				x		x	x	x	x				x		x	x	x	x
HDC09392	HFA09005	x									x								

HDC09397	HFA09009	x									x							
HDC09479	HFA09047	x									x							
HDC09511	HFA09067	x									x							
HDC09513	HFA09069	x									x							
HDC09523	HFA09076	x									x							
HDC09524	HFA09077	x									x							
HDC09872	HFA09206	x									x							
HDC09939	HFA09230	x									x							
CG32120	HFA09263				x			x				x		x	x	x	x	x
HDC10026	HFA09271	x									x							
HDC10243	HFA09354	x									x							
HDC10342	HFA09394	x									x							
HDC10381	HFA09406	x									x							
HDC10383	HFA09408	x									x							
CG10682	HFA09778		x			x		x	x	x		x		x	x	x	x	x
CG32139	HFA10025				x			x				x		x	x	x	x	x
CG13673	HFA10032	x									x							
CG13675	HFA10034				x			x					x			x		
CG32365	HFA10041				x			x					x			x		
CG32062	HFA10157				x		x	x	x	x			x		x	x	x	x
CG14821	HFA10192				x			x					x			x		
CG32381	HFA10203				x		x	x	x	x			x		x	x	x	x
CG32105	HFA10275				x		x		x	x		x		x	x	x	x	x
CG17689	HFA10280				x			x	x	x			x			x	x	x
CG17742	HFA10285	x									x							
CG32043	HFA10330	x									x							
CG18656	HFA10335	x									x							
CG3982	HFA10394	x									x							
CG4328	HFA10410	x											x		x	x	x	x
CG5151	HFA10487				x			x					x			x		
CG6064	HFA10578				x				x	x			x				x	x
CG32180	HFA10613				x		x		x	x			x		x	x	x	x
CG32139	HFA10634				x			x				x		x	x	x	x	x
CG6694	HFA10696				x			x		x		x		x	x	x	x	x
CG6846	HFA10726		x			x	x	x	x	x		x		x	x	x	x	x
CG6884	HFA10739				x		x	x	x	x			x			x	x	x
CG7283	HFA10798		x			x	x	x	x	x		x		x	x	x	x	x
CG32365	HFA10977				x			x					x			x		
CG8615	HFA11016		x			x	x	x	x	x		x		x	x	x	x	x
CG9007	HFA11051		x			x	x	x	x	x		x		x	x	x	x	x
CG8610	HFA11112		x			x	x	x	x	x		x		x	x	x	x	x
CG6292	HFA11124				x		x	x	x	x		x		x	x	x	x	x
CG8522	HFA11182				x		x	x	x	x			x		x	x	x	x
CG4097	HFA11256		x			x	x	x	x	x		x		x	x	x	x	x
CG3329	HFA11257		x			x	x	x	x	x		x		x	x	x	x	x
CG6253	HFA11269		x			x	x	x	x	x		x		x	x	x	x	x
CG11276	HFA11272		x			x	x	x	x	x		x		x	x	x	x	x

CG3395	HFA11273			x			x	x	x	x	x			x		x	x	x	x	x
CG7762	HFA11274			x			x	x	x	x	x			x		x	x	x	x	x
CG4157	HFA11275			x			x	x	x	x	x			x		x	x	x	x	x
CG9343	HFA11308				x				x						x		x	x	x	x
CG10571	HFA11322				x				x					x		x	x	x	x	x
CG10488	HFA11344				x				x						x		x	x	x	x
CG32381	HFA11355				x			x	x	x	x				x		x	x	x	x
CG12284	HFA11404				x				x						x			x	x	x
HDC11277	HFA11462	x										x								
CG11100	HFA11593				x			x				x								
CG11451	HFA11663	x													x		x			
CG12977	HFA11687				x				x			x								
CG32432	HFA11703				x				x						x			x		x
CG32432	HFA11704				x				x						x			x		x
CG14459	HFA11719	x										x								
CG32425	HFA11739				x				x	x	x				x			x	x	x
CG7177	HFA11813				x			x	x	x	x			x		x	x	x	x	x
CG18023	HFA11864	x													x		x	x	x	x
CG7619	HFA11876			x			x	x	x	x	x			x		x	x	x	x	x
HDC11470	HFA11895	x										x								
CG17521	HFA11947			x			x	x	x	x	x			x		x	x	x	x	x
HDC11773	HFA12011	x										x								
HDC11852	HFA12050	x										x								
HDC11908	HFA12065	x										x								
CG1161	HFA12180				x			x	x	x	x				x		x	x	x	x
CG12000	HFA12186			x			x	x	x	x	x			x		x	x	x	x	x
CG1475	HFA12265			x			x	x	x	x	x			x		x	x	x	x	x
CG2099	HFA12302			x			x	x	x	x	x			x		x	x	x	x	x
CG9805	HFA12339			x			x	x	x	x	x			x		x	x	x	x	x
CG2534	HFA12374				x			x	x	x	x				x		x	x	x	x
CG2925	HFA12383			x			x	x	x	x	x			x		x	x	x	x	x
HDC12226	HFA12447	x										x								
CG32466	HFA12500				x			x	x	x	x			x		x	x	x	x	x
CG1030	HFA12604				x			x	x	x					x		x	x	x	x
CG1913	HFA12622			x			x	x	x	x	x			x		x	x	x	x	x
CG2512	HFA12623	x												x		x	x	x	x	x
HDC12421	HFA12666				x				x						x			x		
HDC12613	HFA12723	x										x								
CG31395	HFA12788	x										x								
HDC12872	HFA12796	x										x								
CG3143	HFA13017				x			x	x	x	x				x		x	x	x	x
CG3563	HFA13053				x				x						x			x		
HDC13904	HFA13139	x										x								
HDC13910	HFA13145	x										x								
HDC14001	HFA13174	x										x								
HDC14022	HFA13190	x										x								
HDC14221	HFA13271	x										x								

HDC14245	HFA13286	x									x								
HDC14318	HFA13298	x									x								
CG31353	HFA13530	x									x								
HDC15264	HFA13640	x									x								
CG13598	HFA13643				x			x	x	x				x			x	x	x
HDC15395	HFA13670	x									x								
HDC15690	HFA13740	x									x								
HDC15811	HFA13791	x									x								
CG7610	HFA14094			x		x	x	x	x	x			x		x	x	x	x	x
CG7340	HFA14165				x		x	x	x	x				x		x	x	x	x
CG11522	HFA14323			x		x	x	x	x	x			x		x	x	x	x	x
CG11603	HFA14333	x									x								
CG31258	HFA14371	x									x								
CG11769	HFA14378	x									x								
CG12054	HFA14467			x		x		x	x	x			x		x		x	x	x
CG12071	HFA14471				x			x						x		x	x	x	x
CG12207	HFA14477				x		x	x	x	x				x			x	x	x
CG12254	HFA14483				x				x	x				x				x	x
CG31243	HFA14503				x		x	x	x	x				x		x	x	x	x
CG12425	HFA14515				x			x						x			x		
CG12852	HFA14558	x									x								
CG13820	HFA14670	x									x								
CG13824	HFA14674	x									x								
CG33110	HFA14694				x			x					x		x	x	x	x	x
CG13847	HFA14697	x									x								
CG14236	HFA14730	x									x								
CG31475	HFA14762				x		x	x	x	x				x		x	x	x	x
CG14307	HFA14786	x									x								
CG32474	HFA14897				x		x	x	x	x				x		x	x	x	x
CG14712	HFA14935	x											x		x	x	x	x	x
CG15504	HFA15045				x		x	x	x	x				x		x	x	x	x
CG15507	HFA15048	x									x								
CG15567	HFA15105	x									x								
CG16777	HFA15152	x									x								
CG16918	HFA15165	x												x			x	x	x
CG16918	HFA15165	x												x			x	x	x
CG16941	HFA15166				x		x	x	x	x			x		x	x	x	x	x
CG17622	HFA15247	x									x								
CG17836	HFA15282	x									x								
CG11502	HFA15311				x		x	x	x	x				x		x	x	x	x
CG31243	HFA15327				x		x	x	x	x				x		x	x	x	x
CG1883	HFA15394			x		x	x	x	x	x			x		x	x	x	x	x
CG4759	HFA15638			x		x	x		x	x			x		x	x		x	x
CG31209	HFA15701	x												x			x	x	x
CG5079	HFA15713	x									x								
CG31302	HFA15724				x		x	x	x	x				x		x	x	x	x
CG5166	HFA15727				x		x	x	x	x				x			x	x	x

CG5466	HFA15808				x			x						x			x		
CG31058	HFA15840	x									x								
CG5844	HFA15890				x		x	x				x			x	x	x	x	x
CG6118	HFA15968				x			x						x			x	x	x
CG7031	HFA16178	x									x								
CG7305	HFA16225	x												x			x		
CG7552	HFA16257	x												x		x		x	x
CG7808	HFA16318			x		x	x	x	x	x		x			x	x	x	x	x
CG7913	HFA16337			x		x	x	x	x	x		x			x	x	x	x	x
CG9381	HFA16484				x			x	x	x				x		x	x	x	x
CG9836	HFA16572			x		x	x	x	x	x		x			x	x	x	x	x
CG9930	HFA16585				x		x		x	x				x		x	x	x	x
CG6376	HFA16655				x		x	x	x	x				x		x	x	x	x
CG5887	HFA16668			x		x	x	x	x	x		x			x	x	x	x	x
CG3379	HFA16703			x		x	x	x	x	x		x			x	x	x	x	x
CG1775	HFA16737				x			x	x	x				x		x	x	x	x
CG5266	HFA16798			x		x	x	x	x	x		x			x	x	x	x	x
CG5289	HFA16799			x		x	x	x	x	x		x			x	x	x	x	x
CG11981	HFA16801			x		x	x	x	x	x		x			x	x	x	x	x
CG4863	HFA16834			x		x	x	x	x	x		x			x	x	x	x	x
CG7939	HFA16835			x		x	x	x	x	x		x			x	x	x	x	x
CG7939	HFA16835			x		x	x	x	x	x		x			x	x	x	x	x
CG6779	HFA16838			x		x	x	x	x	x		x			x	x	x	x	x
CG11888	HFA16839			x		x	x	x	x	x		x			x	x	x	x	x
CG5378	HFA16841			x		x	x	x	x	x		x			x	x	x	x	x
CG10370	HFA16842			x		x	x	x	x	x		x			x	x	x	x	x
CG10325	HFA16897				x			x						x		x	x	x	x
CG9476	HFA16899	x										x			x	x	x	x	x
CG4608	HFA16913				x		x	x	x	x				x		x	x	x	x
CG10498	HFA16921				x				x	x		x			x	x	x	x	x
CG31243	HFA16926				x		x	x	x	x				x		x	x	x	x
CG12706	HFA16952	x									x								
CG9423	HFA16976				x		x	x	x	x		x			x	x	x	x	x
CG5099	HFA17003			x		x	x	x	x	x		x			x	x	x	x	x
CG10868	HFA17021				x		x	x	x	x				x		x	x	x	x
CG3992	HFA17068			x		x		x				x			x	x	x	x	x
CG1395	HFA17071			x		x	x	x	x	x		x			x	x	x	x	x
CG12072	HFA17096				x		x	x	x	x		x			x	x	x	x	x
CG31992	HFA17135				x			x	x	x				x			x	x	x
CG31992	HFA17160				x			x	x	x				x			x	x	x
CG2168	HFA17168			x		x	x	x	x	x		x			x	x	x	x	x
CG11154	HFA17194			x		x	x	x	x	x		x			x	x	x	x	x
CG11405	HFA17225				x			x	x	x				x			x	x	x
HDC17206	HFA17248	x									x								
HDC17481	HFA17379	x									x								
HDC17524	HFA17394	x									x								
HDC17815	HFA17506	x									x								

HDC17859	HFA17532	x										x							
CG12650	HFA17609	x										x							
HDC18154	HFA17612	x										x							
HDC17114	HFA17708	x										x							
CG12218	HFA17735				x			x						x		x	x	x	x
CG8264	HFA17743			x		x	x	x	x	x			x		x	x	x	x	x
CG11700	HFA17794	x											x		x	x	x	x	x
CG12236	HFA17830				x			x						x			x	x	x
CG12632	HFA17843				x			x					x		x	x	x	x	x
CG12683	HFA17874	x										x							
CG32791	HFA17892				x			x						x		x	x	x	x
CG14447	HFA17940				x			x	x	x				x		x	x	x	x
CG14801	HFA17958			x		x	x						x		x	x	x	x	x
CG32717	HFA18026				x		x	x	x	x			x		x	x	x	x	x
CG15365	HFA18038				x		x	x	x	x				x			x	x	x
CG32772	HFA18052				x			x					x		x	x	x	x	x
CG15470	HFA18053	x										x							
CG15783	HFA18089	x										x							
CG32776	HFA18119	x											x		x	x	x	x	x
CG32688	HFA18138			x		x		x	x	x			x		x	x	x	x	x
CG3193	HFA18184			x		x	x	x	x	x			x		x	x	x	x	x
CG3075	HFA18272			x		x	x	x	x	x			x		x	x	x	x	x
CG3918	HFA18331				x		x	x	x	x				x		x	x	x	x
CG4136	HFA18349				x		x		x	x				x		x	x	x	x
CG32778	HFA18383				x		x	x	x	x				x		x	x	x	x
CG32676	HFA18442				x				x					x				x	
CG9817	HFA18446				x			x					x		x	x	x	x	x
CG16902	HFA18495				x		x	x						x		x	x	x	x
CG4325	HFA18516	x												x		x	x	x	x
CG11412	HFA18583			x		x	x	x	x	x			x		x	x	x	x	x
CG7434	HFA18707			x		x	x	x	x	x			x		x	x	x	x	x
CG7622	HFA18708			x		x	x	x	x	x			x		x	x	x	x	x
CG3314	HFA18709			x		x	x	x	x	x			x		x	x	x	x	x
CG1527	HFA18710			x		x	x	x	x	x			x		x	x	x	x	x
CG1527	HFA18711			x		x	x	x	x	x			x		x	x	x	x	x
CG3455	HFA18713			x		x	x	x	x	x			x		x	x	x	x	x
CG10798	HFA18762				x			x	x	x				x			x	x	x
CG15319	HFA18801				x		x	x	x	x			x		x	x	x	x	x
CG7803	HFA18855	x										x							
HDC18647	HFA18953	x										x							
HDC18670	HFA18963	x										x							
HDC18812	HFA18999	x										x							
CG32606	HFA19001	x										x							
HDC18875	HFA19029	x										x							
CG12348	HFA19214				x		x	x	x	x				x		x	x	x	x
HDC19512	HFA19236	x										x							
HDC19589	HFA19267	x										x							

CG5488	HFA19335	x										x		x	x	x	x
CG12454	HFA19458	x								x							
CG4013	HFA19495		x		x	x	x	x	x		x		x	x	x	x	x
CG12720	HFA19496	x								x							
CG14200	HFA19555	x								x							
CG15063	HFA19637	x								x							
CG15223	HFA19661	x								x							
CG15740	HFA19706	x								x							
CG15753	HFA19718	x								x							
CG32632	HFA19724			x		x	x	x	x			x		x	x	x	x
CG18646	HFA19816	x										x		x	x	x	x
CG32662	HFA19821	x								x							
CG2033	HFA19831		x		x	x	x	x	x		x		x	x	x	x	x
CG32575	HFA19902	x										x		x	x	x	x
CG4453	HFA19904			x		x	x					x		x	x	x	x
CG4453	HFA19904			x		x	x					x		x	x	x	x
CG32560	HFA19967		x		x	x	x	x	x		x		x	x	x	x	x
CG8198	HFA20092		x		x	x	x	x	x		x		x	x	x	x	x
CG5004	HFA20123			x				x	x			x		x	x	x	x
CG9096	HFA20233			x		x	x	x	x		x		x	x	x	x	x
CG4464	HFA20281		x		x	x	x	x	x		x		x	x	x	x	x
CG16916	HFA20283		x		x	x	x	x	x		x		x	x	x	x	x
CG4013	HFA20288		x		x	x	x	x	x		x		x	x	x	x	x
CG12223	HFA20315		x		x	x	x	x	x		x		x	x	x	x	x
CG9533	HFA20367			x			x					x		x	x	x	x
CG1664	HFA20368		x		x	x	x	x	x		x		x	x	x	x	x
HDC19951	HFA20451	x								x							
CG15455	HFA20526	x										x		x	x	x	x
CG17420	HFA20963		x		x	x	x	x	x		x		x	x	x	x	x
CG17949	HFA21265		x		x	x	x	x	x		x		x	x	x	x	x
CG31613	HFA21267			x		x	x	x	x		x		x	x	x	x	x

Table S6: Human disease homologs of Drosophila genes with RNAi cell viability phenotypes

Drosophila Gene	BlastP	Identity [%]	Human Gene	RefSeq	Disease
foxo (HFA13017)	5.80E-21	85	*FOXO1A*	NP_002006	Rhabdomyosarcoma
srp	1.90E-47	80	*GATA3*	NP_002042	Hypoparathyroidism, and renal dysplasia
Med	5.90E-96	79	*MADH4*	NP_005350	Pancreatic cancer
HDC19469	2.00E-146	75	*KCNA1*	NP_000208	Episodic Ataxia
CG15455	1.10E-53	73	*RUNX1*	NP_001745	Acute myeloid leukemia
Pabp2	9.50E-56	72	*PAPBN1*	NP_004634	Muscular dystrophy
CG4136	6.00E-38	71	*VSX-1*	NP_055403	Posterior polymorphous corneal dystrophy
nej	0	68	*CREBBP*	NP_004371	Rubenstein-Taybi Syndrom
RpS19	1.10E-48	66	*RPS19*	NP_001013	Anemia, Diamond-Blackfan
CG9930	7.20E-30	66	*EMX2*	NP_004089	Schizencephaly
CG10614	8.50E-28	64	*ARX*	NP_620689	Infantile spasm syndrome
CG11121	1.00E-65	64	*SIX-3*	NP_005404	holoprosencephaly 2
CG11198	0	62	*ACACA*	NP_000655	ACC deficiency
CG32139	9.00E-24	61	*SOX-9*	NP_000337	campomelic dysplasia and autosomal XY sex reversal syndrome
CG12208	1.20E-26	60	*RARA*	NP_000955	Leukemia, acute promyelocytic
abd-A	2.60E-15	52	*IPF1*	NP_000200	Agenesis, diabetes
B-H2	2.60E-16	51	*TLX1*	NP_005512	Leukemia, T-cell acute lymphocytic
aop	2.50E-23	50	*ETV6*	NP_001978	Leukemia, acute lymphoblastic
CG7468	1.10E-64	50	*NPHS2*	NP_055440	Nephrotic syndrome, steroid-resistant
CG17615	2.40E-54	49	*LMX1B*	NP_002307	Nail-patella syndrome
CG9381	3.70E-13	47	*RNF6*	NP_005968	Esophageal carcinoma, somatic
CG7177	2.20E-95	46	*PRKWNK1*	NP_061852	Pseudohypoaldosteronism
CG5166	2.20E-37	45	*SCA2*	NP_002964	Spinocerebellar ataxia-2
CG6273	9.30E-20	44	*ETV6*	NP_001978	Leukemia, acute lymphoblastic
cdc2c	1.50E-55	44	*CDK4*	NP_000066	Melanoma
CG18158	2.90E-74	43	*NR2E3*	NP_055064	Enhanced S-cone syndrome
CG13844	1.60E-53	41	*ELOVL4*	NP_073563	Stargadt disease
eyg	5.70E-43	40	*PAX6*	NP_000271	Aniridia, Keratitis
CG15410	5.90E-42	39	*ABCG8*	NP_071882	Sitosterolemia
scrt	5.20E-25	39	*ZNF145*	NP_005997	Promyelocytic leukemia
Rpt4	1.00E-46	38	*PEX1*	NP_000457	Zellweger syndrome-1
wts	5.80E-67	38	*DMPK*	NP_004400	Myotonic dystrophy
Rpt5	2.90E-42	38	*PEX6*	NP_000278	Peroxisomal biogenesis disorder
CG12188	6.10E-27	38	*MKL1*	NP_065882	Megakaryoblastic leukemia, acute
CycD	2.50E-37	37	*CCND1*	NP_444284	Leukemia/lymphoma, B-cell, 1
CG8222	9.50E-73	37	*PDGFRA*	NP_006197	Gastrointestinal stromal tumor, somatic
Lam	8.00E-84	37	*LMNA1*	NP_733821	Cardiomyopathy, dilated, 1A
CG12071	3.20E-10	34	*SALL1*	NP_002959	Townes-Brocks syndrome
rut	3.60E-26	33	*GUCY2D*	NP_000171	Leber congenital amaurosis, type I
CG15665	1.10E-48	33	*ANK1*	NP_065210	Spherocytosis-2
CG18646	1.50E-11	32	*TSC2*	NP_066400	Lymphangioleiomyomatosis, somatic
Eip78C	2.30E-19	31	*PPARG2*	NP_619726	Glioblastoma, susceptibility to
CG8926	1.50E-10	30	*KIF1B*	NP_055889	Charcot-Marie-Tooth neuropathy, type 2A
CG13020	4.50E-22	30	*TTN*	NP_596869	Cardiomyopathy, dilated
CG9811	1.50E-14	30	*RRAS2*	NP_036382	Ovarian carcinoma
CG15365	2.70E-13	30	*LZTS1*	NP_066300	Esophageal squamous cell carcinoma
CG13755	3.00E-140	30	*NPHS1*	NP_004637	Nephrosis-1, congenital, Finnish type
ken	2.60E-15	30	*EVI1*	NP_005232	acute myeloid leukemia
bnl	6.70E-10	30	*FGF14*	NP_787125	Cerebellar ataxia, autosomal dominant
CG5844	2.10E-17	29	*AUH*	NP_001689	3-methylglutaconic aciduria
BcDNA:GH10646	5.40E-34	27	*TIF1*	NP_003843	Thyroid carcinoma, papillary
blw	1.10E-23	27	*ATP6V1B1*	NP_001683	Renal tubular acidosis
CG5960	1.70E-35	25	*RASA1*	NP_072179	Basal cell carcinoma

Shown are human homologs of Drosophila genes with a BlastP E value of 10^{-10} or less.
Sequences for human disease genes were obtained from LocusLink (http://www.ncbi.nlm.nih.gov/LocusLink) .