Supplementary Information for Friedman and Perrimon, "A functional genomic RNAi screen for novel regulators of RTK and ERK signaling," 2006.

Supplementary Methods

Genome-wide RNAi Screening

The genome-wide dsRNA screening collection of the Drosophila RNAi Screening Center (DRSC) covering >95% of the Drosophila genome has been described previously[31]. This set includes 13,589 annotated (BDGP 3.2) targets and 6,831 predicted genes[32]. All *Drosophila* cell culture methods have been previously described[33].

As a screening cell line, we used S2R+ cells, a variant adherent S2 line[34]. We established a stable cell line of S2R+ cells overexpressing cDNAs for the *Drosophila* ERK ortholog Rolled, tagged at its C-terminus with YFP, and *Drosophila* MEK, Dsor1, tagged at the N-terminus with CFP. These constructs were cloned into pUAST and expressed under actinGal4 control. We observed similar kinetics of ERK activation in response to stimulus (compare Sup. Figs. 1b and 2b) and similar effects of dsRNA knockdown of known RTK/ERK pathway components in this cell line compared to wild-type S2R+ cells (compare Sup. Figs. 1d and 3d). Furthermore, all secondary screens validating our primary screen results were performed in cells not expressing these constructs.

We adapted traditional immunocytochemistry techniques and previous HTS assays[35]. Sixty-two 384-well plates pre-aliquoted with the DRSC library were seeded with 4 x 10^4 cells and incubated in serum-free medium for 45 minutes. Serum-containing medium was added and the cells grown for four days at 25ºC. Subsequently, the medium was replaced with phosphate-buffer saline (PBS) ("baseline") or PBS containing 25μg/mL bovine insulin (Sigma) ("Stimulated") for ten minutes, which we previously established was the peak of insulin-stimulated ERK activation in this cell line. Prior to fixation, we measured total YFP fluorescence from the live cells using an Analyst GT plate reader (Molecular Devices) as an approximate measure of cell number for use during gene selection (see below). The cells were then fixed with 4% formaldehyde for 10 minutes and washed with PBS+0.1%Triton (PBST) twice.

Monoclonal antibodies directed towards the dually phosphorylated mammalian ERK1/2 were obtained from Cell Signaling Technologies, Inc. This antibody was raised against the identical peptide, highly conserved among metazoans, used to generate antibodies previously described to be sensitive and specific in *Drosophila* tissues and cells[36, 37]. The antibody was directly conjugated to Alexa 647 dye using the Alexa Fluor 647 Protein Labeling Kit (Molecular Probes). Following washing, the screening plates were incubated with 750ng/mL conjugated dpERK antibody in PBST supplemented with 3% bovine serum albumin at 4ºC, overnight. Antibody solution was removed and the plates washed with PBST twice before reading YFP (which approximates total ERK, excluding endogenous protein) and Alexa 647 (dpERK) channels. Each time point (baseline and stimulated) was screened in duplicate, totaling over 92,000 dsRNAs.

Data Analysis

Our data analysis procedure was directed towards removing as many sources of noise as possible so that robust genomic information could be extracted from the screen. Many of these issues have been recently reviewed[38], some variations of which were applied here

Raw dpERK and ERK channel intensities were first background subtracted to account for autofluorescence of the plastic screening plates. dpERK values were then divided by ERK values for each channel to yield a normalized dpERK value. To correct spatial irregularities in the fluorescence measurements across individual screening plates, normalized dpERK values were corrected by dividing by a moving median of each well's column and row normalized dpERK values. Corrected, normalized dpERK values were converted to Z-scores, the relative number of standard deviations from the plate mean. We filtered the dataset for outliers by converting Z-scores to ranks for each plate, the replicates summed, and used the top and bottom rank-sums for future analysis. The replicate Z-scores for these wells were then averaged, and a cutoff of +/- 1.5 was chosen for the primary "hit list." This cutoff was chosen to include as many novel regulators as possible at this initial stage; only a small decrease in validation rate in secondary screening was observed for genes with a Z-score +/-1.5-2.0 compared to those with a Z-score +/- 2.0 and above (87% vs. 92%). This suggests that a continuum exists between strong regulators and those having no effect on the pathway, and other regulators, important under particular conditions or

contexts, may have been excluded. This hit list was further pruned for duplicate genes and for genes which appeared to have significant off-target effects (OTE). OTE predictions were based on our observation of a trend in amplicons of correlated lethality and increasing number of predicted 21nt overlaps with other genes (not shown). In general, 19-21nt homology with other genes appear to be a major source of false positives in previous *Drosophila* dsRNA screens[39]. Based on this trend, we removed all genes with >10 21nt OT and >35nt maximum OT length. Since this original curation, the annotation of the *Drosophila* genome has changed and may have altered OT number; however, our filtering nevertheless removed those amplicons most likely to be false-positives. In total, 1,644 amplicons are represented in the pruned primary hit list. The complete primary screen gene list and amplicon sequences are available in Sup. Table 1 and at the DRSC website[33]. Isolation of 476 predicted genes[32] suggests that these may be valid transcripts, and that genome-wide functional analyses such as these can be used to validate gene predictions. Indeed, 11% of these genes scored in recent *Drosophila* gene tiling experiments[40]. We found predicted genes at a slightly lower frequency than the DRSC collection overall ($p < 0.042$ by hypergeometric distribution).

We annotated our hit list for molecular function, conservation, and other features by cross-referencing publicly-available databases. GO terms were downloaded from FlyBase [41], and subgroups as displayed in the text were manually curated from these specific terms; conflicts were resolved on a case-by-case basis. "Receptors" subgroup includes many classes of GO-labeled "receptors," including RTKs. Conservation was determined using the HomoloGene[42] and InParanoid[43] databases of orthologous groups; since a systematic, rather than *de novo* or hand curation was used, there may be orthologs known from specific experimental work that are not listed in our tables, and vice-versa. Human disease relevance was extracted using the Ensembl database[44] and from the Homophila database cross-referencing the *Drosophila* genome with the NCBI Online Mendelian Inheritance in Man (OMIM) project[45]; an $E < 1 \times 10^{-10}$ cutoff was used for this reference.

Enrichment *p* values for GO term categories provided in Sup. Table 2 was performed using the hypergeometric test function implemented by the GeneNotes software previously published[46]. Enrichment for pathway components and particular conservation patterns represented in Sup. Fig. 2 was similarly performed using the hypergeometric distribution. For both, the DRSC collection, not the *Drosophila* genome, was used as the background population,

in order to account for collection bias, although our library is > 95% complete. Median absolute value Z-scores for all hits at baseline (1.9, n=651) were compared to those of those hits at baseline conserved in humans (2.0, n=408) and in humans, mice, worms, and yeast (2.1, n=200), and p values calculated from the raw Z-score cumulative distributions using the Kolmogorov-Smirnov test. Genes conserved across all four species examined had a more significant effect on ERK signaling at baseline ($p < 0.001$) than isolated genes overall. This result implies that the core, conserved signaling cascade is modulated by proteins recently added to the regulatory network with more moderate individual effects on signal propagation. We detected no significant enrichment for genes in our primary hit list based on chromosomal location. Median Z-score of hits with particular GO term categories was compared to all hits at baseline or under stimulation (Fig. 2c) and p values calculated using the Mann-Whitney U test, FDR corrected for multiple hypotheses.

Hierarchical clustering was performed in the Cluster program using Pearson correlation or Euclidean distance, and displayed using TreeView.

Secondary Screening

The 362 genes were chosen for secondary screening/validation by selecting for particular annotation categories with known involvement in the RTK/ERK pathway, such as kinases and phosphatases; pruning all but representative members of entire molecular machines (e.g., catalytic and regulatory proteasome components, ribosomal proteins, etc.), since components of a single complex likely influence pathway activity similarly; selection for conserved components and genes with possible human disease relevance; and pruning the predicted genes[32] to 10% of our list (from 29%), since only a small percentage of these have been validated by experimental data. We estimated cell number using our total ERK-YFP pre-fixation fluorescence value and biased against genes with large effects on viability, as *bona fide* pathway components did not affect this value.

dsRNA targeting these genes were resynthesized and 250ng aliquoted randomly in quintuplicate in 384-well plates along with luciferase dsRNA negative controls. In order to use wild-type cells, we modified our screening assay to use total ERK rabbit antibody (Cell Signaling), along with unconjugated dpERK mouse primary antibody. Similar plate

manipulations were used as in the primary screen, with the addition of secondary antibody and DAPI incubations. DAPI, total ERK (Alexa 488) and dpERK (Alexa 647) channels were recorded on the plate reader. Similar data analysis/normalization procedures were used as above. Normalized, corrected dpERK values are represented as percent of controls, and *p* values were calculated for each gene and assay using the Student's *t*-test. These 362 genes were screened in this format in triplicate (S2R+ baseline and insulin stimulus) or duplicate (all other conditions), and the results of the replicates combined, yielding seven assay points and corresponding *p* values for each gene. Multiple hypothesis correction (370 genes x 7 assays) was applied using the False Discovery Rate (FDR) criteria ($q < 0.05$), as previously described[47, 48]. The FDR-corrected *p* values were used to identify hits in secondary screens which significantly affect dpERK levels in any of the given conditions. Directly comparing replicates of the same amplicons performed in parallel secondary screens resulted in validation by FDR criteria of 80-90% of the genes. Of 362 genes tested, 91% (331) had a significant effect on RTK/ERK signaling under one or more stimuli, indicating a low false-positive rate due to assay noise in the primary screen.

As stated in the text, we performed a separate series of experiments to test the contribution of "off-target effects," e.g., knockdown of non-targeted genes due to homology[39, 49]. We reasoned that non-overlapping amplicons targeting the same gene likely have non-overlapping OTE. Although we could not design additional amplicons for some genes, for many of our hits (~300) we designed one or two amplicons in addition to those used in the DRSC collection, and screened these in parallel to the original amplicon at baseline and under insulin stimulus in S2R+ cells. 85% of the tested genes could be validated with at least one additional amplicon. As this validation rate is equivalent to the validation rate between tests of the same amplicon, we conclude that OTE are not a significant contribution to noise in our assay. Importantly, we believe this high validation rate is a result of our pre-filtering of our primary hit list for genes with >10 21nt predicted off-targets. We have indicated the genes which validated through this assay in Sup. Table 4; other genes either did not validate (also marked) or, more often, were not tested in these additional amplicon experiments.

We used wild-type S2R+ and Kc167 cells for secondary screening of these amplicons at baseline and under insulin stimulus. Kc167 cells have a transcriptional profile distinct from the S2 cell line[50]. We detected similar maximal dpERK elevation in Kc cells at 10 minutes following

insulin stimulation (Sup. Fig. 3a). For analysis under additional RTK stimuli, two stable cell lines were created (S2R+ and Kc167) which express the *Drosophila* EGF receptor, type II (DER) under the metallothionein promoter, similar to a S2 line previously described[51], maintained in puromycin or hygromycin, respectively. No DER was detectable by Western blotting in wild-type S2R+ and Kc cells and no robust dpERK elevation was detected following sSpi stimulus. We observed weak but detectable DER expression by Western blotting without copper stimulus in both stable cell lines (Sup. Fig. 3b-c); this level of DER did not elevate dpERK levels compared to wild-type cells through autoactivation, but still led to DER-dependent dpERK elevation following Spitz stimulation (Sup. Fig. 3b-c). As we observed in the screening cell line, wild-type S2R+ cells stimulated with insulin and S2R+DER cells stimulated with conditioned media collected from cells expressing secreted Spitz (sSpi)[51] demonstrated similar, predicted effects of knockdown of known ERK components (Sup. Fig. 3d and e, respectively). As the primary source of baseline ERK activation is through PVR[52], genes isolated from our screen at baseline but not under insulin stimulus may be specific effectors of PVR but not InR activity (Sup. Fig. 2a). In order to extend these observations, we also screened our secondary gene list in S2R+ cells under hyper-stimulation of PVR. For PVR hyper-stimulation, we unexpectedly observed an increase in dpERK in S2R+ cells following application of serum-free medium conditioned from growth in Kc cells, compared to serum-free medium alone (Sup. Fig. 3f). This increase was reduced by knockdown of *Pvf1* and/or *Pvf2* in the Kc cells and *PVR* in the S2R+ cells, suggesting PVR hyper-stimulation, although other stimuli may also be present.

S2 cells expressing Ras^{V12} under the control of a metallothionein promoter inducible with heavy metals was kindly provided by M. Therrien[53]. Secondary screening was performed in this cell line as before in S2R+ cells. For Ras^{V12} induction, the cells were incubated in 700μM $CuSO_4$ following three days of RNAi, and the cells fixed after 24 hours induction. Ras^{V12} was not induced before insulin stimulation in these cells. For *Gap1* epistasis, S2R+ cells were exposed to either *luciferase* or *Gap1* dsRNA and grown for two days in flasks. Each population was then plated in secondary screening plates as before. *Luciferase* dsRNA pre-incubated cells were stimulated with insulin and compared to unstimulated *Gap1* dsRNA pre-incubated cells. We found a ~30% increase in baseline dpERK levels in the *Gap1*-treated cells. Several epistatic relationships between known components and *Gap1* were not recapitulated in this data set, unlike the Ras^{V12} data; we believe this is due to the hypomorphic nature of the *Gap1* and other

gene depletions, or the strength of the hit itself, rather than the true hierarchy in the pathway. Nevertheless, many positive regulators were clearly suppressed by *Gap1* dsRNA, including 55 of the 85 genes which suppress Ras^{V12}-mediated ERK activation. Further investigation is required for genes which suppress *Gap1*- but not Ras^{V12}-mediated ERK activation.

Constructs, Immunoprecipitation and Western Blotting

Full–length cDNA clones for ERK (*Rl*), MEK (*Dsor1*), *CG5169/dGCKIII*, *CG12169/dPPM1*, and PP2A (*Mts*) were obtained from the Berkeley Drosophila Genome Project (BDGP) clone collections[54] (RE08694, LD41207, RE38276, AT28366, and LD26077, respectively); Raf (*Phl*) was a gift of L. Kockel. cDNAs were cloned using PCR (Phusion polymerase, New England Biolabs) with appropriate C-terminal tags on 3' primers (HA or Myc, as indicated) and transferred to pUAST expression vector. For ERK-YFP and CFP-MEK, cDNA for ERK and EYFP (Clontech) or MEK and ECFP (Clontech) were PCR cloned with appropriate linkers into pUAST. Mutation of dGCKIII and dPPM1 was performed using the Site-directed Mutagenesis Kit (Stratagene). Similar mutations as dGCKIII K42R was previously shown to be kinase dead in the Mst3[55, 56] and Mst4[57, 58] mammalian orthologs. The dPPM1 D228A mutation in a conserved metal ion binding site was previously shown inactive the mammalian[59] and yeast[60] orthologs. Stable RNAi hairpin transgenics *in vivo* were created using the DRSC amplicon for each gene cloned in a 3'-5', 5'-3' orientation using the pWiz vector[61]. We failed to identify significant effects of expression of a dPPM1 transgenic RNAi hairpin. All constructs were sequence verified.

For co-immunoprecipitation and Western blotting, indicated constructs were transfected with actinGal4 for constitutive expression using Effectene into 3-4 x 10^6 S2R+ or Kc cells in 6-well dishes. After 3-4 days, cells were lysed in RIPA buffer and Western blotting performed essentially as described[36]. Total protein levels were quantified using the BCA Protein Assay Kit (Pierce). For RNAi experiments in 6-well dishes, we used 25μg per well and scaled all other reagents similarly from 384-well plates. Primers for *dPPM1* alternate amplicon are 5' GGCCCTTTCCCGCGCCCTC 3' and 5' AAGATTAGCCTCGGGAGTTTCGCTTTCGCGAAG 3'; partial overlap with the DRSC amplicon was necessary due to the small size of this gene. Primers for the *dGCKIII* alternate

amplicon are 5' CACAAGCCAGTCGAGAGCCAG 3' and 5' CAGCTTGCGCTCCGAGTGCAG 3'. Amplicon sequences for other dsRNAs are available from the DRSC.

For cell culture immunofluorescence, cells were fixed in 4% formaldehyde for 10 minutes, washed in PBS+0.1%Triton, and incubated overnight in PBST+3%BSA at 4ºC with rat anti-HA (Roche). Samples were subsequently washed in PBST, incubated in fluorescently-conjugated secondary antibodies and DAPI for 1-2hr at room temperature, washed, and mounted for imaging on a Leica TCS SP2 AOBS confocal microscope.

Co-immunoprecipitation was performed according to standard protocols using agarose-conjugated monoclonal anti-Myc antibodies (Santa Cruz Biotechnology, Inc.). The following primary antibodies were used: rabbit polyclonal total ERK (Cell Signaling Technology), monoclonal mouse dpERK (Cell Signaling technology), Rat anti-HA (Roche Applied Science), rabbit anti-Myc (Santa Cruz Biotechnology), rabbit anti-DER (gift of E. Bach), rat anti-Spitz (gift of E. Bach), and guinea pig anti-PVR (gift of K. Brückner). Western blotting detection was performed using HRP-conjugated secondary antibodies (Fig. 1a and Sup. Fig. 1b) or fluorescently-conjugated secondary antibodies (Molecular Probes Alexa 680 and Rockland IRDye 800CW) and the Li-Cor Biosciences Aerius imaging system for digital, quantitative, two-channel Western blotting detection. Quantitation was performed by analyzing the integrated signal intensities for the indicated detected bands using the Li-Cor software.

Genetics

Standard *Drosophila* culture and genetic procedures were used, and all stocks except where noted are available through public stock centers or by request. We generated dGCKIII^{pWiz}, UAS-dPPM1-HA, dPPM1^{pWiz}, UAS-dPPM1-HA D228A, UAS-dGCKIII-HA, and UAS-dGCKIII K42R transgenic flies through traditional transformation techniques in the w^{1118} background. The deficiency uncovering *CG5169/dGCKIII*, w^{1118}; *Df(3R)Exel6176, P{w[+mC]=XP-U}Exel6176 / TM6B*, Tb^{1}, is predicted to disrupt approximately twenty-one genes in this region[41]; none of the twenty other genes appeared as hits in our genome-wide screen. *y, w*; *apterousGal4, UAS-mCD8-GFP* / *CyO* was a gift of C. Micchelli. Other lines discussed in the text include Elp^{B1} / *CyO* and FRT^{2A}, $Gap1^{B2}$ / *TM3*, *ftz-lacZ*.

For imaginal disc immunofluorescence, wing discs from third instar larvae were dissected in cold PBS, fixed for 20 minutes in 4% formaldehyde, and washed in PBS+0.1%Triton. Staining was performed overnight in PBST+3% BSA at 4ºC. Discs were washed in PBST and incubated with secondary antibodies for 1-2hr at room temperature. They were subsequently washed, and mounted for imaging on a Leica TCS SP2 AOBS confocal microscope. Primary antibodies used were rabbit anti-phospho HistoneH3 (Upstate Biotechnology), rabbit anti-activated Caspase3 (Cell Signaling), and mouse anti-dpERK (Cell Signaling).

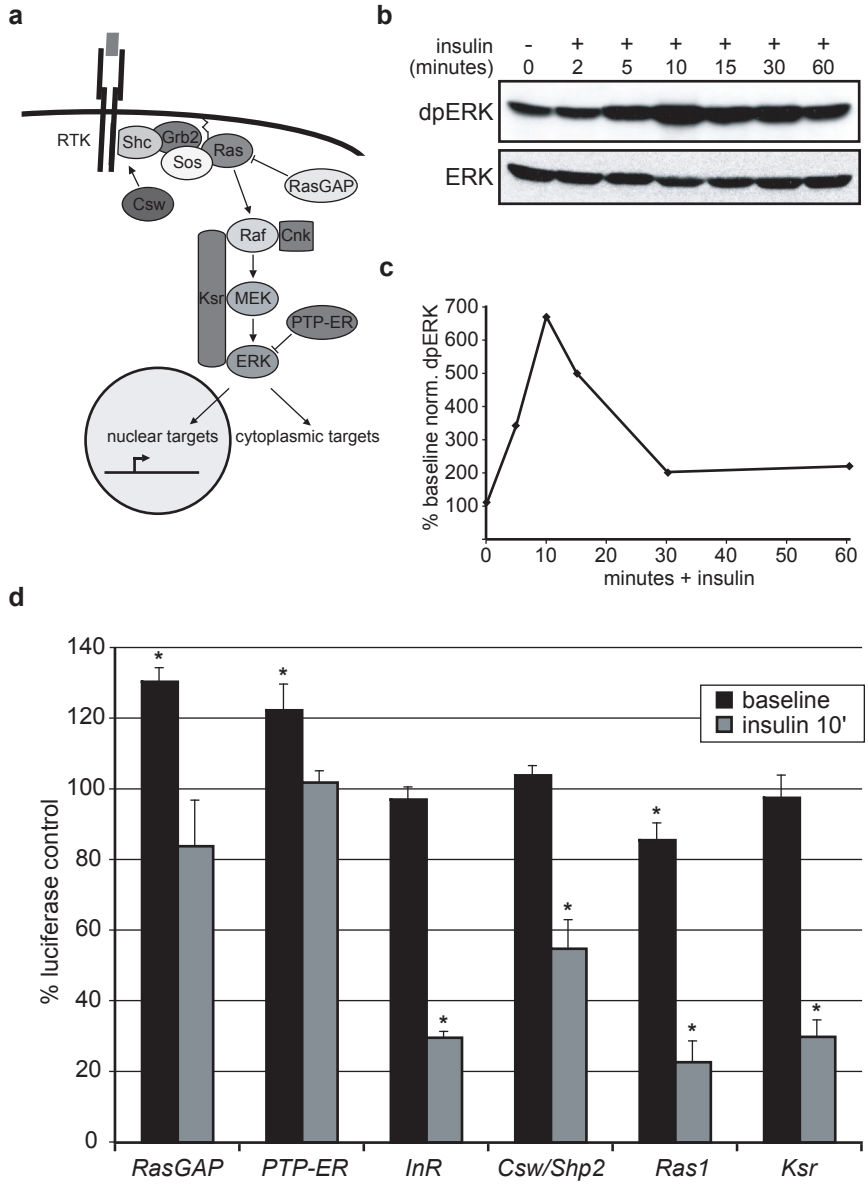
Wings of the indicated genotype were mounted in a 1:1 mixture of Permount (Fisher Scientific) and xylenes (C. Micchelli, unpublished) and imaged using a Zeiss Axioskop2 motplus. Scanning electron microscope imaging of fly eyes was performed according to standard protocols. *In situ* hybridizations of *dGCKIII* in wing discs was performed according to standard protocols.

Mammalian Cell Culture, RNAi, and RT-PCR

DU145 and LNCaP prostate cancer cell lines were a gift of M. Freeman and maintained in DMEM+10%FBS or RPMI+10%FBS, respectively. *Raf-1*, *Mst4*/*MASK*, and *Ppm1*α siRNAs were produced from PCR products obtained from the RZPD esiRNA library and processed into dsRNA-free siRNAs using the Dicer eXtreme gene kit (Roche); we designed and processed similarly *Mst3/STK24* dsRNA targeting comparable regions as Mst4 using the following primers: 5' TGAATTCTGTGACCAGTGATTTG 3' and 5' TTCAGACATTTTTAGGTGGGAAA 3'. siRNAs were transfected into cell lines at the indicated concentration using HiPerfect transfection reagent (Qiagen). After 72 hours, overnight-starved cells were lysed or stimulated with 100ng/mL EGF (Calbiochem) and lysed in RIPA buffer. Following total protein quantitation, Western blotting to dpERK and ERK were performed as described for *Drosophila* cells.

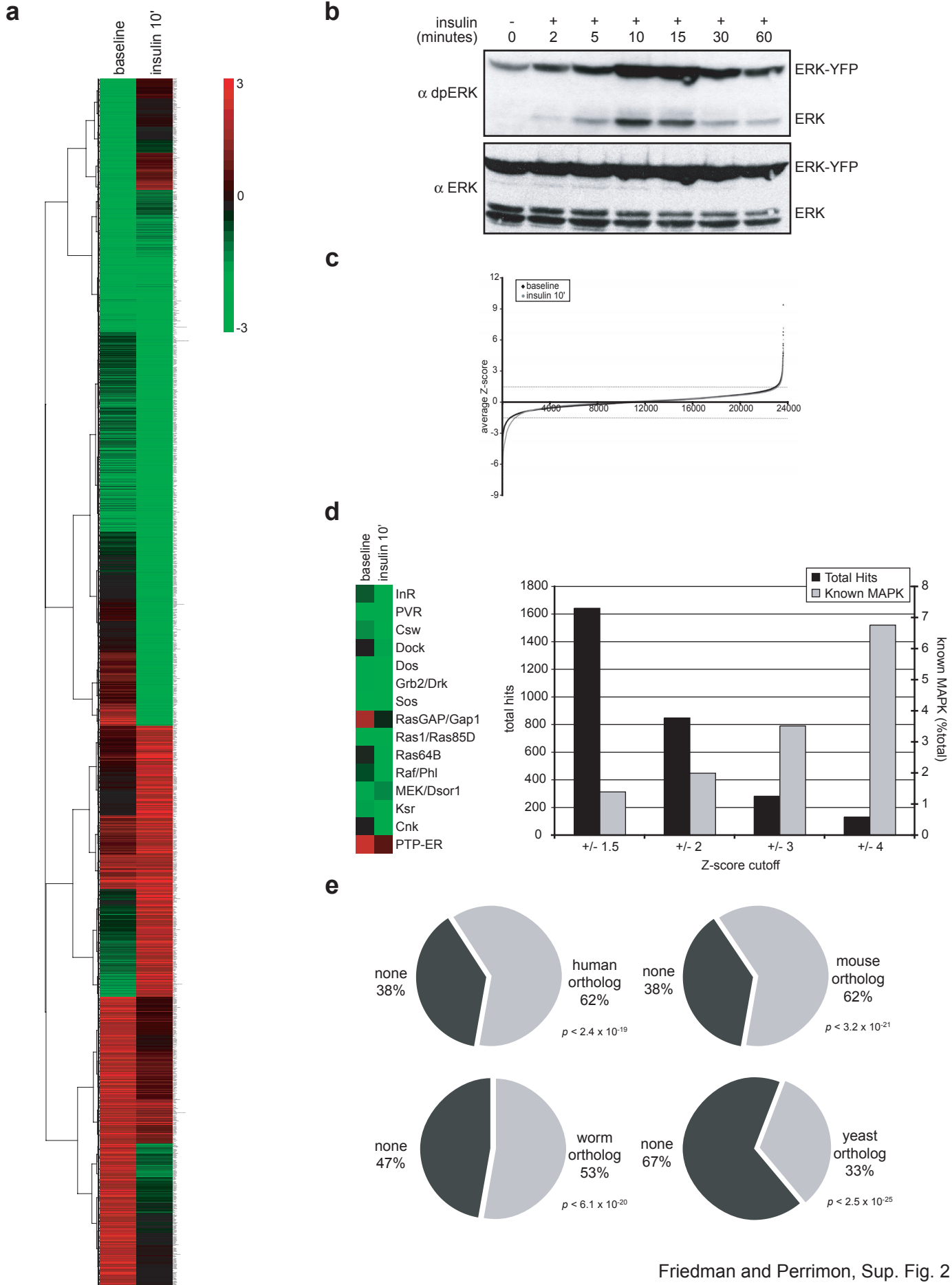
For analysis of efficiency of siRNA knockdown, we extracted total RNA from cells 72 hours after transfection using Trizol (Invitrogen), followed by phenol:chloroform extraction and purification on RNeasy columns (Qiagen). Reverse transcription was performed using random decamer-primed AMV reverse transcriptase (Promega). PCR primers were designed spanning introns, although we did not observe any product in control reactions performed from samples

prepared without reverse transcriptase. All amplifications were within the linear range for each primer pair as determined by testing of multiple cycle numbers. Primers used were as follows: *β-actin* 5' GGCATCCTCACCCTGAAGTA 3' and 5' AGGGCATACCCCTCGTAGAT 3', *GAPDH* 5' GAAGGTGAAGGTCGGAGTCA 3' and 5' TTCACACCCATGACGAACAT 3', *Raf-1* 5' ACAAAGGACAACCTGGCAAT 3' and 5' CCCCCGTCATCAGTTCATAC 3', *Mst3* 5' AACTTGGGGTCCATTGAAGA 3' and 5' AAAATGATCGCTGGAAGGAG 3', and *Mst4* 5' CTTGTGCAAACCCTGAGTTG 3' and 5' TGATAAACGCCATCACAAAA 3'.



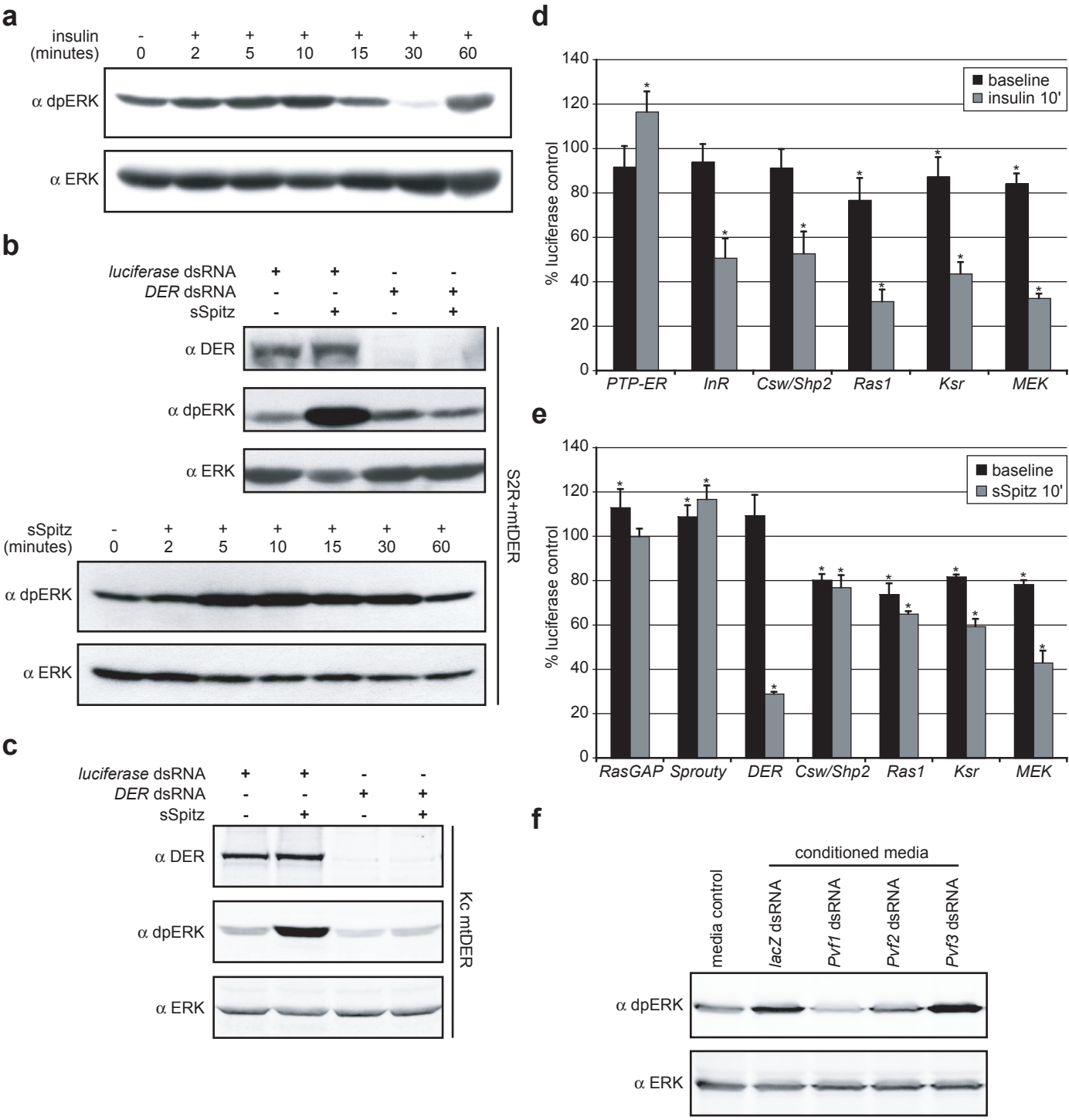
Friedman and Perrimon, Sup. Fig. 1

Supplementary Figure 1 | High-throughput RTK/ERK screening assay. a, RTK/ERK signaling pathway in *Drosophila*. Ligand binding to RTKs induces intracellular tyrosine phosphorylation and recruitment of Src-homology-2 (SH2)-containing adaptors such as Grb2 and the Ras guanine exchange factor (GEF) Sos, activating the small GTPase Ras by GTP exchange. Ras activation results in the membrane recruitment and activation of Raf, which in turn phosphorylates MEK, which finally activates ERK1/2 through dual phosphorylation of a TEY motif within an activation loop[62]. This core signaling cassette is additionally regulated by the phosphatase corkscrew/Shp2, the scaffolds Ksr and Cnk, and negative regulators such as those of the Sprouty family, Ras GTPase activating proteins (RasGAPs), and MAPK phosphatases such as PTP-ER. Although the most well-studied effectors of ERKs are transcription factors such as the ETS family members, a wide range of nuclear and cytoplasmic targets of ERK have been documented, including components of translational control machinery and other kinases such as the ribosomal S6 kinases (RSKs) and MSKs, which in turn have a variety of downstream targets[63, 64]. **b,** Insulin induces elevation of dpERK in wild-type S2R+ cells by Western blotting, with maximal activation at 10 minutes. **c,** Screening cell line and dpERK assay in 384-well plate format demonstrates similar activation kinetics as in **b**. dpERK values are normalized to total ERK (YFP) fluorescence. Hemocytes expressing ERK-YFP displayed identical kinetics of ERK activation and sensitivity to knockdown of known pathway components as wild-type S2R+ cells (see Methods and Sup. Fig. 2b and 3d); subsequent secondary screens were all conducted in wild-type cells. **d,** RNAi of known pathway components (negative regulators *RasGAP*/*Gap1* and *PTP-ER*, and positive regulators *InR*, *Csw*, *Ras1*, and *Ksr*) in screening format results in expected effects on normalized dpERK values, expressed as percent of a luciferase dsRNA negative control. * $p < 0.05$ by Student's *t* test.



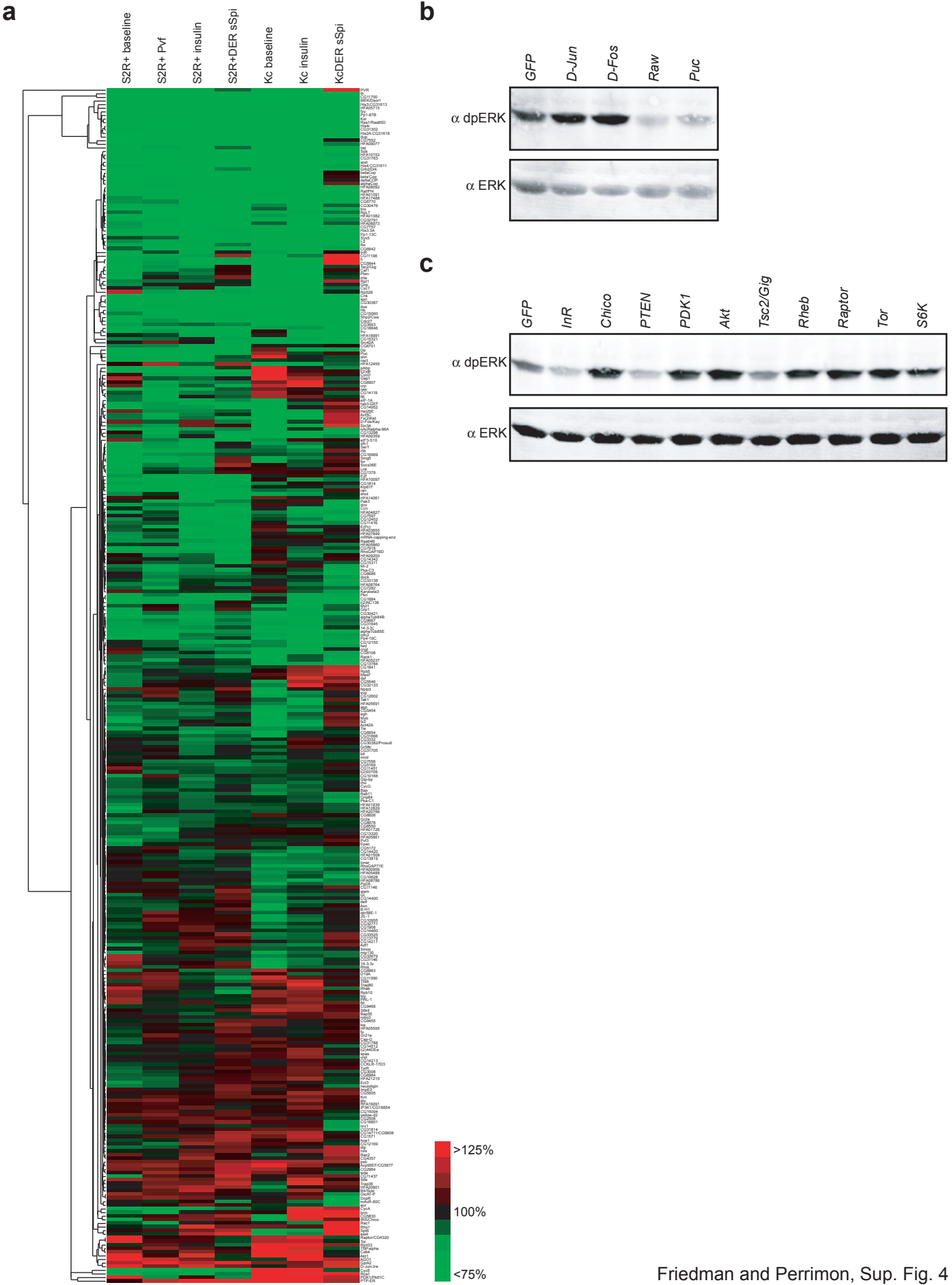
Friedman and Perrimon, Sup. Fig. 2

Supplementary Figure 2 | Primary RTK/ERK Screen Results. a, Hierarchical clustering of all primary screen hits (see Sup. Table 1 for complete data and amplicon identifiers to retrieve sequences). Note that many genes exhibit dynamic regulation of ERK signaling, depending on stimulus state. The raw primary screen hit list, except for those 331 genes listed in the text, are non-validated and any given gene should be re-tested with multiple amplicons. **b,** Western blotting of dpERK elevation following insulin stimulus in ERK-YFP/CFP-MEK expressing primary screening S2R+ cells (see Methods). Compare to Sup. Fig. 2b showing similar activation. **c,** Score of each amplicon screened, at baseline and under insulin stimulus; each data point is an average of two replicates. Although similar numbers of negative regulators ($Z > 1.5$) were uncovered under both conditions, more positive regulators ($Z < -1.5$) were observed under insulin stimulation, a significant difference in amplicon score distribution ($p < 2.8 \times 10^{-20}$ by Kolmogorov-Smirnov test). **d,** We isolated all of the known core components of the RTK/ERK signaling pathway, indicated at left, a significant enrichment for pathway components ($p < 1.6 \times 10^{-14}$). As we raised the Z-score cutoff, we further enriched for known pathway components (right), indicating pathway components are preferentially isolated from the primary screen. Other genes previously characterized as regulators of RTK/ERK signaling were identified, including non-receptor tyrosine kinase src family member Src42D[65], the 14-3-3 proteins ε and ζ[66], and PP2A/*mts*[67]. **e,** Conservation of primary screen hits, by organism, determined from HomoloGene and InParanoid databases. *p* values for enrichment of each ortholog category were calculated using the hypergeometric distribution. For comparison, the ortholog conservation rate in the DRSC dsRNA collection, essentially the *Drosophila* genome, is 50%, 50%, 40%, and 21% for the human, mouse, worm, and yeast genomes, respectively.



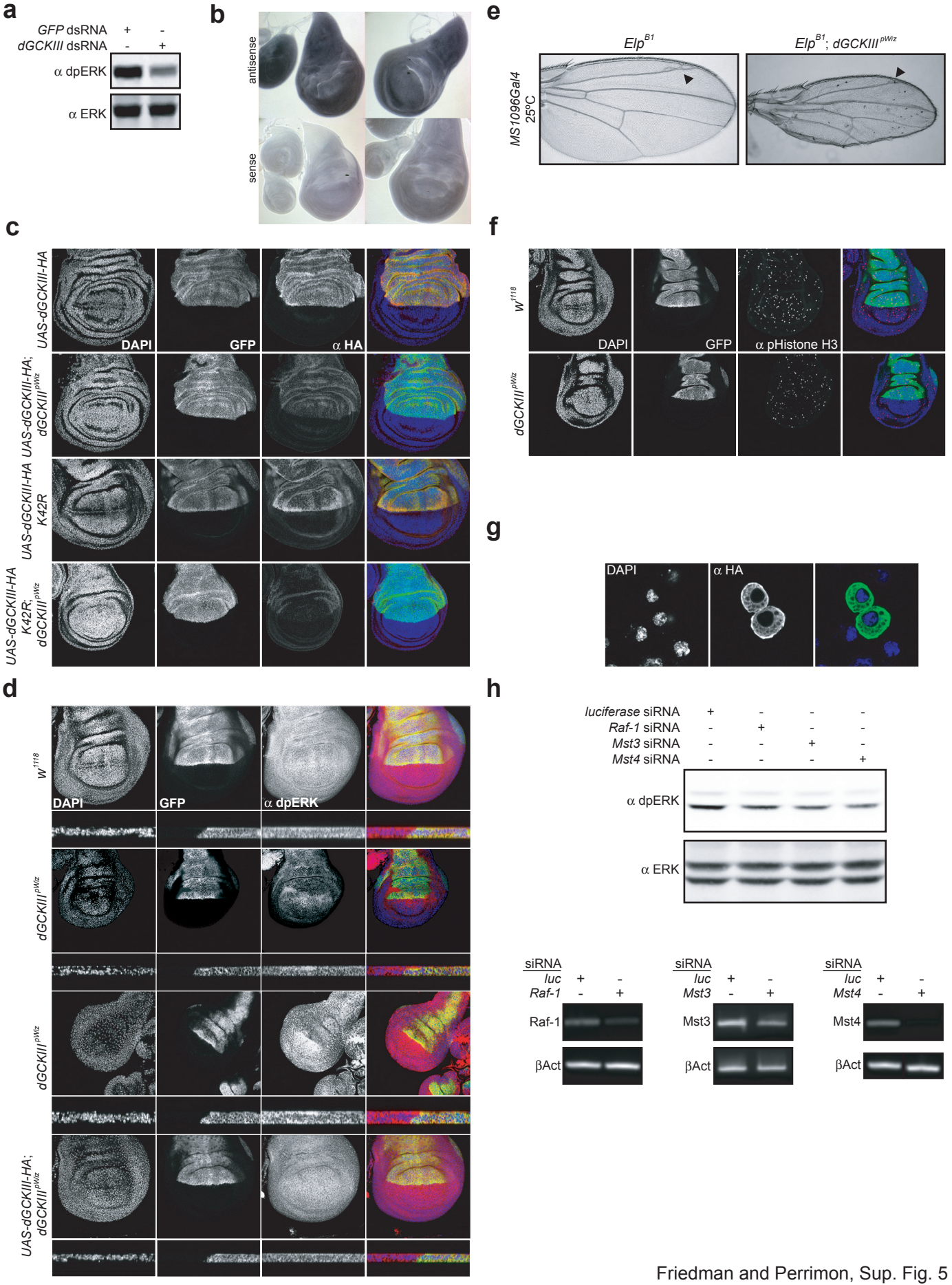
Friedman and Perrimon, Sup. Fig. 3

Supplementary Figure 3 | Secondary screen assay validation. a, Western blotting demonstrating activation of dpERK in Kc cells following insulin stimulus. A similar 10 minute peak activation was seen; these cells also have a secondary peak at 60 minutes. **b,** S2R+ cells stabling expressing DER under metallothionein promoter were stimulated with conditioned media containing secreted Spitz/EGF[51]. A robust dpERK activation was seen after 10 minutes ("+ Spitz" in upper section and see dynamics in lower section). This activation was DER-dependent, as DER knockdown eliminated dpERK elevation. **c,** Kc cells stably expressing DER under metallothionein promoter were stimulated similarly for 10 minutes with sSpi-containing medium, demonstrating robust, DER-dependent ERK activation. **d,** Wild-type S2R+ cells incubated with dsRNAs targeting known pathway components had expected alterations in dpERK levels detected in our secondary screening assay, which normalized dpERK staining to total ERK antibody. Similar results were seen in wild-type Kc cells (not shown). **e,** S2R+mtDER cells at baseline or under sSpi stimulus also displayed expected effects of known pathway components on ERK activation. Similar results were seen in KcmtDER cells in the secondary assays. **f,** Compared to S2R+ cells given serum-free medium, S2R+ cells bathed in serum-free medium conditioned from growth in Kc cells demonstrated elevation of dpERK after 10 minutes. Knockdown of Pvf1 or Pvf2 in the Kc cells reduced this elevation, suggesting Kc cells secrete Pvfs which hyper-stimulate S2R+ cells through PVR activation. * $p < 0.05$ by Student's *t* test.



Friedman and Perrimon, Sup. Fig. 4

Supplementary Figure 4 | Additional secondary screen results. a, Hierarchical clustering of secondary screens, as in Fig. 2a, with gene names. See Sup. Table 5 for complete data and amplicon identifiers. **b,** JNK pathway component knockdown in S2R+ cells at baseline. *D-jun* (*Jra*) and *D-fos* (*kay*) both negatively regulated dpERK levels, while JNK negative regulators *raw* and *puckered* were required for maximal ERK activation. **c,** Akt pathway component knockdown in Kc cells under insulin stimulus. Akt pathway components consistently reflected negative regulation of ERK activation, with positive components of the Akt pathway (*Chico*, *PDK1*, *Akt*, *Rheb*, *Raptor*, *Tor*, and *S6K*) inhibiting ERK activation, and negative components of the Akt pathway (*PTEN* and *Tsc2*) potentiating ERK activation. Note InR is positively required for both pathways. As the Akt pathway in general had little effect on ERK signaling under EGF stimulus (Fig. 2b), determining the *in vivo* role of the interaction of these pathways may require analysis in tissues free of EGFR activity, excluding, for example, imaginal discs.

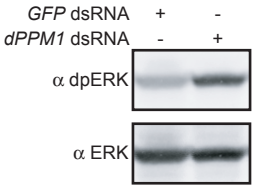


Friedman and Perrimon, Sup. Fig. 5

Supplementary Figure 5 | Additional supporting data for dGCKIII. **a,** Western blotting of S2R+ cells following *dGCKIII* knockdown in insulin-simulated cells. Quantification of this result showed ~19% reduction in normalized dpERK signal intensity (n=5). Reduction in dpERK signal is consistent with our observations in the primary and secondary screens. Three additional amplicons targeting *dGCKIII* gave similar results in these and other dpERK assays. **b,** *In situ* hybridization detecting ubiquitous *dGCKIII* transcripts in third-instar wing imaginal discs. Two examples are shown of antisense- and control sense-probed discs, processed under identical conditions. BDGP microarray data suggests continuous expression of dGCKIII throughout *Drosophila* embryogenesis[54]. Staining in eye discs showed a similar ubiquitous expression. **c,** Relative *apGal4*-directed expression of dGCKIII-HA and dGCKIII-HA K42R in wing imaginal discs visualized by anti-HA staining. *apGal4* dorsal compartment is marked by mCD8-GFP. Composite color images are at right. Both constructs appeared to be expressed at similar levels in wing discs, and similarly could be knocked down by co-expression of the *dGCKIII* hairpin. All discs were dissected and stained simultaneously, and imaging parameters were constant. Note little change in the GFP marker staining, indicating that transgene effects are not likely due to Gal4 titration. **d,** dpERK staining of early third instar wing discs expressing *dGCKIII* hairpin alone or with *dGCKIII* cDNA. Suppression of dpERK levels in the dorsal compartment, more apparent in z-sections, was consistent. Two examples of this suppression are shown. **e,** Suppression of Elp^{B1} ectopic wing vein phenotype with the *dGCKIII* hairpin. **f,** Cell proliferation, as measured by phospho-Histone H3 (pHH3) staining, in wing discs expressing the *dGCKIII* hairpin. We did not observe any decrease in pHH3 staining in the dorsal compartment or control *apGAl4, UAS-GFP* discs. **g,** Immunofluorescent staining of dGCKIII-HA expressed in S2R+ cells indicating a significant proportion of protein localized at the plasma membrane. This localization does not appear to change with insulin stimulus or Raf overexpression (data not shown). **h,** Western blotting of dpERK in DU145 human prostate cancer cells following knockdown of *Raf-1*, *Mst3*, and *Mst4* with 100nM siRNAs. Although there is conflicting evidence for roles of Mst3 and 4 in regulation of ERK signaling[55-58], no study thus far has analyzed Mst3/4 activity by loss-of-function assays. Results are shown following overnight starvation in serum-free medium. Quantification of this change showed ~20% and ~10% average reduction (n=3) in dpERK2 signal intensity normalized to total ERK2 for Mst3 and 4, respectively, comparable to the results with *dGCKIII* RNAi in S2R+ cells. RT-PCR results,

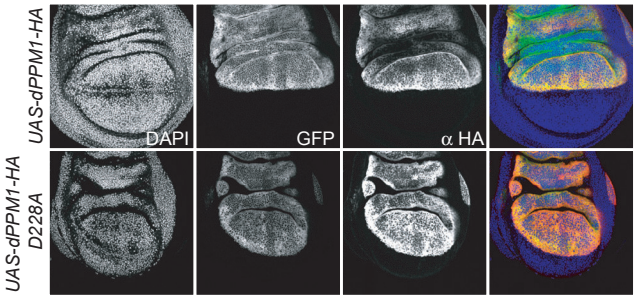
below, demonstrate reduction in indicated transcript abundance compared to β-actin control in DU145 cells. All reactions were within the linear range of amplification. GAPDH controls gave similar results as β-actin (not shown).

a

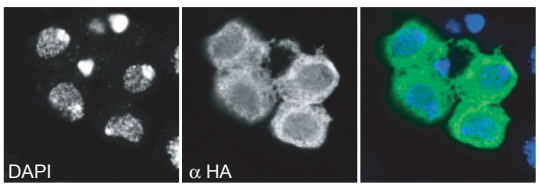


b

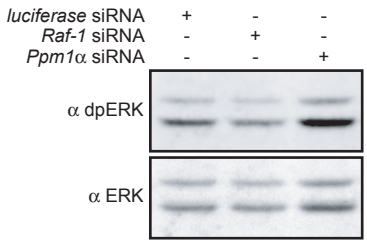
c

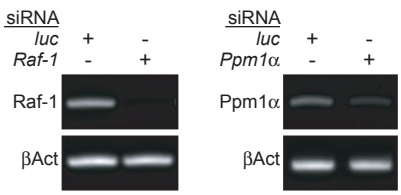


d



e





Friedman and Perrimon, Sup. Fig. 6

Supplementary Figure 6 | Additional supporting data for dPPM1. a, Western blotting of dpERK in S2R+ cells after insulin stimulus following dsRNA knockdown of *dPPM1*; an additional amplicon targeting this gene gave similar results. Quantification of this result showed a 28% increase in normalized dpERK signal intensity (n=9). **b,** Overexpression of dPPM1 but not the D228A mutant in the wing imaginal disc resulted in smaller wings, with significant upward curling, suggesting reduced tissue in the dorsal compartment, where *apGal4* is expressed. Ubiquitous, zygotic overexpression of dPPM1 was embryonic lethal at 29ºC. **c,** Relative *apGal4*-directed expression of dPPM1-HA and dPPM1-HA D228A in wing imaginal discs visualized by anti-HA staining. *apGal4* dorsal compartment is marked by UAS-mCD8-GFP. Both constructs appeared to be expressed at similar levels in wing discs. All discs were dissected and stained simultaneously and imaging parameters were constant. Note we see little consistent change in the GFP marker staining, indicating that transgene effects are not likely due to Gal4 titration. **d,** Immunofluorescent staining of S2R+ cells expressing dPPM1-HA, showing general cytoplasmic staining of the tagged phosphatase. **e,** siRNA knockdown of *Raf-1* and *Ppm1α* in LNCaP prostate cancer cells, following overnight starvation and stimulation with 100ng/mL EGF for 10 minutes; 50nM siRNA was used for this experiment. Quantification of this result showed 44% reduction in normalized dpERK by *Raf-1* siRNA and 53% increase in normalized dpERK2 by *Ppm1α* siRNA (n=5). RT-PCR results, below, demonstrate reduction in indicated transcript abundance compared to β-actin control in LNCaP cells. All reactions were within the linear range of amplification. GAPDH controls gave similar results as β-actin (not shown).

Supplementary Notes

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Supplementary Table 2: Primary Screen hits by Gene Ontology Term Enirchment

GO ID	GO Term (Biological Process)	*p* value
9653	**Morphogenesis**	1.2E-14
8152	**Metabolism**	1.2E-13
8283	**Cell proliferation**	4.6E-13
9887	**Organogenesis**	5.8E-13
7049	**Cell cycle**	2.0E-12
6412	**Protein biosynthesis**	2.4E-12
7444	**Imaginal disc development**	5.5E-12
48477	**Oogenesis**	1.5E-11
6367	**Transcription initiation (Pol II)**	4.1E-11
7276	**Gametogenesis**	1.2E-10
8380	**RNA splicing**	2.6E-08
7067	**Mitosis**	5.9E-08
1654	**Eye morphogenesis**	1.1E-07
6355	**Regulation of transcription**	5.1E-07
30036	**Actin cytoskeleton**	1.3E-06
7399	**Neurogenesis**	3.6E-06
226	**Microtubule cytoskeleton**	4.6E-06
7422	**Peripheral nervous system development**	6.9E-06
30097	**Hemopoiesis**	7.6E-06
16477	**Cell migration**	1.2E-05
42981	**Apoptosis**	5.9E-04

GO ID	GO Term (Molecular Function)	*p value*
3735	**Structural constituent of ribosome**	4.0E-13
16251	**General RNA Pol II transcription factor**	2.1E-12
3723	**RNA binding**	4.5E-12
30528	**Transcription regulator**	2.7E-10
8248	**Pre-mRNA splicing factor**	5.3E-09
45182	**Translation regulator**	1.2E-05
3743	**Translation initiation factor**	1.8E-05
5525	**GTP binding**	5.0E-04
19887	**Protein kinase regulator**	7.7E-04

GO term enrichment calculated using GeneNotes software:

Hong, P. & Wong, W. H. GeneNotes--a novel information management software for biologists. *BMC Bioinformatics* 6, 20 (2005).

Supplementary Table 3: Overlapping hits from RTK/ERK and Morphology RNAi screens

Gene Symbol	Gene Name	FlyBase ID	Avg Baseline Z-score	Avg Insulin Z-score	Kc cells						S2R+ cells					
					A	M	D	S	Z	N	V	A	M	D	S	Z	N	V
Akt1	Akt1	FBgn0010379	1.1	2.7								/			~			
Arc-p34	Arc-p34	FBgn0032859	-1.3	-1.6								-			~			
cact	cactus	FBgn0000250	-1.5	-2.4									O		X	+		
cdc2	cdc2	FBgn0004106	-2.7	-2.6			+		+				O			+	-	
Cdc27	Cdc27	FBgn0012058	3.1	-4.1		<>						-					-	
Cdk4	Cyclin-dependent kinase 4	FBgn0016131	-1.9	-2.7								+		+	S	+	-	
Cdk9	Cyclin-dependent kinase 9	FBgn0019949	-0.5	4.9				S	+				<>		O		-	
CG13503	CG13503	FBgn0034695	0.5	-1.5	•			S				<	+		~		-	
CG14217	CG14217	FBgn0031030	1.1	-2.1											X		-	
CG5169	CG5169	FBgn0038477	0.9	-2.0	X							X	X		X		-	
CG8878	BcDNA:LD23371	FBgn0027504	-0.8	-1.9								/	X		X			
CycE	Cyclin E	FBgn0010382	-4.7	-5.9	•	O			+			A	O		O		-	
CycT	Cyclin T	FBgn0025455	0.0	4.1	•	X		X					<>					
dco	discs overgrown	FBgn0002413	2.0	1.0											X			
Dp	DP transcription factor	FBgn0011763	-3.8	-2.9	+	O	-		+			A			S		-	
drk	downstream of receptor kinase	FBgn0004638	-1.8	-4.7	•	|		|										
Dsor1	Downstream of raf1	FBgn0010269	-1.7	-1.2											X			
ena	enabled	FBgn0000578	0.6	-2.1	•	X		X				X	X		X			
Fs(2)Ket	Female sterile (2) Ketel	FBgn0000986	-2.4	-3.6	-	|		|					X		~			
fwd	four wheel drive	FBgn0004373	1.8	1.9				S										
fzy	fizzy	FBgn0001086	-0.8	-4.0	-	<>	-	S		-		A		-			-	
gig	gigas	FBgn0005198	0.4	-1.8									X		~		-	
His3.3A	Histone H3.3A	FBgn0014857	-1.3	-4.6	-		+	S	Z	-	†	-		-	O	+	-	
Hsp83	Heat shock protein 83	FBgn0001233	0.7	1.8	<	|		|		-								
if	inflated	FBgn0001250	-2.7	-1.1									<>		O			
Klp61F	Kinesin-like protein at 61F	FBgn0004378	1.7	-2.3		<>							<>				-	
ksr	kinase suppressor of ras	FBgn0015402	-1.5	-7.0											X			
mts	microtubule star	FBgn0004177	0.9	-3.4	-	|			+				•		O	+		
Pak3	Pak3	FBgn0044826	0.5	-2.5	<							<						
pav	pavarotti	FBgn0011692	2.4	-0.3			••		+					••		+		
Pten	Pten	FBgn0026379	0.1	-2.7	•	|		-	+			-			O	+	-	
puc	puckered	FBgn0004210	0.1	-3.9	-	|		-	+						~		-	
Pvr	PDGF- and VEGF-receptor related	FBgn0032006	-3.8	-8.4	-	|		|	-			-			~			
Rac2	Rac2	FBgn0014011	-0.1	-2.5								-			X			
ran	ran	FBgn0020255	-1.6	0.1		|		|						O			
Ras85D	Ras oncogene at 85D	FBgn0003205	-2.1	-5.9				-							X			
Rho1	Rho1	FBgn0014020	-1.1	-1.9	+		••	S	Z			•		••				
RhoL	rho-like	FBgn0014380	-0.3	-2.1								/			O			
S6k	RPS6-p70-protein kinase	FBgn0015806	-1.5	2.5	+							+						
Sos	Son of sevenless	FBgn0001965	-2.9	-6.5	-	|		|										
th	thread	FBgn0003691	-0.4	-6.2							†							†

Morphology data from Kiger, *et al.*, 2003

F-ACTIN
- A variable, undefined
- - reduced, non-cortical
- / fibers
- • puncta, dots
- + accumulated
- < polarized
- X processes, ruffles

MICROTUBULE
- M variable, undefined
- - reduced
- • dots
- <> aberrant or frequent mitotic spindles
- + accumulated
- | bipolar extensions or spikes
- X processes
- O disorganized, uniform

DNA
- D variable, undefined
- - small, condensed
- + big, diffuse
- •• multinucleated

CELL SHAPE
- S variable, undefined
- - flat
- ~ retracted
- X processes, spikey, stretchy
- | bipolar
- O round, non-adherant

CELL SIZE
- Z variable, undefined
- - small
- + big

CELL NUMBER
- N variable, undefined
- - sparse

CELL VIABILITY
- V variable, undefined
- † death

Reference:

Kiger, A. *et al.* A functional genomic analysis of cell morphology using RNA interference. *J Biol* 2, 27 (2003).

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Supplementary Table 4: Full Secondary Screen Gene List

			MAPK Phenotype							Conservation							
			S2R+				Kc167										
			Baseline	Pvf Hyperstim	Insulin	sSpitz/EGF	sSpitz/EGF	Baseline	Insulin	Human	Mouse	Worm	Yeast	Human Disease	Annotation	ERK/Viability	Validated
Expected MAPK																	
PVR		PDGF- and VEGF-receptor related													Receptor		
Sos		Son of sevenless													GAP/GEF/GTPase		
PTP-ER		Protein tyrosine phosphatase-ERK/Enhancer of Ras1													Phosphatase		
Cnk		connector enhancer of ksr													Signaling		
Drk		downstream of receptor kinase (Grb2)													Signaling		
Dos		daughter of sevenless													Signaling		
Ras64B		Ras oncogene at 64B													GAP/GEF/GTPase		
Gap1		GTPase-activating protein 1													GAP/GEF/GTPase		
Ksr		kinase suppressor of ras													Kinase		
InR		Insulin-like receptor													Receptor		
Ras85D		Ras oncogene at 85D													GAP/GEF/GTPase		
Dsor1		Downstream of raf1 (MEK)													Kinase		
Csw		corkscrew													Phosphatase		
Phl		pole hole (raf)													Kinase		
Other Canonical Pathway																	
Tor	Akt	Target of rapamycin													Kinase		
Pten	Akt														Phosphatase		
chico	Akt	chico (IRS)													Signaling		
Pk61C	Akt	Protein kinase 61C													Kinase		
S6k	Akt	RPS6-p70-protein kinase													Kinase		
gig	Akt	gigas (Tsc2)													GAP/GEF/GTPase		
Rheb	Akt														GAP/GEF/GTPase		
Akt1	Akt														Kinase		
CG4320	Akt	Raptor													Unknown		
shn	Dpp	schnurri													Transcription Factor		
Src42A	JNK	Src oncogene at 42A													Kinase		
Jra	JNK	Jun-related antigen (D-jun)													Transcription Factor		
Rho1	JNK														GAP/GEF/GTPase		
Rac1	JNK														GAP/GEF/GTPase		
RhoGAP71E	JNK														GAP/GEF/GTPase		
Rac2	JNK														GAP/GEF/GTPase		
RhoL	JNK	rho-like													GAP/GEF/GTPase		
kay	JNK	kayak (D-fos)													Transcription Factor		
puc	JNK	puckered													Phosphatase		
Tak1	JNK	TGF-beta activated kinase 1													Kinase		
RhoGAP19D	JNK														GAP/GEF/GTPase		
Socs36E	STAT	Suppressor of cytokine signaling at 36E													Signaling		
cact	NFκB	cactus (IκB)													Transcription Factor		
fz2	Wnt	frizzled 2													Receptor		
dlp	Wnt	dally-like													Signaling		
Axn	Wnt	Axin													Signaling		
nej	Wnt	nejire (CBP/p300)													Transcription Factor		
Kinase/Phosphatase																	
S6kII		RPS6-protein kinase-II													Kinase		
for		foraging													Kinase		
CG3008															Kinase		
CG18854, IP3K1															Kinase		
CG2049		Protein kinase related to protein kinase N													Kinase		
fwd		four wheel drive													Kinase		
Tie		Tie-like receptor tyrosine kinase													Kinase		
JIL-1															Kinase		
Myt1															Kinase		
Pka-C3		cAMP-dependent protein kinase													Kinase		
Pak3															Kinase		
CG5169															Kinase		
Gprk2		G protein-coupled receptor kinase 2													Kinase		
aur		aurora													Kinase		
dco		discs overgrown													Kinase		
CG14217															Kinase		
CG8878															Kinase		
fu		fused													Kinase		
Pka-C1		cAMP-dependent protein kinase 1													Kinase		
PRL-1															Phosphatase		
mts		microtubule star													Phosphatase		
CG12169															Phosphatase		
CG11437															Phosphatase		
Pp1-87B		Protein phosphatase 1 at 87B													Phosphatase		
flw		flap wing													Phosphatase		
mRNA-capping-enzyme															Phosphatase		
Pp1-13C		Protein phosphatase 1 at 13C													Phosphatase		
Pp4-19C		Protein phosphatase 19C													Phosphatase		
PpD5		Protein phosphatase D5													Phosphatase		
Other Signaling																	
Rack1		Receptor of activated protein kinase C 1													Signaling		
neuroligin		neuroligin													Signaling		
CG30387															Signaling		
14-3-3ζ															Signaling		
Ccn															Signaling		
rols		rolling pebbles													Signaling		
I-2		Inhibitor-2													Signaling		
Krn		Keren													Signaling		
Acp95EF, CG5677		Accessory gland-specific peptide 95EF													Signaling		
14-3-3ε															Signaling		
flfl		falafel													Signaling		
Lnk															Signaling		

spri	sprint	Signaling	
Grp1	General receptor for phosphoinositides 1	GAP/GEF/GTPase	
trio		GAP/GEF/GTPase	
loner		GAP/GEF/GTPase	
Rab11	Rab-protein 11	GAP/GEF/GTPase	
CG18646		GAP/GEF/GTPase	
rab3-GEF		GAP/GEF/GTPase	
ran		GAP/GEF/GTPase	
CG10628		GAP/GEF/GTPase	
Epac		GAP/GEF/GTPase	
Gtp-bp	GTP-binding protein	GAP/GEF/GTPase	
Cell Cycle			
CycE	Cyclin E	Cell Cycle	
Rca1	Regulator of cyclin A1	Cell Cycle	
cdc2	cdc2	Cell Cycle	
fzy	fizzy	Cell Cycle	
l(2)03709		Cell Cycle	
Cdk4	Cyclin-dependent kinase 4	Cell Cycle	
Cdc27		Cell Cycle	
CycA	Cyclin A	Cell Cycle	
CycT	Cyclin T	Cell Cycle	
CG7597		Cell Cycle	
CycG	Cyclin G	Cell Cycle	
CycD	Cyclin D	Cell Cycle	
Transcription Factor			
CG31666		Transcription Factor	
CG6686		Transcription Factor	
crp	cropped	Transcription Factor	
CG10955		Transcription Factor	
l(2)NC136		Transcription Factor	
Adf1	Adh transcription factor 1	Transcription Factor	
Dp	DP transcription factor	Transcription Factor	
CG6854		Transcription Factor	
CG32133		Transcription Factor	
h	hairy	Transcription Factor	
CG14711, CG6808		Transcription Factor	
E2f	E2F transcription factor	Transcription Factor	
fru	fruitless	Transcription Factor	
zfh1	Zn finger homeodomain 1	Transcription Factor	
toy	twin of eyeless	Transcription Factor	
Smox	Smad on X	Transcription Factor	
vnd	ventral nervous system defective	Transcription Factor	
Myb	Myb oncogene-like	Transcription Factor	
CG1379		Transcription Factor	
B-H1	BarH1	Transcription Factor	
D19A		Transcription Factor	
Chromatin/General TF			
His3.3A	Histone H3.3A	Chromatin	
E(Pc)	Enhancer of Polycomb	Chromatin	
Cap-G		Chromatin	
CG6701		Chromatin	
dup	double parked	Chromatin	
Mi-2		Chromatin	
Caf1	Chromatin assembly factor 1 subunit	Chromatin	
His4r	Histone H4 replacement	Chromatin	
Spt6		Chromatin	
His2A:CG31618		Chromatin	
His3:CG31613		Chromatin	
His4:CG31611		Chromatin	
glu	gluon	Chromatin	
kis	kismet	Chromatin	
RpII33	RNA polymerase II 33kD subunit	General TF	
TfIIB	Transcription factor IIB	General TF	
Rpb5		General TF	
Sin3A		General TF	
Trap36		General TF	
brm	brahma	General TF	
TfIIFalpha	Transcription factor IIFalpha	General TF	
Rpb10		General TF	
Med7		General TF	
Trap80		General TF	
Cytoskeletal			
Act42A	Actin 42A	Cytoskeletal	
shot	short stop	Cytoskeletal	
Bap55	Brahma associated protein 55kD	Cytoskeletal	
Klp61F	Kinesin-like protein at 61F	Cytoskeletal	
Gl	Glued	Cytoskeletal	
alphaTub84B	alpha-Tubulin at 84B	Cytoskeletal	
alphaTub85E	alpha-Tubulin at 85E	Cytoskeletal	
Act5C	Actin 5C	Cytoskeletal	
CG1571		Cytoskeletal	
ctp	cut up	Cytoskeletal	
fliI	flightless I	Cytoskeletal	
Grip84	gamma-tubulin ring protein 84	Cytoskeletal	
Metabolic/Enzyme			
CG2964		Metabolism	
CG9468		Metabolism	
CG11198		Metabolism	
CG1814		Metabolism	
CG6984		Metabolism	
GlcAT-P		Metabolism	
CG10168		Metabolism	
CG5844		Metabolism	
mnd	minidiscs	Metabolism	
CG33138		Metabolism	

l(2)44DEa		Metabolism	
rho	rhomboid	Enzyme	
CG31146		Enzyme	
egh	egghead	Enzyme	
Ect3		Enzyme	
RNA Processing/Translation			
RpL7	Ribosomal protein L7	Ribosome	
RpS26	Ribosomal protein S26	Ribosome	
aret	arrest	RNA Processing	
Smg5		RNA Processing	
AGO1	Argonaute 1	RNA Processing	
CG1884		RNA Processing	
pAbp	polyA-binding protein	RNA Processing	
CG13298		RNA Processing	
qkr58E-1	quaking related 58E-1	RNA Processing	
Hel25E	Helicase at 25E	Splicing	
CG7757		Splicing	
CG5454		Splicing	
CG11146		Signaling	
dock	dreadlocks	Signaling	
ImpE3	Ecdysone-inducible gene E3	Signaling	
Nplp3	Neuropeptide-like precursor 3	Signaling	
Pvf3	PDGF- and VEGF-related factor 3	Signaling	
eIF3-S10		Translation	
eIF-1A	Eukaryotic initiation factor 1A	Translation	
Ubiquitylation/Proteasomal			
CG30382, Prosalpha6	Proteasome alpha6 subunit	Proteasome	
Rpt1		Proteasome	
CG2508		Ubiquitylation	
CG30421		Ubiquitylation	
ago	archipelago	Ubiquitylation	
CG5505		Ubiquitylation	
th	thread	Ubiquitylation	
CG11700		Ubiquitylation	
Receptor/Ion Transport			
CG31645		Receptor	
dsf	dissatisfaction	Receptor	
Gr58c	Gustatory receptor 58c	Receptor	
CG16801		Receptor	
TyrR	Tyramine receptor	Receptor	
CG7918		Receptor	
DopR	Dopamine receptor	Receptor	
svp	seven up	Receptor	
btl	breathless	Receptor	
CCKLR-17D3	CCK-like receptor at 17D3	Receptor	
Gr21a	Gustatory receptor 21a	Receptor	
Gr2a	Gustatory receptor 2a	Receptor	
mAcR-60C	muscarinic Acetylcholine Receptor 60C	Receptor	
robo3		Receptor	
hoe1	hoepel1	Ion Transport	
CG12602		Ion Transport	
nrv1	nervana 1	Ion Transport	
SerT	Serotonin transporter	Ion Transport	
CG4357		Ion Transport	
nAcRalpha-96Aa	nicotinic Acetylcholine Receptor alpha 96Aa	Ion Transport	
CG6608		Ion Transport	
Hk	Hyperkinetic	Ion Transport	
cngl	CNG channel-like	Ion Transport	
CG15094		Ion Transport	
Other			
CG9455		Other	
spas	spastin	Other	
CG6842		Other	
Fs(2)Ket	Female sterile (2) Ketel	Protein Transport	
Karybeta3	Karyopherin beta 3	Protein Transport	
Syx5	Syntaxin 5	Trafficking	
beta'Cop	beta'-coatomer protein	Trafficking	
alphaCop	alpha-coatomer protein	Trafficking	
deltaCOP	delta-coatomer protein	Trafficking	
Bap	Beta Adaptin	Trafficking	
betaCop	beta-coatomer protein	Trafficking	
Unknown Function			
CG31814		Unknown	
CG11990		Unknown	
CG32791		Unknown	
CG3332		Unknown	
CG6550		Unknown	
defl	deflated	Unknown	
CG5546		Unknown	
CG5830		Unknown	
CG31302		Unknown	
CG9667		Unknown	
CG32679		Unknown	
twit	twilight	Unknown	
CG3878		Unknown	
CG6907		Unknown	
CG30476		Unknown	
CG8963		Unknown	
CG14447	Glutamate receptor binding protein	Unknown	
CG13784		Unknown	
CG11416		Unknown	
CG1841		Unknown	
CG13779		Unknown	
side	sidestep	Unknown	
B4		Unknown	

CG11451														Unknown	
CG12155														Unknown	
CG12452														Unknown	
CG13819														Unknown	
CG14119														Unknown	
CG14342														Unknown	
CG14420														Unknown	
CG14952														Unknown	
CG15060														Unknown	
CG15321														Unknown	
CG16969														Unknown	
CG17665														Unknown	
CG1908														Unknown	
CG31705														Unknown	
CG31763														Unknown	
CG32771														Unknown	
CG3563														Unknown	
CG6770														Unknown	
CG7282														Unknown	
CG7552														Unknown	
CG8108														Unknown	
east	enhanced adult sensory threshold													Unknown	
l(2)dtl	lethal-(2)-denticleless													Unknown	
pxb														Unknown	
raw	raw													Unknown	
CG13320														Unknown	
CG14213														Unknown	
CG14400														Unknown	
CG14460														Unknown	
CG14512														Unknown	
CG15311														Unknown	
CG31788														Unknown	
CG5172														Unknown	
CG7556														Unknown	
slam	slow as molasses													Unknown	
yellow-d2														Unknown	
CG40099														Unknown	
No Annotation															
HFA00956														No annotation	
HFA01082														No annotation	
HFA01091														No annotation	
HFA01508														No annotation	
HFA01639														No annotation	
HFA01726														No annotation	
HFA03655														No annotation	
HFA04827														No annotation	
HFA05237														No annotation	
HFA05488														No annotation	
HFA05595														No annotation	
HFA05691														No annotation	
HFA05715														No annotation	
HFA05860														No annotation	
HFA05861														No annotation	
HFA07849														No annotation	
HFA08092														No annotation	
HFA08764														No annotation	
HFA08786														No annotation	
HFA08973														No annotation	
HFA09077														No annotation	
HFA09200														No annotation	
HFA09359														No annotation	
HFA10097														No annotation	
HFA10152														No annotation	
HFA12455														No annotation	
HFA12829														No annotation	
HFA14061														No annotation	
HFA17488														No annotation	
HFA18991														No annotation	
HFA19091														No annotation	
HFA20801														No annotation	

Conservation calls are based strictly on InParanoid and Homologene ortholog queries (October 2004) and therefore may not reflect more detailed analyses.

Annotation categories are manual groupings of Gene Ontology data queried from FlyBase (October 2004) and therefore may not reflect more comprehensive studies of function.

"Validated"

	indicates an additional amplicon targeting the given gene affected ERK activation in S2R+ cells at baseline or under insulin stimulation. See Methods for details.
	indicates an additional amplicon targeting the given gene did not affect ERK activation in S2R+ cells at baseline or under insulin stimulation. See Methods for details.
	indicates no additional amplicons targeting the given gene were tested

dpERK	%Control
	>125%
	110-125%
	105-110%
	95-105%
	90-95%
	70-90%
	<70%

Viability	%Control
	>110%
	<90%

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Supplementary Table 6: Human Orthologs of Secondary Screen Gene List

		Human Protein (InParanoid)	Human Protein (Homologene)	InParanoid Group	Homologene Group	MAPK Phenotype							Conservation				Annotation	ERK/Viability
						S2R+				Kc167								
						Baseline	Pvf Hyperstim	Insulin	sSpitz/EGF	sSpitz/EGF	Baseline	Insulin	Human	Mouse	Worm	Yeast	Human Disease	
Possible Human Disease Ortholog (via Homophila <E^{-10})																		
Pka-C1	cAMP-dependent protein kinase 1	PRKACA	PRKACA	896	55518												Kinase	
Rack1	Receptor of activated protein kinase C 1	RACK1	GNB2L1	1245	4446												Signaling	
beta'Cop	beta'-coatomer protein	COPB2	COPB2	134	3499												Trafficking	
cdc2	cdc2	CDC2	CDC2, CDK2	1560	55603												Cell Cycle	
Act42A	Actin 42A	ACTG1		540													Cytoskeletal	
CG11198		ACACA	ACACB	18	37438												Metabolism	
CG33138		GBE1	GBE1	360	129												Metabolism	
Bap55	Brahma associated protein 55kD	ACTL6B	ACTL6B	1348	3176												Cytoskeletal	
l(2)44DEa		ACSL3	ACSL3	754	3278												Metabolism	
Rho1		RHOA	RHOA	2234	1257												GAP/GEF/GTPase	
Rpt1		PSMC2		461													Proteasome	
ago	archipelago	FBXW7		489	62613												Ubiquitylation	
Klp61F	Kinesin-like protein at 61F	KIF11	KIF11	1071	3322												Cytoskeletal	
Pk61C	Protein kinase 61C	PDPK1	PDPK1	2893	37643												Kinase	
Ras64B	Ras oncogene at 64B	RRAS2	RRAS2	2910	6945												GAP/GEF/GTPase	
alphaCop	alpha-coatomer protein	COPA	COPA	54	3218												Trafficking	
CG7757		PRPF3	PRPF3	1212	3447												Splicing	
Gl	Glued	DCTN1	DCTN1	714	3011												Cytoskeletal	
Mi-2		CHD4	CHD4	44	55568												Chromatin	
Myt1		NP_004194	PKMYT1	3223	31227												Kinase	
Pka-C3	cAMP-dependent protein kinase	PRKX	PRKX	1592	55868												Kinase	
S6k	RPS6-p70-protein kinase	RPS6KB1	RPS6KB1	1069	2938												Kinase	
brm	brahma	SMARCA2	SMARCA2	74	2308												General TF	
CG7597		CDC2L5	CRK7	941	56360												Cell Cycle	
Rheb		RHEB	RHEB	3324	4101												GAP/GEF/GTPase	
CG5169		STK24		1229	49473												Kinase	
spas	spastin	SPG4	SPG4	1417	8970												Other	
Caf1	Chromatin assembly factor 1 subunit	RBBP4	RBBP4	457	21153												Chromatin	
Rab11	Rab-protein 11	RAB11A	RAB11A	2094	37903												GAP/GEF/GTPase	
Ras85D	Ras oncogene at 85D	HRAS	KRAS	2695	37990												GAP/GEF/GTPase	
aur	aurora	STK6	STK6	2093	2670												Kinase	
Act5C	Actin 5C	ACTG1		540	37501												Cytoskeletal	
Dsor1	Downstream of raf1 (MEK)	MAP2K1	MAP2K1	1388	2063												Kinase	
Bap	Beta Adaptin	AP1B1		116	50322												Trafficking	
CG6842		VPS4B	VPS4B	707	37976												Other	
betaCop	beta-coatomer protein	COPB	COPB	152	5664												Trafficking	
ran		RAN	RAN	1901	4612												GAP/GEF/GTPase	
for	foraging	PRKG1	PRKG2	409	4556												Kinase	
kis	kismet	CHD7	CHD7	89	19067												Chromatin	
robo3			ROBO1		2206												Receptor	
dock	dreadlocks	NCK1	NCK2	2282	20794												Signaling	
CG31814		NTRI_HUMAN		4440	65144												Unknown	
PVR	PDGF- and VEGF-receptor related	FLT1		1776	62684												Receptor	
CG9468			MAN2B2		7411												Metabolism	
CycE	Cyclin E	CCNE1	CCNE1	2928	14452												Cell Cycle	
Pten		PTEN	PTEN	2777	265												Phosphatase	
Sos	Son of sevenless	SOS2	SOS2	244	38253												GAP/GEF/GTPase	
cact	cactus (IκB)	NFKBIA	NFKBIA	4546	7863												Transcription Factor	
dsf	dissatisfaction	NR2E1		2373	62223												Receptor	
neuroligin	neuroligin	NLGN4X; NLG	NLGN1	2183	56690												Signaling	
Grp1	General receptor for phosphoinositides 1	PSCD1	PSCD1	1040	31262												GAP/GEF/GTPase	
CG30387		Q9ULH0	KIDINS220	367	14254												Signaling	
SerT	Serotonin transporter	SLC6A4		718	62735												Ion Transport	
mAcR-60C	muscarinic Acetylcholine Receptor 60C	CHRM3	CHRM5	1948	22697												Receptor	
Src42A	Src oncogene at 42A	FRK	FRK	948	48065												Kinase	
Epac		RAPGEF4	RAPGEF4	460	4451												GAP/GEF/GTPase	
shot	short stop	DST	MACF1	32	57199												Cytoskeletal	
CG16801		NR2E3	NR2E3	2111	56601												Receptor	
Cdk4	Cyclin-dependent kinase 4	CDK6	CDK6	2811	963												Cell Cycle	
Drk	downstream of receptor kinase (Grb2)	GRB2	GRB2	2801	1576												Signaling	
trio		TRIO	TRIO	56	20847												GAP/GEF/GTPase	
Rac1		RAC2		2122	38000												GAP/GEF/GTPase	
fz2	frizzled 2	FZD8	FZD8	774	40606												Receptor	
CG4357		SLC12A2	SLC12A2	283	20283												Ion Transport	
CycA	Cyclin A	CCNA1	CCNA2	3157	55562												Cell Cycle	
Gap1	GTPase-activating protein 1	RASA3	RASA3	776	7217												GAP/GEF/GTPase	
Rac2		RAC3	RAC2	2122	55699												GAP/GEF/GTPase	
btl	breathless	FGFR2	FGFR1	1465	32087												Receptor	
loner		IQSEC1		1253	8911												GAP/GEF/GTPase	
TyrR	Tyramine receptor	HTR1A	HTR1A	2663	20148												Receptor	
Ksr	kinase suppressor of ras	NP_775869		1957													Kinase	
nAcRalpha-96Aa	nicotinic Acetylcholine Receptor alpha 96Aa	CHRNA4	CHRNB3	1452	36035												Ion Transport	
Akt1		AKT3	AKT3	802	55904												Kinase	
CG10168		UGT2B11		3028													Metabolism	
CG11990		HRPT2	HRPT2	994	11571												Unknown	
Ect3		GLB1	GLB1L	1356	56991												Enzyme	
CG6608		SLC25A19		3613	43063												Ion Transport	
DopR	Dopamine receptor	DRD1	DRD5	3278	20216												Receptor	
Gprk2	G protein-coupled receptor kinase 2	GRK6	GRK6	636	37570												Kinase	
InR	Insulin-like receptor	IGF1R	INSR	453	20090												Receptor	
RhoL	rho-like		RAC1, RAC3		38000												GAP/GEF/GTPase	
svp	seven up	NR2F1	NR2F1	1435	21158												Receptor	
CG32791		NTRI_HUMAN		4440	45284												Unknown	
Smox	Smad on X	SMAD3	SMAD3	989	55937												Transcription Factor	
Csw	corkscrew	PTPN11	PTPN11	1222	2122												Phosphatase	
nej	nejire (CBP/p300)	EP300	EP300	98	1094												Transcription Factor	
Phl	pole hole (raf)	BRAF	BRAF	960	3197												Kinase	
B-H1	BarH1	BARHL1	BARHL2	4215	10572												Transcription Factor	
CG14217		TAOK1	TAOK1	280	27041												Kinase	
rab3-GEF		MADD	MADD	170	14249												GAP/GEF/GTPase	
CCKLR-17D3	CCK-like receptor at 17D3	CCKAR	CCKAR	2960	37337												Receptor	
CycD	Cyclin D	CCND2	CCND2	3952	37525												Cell Cycle	
Tak1	TGF-beta activated kinase 1	MAP3K7	MAP3K7	2207	37743												Kinase	
flil	flightless I	FLII	FLII	100	11092												Cytoskeletal	
S6kII	RPS6-protein kinase-II	RPS6KA2		435													Kinase	
fzy	fizzy	CDC20	CDC20	1284	37459												Cell Cycle	
CG13320		USH1G	USH1G	3316	56113												Unknown	
CG5505		USP36		2734	63622												Ubiquitylation	
toy	twin of eyeless	PAX6		1684	62773												Transcription Factor	
hoe1	hoepel1	OCA2		1230	51214												Ion Transport	
PTP-ER	Protein tyrosine phosphatase-ERK/Enhancer of Ras1		PTPN5		8423												Phosphatase	
CG2049	Protein kinase related to protein kinase N	PKN1	PKN1	349	48130												Kinase	
rols	rolling pebbles	Q9NXY9	DKFZP564D16	381	64680												Signaling	
JIL-1		RPS6KA5	RPS6KA5	1139	48302												Kinase	
gig	gigas (Tsc2)	TSC2	TSC2	544	462												GAP/GEF/GTPase	
zfh1	Zn finger homeodomain 1	TCF8		2824	50514												Transcription Factor	
CG1379		RUNX2		3502													Transcription Factor	
mnd	minidiscs	SLC7A5		1342	62590												Metabolism	

RhoGAP19D		ARHGAP15		4569	34421												GAP/GEF/GTPase	
CG31146			NLGN2		56901												Enzyme	
Others with Human Orthologs																		
CG2508		CDC23	CDC23	1264	3426												Ubiquitylation	
Tor	Target of rapamycin	FRAP	FRAP1	25	3637												Kinase	
Fs(2)Ket	Female sterile (2) Ketel	KPNB1	KPNB1	225	1707												Protein Transport	
Hel25E	Helicase at 25E	ATP6V1G2	BAT1, DDX39	586	48376												Splicing	
His3.3A	Histone H3.3A	H3F3A		2957	38374												Chromatin	
RpII33	RNA polymerase II 33kD subunit	POLR2C	POLR2C	1738	2017												General TF	
RpL7	Ribosomal protein L7	RPL7	RPL7	2805	37375												Ribosome	
RpS26	Ribosomal protein S26	RPS26	RPS26	3941	37420												Ribosome	
Syx5	Syntaxin 5	STX5A	STX5A	2418	2381												Trafficking	
TfIIB	Transcription factor IIB	GTF2B	GTF2B	1357	1158												General TF	
glu	gluon	SMC4L1	SMC4L1	356	4015												Chromatin	
mts	microtubule star	PPP2CB	PPP2CA	816	37660												Phosphatase	
CG10955		KIAA0252	KIAA0252	1588	5871												Transcription Factor	
l(2)NC136		CNOT3	CNOT3	974	7149												Transcription Factor	
Rpb5		POLR2E	POLR2E	2118	2018												General TF	
l(2)03709		REA	REA	2320	5263												Cell Cycle	
CG1884		NP_057368	CNOT1	66	9453												RNA Processing	
CG30382, Prosal	Proteasome alpha6 subunit	PSMA6	PSMA6	2316	2085												Proteasome	
pAbp	polyA-binding protein	PABPC4	PABPC1	626	37638												RNA Processing	
fwd	four wheel drive	PIK4CB	PIK4CB	911	6741												Kinase	
CG6854		CTPS	CTPS	456	20446												Transcription Factor	
CG5830		CTDSP1		2425	64690												Unknown	
CG32133		PAXIP1L	PAXIP1L	2329	7202												Transcription Factor	
Cdc27		CDC27	CDC27	918	960												Cell Cycle	
eIF3-S10		EIF3S10	EIF3S10	328	2779												Translation	
Karybeta3	Karyopherin beta 3	RANBP5	RANBP5	173	1710												Protein Transport	
alphaTub84B	alpha-Tubulin at 84B	TUBA1		345	38127												Cytoskeletal	
Rpb10		POLR2L	POLR2L	4502	6983												General TF	
CG14512		GLT28D1	GLT28D1	4823	44843												Unknown	
Med7		CRSP9	CRSP9	3482	3153												General TF	
fifl	falafel	KIAA2010	KIAA2010	407	41377												Signaling	
CG9667		RAB43	KIAA1160	2541	6283												Unknown	
His4r	Histone H4 replacement	HIST1H4E		3659	38374												Chromatin	
Pp1-87B	Protein phosphatase 1 at 87B	PPP1CA		910	59064												Phosphatase	
alphaTub85E	alpha-Tubulin at 85E		TUBA1		38127												Cytoskeletal	
dco	discs overgrown	CKIe	CSNK1D	1074	58362												Kinase	
eIF-1A	Eukaryotic initiation factor 1A	EIF1AY		3392	56473												Translation	
CG4320	Raptor	RPOR_HUMAN		243													Unknown	
ctp	cut up	NP_542408	Dlc2	3909	2777												Cytoskeletal	
deltaCOP	delta-coatomer protein	ARCN1	ARCN1	965	1250												Trafficking	
Spt6		SUPT6H	SUPT6H	86	40661												Chromatin	
CG14213		RQCD1	RQCD1	1599	3973												Unknown	
mRNA-capping-enzyme		RNGTT	RNGTT	1031	37851												Phosphatase	
CG7556		DNAJC1	DNAJC1	3295	7293												Unknown	
Gtp-bp	GTP-binding protein	ENSP0000034	SRPR	899	2364												GAP/GEF/GTPase	
His2A:CG31618		HIST2H2AB	HIST*	3483	38374												Chromatin	
His3:CG31613		NP_066403		2981	38374												Chromatin	
His4:CG31611		HIST1H4E		3659	38374												Chromatin	
CG3008		RIOK3	RIOK3	1982	2843												Kinase	
CG3332		OTOP1; OTOF	OTOP1	4508	35244												Unknown	
aret	arrest	CUGBP1	CUGBP2	1742	4783												RNA Processing	
CG10628		Q8N982	LOC85865	2558	8114												GAP/GEF/GTPase	
CG6686		SART1	SART1	1582	38015												Transcription Factor	
nrv1	nervana 1	ATP1B1	ATP1B1	4468	37509												Ion Transport	
PRL-1		PTP4A1	PTP4A3	3645	22500												Phosphatase	
qkr58E-1	quaking related 58E-1	KHDRBS3	KHDRBS2	3597	15772												RNA Processing	
14-3-3ζ		YWHAZ		1850	62217												Signaling	
AGO1	Argonaute 1	EIF2C1	EIF2C2	130	49297												RNA Processing	
CG6550		CDKAL1	CDKAL1	736	9830												Unknown	
CG6984		ECHDC3	ECHDC3	3467	11484												Metabolism	
dup	double parked	NP_112190		3319	62783												Chromatin	
Dp	DP transcription factor	DRTF1	TFDP1	2850	38276												Transcription Factor	
Jra	Jun-related antigen (D-jun)	JUND	JUN	4511	1679												Transcription Factor	
CG13298		PM14_HUMAN	SF3B14	3797	9360												RNA Processing	
dlp	dally-like	GPC4	GPC6	2027	55922												Signaling	
defl	deflated	NP_056249	DKFZP434B16	1258	9136												Unknown	
CG5546		NP_703151	MED19	4697	14684												Unknown	
Trap36		VDRIP	MED4	3809	8568												General TF	
CycT	Cyclin T	CCNT2; CCNT	CCNT1	2378	947												Cell Cycle	
I-2	Inhibitor-2	PPP1R2	PPP1R2	4709	4546												Signaling	
TfIIFalpha	Transcription factor IIFalpha	GTF2F1	GTF2F1	2022	1585												General TF	
CG31302		RIP2_HUMAN		1832	18077												Unknown	
CG5454		SNRPC	SNRPC	4046	31131												Splicing	
Acp95EF, CG567	Accessory gland-specific peptide 95EF		SPCS3		41454												Signaling	
kay	kayak (D-fos)	NP_569736		5313													Transcription Factor	
CG32679		NP_113649		5389	62776												Unknown	
spri	sprint	RIN2	RIN2	3293	32430												Signaling	
twit	twilight	NP_775106	LIN9	2579	35252												Unknown	
flw	flap wing	NP_002700	PPP1CB	923	37659												Phosphatase	
CG1841			LOC388419		46324												Unknown	
CG3878		RCOR1		3442													Unknown	
Pp4-19C	Protein phosphatase 19C	PPP4C	PPP4C	929	2038												Phosphatase	
Cap-G		CND3_HUMAN	HCAP-G	3285	44071												Chromatin	
14-3-3ε		YWHAE	YWHAE	1698	38223												Signaling	
puc	puckered		DUSP16		15604												Phosphatase	
Hk	Hyperkinetic	KCNAB2	KCNAB2	2366	56492												Ion Transport	
CG31666		ZBTB8		5296	44261												Transcription Factor	
Socs36E	Suppressor of cytokine signaling at 36E	SOCS1		5305													Signaling	
CG6907		ELP4	ELP4	3577	32433												Unknown	
Smg5		KIAA1089		3755	51246												RNA Processing	
CG30421		USP31	USP31	1573	44504												Ubiquitylation	
E(Pc)	Enhancer of Polycomb	EPC1		1708	37048												Chromatin	
CG30476		SAMD12		5251	35104												Unknown	
CG1814		NP_057659	TU12B1-TY	1914	9571												Metabolism	
CG8963		PAIP1		4802	51327												Unknown	
Sin3A		SIN3B	SIN3B	389	49196												General TF	
Cnk	connector enhancer of ksr	CNKSR2	CNKSR1	2965	4604												Signaling	
Ccn		CTGF		4702													Signaling	
CycG	Cyclin G	CCNG2	CCNG2	4879	3208												Cell Cycle	
E2f	E2F transcription factor	E2F4		4263													Transcription Factor	
Lnk		NP_056318		2641	52075												Signaling	
Trap80		CRSP6	CRSP6	1349	3151												General TF	
fru	fruitless	ZNF297		5020	66145												Transcription Factor	
CG14447	Glutamate receptor binding protein	GRIP2	GRIP2	2031	16327												Unknown	
Grip84	gamma-tubulin ring protein 84	TUBGCP2	ADAM8	1457	55554												Cytoskeletal	
Myb	Myb oncogene-like	MYB	MYB	2515	31311												Transcription Factor	
CG40099		Q9P225		3450													Unknown	
CG13784		PQLC1		4586													Unknown	
CG11146		SHB		4689	34289												Signaling	

* - Many Histone Orthologs/Paralogs in this group

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Annotation categories are manual groupings of Gene Ontology data queried from FlyBase (October 2004) and therefore may not reflect more comprehensive studies of function.

dpERK	%Control
	>125%
	110-125%
	105-110%
	95-105%
	90-95%
	70-90%
	<70%

Viability	%Control
	>110%
	<90%

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Supplementary Table 7: Validated regulators induced by RTK/ERK signaling *in vivo*

		Asha, *et al.*, 2003 RasV12 Expression Dataset (Fold Induction)	Jordan, *et al.*, 2005 EGFR Ovary Dataset*	MAPK Phenotype							Conservation					Annotation	ERK/Viability	Validated
				S2R+				Kc167										
				Baseline	Pvf Hyperstim	Insulin	sSpitz/EGF	sSpitz/EGF	Baseline	Insulin	Human	Mouse	Worm	Yeast	Human Disease			
Act42A	Actin 42A	-0.98														Cytoskeletal		
Act5C	Actin 5C	-1.4														Cytoskeletal		
Adf1	Adh transcription factor 1	1.16														Transcription Factor		
aur	aurora	1.02														Kinase		
Axn	Axin	0.93														Signaling		
B4		0.75														Unknown		
Bap55	Brahma associated protein 55kD	0.69	GOFupLOFdown													Cytoskeletal		
Caf1	Chromatin assembly factor 1 subunit	1.68	GOFupLOFdown													Chromatin		
Cap-G		1.23	GOFupLOFdown													Chromatin		
cdc2	cdc2	2.97														Cell Cycle		
Cdk4	Cyclin-dependent kinase 4	1.24														Cell Cycle		
CG11198		1														Metabolism		
CG11451		2.98														Unknown		
CG12155		1.39														Unknown		
CG13298		1.12														RNA Processing		
CG13779		-1.03														Unknown		
CG13784		-1.33														Unknown		
CG14217		0.98														Kinase		
CG14512		1.16														Unknown		
CG16969		0.89														Unknown		
CG1841		0.82														Unknown		
CG1884			GOFupLOFNC													RNA Processing		
CG18854, IP3K1		-1.05														Kinase		
CG30421		1.37														Ubiquitylation		
CG31666		3.03														Transcription Factor		
CG3563			GOFupLOFdown													Unknown		
CG3878			GOFdownLOFup													Unknown		
CG6686		0.57	GOFdownLOFup													Transcription Factor		
CG6701		0.28														Chromatin		
CG6770		-1.2														Unknown		
CG6854		0.86														Transcription Factor		
CG6984			GOFdownLOFup													Metabolism		
CG8878		1.02														Kinase		
CG9455		0.74														Other		
CG9667		0.77														Unknown		
crp	cropped	1.82														Transcription Factor		
ctp	cut up	1.11														Cytoskeletal		
CycA	Cyclin A	1.52														Cell Cycle		
CycE	Cyclin E	2.77														Cell Cycle		
dock	dreadlocks	0.54														Signaling		
Dp	DP transcription factor	1.22	GOFupLOFdown													Transcription Factor		
Drk	downstream of receptor kinase (Grb2)		GOFupLOFdown													Signaling		
egh	egghead	0.63														Enzyme		
flw	flap wing	1.98														Phosphatase		
for	foraging	1.03	GOFupLOFdown													Kinase		
Fs(2)Ket	Female sterile (2) Ketel	0.66														Protein Transport		
fwd	four wheel drive	0.72														Kinase		
fzy	fizzy	2.79														Cell Cycle		
glu	gluon	2.25	GOFupLOFdown													Chromatin		
Gprk2	G protein-coupled receptor kinase 2	0.42														Kinase		
Grip84	gamma-tubulin ring protein 84	0.56														Cytoskeletal		
hoe1	hoepel1	-2.59														Ion Transport		
JIL-1		1.12														Kinase		
kis	kismet	0.93	GOFupLOFNC													Chromatin		
Klp61F	Kinesin-like protein at 61F	2.99														Cytoskeletal		
l(2)NC136		0.7	GOFupLOFdown													Transcription Factor		
mnd	minidiscs	1.08	GOFdownLOFup													Metabolism		
Myb	Myb oncogene-like	0.9														Transcription Factor		
nrv1	nervana 1	0.87														Ion Transport		
Pk61C	Protein kinase 61C	0.87														Kinase		
Pten		1.4														Phosphatase		
PTP-ER	Protein tyrosine phosphatase-ERK/Enhancer of Ras1	0.51														Phosphatase		
puc	puckered		GOFupLOFdown													Phosphatase		
qkr58E-1	quaking related 58E-1		GOFdownLOFup													RNA Processing		
Rac1		0.86														GAP/GEF/GTPase		
Rack1	Receptor of activated protein kinase C 1	-1.28														Signaling		
Ras85D	Ras oncogene at 85D	0.92														GAP/GEF/GTPase		
Rca1	Regulator of cyclin A1	0.63														Cell Cycle		
RhoGAP19D			GOFupLOFdown													GAP/GEF/GTPase		
RhoGAP71E		1.57														GAP/GEF/GTPase		
Rpb5		0.51														General TF		
RpL7	Ribosomal protein L7	-1.19														Ribosome		
RpS26	Ribosomal protein S26	-1.52														Ribosome		
shn	schnurri	0.93														Transcription Factor		
shot	short stop	1.02														Cytoskeletal		
Sin3A		1.99														General TF		
Smg5			GOFupLOFdown													RNA Processing		
Smox	Smad on X	1.07														Transcription Factor		
spas	spastin	0.73														Other		
Spt6		0.58														Chromatin		
Tak1	TGF-beta activated kinase 1	1.47														Kinase		
TfIIB	Transcription factor IIB	1.51														General TF		

* "GOF": gain-of-function RTK/ERK pathway components; "LOF": loss-of-function RTK/ERK pathway components; "down"/"up": transcripts induced or repressed by GOF or LOF analysis; "NC": no change

References

Asha, H. *et al.* Analysis of Ras-induced overproliferation in *Drosophila* hemocytes. *Genetics* 163, 203-15 (2003).

Jordan, K. C. *et al.* Genome wide analysis of transcript levels after perturbation of the EGFR pathway in the *Drosophila* ovary. *Dev Dyn* 232, 709-24 (2005).

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Annotation categories are manual groupings of Gene Ontology data queried from FlyBase (October 2004) and therefore may not reflect more comprehensive studies of function.

"Validated"

	indicates an additional amplicon targeting the given gene affected ERK activation in S2R+ cells at baseline or under insulin stimulation. See Methods for details.
	indicates an additional amplicon targeting the given gene did not affect ERK activation in S2R+ cells at baseline or under insulin stimulation. See Methods for details.
	indicates no additional amplicons targeting the given gene were tested

dpERK	%Control
	>125%
	110-125%
	105-110%
	95-105%
	90-95%
	70-90%
	<70%

Viability	%Control
	>110%
	<90%

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Supplementary Table 8: Genes which suppress RasV^{12}-mediated ERK activation

	S2 mtRas^{V12}	
Gene	Insulin	$CuSO_4$ - Induced
14-3-3epsilon		
14-3-3zeta		
ago		
alphaCop		
aur		
betaCop		
beta'Cop		
cdc2		
CG11198		
CG11416		
CG11451		
CG11700		
CG15060		
CG1814		
CG18646		
CG2049		
CG30387		
CG30476		
CG31302		
CG31763		
CG32183		
CG33138		
CG3563		
CG5172		
CG5546		
CG5844		
CG6550		
CG6686		
CG6701		
CG6842		
CG7282		
CG7757		
CG7918		
CG8878		
cnk		
CycA		
CycE		
CycT		
deltaCOP		
Dp		
Dsor1		
dup		
flw		
for		
fru		
Fs(2)Ket		
fzy		

Grp1		
h		
HFA01082		
HFA01091		
HFA05237		
HFA05715		
HFA08092		
HFA09077		
HFA09359		
HFA10152		
HFA17488		
His2A:CG31618		
His3:CG31613		
His4r		
Hk		
Klp61F		
ksr		
l(2)03709		
mRNA-capping-enzyme		
Pak3		
phl		
Pp1-13C		
Pp1-87B		
puc		
Rab11		
rab3-GEF		
ran		
Ras85D		
Rpb10		
Rpb5		
RpII33		
RpL7		
Rpt1		
shn		
spri		
th		
Tie		
trio		

dpERK	%Control
	>125%
	110-125%
	105-110%
	95-105%
	90-95%
	70-90%
	<70%