© 2015. Published by The Company of Biologists Ltd | Development (2015) 142, 1502-1515 doi:10.1242/dev.116715

RESEARCH ARTICLE

Genome-wide identification of phospho-regulators of Wnt signaling in *Drosophila*

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ABSTRACT

Evolutionarily conserved intercellular signaling pathways regulate embryonic development and adult tissue homeostasis in metazoans. The precise control of the state and amplitude of signaling pathways is achieved in part through the kinase- and phosphatase-mediated reversible phosphorylation of proteins. In this study, we performed a genome-wide *in vivo* RNAi screen for kinases and phosphatases that regulate the Wnt pathway under physiological conditions in the *Drosophila* wing disc. Our analyses have identified 54 high-confidence kinases and phosphatases capable of modulating the Wnt pathway, including 22 novel regulators. These candidates were also assayed for a role in the Notch pathway, and numerous phospho-regulators were identified. Additionally, each regulator of the Wnt pathway was evaluated in the wing disc for its ability to affect the mechanistically similar Hedgehog pathway. We identified 29 dual regulators that have the same effect on the Wnt and Hedgehog pathways. As proof of principle, we established that Cdc37 and Gilgamesh/CK1γ inhibit and promote signaling, respectively, by functioning at analogous levels of these pathways in both *Drosophila* and mammalian cells. The Wnt and Hedgehog pathways function in tandem in multiple developmental contexts, and the identification of several shared phospho-regulators serve as potential nodes of control under conditions of aberrant signaling and disease.

KEY WORDS: Wnt, Wingless, *In vivo* RNAi screen, Hedgehog, Notch

INTRODUCTION

The canonical Wnt signaling pathway is evolutionarily conserved and regulates essential biological processes such as cell fate specification, proliferation and migration during metazoan development. As a consequence, aberrant Wnt signaling can result in diverse human developmental disorders and cancers (MacDonald et al., 2009; Wodarz and Nusse, 1998). Cells use the reversible phosphorylation of proteins to control the state and amplitude of signaling pathways (Cohen, 1992; Hunter, 1995; Salazar and Höfer, 2009). In the silent state of the Wnt pathway, the transcriptional effector β-catenin is phosphorylated within a cytosolic Axin (Axn) complex by Casein Kinase 1α (CK1α) and Glycogen Synthase Kinase 3β (GSK3β). This modification facilitates its poly-ubiquitination and degradation (Aberle et al., 1997; Amit et al., 2002; Kitagawa et al., 1999; Liu et al., 1999, 2002). In the absence of stabilized β-catenin, the DNA-binding protein TCF represses target gene expression (Cavallo et al., 1998; Roose et al., 1998). Binding of secreted Wnt ligand to its transmembrane Frizzled (Fz) receptor and LRP co-receptor initiates pathway activity through the recruitment of the Axn complex to the cell surface (Cliffe et al., 2003; Tamai et al., 2004; Umbhauer et al., 2000; Wong et al., 2003; Yang-Snyder et al., 1996). This induces the phosphorylation of LRP by CK1α, GSK3β and the plasma membrane-associated CK1γ (Davidson et al., 2005; Tamai et al., 2004; Zeng et al., 2005), which then reciprocally facilitates the disassembly of the Axn complex to prevent β-catenin degradation (Cselenyi et al., 2008; Kim et al., 2013; Mi et al., 2006; Willert et al., 1999). Stabilized β-catenin translocates to the nucleus to form a transcriptional complex with TCF to direct expression of target genes (Behrens et al., 1996; Molenaar et al., 1996; van de Wetering et al., 1997). Preceding these events in signal-receiving cells, the secretion, diffusion and reception of Wnt itself is phospho-regulated in ligand-producing cells and in the extracellular environment (Buechling and Boutros, 2011).

Although several kinases and phosphatases are known to regulate Wnt signaling (Gao et al., 2014; Verheyen and Gottardi, 2010), our current knowledge of these enzymes remains incomplete. In fact, differential phospho-proteome analyses have identified both novel phospho-regulators and novel phosphorylation sites on known regulators of the pathway (Bodenmiller et al., 2007; Tang et al., 2007). It is unlikely that the reversible phosphorylation of all of these newly identified phospho-epitopes is catalyzed by the current subset of known kinases and phosphatases of Wnt signaling, thereby suggesting that there are additional unidentified phospho-regulators of the pathway.

Several large-scale *in vitro* screening analyses have been performed in various *Drosophila* and mammalian cell lines to identify regulators of the Wnt pathway. These high-throughput loss- and gain-of-function studies quantitated the response of exogenous Wnt pathway reporters under conditions of elevated signaling (Anton et al., 2011; Buechling et al., 2011; Caspi and Rosin-Arbesfeld, 2008; DasGupta et al., 2005; Firestein et al., 2008; Groenendyk and Michalak, 2011; Jacob et al., 2011; James et al., 2009; Kategaya et al., 2009; Major et al., 2008; Miller et al., 2009; Port et al., 2011; Tang et al., 2008). In this study, we have performed a comprehensive genome-wide *in vivo* RNAi screen for kinases and phosphatases in *Drosophila* to build a phospho-regulatory network of the Wnt pathway. *Drosophila* has significantly contributed to our understanding of the molecular mechanism of the Wnt pathway (Bejsovec, 2006), and has low functional redundancy but high functional conservation of the genes with humans (Fortini et al., 2000; Reiter et al., 2001). The strength of our approach is that we assayed the effects of putative phospho-regulators of the Wnt pathway on endogenous targets under physiological levels of signaling in an intact tissue. Wing discs from third instar larvae were immunostained against direct high- and low-threshold targets of the pathway, allowing us to evaluate the entire ligand-induced gradient of signaling (Barolo, 2006). As the wing disc comprises distinct

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Received 17 August 2014; Accepted 2 March 2015

Development (2015) 142, 1502-1515 doi:10.1242/dev.116715

cells that either produce or respond to the ligand, we could identify phospho-regulators at discrete positions in the pathway. Our analyses of the Wnt pathway have yielded a large subset of high-confidence kinases and phosphatases, including 22 previously unidentified regulators of signaling. Among candidate Wnt regulators, we determined which acted indirectly through the Notch pathway to modulate *wg* transcription. Furthermore, we established those shared between the Wnt and Hh pathways. Last, we validated two dual regulators of the Wnt and Hh pathways using biochemical assays to demonstrate that their roles are evolutionarily conserved from *Drosophila* to mammalian cells.

RESULTS

Design of an *in vivo* screen to identify phospho-regulators of the Wnt pathway

In the wing imaginal disc, Wingless (Wg) (*Drosophila* Wnt) produced from cells at the dorsal/ventral (D/V) compartment boundary induces the nested expression of pathway target genes *senseless* (*sens*) (high-threshold) and *Distal-less* (*Dll*) (low-threshold) in adjacent non-boundary cells that receive the ligand (Neumann and Cohen, 1997; Zecca et al., 1996) (Fig. 1). We compiled a list of all protein kinases and phosphatases, non-protein kinases and phosphatases, as well as factors that associate with these enzymes, such as cyclins and regulatory subunits, which we will collectively refer to as the kinome and phosphatome (supplementary material Figs S1, S2). Using transgenic RNAi libraries of UAS-driven inverted repeats (IRs) and tissue-specific Gal4 drivers, we knocked down in a spatially restricted fashion the expression of each of 385 and 205 genes present in the *Drosophila* kinome and phosphatome, respectively, to assay their effect on Wnt signaling (Fig. 1). At least two non-overlapping IRs per gene were independently tested to minimize positive and negative false discovery.

The primary screen was performed using a combination of *decapentaplegic* (*dpp*)-*Gal4* and *hedgehog* (*hh*)-*Gal4* that are expressed along the anterior/posterior compartment boundary and in the posterior compartment of the wing disc, respectively. These *Gal4* drivers were used to knock down gene function in both the ligand-producing and ligand-receiving cells (Fig. 1). A *UAS-dicer-2* transgene was used in combination with the *Gal4* drivers for all screening analyses to enhance the efficiency of RNAi-mediated gene knockdown (Dietzl et al., 2007). Wing discs from every genotype were immunostained against Sens, Dll and Wg to assay for pathway activity and status of the ligand. A subset of known regulators of the Wnt pathway was knocked down in a pilot screen to validate our experimental design (supplementary material Fig. S3). We consistently observed reproducible and expected defects in pathway targets (and Wg protein in certain cases) with known components of signaling, although some effects were subtle, yet highly penetrant and reproducible (e.g. supplementary material Fig. S3).

The wing disc is specified by Wnt and other signaling pathways (Couso et al., 1994; Rulifson et al., 1996), and disruption of this process may induce compensatory mechanisms that form a relatively normal adult wing (Herrera et al., 2013; Ryoo et al., 2004; Wells et al., 2006). Discs were therefore immunostained from every genotype irrespective of the presence of an adult wing or other phenotype. A gene that modified the levels of Sens and/or Dll when knocked down independently with at least two non-overlapping IRs using any combination of *Gal4* drivers was classified as a candidate of the Wnt pathway (examples of modifiers are shown in Fig. 2). We identified 90 candidates from the primary screen (Fig. 3). Scoring of

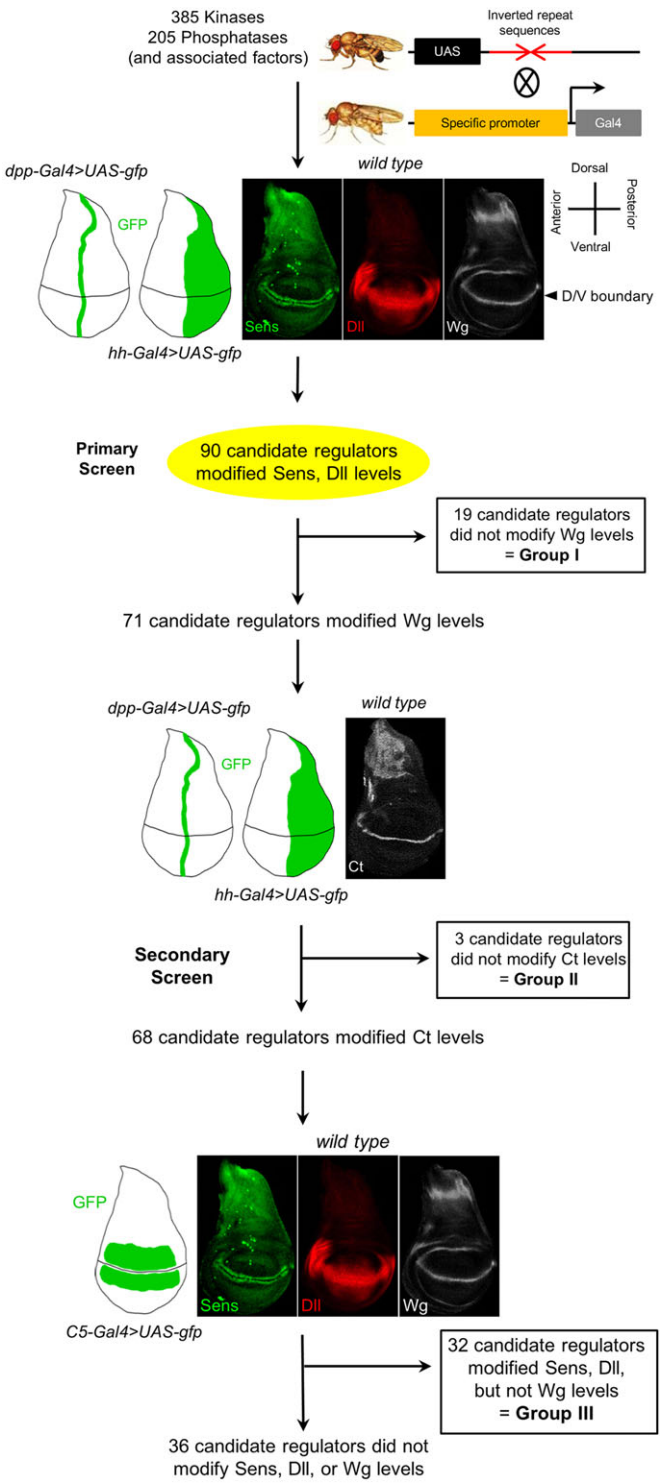

Fig. 1. Design of Wnt pathway screen in the *Drosophila* wing disc. Schematic illustration of *in vivo* Wnt pathway screen. Five-hundred and ninety kinases, phosphatases and associated factors were knocked down in a spatially restricted fashion in the wing disc through the use of *Gal4* drivers/*UAS*-IRs. Secondary screens were performed to further functionally classify the regulators, as described in the text. Ninety primary screen candidates were classified into 54 high-confidence Group I, Group II and Group III regulators based on secondary screens.

all crosses with multiple RNAi lines is provided in supplementary material Figs S4, S5. No gene, when knocked down, modified the levels of only Sens or Dll, although in some cases one pathway

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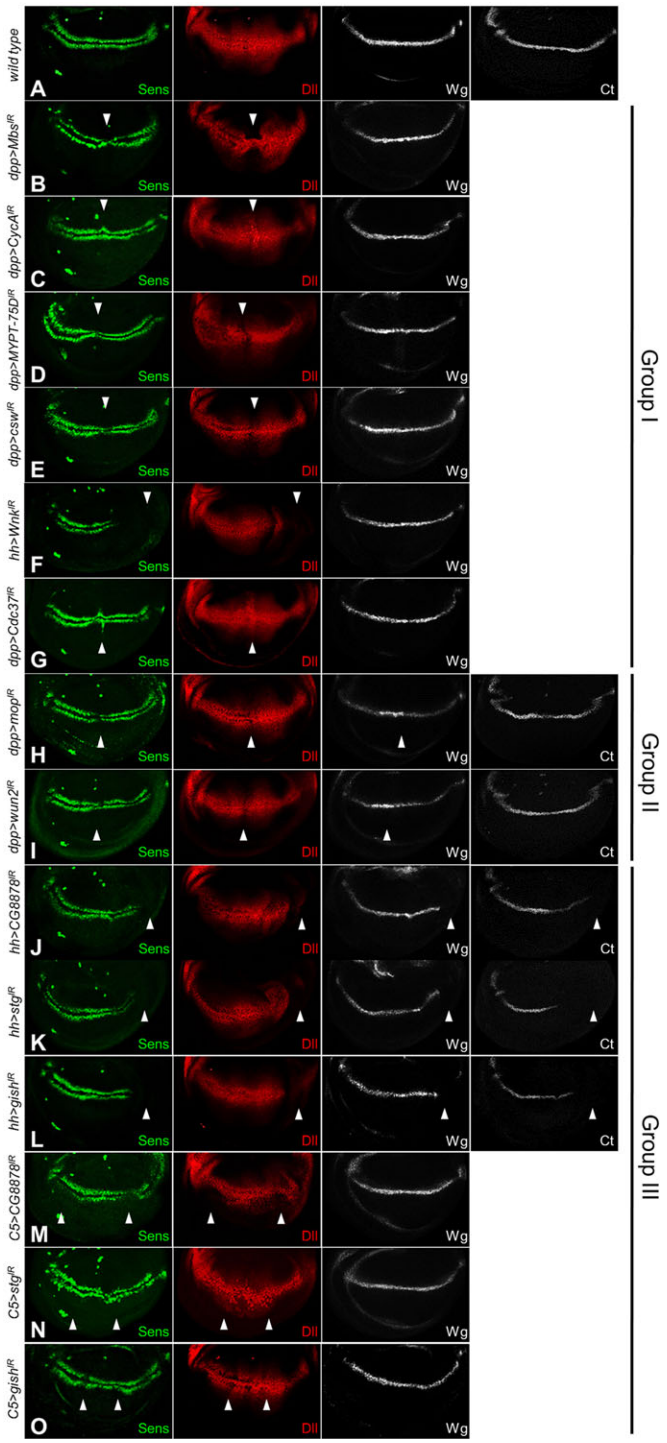

Fig. 2. High-confidence regulators of the Wnt pathway identified in the *Drosophila* wing disc. (A) A wild-type wing disc that displays the levels of Sens, Dll, Wg and Ct. (B-G) Group I regulators knocked down using *dpp-Gal4* or *hh-Gal4* modified the levels of Sens and Dll, but not Wg. Knockdown of *Mbs*, *MYPT-75D*, *csw* and *Wnk* decreased the levels of Sens and Dll (arrowheads), while knockdown of *CycA* and *Cdc37* increased the levels of Sens and Dll (arrowheads). (H,I) Group II regulators modified the levels of Sens, Dll and Wg, but not of Ct. Knockdown of *mop* and *wun2* decreased the levels of Sens and Dll (arrowheads), and increased Wg levels (arrowheads). (J-O) Group III regulators, such as *CG8878*, *stg* and *gish*, modified the levels of Sens, Dll, Wg and Ct (arrowheads). (M-O) Knockdown of these regulators in only the ligand-receiving cells with *C5-Gal4* modified the levels of Sens and Dll (arrowheads), but not Wg.

target was affected to a greater extent than the other (Figs 2, 3). Of note, most candidates had mild to moderate effects on targets, consistent with results obtained in our pilot screen with known Wnt pathway components, suggesting that under these assay conditions relatively subtle effects are valid, as confirmed by further analyses.

Nineteen of the 90 candidates had no observable effect on Wg levels or distribution, and thus do not function upstream of or at the level of the ligand-receptor interaction to affect the secretion, diffusion or reception of Wg. These candidates were classified as Group I high-confidence regulators of the Wnt pathway that function downstream of the ligand-receptor interaction in the ligand-receiving cells (Figs 2, 3). Group I includes known regulators of signaling such as *Wnk* (Serysheva et al., 2013) and novel regulators such as *Myosin binding subunit* (*Mbs*), *Cyclin A* (*CycA*), *MYPT-75D*, *corkscrew* (*csw*) and *Cdc37* (Fig. 2A-G).

Seventy-one of the 90 candidates modified the levels of Wg (Fig. 2; supplementary material Figs S4, S5). The Notch pathway signals from non-boundary cells to induce the expression of *wg* in cells at the D/V boundary of the wing disc (Diaz-Benjumea and Cohen, 1995; Neumann and Cohen, 1996; Rulifson and Blair, 1995; supplementary material Fig. S6). To test whether the candidates that affect Wg levels do so as a result of their regulation of the ligand after translation or by affecting expression of the ligand via the Notch pathway, they were re-analyzed in a secondary screen. To address transcriptional regulation by Notch signaling, we determined whether they also modified the levels of another Notch pathway target, Cut (Ct) (Micchelli et al., 1997; Fig. 1). Only three of the 71 candidates when knocked down with either *dpp-Gal4* or *hh-Gal4* had no effect on Ct levels. We inferred that these three candidates have no effect on the Notch pathway and *wg* expression, but rather modify the secretion, diffusion or reception of Wg, which was detected as a change in its levels. These candidates were classified as Group II high-confidence regulators of the Wnt pathway that function upstream of or at the level of the ligand-receptor interaction in the ligand-producing cells or extracellular environment, respectively (Figs 2, 3). Group II includes a known regulator of signaling, *myopic* (*mop*) (Miura et al., 2008; Pradhan-Sundd and Verheyen, 2014), and a novel regulator, *wunen2* (*wun2*) (Fig. 2H-I). As further evidence of the functional role of Group II candidates, we and others have confirmed that two of the three Group II candidates (CG3530, Mop) have no effect on *wg-lacZ* but do affect the Wg protein (Silhankova et al., 2010; Pradhan-Sundd and Verheyen, 2014).

The remaining 68 of the 71 candidates modified Ct levels and were inferred to regulate *wg* expression as a result of their effect on multiple Notch targets (Fig. 3). These candidates were further tested to determine whether they also affected the Wnt pathway independently of their regulation of *wg* expression. The majority of regulators of developmental pathways function in the ligand-receiving cells and not the ligand-producing cells. Therefore, if a gene independently affects the Wnt and Notch pathways in the wing disc, it would likely do so in the ligand-receiving cells of both pathways, regulating Wnt signaling in the non-boundary cells and Notch signaling in the boundary cells. We knocked down the 68 candidates that we identified as regulators of the Notch pathway using the *C5-Gal4* driver, which is expressed in only non-boundary cells adjacent to the D/V compartment boundary (Fig. 1). By knocking down gene function in only the ligand-receiving cells of the Wnt pathway, which correspond to the ligand-producing cells of the Notch pathway (supplementary material Fig. S6), we could distinguish candidates that independently regulate both Wnt and Notch signaling from those that regulate only Notch signaling in the

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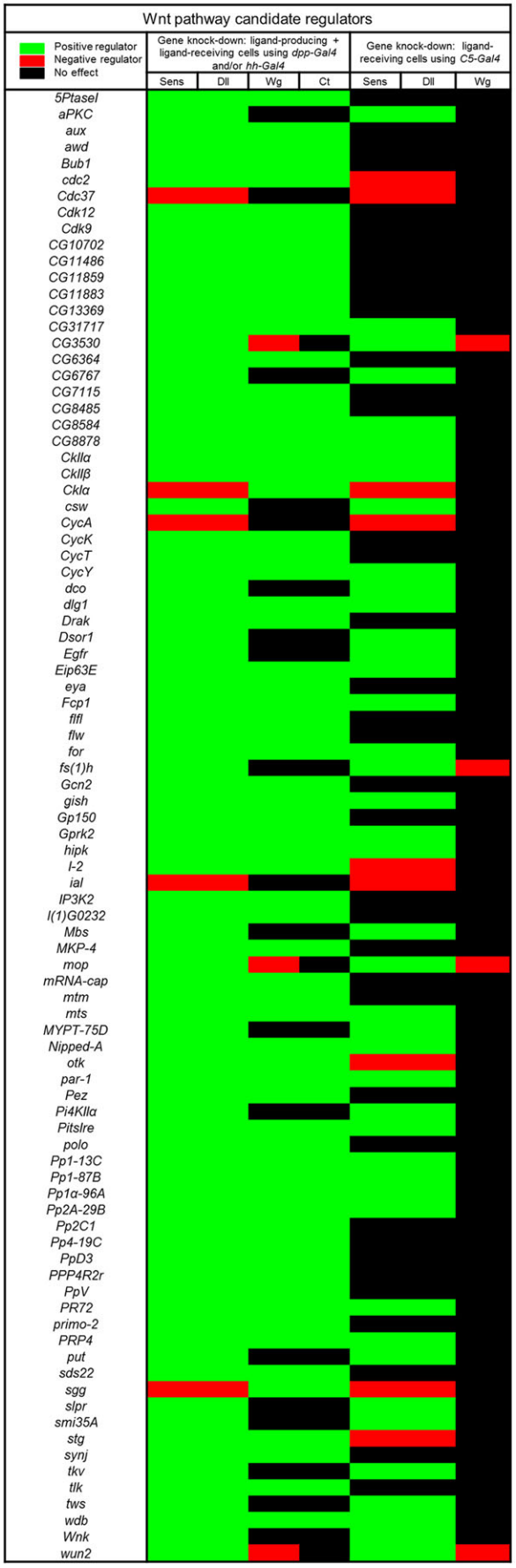

Fig. 3. Results of Wnt pathway screen in the *Drosophila* wing disc. Graphical summary that displays whether knockdown of candidates decreased (green), increased (red) or had no effect (black) on the levels of Sens, Dll, Wg and Ct. Summary reflects results obtained with at least two unique RNAi lines (see supplementary material Figs S4 and S5 for all data) knocked down in signal-producing and signal-receiving cells (using either *dpp-Gal4* or *hh-Gal4*) or in ligand-receiving cells only (using *C5-Gal4*) of the wing disc.

wing disc. When knocked down in the non-boundary cells, 32 of the 68 candidates modified the levels of the Wnt pathway targets Sens and Dll, but not Wg, and thus did not affect the Notch pathway in these cells to regulate *wg* expression at the D/V compartment boundary. These 32 candidates were classified as Group III high-confidence regulators of the Wnt pathway that function downstream of the ligand-receptor interaction in the signal-receiving cells and that affect the Notch pathway independently (Figs 2, 3). Group III includes known regulators of signaling, such as *string* (*stg*) (Davidson et al., 2009) and *gilgamesh* (*gish*) (Davidson et al., 2005; Zhang et al., 2006), and a novel regulator, *CG8878* (Fig. 2J-O). The remaining 36 candidates when knocked down in the non-boundary cells had no effect on the levels of Sens, Dll or Wg, and thus do not regulate the Wnt or Notch pathway in these cells (Fig. 3). Although these 36 candidates (supplementary material Fig. S7) regulate the Notch pathway and *wg* expression in the boundary cells, through our analyses we could not determine whether any of these candidates, albeit unlikely, also independently regulate Wg secretion from these cells to affect the Wnt pathway.

In summary, the 590 phospho-regulators screened in the wing disc yielded 90 candidates that were classified into 54 high-confidence regulators of the Wnt pathway and 36 candidates that regulate *wg* expression to indirectly affect the Wnt pathway. The high-confidence regulators were further classified into three phenotypic categories, referred to as Groups I, II and III, each of which comprise known and novel regulators of signaling (Fig. 4). Of the high-confidence regulators, 33 are kinases (and associated factors) and 21 are phosphatases (and associated factors). Forty-five of the 54 high-confidence regulators promote (blue in Fig. 4A), while the remaining nine inhibit (yellow in Fig. 4A), signaling.

Remarkably, 32 of the 90 candidates are known regulators of the Wnt pathway (indicated by gray shading in Fig. 4). This large subset of known regulators is reflective of the robustness and low false-negative error rate of our screen design. Moreover, based on our unbiased genetic analyses, all 32 of these known regulators cluster together in the category of high-confidence Groups I, II and III regulators. This strongly suggests that at least some of the 22 novel high-confidence regulators identified are bona fide regulators of the Wnt pathway. We classified the high-confidence regulators of the Wnt pathway according to their respective kinase and phosphatase groups. The *Drosophila* kinome comprises 13 groups (defined in supplementary material Fig. S1), of which 12 are represented by the high-confidence regulators of the Wnt pathway (Fig. 4B; supplementary material Fig. S1). The *Drosophila* phosphatome comprises nine groups (defined in supplementary material Fig. S2), of which six are represented by the high-confidence phosphatases (and associated factors) of the Wnt pathway (Fig. 4C; supplementary material Fig. S2).

Hh pathway counterscreen identifies Cdc37 and Gish as dual regulators of signaling

The Hh pathway has diverse functions during metazoan development, such as the regulation of organogenesis and stem

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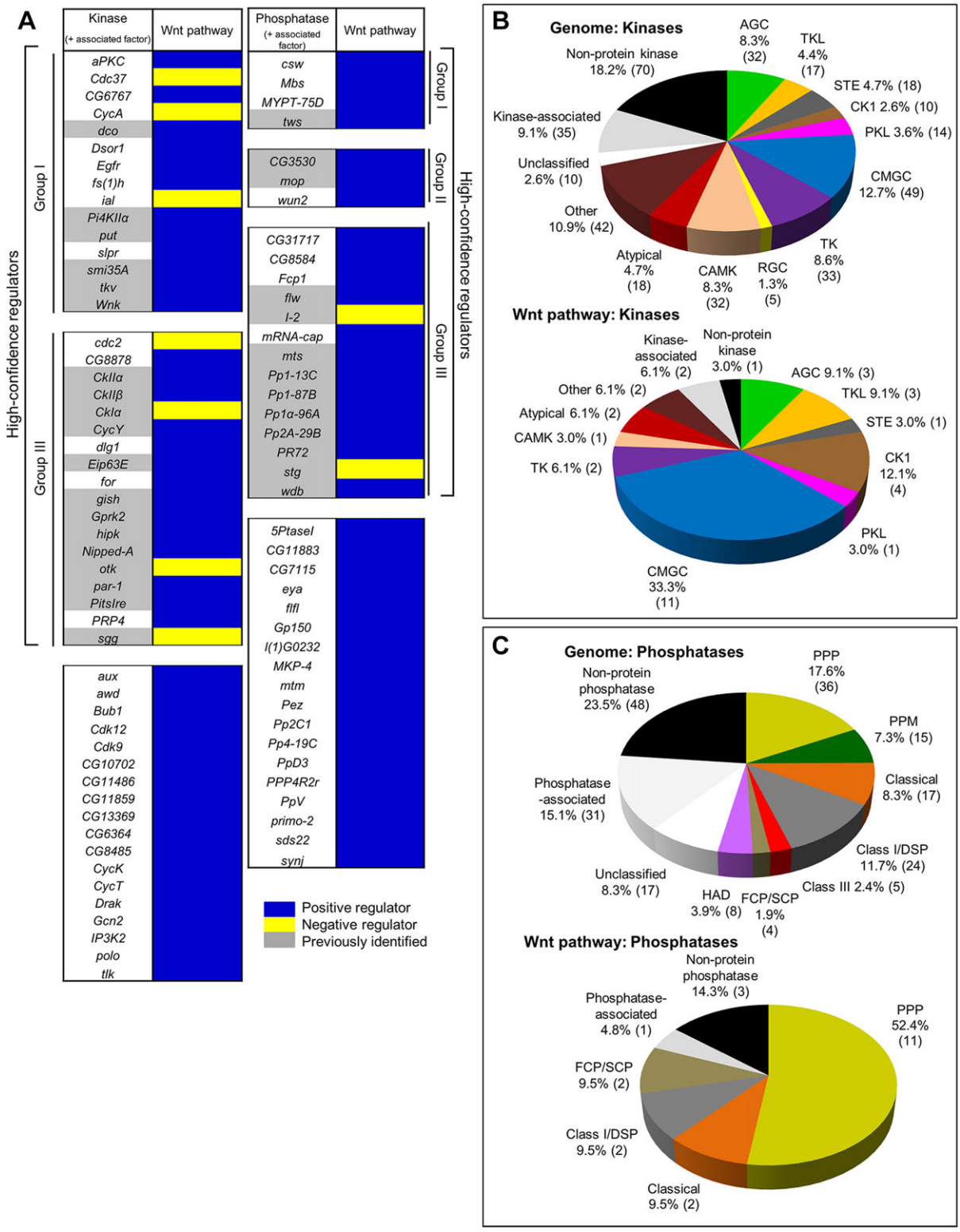

Fig. 4. Summary of phospho-regulators of the Wnt pathway identified in the *Drosophila* wing disc. (A) Ninety candidates of the Wnt pathway were refined into 54 high-confidence regulators (Group I, Group II and Group III). Forty-five high-confidence regulators promote (blue) and nine inhibit (yellow) the Wnt pathway. Three high-confidence regulators function upstream or at the level of the ligand-receptors interaction (Group II), whereas the remaining 51 function downstream of the ligand-receptor interaction (Group I, Group III). Thirty-two of the 90 candidates identified are previously validated regulators of the Wnt pathway (gray). (B,C) Graphical summary of the kinase (B) and phosphatase (C) groups in the *Drosophila* genome, and the subset of these that regulate the Wnt pathway.

cell homeostasis (Varjosalo and Taipale, 2008). Similar to the Wnt pathway, the Hh pathway is also subject to reversible phosphorylation in its silent and active states. In the absence of signaling, the transcriptional effector GLI is phosphorylated by Protein Kinase A (PKA), GSK3β and CK1α within a cytosolic Kif7 complex (Chen et al., 1998; Jia et al., 2002, 2005; Price and

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Kalderon, 1999, 2002; Zhang et al., 2005). The phosphorylation of GLI triggers its poly-ubiquitylation and partial degradation to yield a truncated form of the protein that represses target gene expression (Aza-Blanc et al., 1997; Jia et al., 2005; Jiang and Struhl, 1998; Méthot and Basler, 1999; Smelkinson and Kalderon, 2006; Smelkinson et al., 2007; Tempé et al., 2006). Binding of the Hh ligand to its receptor Patched (Ptc) recruits the Kif7 complex to the transmembrane signal transducer Smoothened (Smo) (Lum et al., 2003; Ogden et al., 2003; Ruel et al., 2003; Zheng et al., 2010). This induces Smo phosphorylation by PKA and CK1α, which promotes its accumulation at the cell surface (Apionishev et al., 2005; Denef et al., 2000; Jia et al., 2004; Zhang et al., 2004; Zhu et al., 2003). The interaction between Kif7 and phospho-Smo disassembles the complex to stabilize full-length GLI that directs target gene expression (Aza-Blanc et al., 1997; Jia et al., 2003; Liu et al., 2007; Ohlmeyer and Kalderon, 1998). Although the evolutionary relationship between Wnt and Hh signaling remains unclear, these pathways have a similar phospho-regulatory mechanism of signal transduction and comprise similar or identical regulators that exert the same effect on signaling by functioning at analogous levels of the relays (Kalderon, 2002).

We evaluated the ability of all 90 candidates of the Wnt pathway to regulate the Hh pathway *in vivo*. We performed this counter-screen to identify shared phospho-regulators that exert the same effect to either promote or inhibit signaling, and thereby potentially function at analogous levels of these pathways. Hh signaling stabilizes full-length Cubitus interruptus (Ci) (*Drosophila* GLI) to regulate expression of the target gene *ptc* along the anterior/posterior (A/P) boundary in the anterior compartment of the wing disc (Strigini and Cohen, 1997) (Fig. 5A). Each candidate was knocked down using *MS1096-Gal4* (with *UAS-dicer-2*) and wing discs were immunostained to detect Ci and Ptc. The *MS1096-Gal4* domain is in the center of the wing disc with stronger expression in the dorsal half relative to the ventral half (supplementary material Fig. S8A). The enhancer of *ptc*, unlike that of other Hh pathway targets, responds in a cooperative manner to both the levels and active state of Ci; thus, a change in the levels of Ci that modulates the amplitude of Hh signaling might not necessarily result in an effect on the expression of *ptc* (Parker et al., 2011). Any gene that modified the levels of Ci, but not necessarily Ptc, when independently knocked down with at least two non-overlapping IRs was classified as a regulator of the Hh pathway (Fig. 5B; supplementary material Fig. S8B). A limitation of this approach is that the counter-screen cannot identify regulators of the Hh pathway that do not affect the levels of Ci, but function downstream of its stabilization to modulate the expression of targets other than *ptc*. Sixty-six of the 90 candidates of the Wnt pathway modified the levels of Ci (and Ptc in most cases) to regulate the Hh pathway (supplementary material Figs S4, S5). The remaining 24 candidates of the Wnt pathway had no effect on the levels of Ci or Ptc (supplementary material Figs S4, S5). While the majority of Wnt regulators promoted signaling, 45 of 66 regulators of the Hh pathway inhibited signaling (supplementary material Fig. S8B). Nevertheless, 29 Wnt candidates exerted the same effect on the Hh pathway to either promote or inhibit signaling in the wing disc (Fig. 5B). Of these 29 dual regulators that we propose function at analogous levels of these pathways, 25 are high-confidence regulators and eight are novel regulators of the Wnt pathway. As proof of principle, we recovered *hipk* in our screen, which we have previously shown to be a dual regulator of Wnt and Hh acting on the E3 ubiquitin ligase Supernumerary limbs (Slimb) at analogous levels of the pathways (Swarup and Verheyen, 2011).

We identified *Cdc37* and *gish* as novel negative and positive regulators, respectively, of the Hh pathway. Knockdown of a negative control, *lacZ* (supplementary material Fig. S8A), displayed normal levels of Ci and Ptc, as seen in wild-type tissue (Fig. 5C). Knockdown of *Cdc37* resulted in the robust enhancement of Ci levels, both within and away from the signaling domain in the anterior compartment of the wing disc (Fig. 5D). Knockdown of *Cdc37* consistently led to the distortion of the morphology of the disc, which precluded an accurate evaluation of Ptc levels. Based on our data, *Cdc37* knockdown does not seem to appreciably alter Ptc levels, but we cannot definitively rule out this possibility. *Cdc37* knockdown phenocopies loss-of-function mutants of other negative regulators of the Hh pathway that strongly enhance the levels of Ci but have minimal or no effect on Ptc levels, such as *sgg* (*Drosophila* GSK3β) (Jia et al., 2002; Price and Kalderon, 2002) and *slimb* (Wang et al., 1999). Knockdown of *gish* resulted in the reduction of Ci and a reduction in the levels of Ptc, as indicated by the width of its expression domain in the central region of the wing disc (Fig. 5E).

In our screening analyses, all 90 candidates of the Wnt pathway were evaluated against Notch (as part of the secondary screen) and Hh (as part of the counter-screen) pathways in the wing disc (Fig. 6A). This allowed us to distinguish between candidates that are specific to the Wnt pathway from those that are shared between the Wnt and other signaling pathways (Fig. 6B; Tabata and Takei, 2004). These analyses also allowed us to evaluate whether a candidate of the Wnt pathway does so only indirectly as a result of its effect on cell death, cell proliferation or non-specific gene transcription. For example, if knockdown of a candidate decreases Wnt signaling but does not affect or increases Notch and/or Hh signaling, we inferred that this candidate does not affect cell death or non-specific gene transcription. However, if knockdown of a candidate decreases Wnt, Notch and Hh signaling, it is possible that it indirectly regulates the Wnt pathway due to an effect on cell death or non-specific gene transcription. Twelve of the 90 candidates of the Wnt pathway when knocked down had the same effect (i.e. positive or negative regulator of both pathways) on both the Notch and Hh pathways (Fig. 6C). This does not necessarily imply that these 12 candidates indirectly regulate the Wnt pathway, but that further analysis of these candidates is required. Coincidentally, 11 of these 12 candidates have been previously described to regulate the Wnt pathway (Fig. 6C).

Cdc37 inhibits the Wnt and Hh pathways by destabilizing their transcriptional effectors

As proof of concept for dual regulators that operate at analogous levels of the Wnt and Hh pathways, we further characterized the functions of *cdc37* and *gish*. Cdc37 is a chaperone that functions to regulate the folding and biogenesis of diverse kinases (Caplan et al., 2007). We identified *cdc37* as a novel negative regulator whose knockdown resulted in increased pathway activity within the signaling domain and ectopic pathway activity outside the signaling domain (Fig. 2G). The *Drosophila* eye has frequently been used to screen for regulators of the Wnt pathway using a *sevenless* (*sev*)>*wg* (or equivalent) gain-of-function genetic background in which eye development is impaired (Fig. 7A; Greaves et al., 1999; Port et al., 2011). Heterozygosity for a loss-of-function allele of *cdc37* caused an enhancement of the *sev>wg* phenotype (Fig. 7B), suggesting that reduction of *cdc37* enhances the aberrant eye phenotype caused by Wg signaling. We examined embryos homozygous for the $cdc37^{eD4}$ allele. Wg signaling is required for specification of regions of naked cuticle

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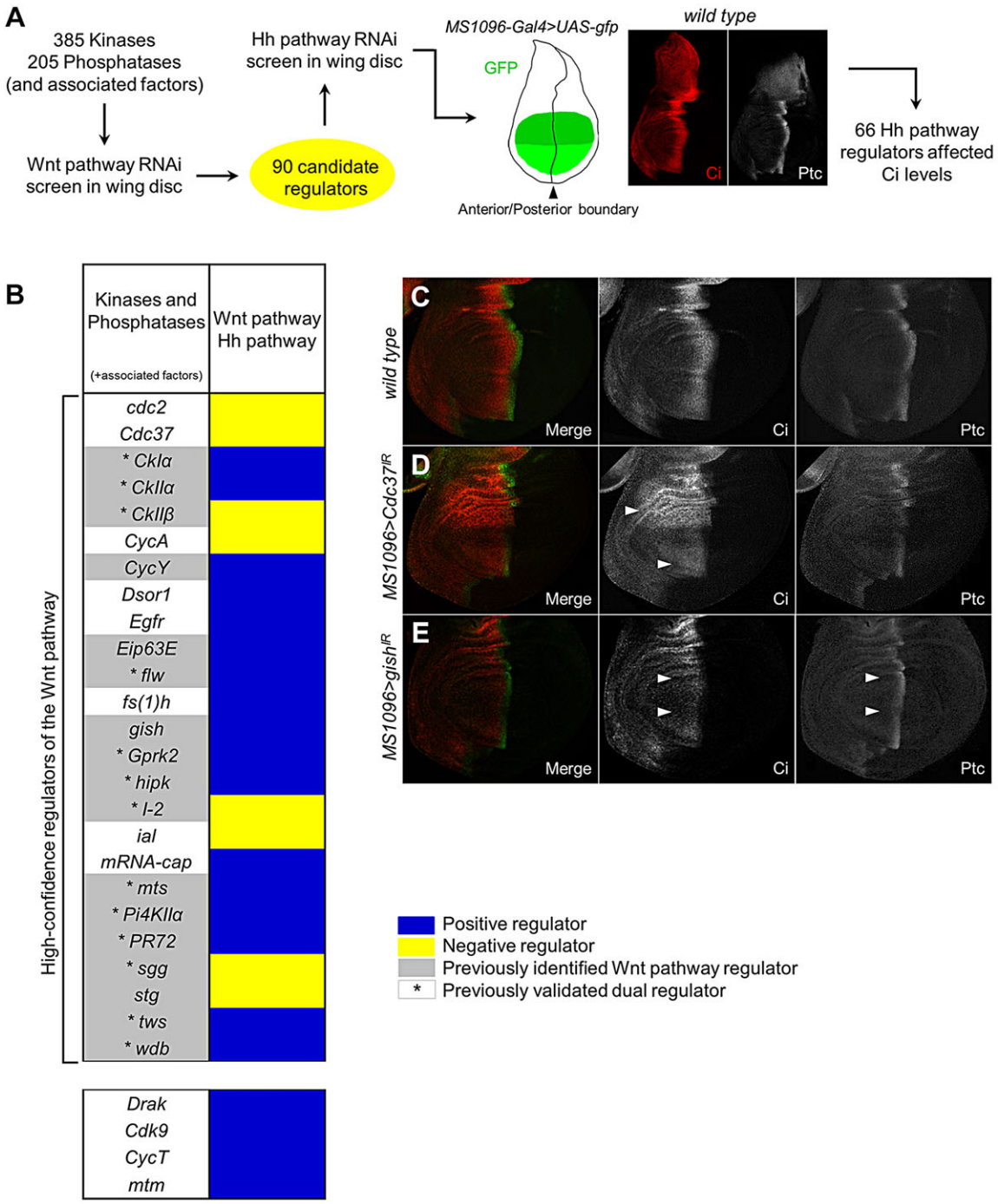

Fig. 5. Hh pathway counter-screen in the *Drosophila* wing disc. (A) Ninety candidates of the Wnt pathway were knocked down throughout the wing pouch using *MS1096-Gal4* and wing discs were immunostained against Ci and Ptc. Sixty-six candidates modified Ci levels to regulate the Hh pathway. (B) Twenty-nine candidates of the Wnt pathway were identified to have the same effect on the Hh pathway to either promote (blue) or inhibit (yellow) signaling. All but four of these 29 candidates are high-confidence regulators and 17 are known regulators (gray) of the Wnt pathway. Thirteen of the 29 candidates have been previously validated as dual regulators (asterisks) of the Wnt and Hh pathways. (C) The levels of Ci and Ptc along the anterior/posterior compartment boundary of a wild-type wing disc. (D) Knockdown of *cdc37* increased the levels of Ci (arrowheads), but did not affect Ptc. (E) Knockdown of *gish* decreased the levels of Ci and Ptc (arrowheads).

between denticle belts in the cuticle (Fig. 7C). Enhanced Wg signaling caused loss of denticle belts, such as was seen in *cdc37* mutant embryos, suggesting loss of *cdc37* promotes Wg signaling (Fig. 7D,E). Somatic loss-of-function clones of *cdc37* do not survive in the wing disc due to its requirement for cell viability (Lange et al., 2002). We therefore generated MARCM loss-of-function clones of *cdc37* (positively marked with nuclear GFP) that overexpress the apoptosis inhibitor *p35*. These MARCM clones, although small in size, displayed a cell-autonomous upregulation of Wnt and Hh signaling, as indicated by an increase in Dll and Ci levels, respectively. Similar to the effect seen with RNAi (Figs 2G, 5D), ectopic Dll (Fig. 7F) and Ci (Fig. 7G) were observed in MARCM clones away from the signaling domains of these pathways. This result suggests that Cdc37 may normally destabilize the effectors to function

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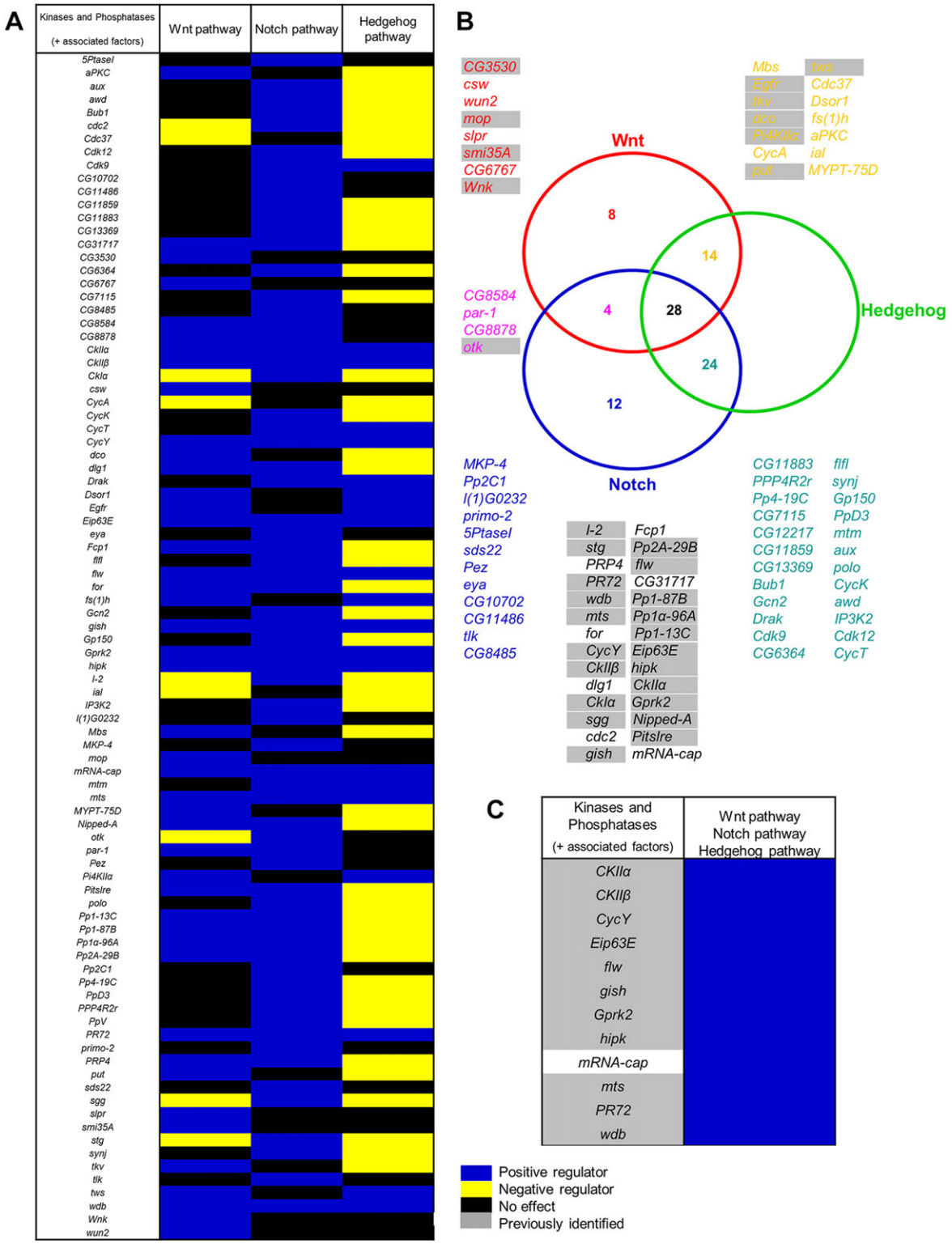

Fig. 6. Specific and shared phospho-regulators of the Wnt, Notch and Hh pathways in *Drosophila*. (A) Graphical summary that displays whether a regulator promotes (blue), inhibits (yellow) or has no effect (black) on the Wnt, Notch and Hh pathways in the wing disc. (B) Venn diagram showing specific or shared regulators of the Wnt, Notch and Hh pathways. Previously known regulators of the Wnt pathway are indicated with gray shading. (C) Twelve of the 90 candidates of the Wnt pathway identified also had the same effect on the Notch and Hh pathways in the wing disc.

as a negative regulator of signaling in the silent and active states of the Wnt and Hh pathways. Indeed, when we assayed for levels of stabilized Armadillo (Arm) (*Drosophila* β-catenin), we found more in wing discs reduced for *cdc37* function compared with wild type (Fig. 7I). Although *cdc37* RNAi did not significantly affect *ptc* expression, it did result in increased expression of another target gene of the Hh pathway, *dpp-lacZ* (supplementary material Fig. S9A). Although loss of *cdc37* clearly stabilizes Ci, studies suggest that there

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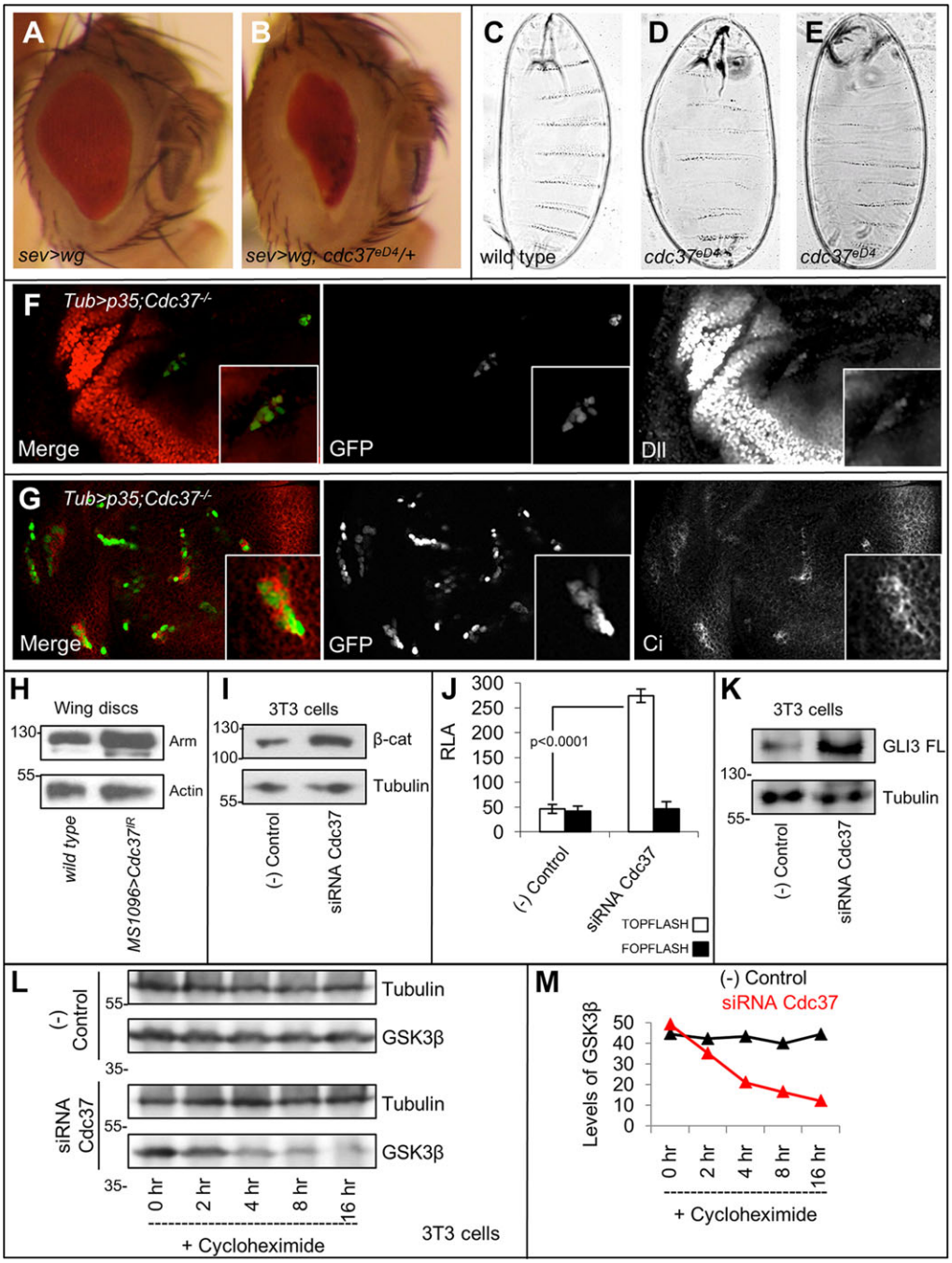

Fig. 7. Cdc37 destabilizes the effectors of the Wnt and Hh pathways to inhibit signaling. (A,B) Expression of *wg* from the *sev* promoter (*sev>wg*) results in a small rough eye, which is enhanced upon heterozygosity for the $cdc37^{eD4}$ allele. (C-E) Homozygous mutant $cdc37^{eD4}$ embryos display cuticle defects reminiscent of *wg* gain-of-function defects, including loss of denticle belts. (F,G) MARCM clones (with *p35* transgene) of $cdc37^{eD4}$ (marked by nuclear GFP) in the wing disc resulted in an increase in Dll (F) and Ci (G) levels. (H) Protein lysate from wing discs reduced for *Cdc37* function had elevated levels of stabilized Arm compared with wild-type discs. (I) Compared with the mock-transfected control, siRNA-mediated knockdown of *Cdc37* in 3T3 cells enhanced the levels of stabilized β-catenin. (J) Increased Wnt pathway activity is observed following siRNA-mediated knockdown of *Cdc37*, as measured from the TOPFLASH reporter but not FOPFLASH (negative control) (RLA=Relative Luciferase Activity). Student's *t*-test was performed and s.d. was calculated. (K) siRNA-mediated knockdown of *Cdc37* in 3T3 cells enhanced the levels of full-length GLI3 compared with the mock-transfected control. (L,M) siRNA-mediated knockdown of *Cdc37* in cycloheximide-treated 3T3 cells displayed progressively lower levels of GSK3β over the course of 16 h, compared with the mock-transfected control, as seen in western blot (L) and following densitometry (M). The levels of GSK3β were normalized to the loading control. Molecular weights are indicated in kDa next to each blot.

is an additional step that regulates Ci import into nucleus. We propose that only a small amount of Ci enters the nucleus in *cdc37* clones, which may explain why there are no apparent changes in *ptc* (which requires high-level Hh for expression), although we do observe a change in *dpp*, which is a low level target. Knockdown of *cdc37* caused increased cell proliferation in discs, as indicated by increased levels of phospho-Histone H3 (PH3; supplementary material Fig. S9B), but this proliferation cannot solely explain the expression of targets in regions outside the normal domain of signaling. Thus, these results indicate that loss of *cdc37* enhances both Wg and Hh signaling outputs.

To examine whether the function of Cdc37 is evolutionarily conserved, we carried out biochemical studies in mammalian cells. Knockdown of *Cdc37* with siRNA in 3T3 cells strongly enhanced levels of stabilized β-catenin (Fig. 7J). Furthermore, knockdown of *Cdc37* also induced signaling in unstimulated 3T3 cells, as measured using the Wnt pathway-specific TOPFLASH reporter, compared with mock-transfected 3T3 cells (Fig. 7K). Knockdown of *Cdc37* also enhanced the levels of full-length GLI3 (Fig. 7L). We were unable to detect the truncated form of GLI3 in our assay. These effects of Cdc37 in mammalian cells mimic those of negative regulators of the Wnt and Hh pathways, such as GSK3β and CK1α. As Cdc37 is a kinase-associated chaperone, we propose that it functions to promote the stability of GSK3β and/or CK1α, which both constitutively destabilize the effectors of the Wnt and Hh pathways. Accordingly, cycloheximide-treated 3T3 cells reduced for *Cdc37* displayed progressively lower levels of GSK3β due to its shorter half-life, compared with mock-transfected cells (Fig. 7M,N). We did not

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evaluate whether Cdc37 regulates the stability of CK1α in our assay. Thus, our analyses suggest that Cdc37 has a novel evolutionarily conserved function from *Drosophila* to mammalian cells to promote the stability of GSK3β and inhibit both the Wnt and Hh pathways.

Gish/CK1γ promotes the Hh pathway by phosphorylating Smo

Gish (*Drosophila* CK1γ) is a plasma membrane-associated kinase that has been described to promote the Wnt pathway by phosphorylating the co-receptor LRP (Davidson et al., 2005; Zhang et al., 2006). Consistently, it was recovered as a high-confidence Wnt regulator (Fig. 2L,O). Thus far, no role has been ascribed to this kinase in the regulation of the Hh pathway. Although knockdown of *gish* decreased the expression of targets of the Wnt, Notch and Hh pathways in the wing disc (Fig. 6A), the levels of Delta (Dl) and cleaved-Caspase 3 (Casp 3) were unaffected (supplementary material Fig. S9C). Thus, *gish* does not have a non-specific effect on gene transcription or cell death. Compared with wild type, a *gish* mutant wing disc displayed lower levels of Ci (Fig. 8A). Hh signaling stabilizes Ci to regulate expression of *dpp-lacZ* within the morphogenetic furrow (MF) of the eye disc (Fig. 8B). A somatic clone of *gish* in the eye disc had decreased levels of Ci and correspondingly *dpp-lacZ* expression (Fig. 8C), thereby confirming its role as a positive regulator of Hh signaling across multiple tissues.

We propose that Gish/CK1γ regulates the phosphorylation of the transmembrane protein Smo to promote the Hh pathway, analogous to its role in regulating the phosphorylation of LRP in the Wnt pathway. We tested this hypothesis using protein lysates from wing discs in a gel mobility shift assay (Fig. 8D). Knockdown of *gish* throughout the wing blade using *71B-Gal4* reduced the phosphorylation of Smo in disc protein lysates (as detected by a faster migrating band). As Smo phosphorylation is a prerequisite to its accumulation, knockdown of *gish* also decreased Smo levels. Conversely, overexpression of the Hh ligand, *hh-N*, expectedly enhanced the phosphorylation (as detected by a slower migrating band in lane 3) and levels of Smo (Fig. 8D). The simultaneous knockdown of *gish* in the presence of ectopic *hh-N* reduced the phosphorylation (migration) and levels of Smo, compared with ectopic *hh-N* alone (determined by densitometry), thus indicating that Gish acts downstream of the Hh ligand.

To determine whether the effect of Gish on the Hh pathway is evolutionarily conserved, the effect of CK1γ on signaling was assessed in mammalian cells. 3T3 cells transfected with CK1γ displayed increased levels of full-length GLI3, compared with mock-transfected cells (Fig. 8E). When exogenous CK1γ and Smo were co-transfected into 3T3 cells, they were detected in a complex in a co-immunoprecipitation assay (Fig. 8F). Furthermore, CK1γ robustly phosphorylated Smo at one or more serine and/or threonine residues (as detected by phospho-specific antibodies) in the presence of ATP in an *in vitro* kinase assay (Fig. 8G). Thus, Gish/CK1γ regulates the phosphorylation of Smo to promote the Hh pathway in *Drosophila* and mammalian cells.

DISCUSSION

Divergent disease states have been attributed to be a cause or consequence of aberrant protein phosphorylation (Reiter et al.,

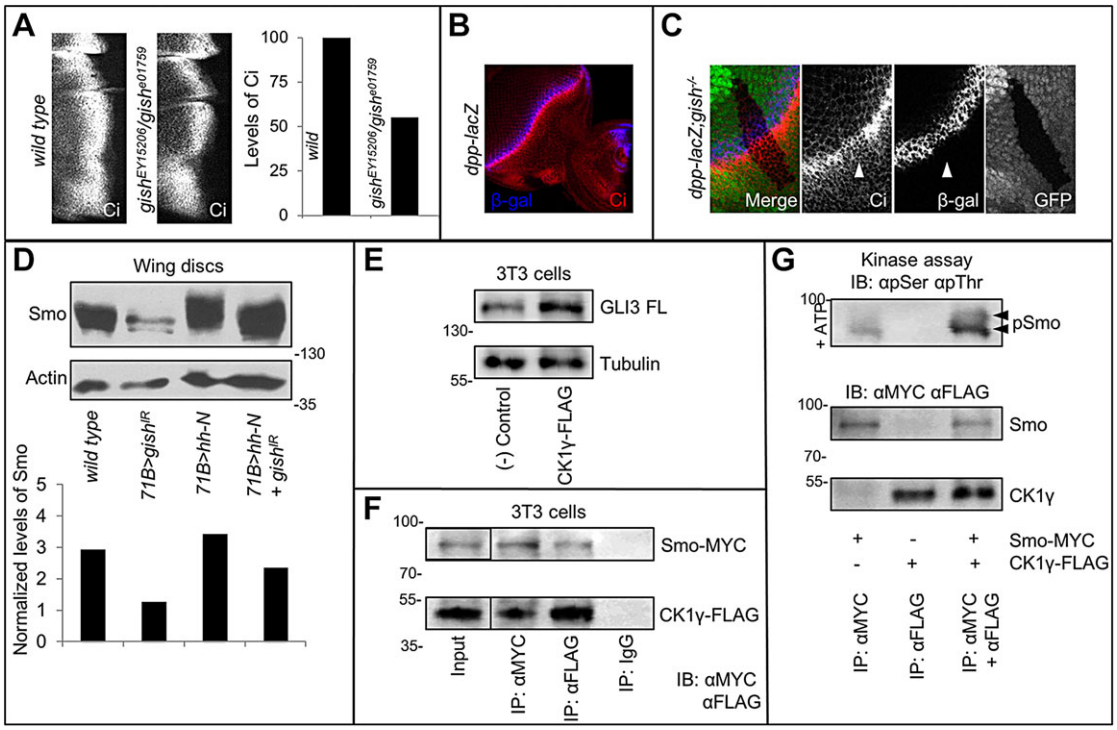

Fig. 8. Gish/CK1γ promotes the Hh pathway by phosphorylating Smo. (A) Ci protein levels are reduced in a *gish* mutant wing disc compared with wild type. (B) The Hh pathway stabilizes Ci to promote *dpp-lacZ* expression within the MF of the eye disc. (C) A *gish* somatic clone (GFP negative) in the eye disc had decreased levels of Ci and *dpp-lacZ* (detected by anti-β-gal; arrowheads). (D) Knockdown of *gish* using *71B-Gal4* in wing discs reduced the phosphorylation (detected by mobility shift) and levels of Smo, compared with wild type. Expression of *hh-N* with *71B-Gal4* enhanced the phosphorylation (as detected by mobility shift) and levels of Smo. The simultaneous knockdown of *gish* in the presence of ectopic *hh-N* reduced the phosphorylation (as detected by mobility shift) and levels of Smo. (E) Overexpression of CK1γ in 3T3 cells resulted in increased levels of full-length GLI3 compared with the mock-transfected control. (F) CK1γ co-precipitated with Smo when transfected into 3T3 cells. Epitope-tagged exogenous proteins were precipitated with corresponding antibodies or with IgG (negative control). (G) Smo was phosphorylated by CK1γ in an *in vitro* kinase assay, as detected by antibodies against phosphorylated serine and threonine residues. The loading control had equal amounts of immunoprecipitated proteins. Molecular weights are indicated in kDa next to each blot.

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2001). Wnt signaling is phosphor-regulated both in its silent and active states, but thus far our understanding of kinases, phosphatases and associated factors of the pathway has been limited. In this study, we performed the first genome-wide *in vivo* screen under physiological conditions in the *Drosophila* wing disc for phospho-regulators of the Wnt pathway. We identified 54 high-confidence regulators, 22 of which are novel. The results of our analyses do not indicate whether a high-confidence regulator has a direct or indirect effect on signaling. However, as ∼60% of the high-confidence regulators identified have been previously validated to have a direct effect on Wnt signaling, we predict that at least some of the novel high-confidence regulators identified would also have a direct effect on the pathway. Indeed, subsequent analyses of Myopic revealed a novel role in regulating Wg secretion (Pradhan-Sundd and Verheyen, 2014). Although the mechanism and components of the Wnt pathway are for the most part conserved between *Drosophila* and humans, there are possibly vertebrate-specific phospho-regulators of signaling that would not have been identified in our analyses. Our dataset represents the largest list of putative phospho-regulators of the Wnt pathway identified to date, almost all of which have identified human orthologs (supplementary material Figs S1, S2) and are therefore likely to be functionally conserved.

As part of this study, we also established previously unknown relationships between the Wnt and Hh pathways *in vivo* by identifying 12 novel dual regulators that we propose function at analogous levels of signaling (Fig. 5B). As proof of concept, we biochemically characterized the roles of Cdc37 and Gish/CK1γ to demonstrate that their functions are conserved from *Drosophila* to mammalian cells. We also describe an initial analysis of candidate regulators of Notch signaling during wing disc development. Although these findings are preliminary, they highlight an emerging theme of phospho-regulation of Notch that likely hold parallels in vertebrate biology. The comparison of signaling pathways *in vivo* and the identification of specific versus shared phospho-regulators facilitate our understanding of human development and disease states.

MATERIALS AND METHODS

Drosophila genetics

The following *Drosophila* strains were used: w^{1118} (wild type), *dpp-Gal4/TM6B*, *C5-Gal4*, *MS1096-Gal4*, *omb-Gal4*, *71B-Gal4*, *UAS-flp*, *UAS-dicer-2*, *UAS-p35*, *dpp-lacZ/CyO*, $Cdc37^{eD4}$ *FRT79/TM6B*, *FRT82,GFP/TM6B*, *MARCM79*, *hs-flp;;FRT82,GFP/TM6B* and $gish^{EY15206}$ (Bloomington *Drosophila* Stock Center); *hh-Gal4/TM6B* and *eyFlp;ey-Gal4,GMR-Gal4; sev>y+>wg* (Port et al., 2011); *UAS-hhN* (Su et al., 2011); and *FRT82* $gish^{e01759}$*/TM6B* (Gault et al., 2012). The transgenic RNAi strains used for the screens were obtained from the Vienna Drosophila RNAi Center (Dietzl et al., 2007), National Institute of Genetics and Harvard Transgenic RNAi Project (supplementary material Fig. S4, Fig. 5). The percentage of inverted repeats with predicted sequence-dependent off-target effects are as follows: 65% (0), 27% (1-2) and 8% (>2). All genes (at least two independent RNAi lines per gene) were first tested with the *dpp-Gal4* driver. If a phenotype was only observed with one RNAi line for *dpp-Gal4* or with two lines targeting the same region of the mRNA, the gene was re-tested with *hh-Gal4*. The one or more RNAi lines that were re-tested with *hh-Gal4* were either the same lines that were used with *dpp-Gal4* or additional lines (if new ones were available). If a RNAi line displayed a phenotype with both the *dpp-Gal4* and *hh-Gal4* drivers in the primary screen, the *hh-Gal4 driver* was used in the secondary screen. Wing discs from 20 larvae of each genotype were immunostained (30 larvae for genotypes that were non-homozygous with a balancer chromosome). Knockdown of no gene precluded analysis due to lethality before the third instar stage. Twenty flies of each genotype were scored for adult phenotypes. The penetrance of all phenotypes indicated is between 80 and 100%. False-positive results due to cell death, cell proliferation or non-specific gene transcription were evaluated through testing the ability of candidate regulators to affect different signaling pathways in the wing disc. *cdc37* MARCM clones were generated by crossing the *UAS-p35;* $Cdc37^{eD4}$ *FRT79/TM6B* and *MARCM79* strains, and progeny were heat-shocked 48 h after egg laying (AEL) for 2 h at 38°C. *gish* somatic clones in the wing disc were generated by crossing the *UAS-flp;* *FRT82* $gish^{e01759}$*/TM6B* and *omb-Gal4;;FRT82,GFP/TM6B* strains. *gish* somatic clones in the eye disc were generated by crossing the *hs-flp;;FRT82, GFP/TM6B* and *FRT82* $gish^{e01759}$*/TM6B* strains, and progeny were heat-shocked 48 h AEL for 2 h at 38°C.

Immunostaining of wing discs

Drosophila wing and eye discs from third instar larvae were dissected, immunostained and mounted according to standard procedures (Swarup and Verheyen, 2011). The following primary antibodies were used: anti-Ci 2A1 (1:50), anti-Ptc (1:50), anti-Wg 4D4 (1:100), anti-Ct 2B10 (1:75), anti-Delta C594.9B (1:50) (Developmental Studies Hybridoma Bank), anti-Phospho Histone H3 (1:100), anti-β-galactosidase (1:1500), anti-Cleaved Caspase 3 (1:100) (Cell Signaling Technology), anti-Sens (1:1000) (Nolo et al., 2000) and anti-Dll (1:400) (Duncan et al., 1998; Panganiban et al., 1995). Fluorescent secondary antibodies (1:400) were from Jackson Immunolabs. Wing disc images were obtained on a Nikon A1R laser scanning confocal microscope and all images are derived from stacked *z*-series. A fixed-size box (width=width of stabilized Ci domain in wild type, height=bottom of disc to top of wing pouch) was used to compare the integrated density of wild type and *gish* mutant. For quantification of immunostained discs, integrated density (pixel area multiplied by baseline subtracted intensity) was calculated.

Cell culture

NIH-3T3 cells (American Type Culture Collection) were cultured at 37°C in DMEM supplemented with 10% FBS (Invitrogen). 3T3 cells were transfected with CK1γ-FLAG (Davidson et al., 2005), Smo-MYC (Chen et al., 2011), TOPFLASH, FOPFLASH, or Renilla luciferase (Ishitani et al., 2003) using Polyfect Reagent (Qiagen) and harvested 36-48 h post-transfection. Knockdown of Cdc37 was performed with siRNA and Lipofectamine RNAiMAX Reagent (Invitrogen). As a negative control, cells were mock-transfected with empty vector. Cells were treated with cycloheximide (25 µg/ml) (Sigma) 24 h after transfection with Cdc37 siRNA [a mixture of two distinct siRNAs targeted to different parts of the genes from Ambion (136227 and 136228)] and harvested at the indicated time points.

Biochemical assays

Protein lysates were prepared from 3T3 cells and wing discs. Proteins of interest were immunoprecipitated from lysates using antibodies and Protein-G Sepharose beads (Sigma). The kinase assay with non-radiolabeled ATP/kinase assay buffer (Cell Signaling Technology) was performed with immunoprecipitated proteins at 30°C for 30 min. The de-phosphorylation assay was performed on protein lysate using lambda protein phosphatase at 30°C for 1 h (New England Biolabs).

Western blot analyses

Western blot analyses were performed using the following primary antibodies: anti-Tubulin (1:1000), anti-Myc (1:1000), anti-FLAG (1:1000) (Sigma), anti-GSK3β (1:500) (Abcam), anti-GLI3 N19 (1:200) (Santa Cruz Biotechnology), anti-Phosphoserine (1:500), anti-Phosphothreonine (1:500) (Sigma), anti-Smo 20C6 (1:10) and anti-Armadillo N27A1 (1:200) (Developmental Studies Hybridoma Bank). For quantification of western blot bands, integrated density (pixel area multiplied by baseline subtracted intensity) of each band was calculated within an appropriate exposure range and then normalized to the loading control.

Transcriptional assays

Transcriptional assays were performed using TOPFLASH, FOPFLASH and Renilla (control) luciferase reporter plasmids. The experiment was performed using the Dual Luciferase Reporter Assay System (Promega).

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The value for each data point is the average of three individual experiments. The s.d. was calculated and Student's *t*-test was performed for statistical significance.

Acknowledgements
We are grateful to the numerous people who provided fly strains, plasmids and antibodies, especially Hugo Bellen, Ian Duncan, and Sean Carroll. We are also grateful to the RNAi stock centers (Vienna Drosophila RNAi Center, National Institute of Genetics and Harvard Transgenic RNAi Project) for generating the strains used in this study. Thanks to past and present members of the Verheyen lab for discussions, as well as to Hans Clevers, Roel Nusse and Nick Harden for comments on the study.

Competing interests
The authors declare no competing or financial interests.

Author contributions
S.S., E.M.V. designed the experiments. S.S., T.P.-S. performed the experiments. S.S., T.P.-S., E.M.V. analyzed the data. S.S., E.M.V. wrote the manuscript.

Funding
This work was supported by an operating grant from the Canadian Institutes of Health Research.

Supplementary material
Supplementary material available online at
http://dev.biologists.org/lookup/suppl/doi:10.1242/dev.116715/-/DC1

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FIGURE LEGENDS

Fig. S1 Kinases and kinase-associated factors assayed in screen

All genes (sorted by CG number) encoding either kinases or associated factors for which UAS-controlled inverted repeat strains were tested. Kinase group and family is indicated, as well as human orthologs.

Fig. S2 Phosphatases and Phosphatase-associated factors assayed in screen

All genes (sorted by CG number) encoding either phosphatase or associated factors for which UAS-controlled inverted repeat strains were tested. Phosphatase group and family is indicated, as well as human orthologs.

Fig. S3 Wnt pathway pilot screen in the Drosophila wing disc.

Graphical summary of the Wnt pathway pilot screen in the wing disc that displays whether knock-down of pathway components decreased (green), increased (red), or had no effect (black) on the levels of the ligand and downstream targets. A subset of known components of the Wnt pathway were knocked-down using *dpp-Gal4* and *hh-Gal4* in the wing disc to evaluate their effects on the levels of Sens, Dll, and Wg. Knock-down of *lacZ* (negative control) did not affect the levels of Sens, Dll, or Wg. Knock-down of the positive regulator *porcupine (por)* resulted in a decrease of Sens and Dll levels (arrowheads) in the ligand-receiving cells due to the impaired secretion of Wg (arrow) (detected as an increase in levels) from the ligand-producing cells. Knock-down of the negative regulator *Axn* resulted in increased levels of Sens and Dll (arrowheads) in the ligand-receiving cells, but had no effect on Wg levels. Knock-down of the ligand *wg* resulted in the loss of Wg (arrow) in the ligand-producing cells and a corresponding loss of Sens and Dll (arrowheads) in the ligand-receiving cells.

Fig. S4 Results of primary and secondary screening of kinome

Results obtained with indicated RNAi lines in crosses indicated in top line of table, under primary screen and secondary screen headings. Blank infill indicates that there was no change in targets in primary screen. Black infill means there was no change observed in in that particular assay although there was an effect either with another readout or another RNAi line corresponding to the same gene. Coloured infill represents changes to readouts as indicated in legend. Genes that were tested in secondary screen are highlighted by lines above and below the gene. Adult phenotypes (if observed) are indicated. Any indicated lethality occurred after third instar, thus allowing larval discs to be examined. Transgenic RNAi Project strains are in blue font, while National Institute of Genetics (NIG) strains have the letter R in the stock number. All other strains are from Vienna Drosophila RNAi Center (VDRC).

Fig. S5 Results of primary and secondary screening of phosphatome

Results obtained with indicated RNAi lines in crosses indicated in top line of table, under primary screen and secondary screen headings. Blank infill indicates that there was no change in targets in primary screen. Black infill means there was no change observed in in that particular assay although there was an effect either with another readout or another RNAi line corresponding to the same gene. Coloured infill represents changes to readouts as indicated in legend. Genes that were tested in secondary screen are highlighted by lines above and below the gene. Adult phenotypes (if observed) are indicated. Any indicated lethality occurred after third instar, thus allowing larval discs to be examined. Transgenic RNAi Project strains are in blue font, while National Institute of Genetics (NIG) strains have the letter R in the stock number. All other strains are from Vienna Drosophila RNAi Center (VDRC).

Fig. S6 Signaling at the dorsal/ventral compartment boundary of the *Drosophila* wing disc. The Notch pathway signals from non-boundary cells to induce expression of the target genes *wg* (red) and *ct* (orange) in cells at the dorsal/ventral compartment boundary of the wing disc. Wg that is subsequently produced from cells at the dorsal/ventral compartment boundary induces the nested expression of the target genes *sens* (blue) and *Dll* (grey) in non-boundary cells that receive the ligand. The expression domains of *dpp-Gal4*, *hh-Gal4*, and *C5-Gal4* in the wing disc relative to boundary and non-boundary cells are shown in green.

Fig. S7 Identification of phospho-regulators of the Notch pathway.
(A) 36 kinases (and associated factors) [left column] and 32 phosphatases (and associated factors) [right column] of the 590 phospho-regulators screened in the wing disc were shown to regulate the Notch pathway and the levels of Wg and Ct. (B) All 68 phospho-regulators identified positively (blue) regulate the Notch pathway. (C) The kinase and phosphatase groups are displayed in a graphical summary, and family names are as defined in Figs. S1 and S2.

Fig.S8 Identification of phospho-regulators of the Hh pathway.
(A) *MS1096-Gal4* is expressed throughout the central region of the wing disc, but displays stronger expression in the dorsal half. Knock-down of *lacZ* (negative control) using *MS109-Gal4* did not affect Ci or Ptc levels. (B) 66 of the 90 candidates of the Wnt pathway also regulated the Hh pathway to either promote (21; blue) or inhibit (45; yellow) signaling in the

wing disc. 24 candidates of the Wnt pathway did not affect (black) the Hh pathway in the wing disc.

Fig.S9 Effects of *cdc37* and *gish* in the wing disc

(A) Relative to the knock-down of *lacZ* (negative control), knock-down of *Cdc37* with *MS1096-Gal4* in the wing disc resulted in increased levels of expression of the Hh pathway target *dpp-lacZ* (arrowheads) as detected by anti-β-gal. (B) The *dpp-Gal4* driver is expressed along the anterior/posterior compartment boundary of the wing disc (green). Knock-down of *cdc37* with *dpp-Gal4* increases the levels of PH3 (white). (C) The *hh-Gal4* driver is expressed in the posterior compartment of the wing disc (green). Knock-down of *gish* with *hh-Gal4* did not affect Dl (red) or Casp 3 levels (white).

Kinases and Associated factors

Fig S1

Classifications of kinases and associated factors

AGC	Named after Protein Kinase A, G, and C families
Tyrosine Kinase-Like	
STE	Homologs of the yeast STE7, STE11 and STE20 genes
Casein Kinase 1	
Protein Kinase-Like	
CMGC	Named after a set of families (CDK, MAPK, GSK3 and CLK)
Tyrosine Kinase	
Receptor Guanylate Cyclase	
Calcium & Calmodulin-regulated Kinase	
Atypical	
Other	
Unclassified	Diverse group of kinases and candidate kinases
Kinase-associated	Co-factors such as cyclins
Non-protein Kinase	Such as lipid and sugar kinases

Kinase Gene			Gene Group	Gene Family	Human ortholog(s)
Annotation Symbol	Gene Symbol	Gene Name			
CG10023	Fak56D	Focal adhesion kinase	TK	FAK	PTK2, PTK2B
CG10033	for	foraging	AGC	PKG	PRKG1, PRKG2
CG10079	Egfr	Epidermal growth factor receptor	TK	EGFR	ERBB2, ERBB4, EGFR, ERBB3
CG10082			Non-Protein Kinase	Lipid Kinase	
CG10155	Spred	Sprouty-related protein with EVH-1 domain	Kinase-associated		SPRED3, SPRED2, SPRED1
CG10177			CAMK	DCAMKL	
CG10244	Cad96Ca	Cadherin 96Ca	TK		
CG10260			PKL		PI4KA
CG10268			Unclassified		PMVK
CG10295	Pak	PAK-kinase	STE	STE20	PAK3, PAK2, PAK1
CG10308	CycJ	Cyclin J	CMGC	CDK	
CG10498	cdc2c	cdc2c	CMGC	CDK	CDK1, CDK2, CDK3
CG10504	Ilk	Integrin linked kinase	TKL	MLK	ILK
CG10522	sticky		AGC	DMPK	CIT
CG10539	S6k	RPS6-p70-protein kinase	AGC	RSK	RPS6KB1
CG10572	Cdk8	Cyclin-dependent kinase 8	CMGC	CDK	CDK8, CDK19
CG10579	Eip63E	Ecdysone-induced protein 63E	CMGC	CDK	CDK16, CDK14, CDK18
CG10637	Nak	Numb-associated kinase	Other	NAK	BMP2KL, BMP2K, AAK1
CG10673			Other	Bud32	TP53RK
CG10702			Kinase-associated		
CG10738			RGC	RGC	NPR2, NPR1
CG10776	wit	wishful thinking	TKL	STKR	BMPR2, ACVR2A, TGFBR2, ACVR2B
CG10895	lok	loki	CAMK	RAD53	CHEK2
CG10933			Kinase-associated		
CG10951	niki	nimA-like kinase	Other	NEK	
CG10967	Atg1	Autophagy-specific gene 1	Other	ULK	

Kinases and Associated factors

CG1098	Madm	MLF1-adaptor molecule	Other	NRBP	NRBP1, NRBP2
CG1107	aux	auxillin	Other	NAK	DNAJC6, GAK
CG11221			Other	NKF1	
CG11228	hpo	hippo	STE	STE20	STK3, STK4
CG11249			Non-Protein Kinase	Carbohydrate Kinase	Pyruvate Kinase
CG11255			Non-Protein Kinase	Carbohydrate Kinase	ADK
CG11420	png	pan gu	Other		
CG11486			Kinase-associated		PAN3
CG11489	srpk79D	serine-arginine protein kinase at 79D	CMGC	SRPK	SRPK1, SRPK3, SRPK2
CG11525	CycG	Cyclin G	CMGC	CDK	
CG11533	Asator	Asator	CK1	TTBK	TTBK1, TTBK2
CG11594			Non-Protein Kinase	Carbohydrate Kinase	FGGY
CG11621	Pi3K68D	Phosphotidylinositol 3 kinase 68D	Non-Protein Kinase	Lipid Kinase	PIK3C2G, PIK3C2A, PIK3C2B
CG11660			Atypical	RIO	RIOK3, RIOK1
CG11811			Non-Protein Kinase	Nucleic Acid Kinase	GUK1
CG11859			Atypical	RIO	RIOK2
CG12019	Cdc37	Cdc37	Kinase-associated		CDC37
CG12066	Pka-C2	cAMP-dependent protein kinase 2	AGC	PKA	PRKACB
CG12069			AGC	PKA	
CG12072	warts	warts	AGC	NDR	LATS2, LATS1
CG1210	Pdk1	Phosphoinositide-dependent kinase 1	AGC	PDK1	PDPK1, PDPK2
CG12147			CK1	CK1	
CG1216	mri	mrityu	Kinase-associated		KCTD20, BTBD10
CG12229			Non-Protein Kinase	Carbohydrate Kinase	Pyruvate Kinase
CG12244	lic	licorne	STE	STE7	MAP2K4, MAP2K6, MAP2K3
CG1227			Other	NAK	STK16
CG12289			Non-Protein Kinase	Carbohydrate Kinase	KHK
CG12306	polo	polo	Other	PLK	PLK3, PLK2, PLK1
CG12559	rl	rolled	CMGC	MAPK	MAPK3, MAPK1
CG1271			Non-Protein Kinase	Lipid Kinase	GK5
CG13369			Non-Protein Kinase	Carbohydrate Kinase	RBKS
CG13388	Akap200	A kinase anchor protein 200	Kinase-associated		UNC79
CG1344			Other	SCY1	SCYL3
CG13591	Ssl	Suppressor of Stellate-like	CMGC		CSNK2B
CG1362	cdc2rk	cdc2-related-kinase	CMGC	CDK	CDC2L2
CG13688	Ipk2	Ipk2	PKL	IPK	IPMK
CG1389	tor	torso	Atypical	PIKK	
CG14026	tkv	thickveins	TKL	STKR	ACVR1, BMPR1A, ACVR1C, BMPR1B, ACVRL1, ACVR1B
CG14030	Bub1	Bub1 homologue	Other	BUB	BUB1
CG14217	Tao	Tao	STE	STE20	TAOK1, TAOK2, TAOK3
CG14305			CAMK	TSSK	TSSK6, TSSK2, TSSK4, TSSK3, TSSK1

Kinases and Associated factors

CG14396	Ret	Ret oncogene	TK	Ret	FGFR4, FGFR1, FGFR3, RET, FGFR2
CG14895	Pak3	Pak3	STE	STE20	PAK3, PAK1, PAK2
CG14939	CycY	Cyclin Y	CMGC	CDK	CCNYL2, CCNY, CCNYL1, CCNYL3
CG1495	CaMK1	Calcium/calmodulin-dependent protein kinase I	CAMK	CAMK1	PSKH2, CAMK4, CAMK1D, CAMK1D. PSKH1. PNCK. CAMK1
CG14992	Ack	Activated Cdc42 kinase	TK	Ack	TNK2, TNK1
CG1511	Eph	Eph receptor tyrosine kinase	TK	Eph	EPHB6, EPHB3, EPHA1, EPHA10, EPHA4. EPHB2. EPHA8. EPHA2.
CG15218	CycK	Cyclin K	CMGC	CDK	CCNK
CG15224	CkIIβ	Casein kinase II β subunit	CMGC	CK2	CSNK2B
CG15547			Non-Protein Kinase	Nucleic Acid Kinase	
CG15793	Dsor1	Downstream of raf1	STE	STE7	MAP2K1, MAP2K2
CG15862	Pka-R2	cAMP-dependent protein kinase R2	AGC	PKA	PRKAR2A, PRKAR2B
CG1594	hop	hopscotch	TK	Jak	
CG1609	Gcn2	Gcn2	Other	PEK	EIFA2K4
CG16708	Cerk	Ceramide kinase	Non-Protein Kinase	Lipid Kinase	CERK
CG16903			Kinase-associated		CCNL1, CCNL2
CG16910	key	kenny	Kinase-associated		
CG16973	msn	misshapen	STE	STE20	MINK1, MAP4K4, TNIK
CG17010			Non-Protein Kinase	Carbohydrate Kinase	RBKS
CG17090	hipk	homeodomain interacting protein kinase	CMGC	DYRK	HIPK1, HIPK2, HIPK3
CG17146	Adk1	Adenylate kinase-1	Non-Protein Kinase	Nucleic Acid Kinase	AK1, AK5
CG17161	grp	grapes	CAMK	CAMKL	CHEK1
CG17216	KP78b	KP78b	CAMK	CAMKL	MARK2, MARK4, MARK3, MARK1
CG17245	plexB	plexin B	Kinase-associated		PLXNA3, PLXNB1, PLXNA1, PLXND1, PLXNA4, PLXNB3, PLXNB2, PLXNA2
CG1725	dlg1	discs large 1	Kinase-associated		DLG3, DLG2, DLG4, DLG1
CG17256	Nek2	Nek2	Other	NEK	NEK7, NEK2, NEK6
CG17299	SNF4Aγ	SNF4/AMP-activated protein kinase gamma subunit	PKL	PIPK	PRKAG1, PRKAG2, PRKAG3
CG17342	Lk6	Lk6	CAMK	MAPKAPK	MKNK2
CG17348	drl	derailed	TK	Ryk	RYK, TYRO, AXL, MERTK
CG1747	Sk1	Sphingosine kinase 1	Non-Protein Kinase	Lipid Kinase	SPHK1, SPHK2
CG17471			PKL	PIPK	PIP4K2B, PIP4K2C, PIP4K2A
CG17520	CkIIα	casein kinase IIα	CMGC	CK2	CSNK2A1, CSNK2A2
CG17528			CAMK	DCAMKL	DCX, DCLK3, DCLK1, DCLK2
CG17559	dnt	doughnut on 2	TK	Ryk	RYK, TYRO3, AXL, MERTK
CG17596	S6kII	Ribosomal protein S6 kinase II	AGC	RSK	RPS6KA6, RPS6KA5, RPS6KA4, RPS6KA3. RPS6KA2. RPS6KA1
CG17603	Taf1	TBP-associated factor 1	Atypical	TAF1	TAF1L, TAF1
CG17698			Other	CAMKK	CAMKK1, CAMKK2
CG1772	dap	dacapo	Kinase-associated		CDKN1B
CG17998	Gprk2	G protein-coupled receptor kinase 2	AGC	GRK	GRK4, GRK5, GRK6
CG18069	CaMKII	Calcium/calmodulin-dependent protein kinase II	CAMK	CAMK2	CAMK2A, CAMK2D, CAMK2B, CAMK2G
CG18085	sev	sevenless	TK	Sev	ROS1

Kinases and Associated factors

CG18247	shark	SH2 ankyrin repeat kinase	TK	Syk	
CG18255	Strn-Mlck	Stretchin-Mlck	CAMK	MLCK	MYLK4, MYLK2, MYLK, MYLK3
CG1830	PhKγ	Phosphorylase kinase γ	CAMK	PHK	PHKG2
CG18374	Gyk	Glycerol kinase	Non-Protein Kinase	Lipid Kinase	GK, GK2
CG18402	InR	Insulin-like receptor	TK	InsR	IGF1R
CG1848	LIMK1	LIM-kinase1	TKL	LISK	LIMK1, LIMK2
CG18492	Tak1	TGF-β activated kinase 1	TKL	MLK	MAP3K7
CG1851	Ady43A	Ady43A	Non-Protein Kinase	Nucleic Acid Kinase	
CG18582	mbt	mushroom bodies tiny	STE	STE20	PAK6, PAK4, PAK7
CG18854			Non-Protein Kinase	Lipid Kinase	
CG1891	sax	saxophone	TKL	STKR	ACVR1, BMPR1A, ACVR1C, BMPR1B. ACVRL1
CG1915	sls	sallimus	Kinase-associated		TTN
CG1939	Dpck	Dephospho-CoA kinase	Unclassified		DCAKD
CG1951			Other	SCY1	SCYL2
CG1954	Pkc98E	Protein C kinase 98E	AGC	PKC	PRKCG, PRKCB, PRKCE, PRKCQ. PRKCA. PRKCH.
CG1973	yata	yata	Other	SCY1	SCYL1
CG2028	CkIα	Casein kinase Iα	CK1	CK1	CSNK1A1, CSNK1D, CSNK1A1L, CSNK1E
CG2048	dco	discs overgrown	CK1	CK1	CSNK1A1, CSNK1D, CSNK1A1L, CSNK1E
CG2049	Pkn	Protein kinase related to protein kinase N	AGC	PKN	PKN2, PKN3, PKN1
CG2087	PEK	pancreatic eIF-2α kinase	Other	PEK	EIF2AK3
CG2201			Non-Protein Kinase	Lipid Kinase	CHKB-CPT1B, CHKB
CG2210	awd	abnormal wing discs	Atypical	NDK	NME4, NME2, NME1, NME1-NME2, NME3
CG2246			Non-Protein Kinase	Carbohydrate Kinase	PRPSAP2, PRPSAP1
CG2252	fs(1)h	female sterile (1) homeotic	Atypical		BRD2, BRD3, BRDT, BRD4
CG2272	slpr	slipper	TKL	MLK	RP5-862P8.2, MLTK, MAP3K9, MAP3K10, MAP3K11
CG2577			CK1	CK1	CSNK1E, CSNK1A1, CSNK1A1L, CSNK1D
CG2615	ik2	IκB kinase-like 2	Other	IKK	IKBKE, TBK1
CG2621	sgg	shaggy	CMGC	GSK	GSK3B, GSK3A
CG2699	Pi3K21B	Pi3K21B	PKL	PIK	PIK3R3, PIK3R2, PIK3R1
CG2794			Non-Protein Kinase	Carbohydrate Kinase	
CG2845	phl	pole hole	TKL	RAF	ARAF, BRAF, RAF1
CG2846			Non-Protein Kinase	Carbohydrate Kinase	RFK
CG2899	ksr	kinase suppressor of ras	TKL	RAF	KSR2, KSR1
CG2929	Pi4KIIα	Pi4KIIα	PKL	PIK	PI4K2A, PI4K2B
CG2964			Non-Protein Kinase	Carbohydrate Kinase	PKLR, PKM
CG3001	Hex-A	Hexokinase A	Non-Protein Kinase	Carbohydrate Kinase	HK3, HK2, HKDC1, HK1
CG30021	metro	menage a trois	Non-Protein Kinase	Nucleic Acid Kinase	MPP4, MPP5, MPP7, MPP2, MPP1. MPP3
CG3008			Atypical	RIO	RIOK3, RIOK1
CG30184			Kinase-associated		
CG30295	Ipk1	Ipk1	PKL		IPPK
CG3051	SNF1A	SNF1A/AMP-activated protein kinase	CAMK	CAMKL	PRKAA1, PRKAA2
CG3068	aur	aurora	Other	Aur	AURKC, AURKB, AURKA

Kinases and Associated factors

CG3068	aur	aurora	Other	Aur	AURKC, AURKB, AURKA
CG3086	MAPk-Ak2	MAP kinase activated protein-kinase-2	CAMK	MAPKAPK	MAPKAPK2, MAPKAPK5, MAPKAPK3
CG31003	gskt	gasket	CMGC	GSK	GSK3B, GSK3A
CG3105	Pask	PAS kinase	CAMK	CAMKL	PASK
CG31097			Unclassified		
CG31127	Wsck	Wsck	TK		
CG31140			Non-Protein Kinase	Lipid Kinase	DGKQ
CG31183			Kinase-associated		NPR2, NPR1
CG3127	Pgk	Phosphoglycerate kinase	Non-Protein Kinase	Lipid Kinase	PGK1, PGK2
CG31349	pyd	polychaetoid	Kinase-associated		TJP2, TJP1, TJP3
CG3140	Adk2	Adenylate kinase-2	Non-Protein Kinase	Nucleic Acid Kinase	AK4, AK2, AK3
CG31421	Takl1	Tak1-like 1	TKL		MAP3K7
CG31643			Atypical		FASTKD1, TBRG4, FASTKD3, FASTK, FASTKD3
CG3172	twf	twinfilin	TK		TWF2, TWF1
CG31751			Unclassified		AGPHD1
CG31873			Non-Protein Kinase	Lipid Kinase	FLJ10842
CG32019	bt	bent	CAMK		MYLK4, MYLK2, MYLK3, MYLK
CG32031	Argk	Arginine kinase	Unclassified		CKMT2, CKMT1A, CKMT1B, CKM, CKB
CG32134	btl	breathless	TK		FGFR4, FGFR1, FGFR3, RET, FGFR2
CG3216			RGC	RGC	NPR2, NPR1
CG32417	Myt1	Myt1	AGC		PKMYT1
CG32484	Sk2	Sphingosine kinase 2	Non-Protein Kinase	Lipid Kinase	
CG32649			Atypical		ADCK4, ADCK3
CG32666	Drak	Death-associated protein kinase related	CAMK	MLCK	
CG32703			CMGC		MAPK15
CG32717	sdt	stardust	CAMK		CASK, MPP4, MPP5, MPP7, MPP2, MPP1, MPP3
CG32742	l(1)G0148	lethal (1) G0148	Other		CDC7
CG32743	nonC	no-on-and-no-off transient C	PKL		SMG1
CG3277			TK	VEGFR	
CG32849	Hex-t2	Hex-t2	Non-Protein Kinase	Carbohydrate Kinase	HK3, HK2, HKDC1, HK1
CG32944			CAMK		STK32C, STK32B, STK32A
CG33102	Hex-t1	Hex-t1	Non-Protein Kinase	Carbohydrate Kinase	
CG33114	Gyc32E	Guanyl cyclase at 32E	RGC		NPR2, NPR1
CG3319	Cdk7	Cyclin-dependent kinase 7	CMGC	CDK	CDK7, CDK20
CG3324	Pkg21D	cGMP-dependent protein kinase 21D	AGC	PKG	PRKG2, PRKG1
CG33338	p38c	p38c	CMGC	MAPK	MAPK12, MAPK13, MAPK14, MAPK11
CG33519	Unc-89	Unc-89	Unclassified		
CG33531	Ddr	Discoidin domain receptor	TK		DDR1, DDR2
CG33554	Nipped-A	Nipped-A	Atypical	PIKK	TRRAP
CG33671			Non-Protein Kinase	Carbohydrate Kinase	MVK
CG33981			Kinase-associated		ANAPC13
CG3400	Pfrx	6-phosphofructo-2-kinase	Non-Protein Kinase	Carbohydrate Kinase	PFKFB1, PFKFB2, PFKFB4, PFKFB3
CG34344	rdgA	retinal degeneration A	Non-Protein Kinase	Lipid Kinase	DGKZ, DGKI
CG34356			Unclassified		
CG34357			RGC		NPR2, NPR1
CG34359	IP3K2	Inositol 1,4,5-triphosphate kinase 2	Non-Protein Kinase	Lipid Kinase	ITPK1
CG34361	Dgk	Diacyl glycerol kinase	Non-Protein Kinase	Lipid Kinase	DGKA, DGKG, DGKB

Kinases and Associated factors

CG34380			TK		DDR1, DDR2
CG34384			Non-Protein Kinase	Lipid Kinase	DGKH, DGKD
CG34392	Epac	Exchange protein directly activated by cAMP ortholog	Kinase-associated		RAPGEF5, RAPGEF3, RAPGEF4, RAPGEFL1
CG34412	tlk	Tousled-like kinase	Other	TLK	TLK1, TLK2
CG3510	CycB	Cyclin B	CMGC	CDK	CCNB2, CCNB1
CG3525	eas	easily shocked	Non-Protein Kinase	Lipid Kinase	ETNK2, ETNK1
CG3534			Non-Protein Kinase	Carbohydrate Kinase	XYLB
CG3544			Non-Protein Kinase	Carbohydrate Kinase	XYLB
CG3608			Atypical	ABC1	ADCK1
CG3682	PIP5K59B	PIP5K59B	PKL		PIP5K1A, PIP5K1B, PSMD4, PIP5K1C
CG3738	Cks30A	Cyclin-dependent kinase subunit 30A	Kinase-associated		CKS2, CKS1B
CG3809			Non-Protein Kinase	Nucleic Acid Kinase	ADK
CG3837			Kinase-associated		
CG3915	Drl-2	Derailed 2	TK	Ryk	RYK, TYRO3, AXL, MERTK
CG3938	CycE	Cyclin E	CMGC	CDK	CCNE2, CCNE1
CG4006	Akt1	Akt1	AGC	Akt	SGK3, AKT1, SGK1, AKT2, SGK2, AKT3
CG4007	Nrk	Neurospecific receptor kinase	TK	Musk	MUSK, ROR1, ROR2
CG4012	gek	genghis khan	AGC	DMPK	CDC42BPA, CDC42BPG, CDC42BPB
CG40129	Gprk1	G protein-coupled receptor kinase 1	AGC	GRK	ADRBK2, ADRBK1
CG4026	IP3K1	Inositol 1,4,5-triphosphate kinase 1	PKL		ITPKB, ITPKC, ITPKA
CG40293	Stlk	Ste20-like kinase	STE		STRADA, STRADB
CG4032	Abl	Abl tyrosine kinase	TK	Abl	ABL1, ABL2
CG4041			Other	TBCK	TBCK
CG40478	Dyrk3	Dyrk3	CMGC	DYRK	DYRK4, DYRK1B, DYRK1A, DYRK3, DYRK2
CG4132	pkaap	pkaap	Kinase-associated		AKAP10
CG4141	Pi3K92E	Pi3K92E	PKL		PIK3CB, PIK3CD, PIK3CA, PIK3CG
CG4201	ird5	immune response deficient 5	Other	IKK	IKBKB, CHUK
CG42273	mnb	minibrain	CMGC	DYRK	DYRK4, DYRK1B, DYRK1A, DYRK3, DYRK2
CG42317	Csk	C-terminal Src kinase	TK	Csk	MATK, CSK
CG42320	Doa	Darkener of apricot	CMGC	CLK	CLK4, CLK2, CLK3, CLK1
CG42341	Pka-R1	cAMP-dependent protein kinase R1	AGC	PKA	PRKAR1B, PRKAR1A
CG42347			CAMK		MYLK4, MYLK2, MYLK3
CG42349	Pkcδ	Protein kinase C δ	AGC	PKC	PRKCG, PRKCB, PRKCE, PRKCQ, PRKCA, PRKCH,
CG42366			CMGC	CDC2	ICK, MAK
CG42403	Ca-β	Ca2+-channel-protein-β-subunit	Non-Protein Kinase	Nucleic Acid Kinase	CACNB3, CACNB1, CACNB4, CACNB2
CG4252	mei-41	meiotic 41	Atypical	PIKK	ATR
CG42636	Gyc76c	Guanylyl cyclase at 76C	RGC	RGC	NPR2, NPR1
CG4268	PitsIre	PitsIre	CMGC	CDK	CDC2L2
CG42783	aPKC	atypical protein kinase C	AGC	PKC	PRKCZ, PRKCI
CG42856	Sik3	Salt-inducible kinase 3	CAMK	CAMKL	SIK2, SIK3, SIK1
CG4290	Sik2	Salt-inducible kinase 2	CAMK	CAMKL	SIK2, SIK3, SIK1
CG43143			Unclassified		
CG4353	hep	hemipterous	STE	STE7	MAP2K7
CG43729			Kinase-associated		STAC, STAC3, STAC2
CG43741	Ack-like	Activated Cdc42 kinase-like	TK	Ack	

Kinases and Associated factors

CG4379	Pka-C1	cAMP-dependent protein kinase 1	AGC	PKA	PRKACA, PRKACB, PRKACG, PRKX
CG4488	wee	wee	Other	WEE	WEE2, WEE1
CG4523	Pink1	PTEN-induced putative kinase 1	Other	NKF2	PINK1
CG4527	slik	Sterile20-like kinase	STE	STE20	STK10
CG4546			Unclassified		MYCBP
CG4551	smi35A	smell impaired 35A	CMGC	DYRK	DYRK4, DYRK1B, DYRK1A, DYRK3, DYRK2
CG4583	Ire1	Inositol-requiring enzyme-1	Other	IRE	ERN2, ERN1
CG4629			CAMK	CAMKL	NIM1
CG4720	Pk92B	Protein kinase at 92B	STE	STE11	MAP3K6, MAP3K5, MAP3K15
CG4798	l(2)k01209	lethal (2) k01209	Non-Protein Kinase	Nucleic Acid Kinase	UCKL1
CG4803	Takl2	Tak1-like 2	TKL	MLK	MAP3K7
CG4839			AGC	PKG	PRKG1, PRKG2
CG4926	Ror	Ror	TK	Ror	MUSK, ROR1, ROR2
CG4945			Other	NKF1	
CG5072	Cdk4	Cyclin-dependent kinase 4	CMGC	CDK	CDK4, CDK6
CG5092	Tor	Target of rapamycin	Atypical	PIKK	MTOR
CG5125	ninaC	neither inactivation nor afterpotential C	STE	STE20	
CG5144			Non-Protein Kinase	Nucleic Acid Kinase	CKM, CKB, CKMT2, CKMT1B, CKMT1A
CG5169	GckIII	Germinal centre kinase III	STE	STE20	STK25, STK24, MST4
CG5179	Cdk9	Cyclin-dependent kinase 9	CMGC	CDK	CDK13, CDK9, CDK12
CG5182	Pk34A	Pk34A	CMGC	GSK	
CG5288			Non-Protein Kinase	Carbohydrate Kinase	GALK2
CG5310	nmdyn-D6	nmdyn-D6	Atypical		NME6
CG5363	cdc2	cdc2	CMGC	CDK	CDK1, CDK2, CDK3
CG5373	Pi3K59F	Phosphotidylinositol 3 kinase 59F	CMGC	CDK	PIK3C3
CG5387	Cdk5α	Cdk5 activator-like protein	Kinase-associated		CDK5R1
CG5408	trbl	tribbles	CAMK	Trbl	TRIB2, TRIB3, TRIB1
CG5452	dnk	deoxyribonucleoside kinase	Non-Protein Kinase	Nucleic Acid Kinase	TK2
CG5475	Mpk2	Mpk2	CMGC	MAPK	MAPK12, MAPK13, MAPK14, MAPK11
CG5483	Lrrk	Leucine-rich repeat kinase	TKL	LRRK	LRRK2, LRRK1
CG5626			Non-Protein Kinase	Nucleic Acid Kinase	
CG5680	bsk	basket	CMGC	MAPK	MAPK10, MAPK9
CG5725	fbl	fumble	Non-Protein Kinase	Lipid Kinase	PANK2, PANK1, PANK3
CG5757			Non-Protein Kinase	Nucleic Acid Kinase	DTYMK
CG5790			Other	CDC7	CDC7
CG5940	CycA	Cyclin A	CMGC	CDK	CCNA1, CCNA2
CG5974	pll	pelle	TKL	IRAK	IRAK2
CG6027	cdi	center divider	TKL	LISK	TESK1
CG6092	Dak1	Dak1	Non-Protein Kinase	Nucleic Acid Kinase	CMPK
CG6114	sff	sugar-free frosting	CAMK	CAMKL	BRSK1, BRSK2
CG6117	Pka-C3	cAMP-dependent protein kinase 3	AGC	PKA	PRKACG, PRKACA, PRKACB, PRKX
CG6214	MRP	Multidrug-Resistance like Protein 1	Kinase-associated		ABCC1, ABCC2, ABCC4, ABCC3
CG6292	CycT	Cyclin T	CMGC	CDK	CCNT1, CCNT2
CG6297	JIL-1	JIL-1	AGC	RSK	RPS6KA6, RPS6KA5, RPS6KA4, RPS6KA3, RPS6KA2, RPS6KA1
CG6343	ND42	NADH:ubiquinone reductase 42kD subunit precursor	Kinase-associated		NDUFA10

Kinases and Associated factors

CG6355	fab1	fab2	PKL		PIKFYVE
CG6364			Other	UK	UCK2, UCK1
CG6386	ball	ballchen	CK1	VRK	VRK3, VRK1, VRK2
CG6498			AGC	MASR	MAST1, MAST3, MAST4, MAST2
CG6509			Kinase-associated		DLG5
CG6518	inaC	inactivation no afterpotential C	AGC	PKC	PRKCG, PRKCB, PRKCE, PRKCQ, PRKCA, PRKCH,
CG6521	Stam	Signal transducing adaptor molecule	Kinase-associated		STAM2, STAM
CG6535	tefu	telomere fusion	Atypical	PIKK	ATM
CG6551	fu	fused	Other	ULK	STK36
CG6612	Adk3	Adenylate kinase-3	Non-Protein Kinase	Nucleic Acid Kinase	AK4, AK2, AK3
CG6620	ial	IpIl-aurora-like kinase	Other	AUR	AURKC, AURKB, AURKA
CG6622	Pkc53E	Protein C kinase 53E	AGC	PKC	PRKCG, PRKCB, PRKCE, PRKCQ, PRKCA, PRKCH,
CG6703	CASK	CASK ortholog	CAMK	CAMKL	CASK, MPP4, MPP5, MPP7, MPP2, MPP1, MPP3
CG6715	KP78a	KP78a	CAMK	CAMKL	MARK2, MARK4, MARK3, MARK1
CG6767			Non-Protein Kinase	Carbohydrate Kinase	PRPS1L1, PRPS1
CG6772	Slob	Slowpoke binding protein	Other	Slob	
CG6775	rg	rugose	Kinase-associated		NBEA, LRBA
CG6800			CMGC	CDK	CDK7, CDK20
CG6875	asp	abnormal spindle	Kinase-associated		ASPM
CG6963	gish	gilgamesh	CK1	CK1	CSNK1G1, CSNK1G3, CSNK1G2
CG7001	Pk17E	Protein kinase-like 17E	AGC	RSKR	
CG7004	fwd	four wheel drive	PKL		PIK4B
CG7028	PRP4	PRP4	CMGC	DYRK	PRPF4B
CG7069			Non-Protein Kinase	Carbohydrate Kinase	PKM2, PKLR
CG7070	PyK	Pyruvate kinase	Non-Protein Kinase	Carbohydrate Kinase	PKM2, PKLR
CG7094			CK1	CK1	CSNK1E, CSNK1A1, CSNK1A1L, CSNK1D
CG7097	hppy	happyhour	STE	STE20	MAP4K2, MAP4K3, MAP4K1, MAP4K5
CG7103	Pvf1	PDGF- and VEGF-related factor 1	Kinase-associated		VEGFA, VEGFB, VEGFD
CG7125	PKD	Protein Kinase D	CAMK	PKD	PRKD1, PRKD2, PRKD3
CG7156			AGC	RSKL	
CG7177	Wnk	WNK homolog	Other	WNK	WNK1, WNK4, WNK2, WNK3
CG7186	SAK	Sak kinase	Other	PLK	PLK4
CG7207	cert	ceramide transfer protein	Kinase-associated		COL4A3BP
CG7223	htl	heartless	TK	FGFR	FGFR4, FGFR1, FGFR3, RET, FGFR2
CG7236			CMGC	CDKL	CDKL3, CDKL5, CDKL1, CDKL2
CG7281	CycC	Cyclin C	CMGC	CDK	CCNC
CG7328			Non-Protein Kinase	Carbohydrate Kinase	KHK
CG7335			Non-Protein Kinase	Carbohydrate Kinase	KHK
CG7362			Non-Protein Kinase	Carbohydrate Kinase	PKLR, PKM2
CG7393	p38b	p38b	CMGC	MAPK	MAPK12, MAPK13, MAPK14, MAPK11
CG7405	CycH	Cyclin H	CMGC	CDK	CCNH
CG7470			Unclassified		ALDH18A1
CG7524	Src64B	Src oncogene at 64B	TK	Src	HCK, LCK, FRK, SRC, BLK, YES1, FGR, FYN, LYN
CG7525	Tie	Tie-like receptor tyrosine kinase	TK		TIE1
CG7551			Non-Protein Kinase	Carbohydrate Kinase	KHK

Kinases and Associated factors

CG7597	Cdk12	Cdk12	CMGC	CDK	CDK13, CDK9, CDK12
CG7616			Atypical	ABC1	ADCK5
CG7643	ald	altered disjunction	Other	TTK	TTK
CG7693	fray	frayed	STE	STE20	OXSR1
CG7717	Mekk1	Mekk1	STE	STE11	MAP3K4
CG7719	gwl	greatwall	AGC	MAST	MASTL
CG7766			Kinase-associated		PHKA1, PHKA2
CG7838	BubR1	Bub1-related kinase	Other	BUB	BUB1B
CG7873	Src42A	Src oncogene at 42A	TK	Src	HCK, LCK, FRK, SRC, BLK, YES1, FGR, FYN, LYN
CG7892	nmo	nemo	CMGC	MAPK	NLK
CG7904	put	punt	TKL	STKR	BMPR2, ACVR2A, TGFBR2, ACVR2B
CG7995			Non-Protein Kinase	Lipid Kinase	GK, GK2
CG8049	Btk29A	Btk family kinase at 29A	TK	Tec	TEC, ITK, TXK, BMX, BTK
CG8057	alc	alicorn	Non-Protein Kinase	Nucleic Acid Kinase	PRKAB2, PRKAB1
CG8094	Hex-C	Hexokinase C	Non-Protein Kinase	Carbohydrate Kinase	HK3, HK2, HKDC1, HK1
CG8173			Other	TOPK	PBK
CG8174	SRPK	SRPK	CMGC	SRPK	SRPK1, SRPK3, SRPK2
CG8201	par-1	par-1	CAMK	CAMKL	MARK2, MARK4, MARK3, MARK1
CG8203	Cdk5	Cyclin-dependent kinase 5	CMGC	CDK	CDK5
CG8222	Pvr	PDGF- and VEGF-receptor related	TK	VEGFR	VEGFR3, VEGFR2, VEGFR1
CG8224	babo	baboon	TKL	STKR	ACVR1, BMPR1A, ACVR1C, ACVR1B, BMPR1B, ACVRL1
CG8239			Non-Protein Kinase	Carbohydrate Kinase	MVD
CG8250	Alk	Alk	TK	ALK	ALK, LTK, ROS1
CG8286	P58IPK	P58IPK	Kinase-associated		DNAJC3, DNAJC7
CG8298			Non-Protein Kinase	Lipid Kinase	GK, GK2
CG8351	Tcp-1η	Tcp-1η	Kinase-associated		CCT7
CG8362	nmdyn-D7	nmdyn-D7	Atypical		NME7
CG8363	Papss	PAPS synthetase	Non-Protein Kinase	Nucleic Acid Kinase	PAPSS1, PAPSS2
CG8475			CAMK		PHKB
CG8485			CAMK	CAMKL	SNRK
CG8565			CMGC	SRPK	SRPK1, SRPK3, SRPK2
CG8637	trc	tricornered	AGC	NDR	STK38
CG8657	Dgkε	Diacyl glycerol kinase ε	Non-Protein Kinase	Lipid Kinase	DGKE
CG8726			Other	Slob	PXK
CG8767	mos	mos	Other	MOS	MOS
CG8789	wnd	wallenda	TKL	MLK	MAP3K12, MAP3K13
CG8808	Pdk	Pyruvate dehydrogenase kinase	Atypical	PDHK	PDK4, PDK3, PDK1, PDK2
CG8866			Other	ULK	ULK3
CG8874	Fps85D	Fps oncogene analog	TK	Fer	FER
CG8878			CK1	VRK	
CG8914	CK11β2	Casein kinase II β2 subunit	CMGC		CSNK2B
CG8948	Graf	GTPase regulator associated with focal adhesion kinase ortholog	Kinase-associated		OPHN1, ARHGAP42, ARHGAP10, ARHGAP26
CG8967	otk	off-track	TK	CCK4	PTK7
CG9096	CycD	Cyclin D	CMGC	CDK	CCND1, CCND2, CCND3
CG9222			CAMK	TSSK	TSSK6, TSSK2, TSSK4, TSSK3,

Kinases and Associated factors

CG9222			CAMK	TSSK	TSSK1
CG9326	vari	varicose	Non-Protein Kinase	Nucleic Acid Kinase	MPP4, MPP5, MPP7, MPP2, MPP1, MPP3
CG9358	Phk-3	Pherokine 3	Non-Protein Kinase	Lipid Kinase	
CG9374	lkb1	lkb1	CAMK	CAMKL	STK11
CG9541			Non-Protein Kinase	Nucleic Acid Kinase	
CG9738	Mkk4	MAP kinase kinase 4	STE	STE7	MAP2K4, MAP2K6, MAP2K3
CG9746	ird1	immune response deficient 1	Other	VPS15	PIK3R4
CG9774	rok	Rho-kinase	AGC	DMPK	ROCK1, ROCK2
CG9961			Non-Protein Kinase	Lipid Kinase	PGK1, PGK2
CG9962			CK1	CK1	
CG9985	sktl	skittles	PKL		PIP5K1A, PIP5K1B, PSMD4, PIP5K1C

Fig S2

Classifications of phosphatases and associated factors

Phosphoprotein Phosphatase
Protein Phosphatase Mg+/Mn+-dependent
Classical Protein Tyrosine Phosphatase
Class I/Dual Specificity Phosphatase
Class III Protein Tyrosine Phosphatase
Factor IIF-interacting CTD Phosphatase/Small CTD Phosphatase
Haloacid Dehalogenase-like Hydrolase
Unclassified
Phosphatase-associated — Co-factors such as subunits of multi-component PPTases
Non-protein Phosphatase — Such as lipid and sugar phosphatases

Drosophila Phosphatase			Gene Group	Gene Family	Human ortholog(s)
Annotation Symbol	Gene Symbol	Gene Name			
CG10089			PTP	Class I/DSP	DUSP22, DUSP15
CG10138	PpD5	Protein phosphatase D5	STP	PPP	PPP1CC, PPP1CA
CG10371	Plip	PTEN-like phosphatase	PTP	Classical	PTPMT1
CG10376			STP	PPM	PPM1F, PPM1E
CG10417			STP	PPM	PPM1G
CG10426			Non-Protein Phosphatase	Lipid Phosphatase	INPP5E
CG10443	Lar	Leukocyte-antigen-related-like	PTP	Classical	PTPRS, PTPRD, PTPRF
CG10493	Phlpp	PH domain leucine-rich repeat protein phosphatase	STP	PPM	PHLPP1, PHLPP2
CG10574	I-2	Inhibitor-2		Phosphatase-associated	PPP1R2
CG10592				Unclassified	ALPL, ALPPL2, ALPP
CG10827				Unclassified	ALPL, ALPPL2, ALPP
CG10930	PpY-55A	Protein phosphatase Y at 55A	STP	PPP	
CG10975	Ptp69D	Protein tyrosine phosphatase 69D	PTP	Classical	PTPRG, PTPRZ1
CG11217	CanB2	Calcineurin B2	STP	PPP	PPP3R1, WDR92, PPP3R2
CG11425			Non-Protein Phosphatase	Lipid Phosphatase	PPAP2C, PPAP2B, PPAP2A
CG11426			Non-Protein Phosphatase	Lipid Phosphatase	PPAP2C, PPAP2B, PPAP2A
CG11437			Non-Protein Phosphatase	Lipid Phosphatase	PPAP2C, PPAP2B, PPAP2A
CG11438			Non-Protein Phosphatase	Lipid Phosphatase	PPAP2C, PPAP2B, PPAP2A
CG11440	laza	lazaro	Non-Protein Phosphatase	Lipid Phosphatase	PPAP2C, PPAP2B, PPAP2A
CG11516	Ptp99A	Protein tyrosine phosphatase 99A	PTP	Classical	PTPRG, PTPRZ1
CG11597			STP	PPP	
CG11883				Phosphatase-associated	
CG12034				Phosphatase-associated	SMPD2
CG12078			Asp-based catalysis	HAD	
CG12091			STP	PPM	PPTC7
CG12151	Pdp	Pyruvate dehydrogenase phosphatase	STP	PPM	PDP1, PDP2
CG12169	Ppm1	Ppm1	STP	PPM	
CG12173			Asp-based catalysis	HAD	ENOPH1
CG12217	PpV	Protein phosphatase V	STP	PPP	PPP4C, PPP6C, PPP2CA, PPP2CB
CG12252	Fcp1		Asp-based catalysis	FCP/SCP	CTDP1
CG1228	Ptpmeg	Ptpmeg	PTP	Classical	PTPN3, PTPN4

CG12538			Non-Protein Phosphatase	Nucleic Acid Phosphatase	
CG12746			Non-Protein Phosphatase	Lipid Phosphatase	PPAPDC1A, PPAPDC1B
CG13125	TbCMF46	TbCMF47		Phosphatase-associated	LRRC48
CG13197			PTP	Class I/DSP	DUSP11
CG13311				Phosphatase-associated	
CG13570	spag	spaghetti		Phosphatase-associated	
CG1395	stg	string	PTP	Class I/DSP	CDC25A, CDC25B
CG14022			Non-Protein Phosphatase	Lipid Phosphatase	ACYP1, ACYP2
CG14080	Mkp3	Mitogen-activated protein kinase phosphatase 3	PTP	Class I/DSP	DUSP7, DUSP6, DUSP9
CG14211	MKP-4	MAPK Phosphatase 4	PTP	Class I/DSP	DUSP12
CG14297			PTP	Class III	ACP1
CG14411			PTP	Class I/DSP	MTMR11, MTMR10, MTMR12
CG1455	CanA1	Calcineurin A1	STP	PPP	PPP3CB, PPP3CC
CG14616	l(1)G0196	lethal (1) G0196	Non-Protein Phosphatase	Lipid Phosphatase	PPIP5K2, PPIP5K1
CG1462	Aph-4	Alkaline phosphatase 4		Unclassified	ALPL, ALPPL2, ALPP
CG14719	l-t	inhibitor-t		Phosphatase-associated	
CG14903			PTP	Class III	PTRHD1
CG15035			STP	PPM	PPTC7
CG15385			Non-Protein Phosphatase	Nucleic Acid Phosphatase	ACPL2
CG15400			Non-Protein Phosphatase	Carbohydrate Phosphatase	G6PC
CG15528			PTP	Class I/DSP	DUSP14, DUSP21
CG15533				Phosphatase-associated	SMPD1
CG15534				Phosphatase-associated	SMPD1
CG15743			Non-Protein Phosphatase	Lipid Phosphatase	IMPAD1
CG1637			Non-Protein Phosphatase	Nucleic Acid Phosphatase	PAPL
CG16717				Phosphatase-associated	MPPED2, MPPED1
CG16757	Spn	Spinophilin		Phosphatase-associated	
CG16771				Unclassified	ALPL, ALPPL2, ALPP
CG16870	Acyp	Acylphosphatase	Non-Protein Phosphatase	Lipid Phosphatase	ACYP1, ACYP2
CG16928	mre11	meiotic recombination 11		Phosphatase-associated	MRE11A
CG1696	Dd	Dullard	Asp-based catalysis	FCP/SCP	CTDNEP1
CG17026			Non-Protein Phosphatase	Lipid Phosphatase	IMPA1, IMPA2
CG17027			Non-Protein Phosphatase	Lipid Phosphatase	IMPA1, IMPA2
CG17028			Non-Protein Phosphatase	Lipid Phosphatase	IMPA1, IMPA2
CG17029			Non-Protein Phosphatase	Lipid Phosphatase	IMPA1, IMPA2
CG17124				Phosphatase-associated	PPP1R14C, PPP1R14B, PPP1R14D, PPP1R14A
CG17291	Pp2A-29B	Protein phosphatase 2A at 29B	STP	PPP	PPP2R1B, PPP2R1A
CG17598			STP	PPM	PPM1M, PPM1J, PPM1H
CG17746			STP	PPM	
CG1809				Unclassified	ALPL, ALPPL2, ALPP
CG1810	mRNA-cap	mRNA-capping enzyme	PTP	Class I/DSP	RNGTT
CG1817	Ptp10D	Protein tyrosine phosphatase 10D	PTP	Classical	PTPRB, PTPRJ, PTPRO
CG18243	Ptp52F	Ptp52F	PTP	Classical	
CG1906	alph	alphabet	STP	PPM	PPM1A, PPM1B

CG2096	flw	flapwing	STP	PPP	PPP1CB, PPP1CA, PPP1CC
CG2104			PTP	Unclassified	PPP2R4
CG2890	PPP4R2r	Protein phosphatase 4 regulatory subunit 2-related	STP	PPP	PPP4R2
CG2984	Pp2C1	Protein phosphatase 2C	STP	PPM	PP2CM
CG30103				Phosphatase-associated	NT5E
CG30104				Phosphatase-associated	NT5E
CG3028	Ipp	Inositol polyphosphate 1-phosphatase	Non-Protein Phosphatase	Lipid Phosphatase	INPP1
CG3059	NTPase	NTPase	Non-Protein Phosphatase	Nucleic Acid Phosphatase	ENTPD6, ENTPD5
CG31137	twin	twin		Phosphatase-associated	CNOT6L, CNOT6
CG31299	cu	curled		Phosphatase-associated	CCRN4L
CG31469			PTP	Class III	ACP1
CG31692	fbp	fructose-1,6-bisphosphatase	Non-Protein Phosphatase	Carbohydrate Phosphatase	FBP2, FBP1
CG31717			Non-Protein Phosphatase	Lipid Phosphatase	PPAPDC3, PPAPDC2
CG31759				Phosphatase-associated	PDE12
CG3178	Rrp1	Recombination repair protein 1		Phosphatase-associated	APEX1
CG31795	IA-2	IA-2 ortholog	PTP	Classical	PTPRN2, PTPRN
CG32156	Mbs	Myosin binding subunit	STP	PPP	PPP1R12A, PPP1R12B
CG3245	PpN-58A	Protein phosphatase N at 58A	STP	PPP	
CG32487			Asp-based catalysis	HAD	PDXP, PGP
CG32488			Asp-based catalysis	HAD	PDXP, PGP
CG32505	Pp4-19C	Protein phosphatase 19C	STP	PPP	PPP4C, PPP6C, PPP2CA, PPP2CB
CG32568			STP	PPP	
CG3264				Unclassified	ALPL, ALPPL2, ALPP
CG32697	l(1)G0232	lethal (1) G0232	PTP	Classical	PTPN9
CG32812			STP	PPP	
CG3289	Ptpa	Phosphotyrosyl phosphatase activator	PTP	Unclassified	PPP2R4
CG3290				Unclassified	ALPL, ALPPL2, ALPP
CG3292				Unclassified	ALPL, ALPPL2, ALPP
CG33747	primo-2	primo-2	PTP	Class III	ACP2
CG33748	primo-1	primo-1	PTP	Class III	ACP1
CG3376				Phosphatase-associated	SMPD1
CG34099	Mkp	MAP kinase-specific phosphatase	PTP	Class I/DSP	DUSP19
CG34140				Phosphatase-associated	PUSL1
CG3530			PTP	Class I/DSP	MTMR6, MTMR7, MTMR8
CG3573	Ocrl	Oculocerebrorenal syndrome of Lowe ortholog	Non-Protein Phosphatase	Lipid Phosphatase	INPP5B, OCRL
CG3632			PTP	Class I/DSP	MTMR3, MTMR4
CG3705	aay	astray	Asp-based catalysis	HAD	PSPH
CG3954	csw	corkscrew	PTP	Classical	PTPN11, PTPN6
CG3980	Cep97			Phosphatase-associated	CEP97
CG40448	Pp1-Y2	Pp1-Y2	STP	PPP	PPP1CB, PPP1CA, PPP1CC
CG4123	Mipp1	Multiple inositol polyphosphate phosphatase 1	Non-Protein Phosphatase	Lipid Phosphatase	
CG41534	Pp1-Y1	Pp1-Y1	STP	PPP	PPP1CB, PPP1CA, PPP1CC

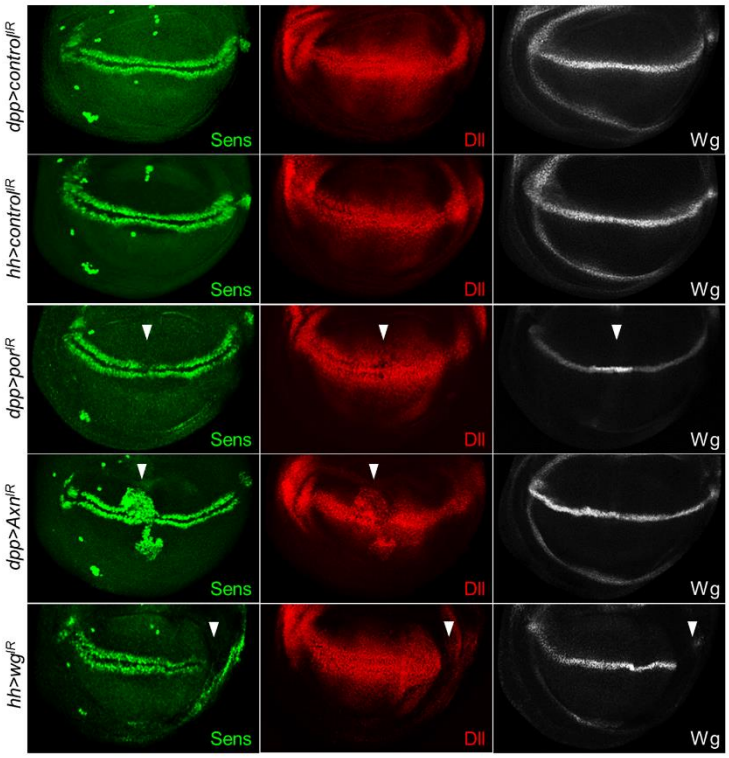
CG4209	CanB	Calcineurin B	STP	PPP	PPP3R1, WDR92, PPP3R2
CG42249				Phosphatase-associated	NT5E
CG42271			Non-Protein Phosphatase	Lipid Phosphatase	INPP4A, INPP4B
CG42283	5Ptasel	5Ptasel	Non-Protein Phosphatase	Lipid Phosphatase	INPP5A
CG42327			PTP	Classical	
CG4317	Mipp2	Multiple inositol polyphosphate phosphatase 2	Non-Protein Phosphatase	Lipid Phosphatase	
CG4733	PR72	PR72	STP	PPP	PPP2R3A, PPP2R3B
CG4827	veil	veil		Phosphatase-associated	NT5E
CG4965	twe	twine	PTP	Class I/DSP	
CG4993	PRL-1	PRL-2	PTP	Class I/DSP	PTP4A1, PTP4A2, PTP4A3
CG5026			PTP	Class I/DSP	MTMR9
CG5150				Unclassified	ALPL, ALPPL2, ALPP
CG5171			Non-Protein Phosphatase	Carbohydrate Phosphatase	
CG5177			Non-Protein Phosphatase	Carbohydrate Phosphatase	
CG5276			Non-Protein Phosphatase	Nucleic Acid Phosphatase	CANT1
CG5361				Unclassified	
CG5567			Asp-based catalysis	HAD	PDXP, PGP
CG5577			Asp-based catalysis	HAD	PDXP, PGP
CG5643	wdb	widerborst	STP	PPP	PPP2R5D, PPP2R5E, PPP2R5C, PPP2R5A
CG5650	Pp1-87B	Protein phosphatase 1 at 87B	STP	PPP	PPP1CB, PPP1CA, PPP1CC
CG5656				Unclassified	ALPL, ALPPL2, ALPP
CG5671	Pten	Pten	PTP	Class I/DSP	PTEN, TPTE, TPTE2
CG5784	Mapmodulin	Mapmodulin		Phosphatase-associated	ANP32E, ANP32D, ANP32A, ANP32B
CG5820	Gp150			Phosphatase-associated	
CG5830			Asp-based catalysis	FCP/SCP	CTDSPL, CTDSP2, CTDSP1
CG5851	sds22	sds22	STP	PPP	PPP1R7
CG6036			STP	PPM	PPM1A, PPM1B
CG6235	tws	twins	STP	PPP	PPP2R2C, PPP2R2B, PPP2R2D, PPP2R2A
CG6238	ssh	slingshot	PTP	Class I/DSP	SSH2, SSH3, SSH1
CG6380			STP	PPP	PPP1R2
CG6542	EDTP	Egg-derived tyrosine phosphatase	PTP	Class I/DSP	MTMR14
CG6562	synj	synaptojanin	Non-Protein Phosphatase	Lipid Phosphatase	SYNJ2, SYNJ1
CG6571	rdgC	retinal degeneration C	STP	PPP	PPEF1, PPEF2
CG6593	Pp1α-96A	Protein phosphatase 1α at 96A	STP	PPP	PPP1CB, PPP1CA, PPP1CC
CG6656			Non-Protein Phosphatase	Nucleic Acid Phosphatase	ACPP, ACP2, ACPT
CG6746			PTP	Unclassified	PTPLA, PTPLB
CG6805			Non-Protein Phosphatase	Lipid Phosphatase	INPP5J, INPP5K
CG6896	MYPT-75D	MYPT-75D	STP	PPP	PPP1R16B, PPP1R16A
CG6899	Ptp4E	Protein tyrosine phosphatase 4E	PTP	Classical	PTPRB, PTPRJ, PTPRO
CG6939	Sbf	SET domain binding factor	PTP	Class I/DSP	SBF2, SBF1

CG7067	NitFhit	Nitrilase and fragile histidine triad fusion protein	Non-Protein Phosphatase	Nucleic Acid Phosphatase	NIT1, NIT2, FHIT
CG7109	mts	microtubule star	STP	PPP	PPP4C, PPP6C, PPP2CA, PPP2CB
CG7115			STP	PPM	PPM1L
CG7134	cdc14	cdc14	PTP	Class I/DSP	CDC14B, CDC14A
CG7180			PTP	Classical	PTPRK
CG7378			PTP	Class I/DSP	DUSP3, DUSP26, DUSP13, DUSP1, DUSP27
CG7615	fig	fos intronic gene	STP	PPM	PPTC7
CG7789			PTP	Class I/DSP	BPNT1
CG7850	puc	puckered	PTP	Class I/DSP	DUSP10
CG7899	Acph-1	Acid phosphatase 1	Non-Protein Phosphatase	Nucleic Acid Phosphatase	ACPP, ACP2, ACPT
CG7913	Pp2A-B'	Pp2A-B'	STP	PPP	PPP2R5D, PPP2R5E, PPP2R5C, PPP2R5A
CG7942	ldbr	lariat debranching enzyme		Phosphatase-associated	DBR1
CG8105				Unclassified	ALPL, ALPPL2, ALPP
CG8147				Unclassified	ALPL, ALPPL2, ALPP
CG8402	PpD3	Protein phosphatase D3	STP	PPP	PPP5C
CG8455			Non-Protein Phosphatase	Lipid Phosphatase	MPPE1
CG8509			PTP	Unclassified	PPP2R4
CG8584			Asp-based catalysis	FCP/SCP	
CG8804	wun	wunen	Non-Protein Phosphatase	Lipid Phosphatase	PPAP2C, PPAP2B, PPAP2A
CG8805	wun2	wunen-2	Non-Protein Phosphatase	Lipid Phosphatase	PPAP2C, PPAP2B, PPAP2A
CG8822	PpD6	Protein phosphatase D6	STP	PPP	PPP1CC, PPP1CA
CG8889	Mppe	Metallophosphoesterase	Non-Protein Phosphatase	Lipid Phosphatase	MPPE1
CG8980	NiPp1	Nuclear inhibitor of Protein phosphatase 1		Phosphatase-associated	PPP1R8
CG9115	mtm	myotubularin	PTP	Class I/DSP	MTMR1, MTM1, MTMR2
CG9128	Sac1	Sac1	Non-Protein Phosphatase	Lipid Phosphatase	SACM1L
CG9151	acj6	abnormal chemosensory jump 6		Phosphatase-associated	POU4F2, POU4F3
CG9156	Pp1-13C	Protein phosphatase 1 at 13C	STP	PPP	PPP1CB, PPP1CA, PPP1CC
CG9181	Ptp61F	Protein tyrosine phosphatase 61F	PTP	Classical	PTN2, PTPN1
CG9236	Cib2	CIB2 ortholog		Phosphatase-associated	CIB1, CIB4, CIB2
CG9238			STP	PPP	PPP1R3C
CG9267			PTP	Classical	PTPLAD1, PTPLAD2
CG9311	mop	myopic	PTP	Classical	PTPN23
CG9351	flfl	falafel	STP	PPP	SMEK2, SMEK1
CG9389			Non-Protein Phosphatase	Lipid Phosphatase	IMPA1, IMPA1
CG9391			Non-Protein Phosphatase	Lipid Phosphatase	IMPA1, IMPA2
CG9449			Non-Protein Phosphatase	Nucleic Acid Phosphatase	ACPP, ACP2, ACPT
CG9451			Non-Protein Phosphatase	Nucleic Acid Phosphatase	ACPP, ACP2, ACPT
CG9452			Non-Protein Phosphatase	Nucleic Acid Phosphatase	ACPP, ACP2, ACPT
CG9493	Pez	Pez	PTP	Classical	PTPN14

CG9554	eya	eyes absent	Asp-based catalysis	HAD	EYA1, EYA4, EYA3, EYA2
CG9601			Non-Protein Phosphatase	Nucleic Acid Phosphatase	PNKP
CG9619	Gbs-76A	Glycogen binding subunit 76A	STP	PPP	PPP1R3A, PPP1R3C, PPP1R3B
CG9764	yrt	yurt		Phosphatase-associated	EPB41L4B, EPB41L5
CG9784			Non-Protein Phosphatase	Lipid Phosphatase	
CG9801			STP	PPM	
CG9819	CanA-14F	Calcineurin A at 14F	STP	PPP	
CG9842	Pp2B-14D	Protein phosphatase 2B at 14D	STP	PPP	PPP3CB, PPP3CC
CG9856	PTP-ER	Protein tyrosine phosphatase-ERK/Enhancer of Ras1	PTP	Class I/DSP	

Fig. S3

Drosophila Gene			PILOT SCREEN							
Annotation Symbol	Gene Symbol	Gene Name	RNAi Stock	UAS-dcr2;;dpp-Gal4			RNAi Stock	UAS-dcr2;hh-Gal4		
				Wing disc				Wing disc		
				Wg	Sens	Dll		Wg	Sens	Dll
CG4889	wg	wingless	13352				104579			
CG6205	por	porcupine	47864				100780			
CG6210	wls	wntless	103812				5215			
CG5912	arr	arrow	36286				6708			
CG11579	arm	armadillo	107344				7767			
CG7926	Axn	Axin	7748							
CG6193	Apc2	Adenomatous polyposis coli tumor suppressor homolog 2	22290				100104			
CG34403	pan	pangolin	108679				25940			



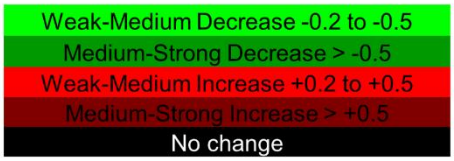


Fig. S4

Kinase Data

Weak-Medium Decrease -0.2 to -0.5
Medium-Strong Decrease > -0.5
Weak-Medium Increase +0.2 to +0.5
Medium-Strong Increase > +0.5
No change

Wing phenotype any visible wing phenotype including notches, blisters, vein spacing, altered size
Lethal No adults recovered

Kinase Gene		PRIMARY SCREEN									SECONDARY SCREEN				HEDGEHOG SCREEN			
Annotation Symbol	Gene Symbol	RNAi Stock	UAS-dcr2;;dpp-Gal4				RNAi Stock	UAS-dcr2;hh-Gal4			RNAi Stock	UAS-dcr2;C5-Gal4		UAS-dcr2; dpp-Gal4 or UAS-dcr2; hh-Gal4	RNAi Stock	MS1096-Gal4;UAS-dcr2		
			Wing disc			Adult		Wing disc			Adult		Wing disc		Adult	Wing disc			Wing disc		Adult
			Wg	Sens	Dll			Wg	Sens	Dll			Wg	Sens	Dll		Cut		Ci	Ptc	
CG10023	Fak56D	17957 108608																			
CG10033	for	38320				Wing phenotype						38320				Wing phenotype		38320			Wing phenotype
		108293																108293			Wing phenotype
							10033R-1				Lethal							10033R-1			Lethal
		10033R-2				Wing phenotype												10033R-2			Wing phenotype/Lethal
CG10079	Egfr	43268				Lethal						43268				Wing phenotype		43268			Wing phenotype
		43267				Wing phenotype															
		107130				Wing phenotype	107130				Lethal							107130			Lethal
		10079R-2																			
CG10082		38327																			
		103749																			
CG10155	Spred	18024																			
		18025																			
CG10177		38349																			
		107848																			
CG10244	Cad96Ca	1091				Wing phenotype															
		8402				Wing phenotype															
CG10260		15993				Wing phenotype															
		105614				Wing phenotype															
CG10268		110640																			
		10268R-2																			
CG10295	Pak	12553				Wing phenotype															
		108937				Wing phenotype															
CG10308	CycJ	31217																			
		110222																			
		104959				Lethal															
CG10498	cdc2c	10498R-2				Wing phenotype															
		34856																			
CG10504	Ilk	16062				Wing phenotype															
CG10522	sticky	47280																			
		100265																			
CG10539	S6k	18126				Wing phenotype															
		104369				Wing phenotype															
CG10572	Cdk8	45370				Wing phenotype															
		107187				Wing phenotype															
CG10579	Eip63E	47860				Wing phenotype	47860				Lethal	47860				Wing phenotype		47860			Wing phenotype
		106824				Wing phenotype															
		50599				Wing phenotype	50599											50599			Wing phenotype
		10579R-3				Wing phenotype															
CG10637	Nak	35482																			
		109507																			
CG10673		27301																			
CG10702		27052				Wing phenotype												27052			Wing phenotype
		3691				Wing phenotype	3691				Lethal	3691						3691			Wing phenotype
		100842					100842														
CG10738		28580																			
		10738R-1																			

CG10776	wit	42244 865 103808 10776R-2									
CG10895	lok	44980 110342									
CG10933		46726 46727 104282		Wing phenotype Wing phenotype Wing phenotype							
CG10951	niki	16120 100823									
CG10967	Atg1	16133 10967R-1									
CG1098	Madm	27347 101758		Wing phenotype							
CG1107	aux	16182 103426		Wing phenotype Wing phenotype				16182			16182 103426	Lethal Lethal
CG11221		100163 42947		Wing phenotype							
CG11228	hpo	29603 7823 104169 27661		Wing phenotype Wing phenotype Wing phenotype Wing phenotype							
CG11249		18196 108319									
CG11255		17533 17534									
CG11420	png	31500 11420R-3									
CG11486		106497 51711 11486R-3 11486R-4		Wing phenotype Wing phenotype	11486R-3 11486R-4		Wing phenotype	106497		Wing phenotype	106497 11486R-3	Wing phenotype Wing phenotype
CG11489	srpk79D	47544 102632									
CG11525	CycG	13655 106846									
CG11533	Asator	45120 45121									
CG11594		28311 105487									
CG11621	Pi3K68D	16240 109582									
CG11660		18526 105395		Wing phenotype Wing phenotype							
CG11811		30915 110740									
CG11859		47401 109296		Wing phenotype Wing phenotype	11859R-2 11859R-3 31580		Lethal Lethal Lethal	109296			47401 109296	Wing phenotype Wing phenotype
CG12019	Cdc37	47776 110727		Lethal Lethal				110727		Wing phenotype	47776 110727	Wing phenotype Wing phenotype
CG12066	Pka-C2	30658 108424									
CG12069		23719 100886									
CG12072	warts	9928 106174 12072R-1		Wing phenotype Lethal Wing phenotype							
CG1210	Pdk1	18736 109812		Wing phenotype Wing phenotype							

CG12147		31659									
		101875									
CG1216	mri	17043									
		101345									
CG12229		31704		Wing phenotype							
		101097									
CG12244	lic	106822									
		20166									
CG1227		38648									
		105610		Wing phenotype							
CG12289		31725									
		105685									
CG12306	polo	20177		Wing phenotype				20177		Wing phenotype		20177		Wing phenotype
		12306R-2			12306R-2									
		12306R-3		Wing phenotype								12306R-3		Wing phenotype
CG12559	rl	43123		Wing phenotype							
		109108									
CG1271		13044									
		110178									
CG13369		17161		Wing phenotype	17161		Lethal					17161		Wing phenotype
		17158		Wing phenotype										
		100747		Wing phenotype				100747				100747		Wing phenotype
		13369R-1			13369R-1							13369R-1		Wing phenotype
CG13388	Akap200	5647		Wing phenotype							
		102374		Wing phenotype							
CG1344		108838									
CG13591	Ssl	17282									
		108692									
CG1362	cdc2rk	32249									
		100299									
CG13688	Ipk2	43824									
		43825									
CG1389	tor	36280									
		101154									
CG14026	tkv	3059		Lethal								3059		Wing phenotype
		105834		Lethal				105834		Wing phenotype		105834		Wing phenotype
CG14030	Bub1	101096			101096		Wing phenotype	101096				101096		
		24833		Wing phenotype	24833		Lethal					24833		Lethal
		14030R-1			14030R-1		Lethal							
		14030R-2		Wing phenotype	14030R-2							14030R-2		Lethal
CG14217	Tao	17432		Wing phenotype							
		107645		Wing phenotype							
CG14305		17477									
		17478									
		14305R-3									
CG14396	Ret	30832									
		107648									
CG14895	Pak3	44607									
		107260									
CG14939	CycY	107010		Wing phenotype	107010		Wing phenotype	107010				107010		
		32335			32335									
					32334		Wing phenotype					32334		
		34009		Wing phenotype										
CG1495	CaMK1	101380									
		1495R-2									
CG14992	Ack	39857		Wing phenotype							
		14992R-1		Wing phenotype/Lethal							
		33899									
CG1511	Eph	4771									
		110448									
CG15218	CycK	36216		Wing phenotype				36216				36216		Wing phenotype
		110774		Wing phenotype								110774		Wing phenotype

CG15224	CkIIβ	32378 106845	Wing phenotype/Lethal Wing phenotype				106845	Wing phenotype		32378 106845	Wing phenotype Wing phenotype
CG15547		32621 104520									
CG15793	Dsor1	40026 40025 107276	Wing phenotype Wing phenotype Wing phenotype	32920	Lethal		40026	Wing phenotype		40026 32920	Wing phenotype Wing phenotype
CG15862	Pka-R2	39437 101763									
CG1594	hop	102830 40037 32966	Wing phenotype Wing phenotype								
CG1609	Gcn2	32664 103976 1609R-2	 Wing phenotype 	32664 1609R-2			103976			32664 103976 1609R-4 1609R-2	 Wing phenotype
CG16708	Cerk	43412 101550	Wing phenotype 								
CG16903		37570 37572	Wing phenotype Lethal								
CG16910	key	7723 100257 101517	 								
CG16973	msn	16973R-2 28791	Lethal Wing phenotype								
CG17010		17010R-2 17010R-3	 								
CG17090	hipk	108254 32855 17090R-1	 Wing phenotype Lethal	108254 32855	Wing phenotype Wing phenotype		32855	Wing phenotype		108254 32855	Wing phenotype Wing phenotype
CG17146	Adk1	25214 104475	 								
CG17161	grp	12680 110076	 								
CG17216	KP78b	51996 105265	 								
CG17245	plexB	27220 12167 46687 17245R-2	 								
CG1725	dlg1	41134 109274 1725R-2 1725R-1	Lethal Lethal 	109274 41136	 Lethal		41136	Wing phenotype		41134 109274	Wing phenotype
CG17256	Nek2	40052 103408	 								
CG17299	SNF4Aγ	17299R-1 17299R-3	 								
CG17342	Lk6	34726 32885	 Wing phenotype								
CG17348	drl	109663 27053	 								
CG1747	Sk1	100039 32932 32930	 								
CG17471		1747R-3									
CG17520	CkIIα	17520R-2 31645	Wing phenotype Wing phenotype	17520R-2 31645	Lethal Lethal		31645			17520R-2 31645	Wing phenotype Wing phenotype
CG17528		26292									
CG17559	dpt	27057									

CG17596	S6kII	106056 5702 101451									
CG17603	Taf1	41099 106119	Lethal Wing phenotype								
CG17698		35634 105884	Wing phenotype								
CG1772	dap	36720									
CG17998	Gprk2	1835 101463 34843	 Wing phenotype	1835 101463	 Wing phenotype	101463		1835 101463 34843	 Wing phenotype Wing phenotype/Lethal		
CG18069	CaMKII	47280 100265									
CG18085	sev	49925 107048									
CG18247	shark	105706 25304									
CG18255	Strn-Mlck	18255R-1 18255R-3 26736 31891	Wing phenotype								
CG1830	PhKγ	110638 33054	Wing phenotype/Lethal								
CG18374	Gyk	52478 110806 18374R-3									
CG18402	InR	991 992	Wing phenotype Wing phenotype								
CG1848	LIMK1	25343 25344									
CG18492	Tak1	101357 1388R-2									
CG1851	Ady43A	33133 1851R-2									
CG18582	mbt	109880 46044 29379									
CG18854		31732									
CG1891	sax	42457 46358	Wing phenotype Wing phenotype								
CG1915	sls	47298 47301	Wing phenotype								
CG1939	Dpck	44765 100276	Wing phenotype								
CG1951		33430 33431									
CG1954	Pkc98E	33434 108151	Wing phenotype								
CG1973	yata	19275 110214									
CG2028	Cklα	13664 110768 25786	 Lethal Wing phenotype/Lethal Lethal			110768	 Wing phenotype 	13664 110768	 Lethal Lethal		
CG2048	dco	9241 2048R-1 2048R-3 27719	 Wing phenotype	9241 2048R-1 2048R-3	 Lethal	9241		9241 2048R-1 2048R-3	 Wing phenotype		
CG2049	Pkn	108870 2055R-2 28335	Lethal								
CG2087	PEK	16427 110278	Wing phenotype								


CG2201
33502
108958
CG2210 awd
110782
2210R-2
Wing phenotype
110782
Wing phenotype
2210R-1
33712
Lethal
2210R-2
110782
Wing phenotype
2210R-2
Wing phenotype
2210R-1
Wing phenotype
33712
Wing phenotype
CG2246
48878
110389
Wing phenotype
CG2252 fs(1)h
51227
Wing phenotype/Lethal
51305
Lethal
108662
Lethal
2252R-2
Lethal
51305
Wing phenotype
51305
108662
Wing phenotype/Lethal
Wing phenotype
CG2272 slpr
33518
106449
2272R-1
Wing phenotype
106449
2272R-1
Wing phenotype
33518
Wing phenotype
33518
2272R-1
Wing phenotype
CG2577
41694
105471
Wing phenotype
CG2615 ik2
49366
103748
CG2621 sgg
101538
Wing phenotype
2621R-3
Wing phenotype
31308
101538
Lethal
2621R-3
Wing phenotype
2621R-1
Wing phenotype
101538
Wing phenotype
101538
2621R-3
Wing phenotype
CG2699 Pi3K21B
104179
33556
Wing phenotype
CG2794
13369
109682
CG2845 phl
20909
107766
Wing phenotype
Wing phenotype
CG2846
100266
CG2899 ksr
45041
110621
Wing phenotype
Wing phenotype
CG2929 Pi4KIIα
40995
110687
40995
110687
Wing phenotype
40995
40995
110687
Wing phenotype
CG2964
42293
109509
Wing phenotype
CG3001 Hex-A
21054
104680
CG30021 metro
29965
110814
CG3008
52634
103828
CG30184
3539
104976
CG30295 Ipk1
47790
109497
CG3051 SNF1A
1827
106200
CG3068 aur
108446
35763
CG3086 MAPk-Ak2
3170
110317
CG31003 gskt
25640
Wing phenotype
107429
CG3105 Pask
25661
107025
CG31097
107652
CG31127 Wsck
33761
CG31140
38936
101347
CG31183
859
4773

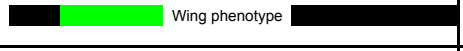
CG3127	Pgk	33798			
		110081			
CG31349	pyd	38863			
		104159			
CG3140	Adk2	25046	Wing phenotype		
		107326	Wing phenotype		
CG31421	Takl1	25760			
		110765			
CG31643		31643R-1			
		31643R-2			
CG3172	twf	25817			
CG31751		21415			
		110319			
CG31873		105621			
		36375			
CG32019	bt	46252			
		46253			
CG32031	Argk	34036			
		34037			
CG32134	btl	27106			
		110277			
CG3216		29915			
		107985			
CG32417	Myt1	34547			
		105157			
CG32484	Sk2	101018			
		41905			
CG32649		26534			
		26536			
CG32666	Drak	107263	Lethal	107263	Wing phenotype
		32961			
		44374		44374	Wing phenotype
		29449		29449	
CG32703		13444			
		109661			
CG32717	sdt	29844			
		100685			
CG32742	l(1)G0148	40716			
		104902			
CG32743	nonC	41990			
		108450			
CG3277		7271			
		108052			
CG32849	Hex-t2	47331			
		100218			
CG32944		29286			
		103642			
CG33102	Hex-t1	46573			
		46574			
CG33114	Gyc32E	108142			
CG3319	Cdk7	10442	Wing phenotype		
		103413	Wing phenotype		
CG3324	Pkg21D	34594			
		103513			
CG33338	p38c	105173			
CG33519	Unc-89	29412			
		106267	Wing phenotype		
CG33531	Ddr	52126			
		101831			
CG33554	Nipped-A	44781	Wing phenotype	44781	Lethal
		52487	Lethal	52487	Wing phenotype
		52486	Lethal		
		2905R-3	Wing phenotype		
CG33671		49772			

CG33981		49773			
CG3400	Pfrx	25958 25959			
CG34344	rdgA	102909 3024			
CG34356		5201 Wing phenotype 28745			
CG34357		109790 Wing phenotype 105185 31983			
CG34359	IP3K2	19159 102772 Wing phenotype	1630R-2 Wing phenotype	102772	19159 Wing phenotype 1630R-2 Wing phenotype 102772 Wing phenotype
CG34361	Dgk	38239 Wing phenotype/Lethal 105753 29459			
CG34380		39447 39446			
CG34384		102481 43739			
CG34392	Epac	110077 Wing phenotype 50373			
CG34412	tlk	105732 Wing phenotype/Lethal 20905 Lethal 2829R-1 Lethal 33983 Lethal		105732 Wing phenotype	105732 Wing phenotype 20905 Lethal
CG3510	CycB	43772 109611			
CG3525	eas	103784 Wing phenotype 34286			
CG3534		109666 41276			
CG3544		21685 105459			
CG3608		106695 3608R-2			
CG3682	PIP5K59B	47027 108104			
CG3738	Cks30A	108401			
CG3809		108975 43780			
CG3837		44576 Wing phenotype 105549			
CG3915	Drl-2	40484 102192			
CG3938	CycE	52662 110204 Wing phenotype			
CG4006	Akt1	2902 Wing phenotype 103703			
CG4007	Nrk	103804 42442			
CG4012	gek	107207 28367			
CG40129	Gprk1	28354			
CG4026	IP3K1	31733			
CG40293	Stlk	27305 34620			
CG4032	Abl	2897 110186			
CG4041		108887 34780			


CG40478 Dyrk3
CG4132 pkaap
21792
106808
Wing phenotype
CG4141 Pi3K92E
38985
107390
Wing phenotype
CG4201 ird5
26427
CG42273 mnb
28628
107066
Wing phenotype
CG42317 Csk
109813
32877
Lethal
CG42320 Doa
20120
102520
Wing phenotype
CG42341 Pka-R1
103720
26329
CG42347
101640
44737
CG42349 Pkcδ
33838
101421
CG42366
108102
Wing phenotype
39904
Lethal
36112
CG42403 Ca-β
27581
105748
CG4252 mei-41
103624
11251
CG42636 Gyc76c
106525
27094
CG4268 PitsIre
107303
45127
Wing phenotype
107303
Lethal
107303
Wing phenotype
45127
Lethal
CG42783 aPKC
2907
105624
Wing phenotype
Wing phenotype
34332
2907
Lethal
105624
Wing phenotype
2907
Lethal
105624
Wing phenotype
CG42856 Sik3
39866
107458
CG4290 Sik2
103739
Wing phenotype
26497
CG43143
16334
106088
CG4353 hep
109277
47509
CG43729
35848
35850
CG43741 Ack-like
26065
100169
3969R-2
CG4379 Pka-C1
31599
Wing phenotype
101524
Wing phenotype
CG4488 wee
106329
26543
CG4523 Pink1
109614
21860
CG4527 slik
43783
Wing phenotype
43784
Wing phenotype
CG4546
34869
34868
CG4551 smi35A
40535
101376
4551R-1
4551R-3
40535
4551R-1
101376
101376
4551R-1
CG4583 Ire1
39561
39562
CG4629
26574
26573
CG4720 Pk92B
110228

CG4720	Pk92B	34892								
CG4798	l(2)k01209	106934								
		26628								
CG4803	Takl2	104701								
		34898								
CG4839		100999								
		26642		Wing phenotype						
CG4926	Ror	932								
		935								
CG4945		24683								
CG5072	Cdk4	40577		Wing phenotype						
		40576								
CG5092	Tor	5092R-1		Wing phenotype						
		5092R-2		Wing phenotype						
		34639		Wing phenotype						
CG5125	ninaC	110702								
		27360								
CG5144		30946								
		104240		Wing phenotype						
CG5169	GckIII	22024		Wing phenotype/Lethal						
		107158		Wing phenotype						
CG5179	Cdk9	30449		Lethal			30449	Wing phenotype		30449 lethal
		103561		Wing phenotype						103561 lethal
CG5182	Pk34A	101146								
		27368								
CG5288		24440								
		103656								
CG5310	nmdyn-D6	39402								
		110565		Wing phenotype						
CG5363	cdc2	106130		Lethal			106130	Wing phenotype		106130 Wing phenotype
		41839		Wing phenotype						41839 Lethal
CG5373	Pi3K59F	100296		Wing phenotype						
		5373R-2		Wing phenotype						
		33384								
CG5387	Cdk5α	34990		Wing phenotype						
		108656		Wing phenotype						
CG5408	trbl	22113								
		106774								
CG5452	dnk	39137		Wing phenotype						
		103385								
CG5475	Mpk2	102484								
		52277								
CG5483	Lrrk	105630								
		22140								
CG5626		27469								
		5626R-1		Wing phenotype						
CG5680	bsk	104569								
		34138		Wing phenotype						
CG5725	fbl	44157		Wing phenotype						
		101437								
CG5757		110460								
		27504								
CG5790		110683								
		45045								
CG5940	CycA	32421		Wing phenotype			32421	Wing phenotype		32421 Wing phenotype
										5940R-4 Wing phenotype
		103595		Wing phenotype/Lethal						103595 Wing phenotype
CG5974	pll	103774								
		2889								
CG6027	cdi	109409								
		43634								
CG6092	Dak1	44165								
		104861								

CG6114	sff	22224									
		100717									
		6117R-1									
CG6117	Pka-C3	6117R-2									
		27569									
CG6214	MRP	105419									
		6214R-3									
CG6292	CycT	37562		Lethal						37562	Wing phenotype/Lethal
		103387		Wing phenotype			103387		Wing phenotype	103387	Wing phenotype
		6292R-3									
CG6297	JIL-1	107001									
		6297R-4									
CG6343	ND42	14444		Wing phenotype							
		110787		Wing phenotype							
CG6355	fab1	27591		Wing phenotype							
		27592									
CG6364		11693		Wing phenotype						11693	Wing phenotype
		108949		Wing phenotype			108494		Wing phenotype	108949	Wing phenotype
CG6386	ball	48980									
		108630		Wing phenotype							
CG6498		109282									
		35101									
CG6509		101596		Wing phenotype							
		46234		Wing phenotype							
CG6518	inaC	101719									
		2895									
CG6521	Stam	22497		Wing phenotype							
		35016		Wing phenotype							
CG6535	tefu	22502									
		108074									
CG6551	fu	27662		Wing phenotype							
		27663		Wing phenotype							
CG6612	Adk3	110382									
		42064									
CG6620	ial	104051		Wing phenotype						104051	Wing phenotype
		35107		Lethal			35107		Wing phenotype	35107	Wing phenotype
		6620R-3		Wing phenotype/Lethal							
CG6622	Pkc53E	27696									
		27699									
CG6703	CASK	104793									
		34184									
CG6715	KP78a	26722		Wing phenotype							
		47657									
CG6767		35111									
		35112			35112					35112	
		109894		Wing phenotype			109894		Wing phenotype	109894	Lethal
		6767R-1		Wing phenotype							
CG6772	Slob	100987									
		30674									
CG6775	rg	107056									
		36404									
CG6800		40394									
		104255									
CG6875	asp	2911		Wing phenotype							
		110177									
CG6963	gish	106826		Wing phenotype	106826		Wing phenotype	106826			106826		Wing phenotype
		26003		Wing phenotype	26003		Wing phenotype				26003		Wing phenotype
		28066		Wing phenotype									
CG7001	Pk17E	101951									
		102179		Wing phenotype							
CG7004	fwd	27786									
		110159									
		27808		Wing phenotype			27808		Wing phenotype	27808	Lethal

CG7028	PRP4	107042		Wing phenotype					107042		Wing phenotype/Lethal
CG7069		101116									
		27834									
CG7070	PyK	49533									
		35165		Wing phenotype							
CG7094		27843									
		108273									
CG7097	hppy	103580									
		35166									
CG7103	Pvf1	6175									
		102699									
CG7125	PKD	22344		Wing phenotype							
		106255		Wing phenotype							
CG7156		26035									
		26036									
CG7177	Wnk	35193			35193					35193	
		35194			35194		Wing phenotype	35194		35194	
		106928		Wing phenotype	106928		Lethal		Wing phenotype	106928	Wing phenotype
CG7186	SAK	27904									
		105102									
CG7207	cert	103563									
		27914									
CG7223	htl	27180									
		6692									
CG7236		27505									
CG7281	CycC	27937		Wing phenotype							
		48834		Wing phenotype							
CG7328		110610									
		27952									
		7328R-1									
CG7335		29035									
		100062									
CG7362		104218									
		7557									
CG7393	p38b	108099									
		7393R-3									
CG7405	CycH	10398									
		104312		Wing phenotype							
CG7470		101476									
		38955									
CG7524	Src64B	35252									
		7524R-3									
CG7525	Tie	27087									
		26879									
CG7551		7551R-1									
		7551R-3									
CG7597	Cdk12	25508		Lethal				25508		25508	
					34838		Lethal		Wing phenotype		Wing phenotype/Lethal
		25510		Wing phenotype						34838	
										25510	Wing phenotype
CG7616		41408									Lethal
		104663									
CG7643	ald	110572									
		7643R-3		Wing phenotype							
CG7693	fray	106919									
		41719									
CG7717	Mekk1	110339									
		25529									
CG7719	gwl	21046									
		7719R-1		Wing phenotype							
CG7766		52572									
		110184		Wing phenotype							
CG7838	BubR1	26109									
		7838R-1		Wing phenotype							

CG7873	Src42A	26019	Wing phenotype								
		100708	Wing phenotype								
CG7892	nmo	3002									
		101545									
		25793									
CG7904	put	37279	Lethal			37279	Wing phenotype			37279	Wing phenotpye
		107071									
		7904R-2	Lethal							7904R-2	Wing phenotype
		7904R-3	Lethal							7904R-3	Wing phenotype
CG7995		101869									
		22652									
CG8049	Btk29A	106962									
		22675									
CG8057	alc	104489									
		8057R-4									
CG8094	Hex-C	35338									
		35337									
CG8173		35846	Wing phenotype								
		105661									
CG8174	SRPK	26933									
		103416	Wing phenotype								
CG8201	par-1	52553		52553							
		52556		52556		52556				52556	
		32410	Wing phenotype	32410	Lethal					32410	Wing phenotype
CG8203	Cdk5	104491									
		35856									
CG8222	Pvr	43459									
		105353									
CG8224	babo	3825									
		106092	Wing phenotype								
CG8239		24254									
		107034									
CG8250	Alk	107083									
		11446									
CG8286	P58IPK	109649									
		14154									
CG8298		43541									
		43539									
		8298R-3									
CG8351	Tcp-1η	108585	Wing phenotpye								
		28895									
CG8362	nmdyn-D7	35903									
		105161									
CG8363	Papss	35904									
		110544									
CG8475		2801	Wing phenotype								
		110591									
CG8485		35939	Wing phenotpye			35939	Wing phenotype			35939	Wing phenotype
		35940		35940						35940	
CG8565		100449									
		8565R-2									
CG8637	trc	35988	Wing phenotype								
		107923	Wing phenotype								
CG8657	Dgkε	4659	Wing phenotype								
		8657R-2									
CG8726		109451									
		40719									
CG8767	mos	110435									
		43526									
CG8789	wnd	103410									
		26910									
CG8808	Pdk	106641									
		37968									
CG8866		103725									

CG8874	Fps85D	44859 36053 107266			
CG8878		28971 Wing phenotype 100985 Wing phenotype		100985 Wing phenotype	28971 Wing phenotype 100985 Wing phenotype
CG8914	CK11β2	102633 26915			
CG8948	Graf	42165 42166			
CG8967	otk	42566 30834 104688 8967R-2	42566 30834 104688 Wing phenotype 8967R-2	104688	30834 104688 Wing phenotype
CG9096	CycD	29024			
CG9222		105361 104259			
CG9326	vari	27010 104548 Wing phenotype			
CG9358	Phk-3	24157 106938			
CG9374	lkb1	49008 34362 108356 Wing phenotype			
CG9541		49212 102912			
CG9738	Mkk4	108561 26929			
CG9746	ird1	9746R-1 Wing phenotype 110706 Wing phenotype			
CG9774	rok	3793 Wing phenotype 104675 Wing phenotype			
CG9961		101702 36176			
CG9962		108721 36473			
CG9985	sktl	6229 Wing phenotype 101624 Wing phenotype			

Weak-Medium Decrease -0.2 to -0.5
Medium-Strong Decrease > -0.5
Weak-Medium Increase +0.2 to +0.5
Medium-Strong Increase > +0.5
No change

Transgenic RNAi Project strains are in blue font
National Institute of Genetics strains have the letter R in the stock number
All other strains are from Vienna Drosophila RNAi Center

Fig. S5

Phosphatase Data

Weak-Medium Decrease -0.2 to -0.5
Medium-Strong Decrease > -0.5
Weak-Medium Increase +0.2 to +0.5
Medium-Strong Increase > +0.5
No change

Wing phenotype any visible wing phenotype including notches, blisters, vein spacing, altered size
Lethal No adults recovered

Drosophila Phosphatase			PRIMARY SCREEN							SECONDARY SCREEN					HEDGEHOG SCREEN			
Annotation Symbol	Gene Symbol	Gene Name	RNAi Stock	UAS-dcr2;;dpp-Gal4				RNAi Stock	UAS-dcr2;hh-Gal4				RNAi Stock	UAS-dcr2;C5-Gal4		UAS-dcr2; dpp-Gal4 or UAS-dcr2; hh-Gal4	RNAi Stock	MS1096-Gal4;UAS-dcr2	
				Wing disc			Adult		Wing disc			Adult		Wing disc		Wing disc		Wing disc	Adult
				Wg	Sens	Dll			Wg	Sens	Dll			Wg	Sens	Dll	Adult	Cut		Ci	Ptc	
CG10089			17991																			
			108744																			
CG10138	PpD5	Protein phosphatase D5	18016				Wing phenotype															
			104452				Wing phenotype															
CG10371	Plip	PTEN-like phosphatase	47624				Wing phenotype															
			104774				Wing phenotype															
CG10376			101335				Wing phenotype															
			35475				Wing phenotype															
CG10417			27259																			
			106180																			
CG10426			16048																			
			34037																			
CG10443	Lar	Leukocyte-antigen-related-like	107996																			
			36270																			
			36269																			
CG10493	Phlpp	PH domain leucine-rich repeat protein phosphatase	110360																			
			45365																			
CG10574	I-2	Inhibitor-2	101547				Wing phenotype						101547					101547			Wing phenotype	
			39054				Wing phenotype											39054			Wing phenotype	
CG10592			104767																			
			38171																			
CG10827			30822																			
			100073																			
CG10930	PpY-55A	Protein phosphatase Y at 55A	102021																			
			16096																			
CG10975	Ptp69D	Protein tyrosine phosphatase 69D	104761																			
			40631																			
CG11217	CanB2	Calcineurin B2	104370																			
			28762																			
CG11425			103037																			
			8458																			
CG11426			42599																			
			42600																			
CG11437			9452				Wing phenotype															

CG11437			31721																
CG11438			11438R-3																
			11438R-2																
CG11440	laza	lazaro	42594																
			109898																
CG11516	Ptp99A	Protein tyrosine phosphatase 99A	103457																
			27208																
CG11597			38540																
			104729																
CG11883			106744					106744			Wing phenotype					106744			Wing phenotype
			38590			Wing phenotype						38590				38590			Wing phenotype
CG12034			107062																
			12034R-1																
CG12078			101274																
			15288																
CG12091			100800																
			13987																
CG12151	Pdp	Pyruvate dehydrogenase phosphatase	31661																
			107271																
CG12169	Ppm1	Ppm1	38631																
			101257																
CG12173			31676																
			31674																
CG12217	PpV	Protein phosphatase V	101997			Wing phenotype						101997				101997			Wing phenotype
			31690			Wing phenotype										31690			Wing phenotype
CG12252	Fcp1		38640			Wing phenotype										38640			Lethal
			106253			Wing phenotype						106253				106253			Wing phenotype
CG1228	Ptpmeg	Ptpmeg	38652																
			103740																
CG12538			102895																
			31957																
CG12746			104989																
			33314																
CG13125	TbCMF46	TbCMF47	17123																
CG13197			36423																
			105122			Wing phenotype													
CG13311			51574																
			101655			Wing phenotype													
CG13570	spag	spaghetti	103353																
			23895																
CG1395	stg	string	17760					17760			Wing phenotype	17760			Wing phenotype	17760			Wing phenotype
			1395R-1			Wing phenotype													
			1395R-2																
			34831					34831			Lethal					34831			Wing phenotype
CG14022			110777																
			31795																
		Mitogen-activated	45415																

CG14080	Mkp3	activated protein kinase phosphatase	23911								
CG14211	MKP-4	MAPK Phosphatase 4	104884 3146 3147 14211R-4		Wing phenotype Lethal Wing phenotype		104884			104884 3147	 Wing phenotype Wing phenotype
CG14297			102071 14297R-4								
CG14411			17579 109622		Wing phenotype						
CG1455	CanA1	Calcineurin A1	32283 32284								
CG14616	l(1)G0196	lethal (1) G0196	106973								
CG1462	Aph-4	Alkaline phosphatase 4	6862 110043								
CG14719	l-t	inhibitor-t	105565 32314								
CG14903			49108 105752								
CG15035			100241 32353								
CG15385			51859 110458								
CG15400			7261 15400R-2								
CG15528			14865 105484								
CG15533			42520 102842								
CG15534			108181 30187								
CG15743			42686 42685		Wing phenotype						
CG1637			101368 33733		Wing phenotype						
CG16717			29199 105831								
CG16757	Spn	Spinophilin	19658 105888								
CG16771			6386 16771R-2								
CG16870	Acyp	Acylphosphatase	16870R-2 16870R-1								
CG16928	mre11	meiotic recombination 11	30474 30476								
CG1696	Dd	Dullard	104785 12941								
CG17026			32813 49199								
CG17027			103270								

CG17027			50075																
CG17028			100100																
			32819																
CG17029			32823																
			49565																
CG17124			19078																
			17124R-3																
CG17291	Pp2A-29B	Protein phosphatase 2A at 29B	49672		Lethal							49672		Wing phenotype		49672		Lethal	
			23886		Wing phenotype											23886		Wing phenotype	
CG17598			32956		Wing phenotype														
			32955																
CG17746			100178																
			40102																
CG1809			43231																
			1809R-1																
CG1810	mRNA-cap	mRNA-capping enzyme	108809		Wing phenotype							108809		Wing phenotype		108809		Wing phenotype/ Lethal	
			32847		Wing phenotype	32847		Lethal								32847		Wing phenotype	
CG1817	Ptp10D	Protein tyrosine phosphatase 10D	1102																
			8010																
CG18243	Ptp52F	Ptp52F	39175																
			3116																
CG1906	alph	alphabet	105483																
			32476																
CG2096	flw	flapwing	29622			29622										29622			
			104677		Wing phenotype	104677		Wing phenotype				104677		Wing phenotype		104677		Wing phenotype	
			15305R-1			15305R-1													
CG2104			30018																
			100216																
CG2890	PPP4R2r	Protein phosphatase 4 regulatory subunit 2-related protein	105399		Wing phenotype											105399		Wing phenotype	
			25445		Wing phenotype							25445				25445		Wing phenotype/ Lethal	
CG2984	Pp2C1	Protein phosphatase 2C	105249			105249		Wing phenotype				105249				105249		Wing phenotype	
			2984R-4													2984R-4		Wing phenotype	
						2984R-3		Wing phenotype											
CG30103			105458																
			4950																
CG30104			10051																
			106640																
CG3028	Ipp	Inositol polyphosphate 1-phosphatase	110775																
			25615																
CG3059	NTPase	NTPase	110510																
			7265																
CG31137	twin	twin	13365																

CG31137	twin	twin	104442																				
CG31299	cu	curled	45442																				
			109759																				
CG31469			102474																				
			23397																				
CG31692	fbp	fructose-1,6-bisphosphatase	108554																				
			21396																				
CG31717			7663			Lethal						7663			Wing phenotype				7663			Wing phenotype	
			7662			Wing phenotype/Lethal													7662			Wing phenotype	
			105379				105379			Wing phenotype									105379			Wing phenotype	
			31717R-1				31717R-1												31717R-1				
																			31717R-3				
CG31759			106772																				
			47321																				
CG3178	Rrp1	Recombination repair protein 1	108661																				
			19819																				
CG31795	IA-2	IA-2 ortholog	7560																				
			110595																				
CG32156	Mbs	Myosin binding subunit	105762			Wing phenotype						105762			Wing phenotype				105762			Wing phenotype	
			32516			Wing phenotype	32516												32516			Wing phenotype	
CG3245	PpN-58A	Protein phosphatase N at 58A	41901																				
			102060																				
CG32487			26154																				
			2077R-1																				
CG32488			46570																				
			100129																				
CG32505	Pp4-19C	Protein phosphatase 19C	25317			Wing phenotype						25317							25317			Lethal	
			27726			Lethal	38372			Lethal									27726			Wing phenotype	
CG32568			103317																				
			46651																				
CG3264			43250																				
			108783																				
CG32697	l(1)G0232	lethal (1) G0232	104427																104427			Wing phenotype	
			21276				21276												21276			Wing phenotype	
							3101R-1			Lethal		3101R-1											
							33744			Lethal													
CG32812			45397																				
			104081																				
CG3289	Ptpa	Phosphotyrosyl phosphatase activator	41913																				
			41912																				
CG3290			104586																				
			52378																				
CG3292			19989																				
			3292R-2																				
CG33747	primo-2	primo-2	23081			Wing phenotype													23081			Wing phenotype/ Lethal	
			23079			Wing phenotype	23079			Lethal									23079			Lethal	
			39004				39004												39004				
							50642			Lethal		50642											


CG33748 primo-1 primo-1
CG3376
CG34099 Mkp MAP kinase-specific phosphatase
CG34140
12226
12227
104374
23452
23454
Wing phenotype
CG3530
110786
26217
26216
Wing phenotype
110786
Wing phenotype
26216
Wing phenotype
110786
Wing phenotype
26216
Wing phenotype
CG3573 Ocrl Oculocerebrorenal syndrome of Lowe ortholog
CG3632
CG3705 aay astray
34649
110796
110167
26254
23179
110661
CG3954 csw corkscrew
108352
21757
Wing phenotype
Wing phenotype
21757
Wing phenotype
108352
Wing phenotype
21757
Wing phenotype
CG3980 Cep97
CG40448 Pp1-Y2 Pp1-Y2
CG4123 Mipp1 Multiple inositol polyphosphate phosphatase 1
CG41534 Pp1-Y1 Pp1-Y1
CG4209 CanB Calcineurin B
CG42249
CG42271
34774
103357
109147
101634
8493
110123
21607
21611
52390
101200
47397
41672
100176
Wing phenotype
CG42283 5PtaseI 5PtaseI
33768
33769
25030
100802
Wing phenotype
Wing phenotype
33768
33769
Lethal
Lethal
100802
Wing phenotype/ Lethal
33769
33768
Wing phenotype
100802
Wing phenotype
CG42327
CG4317 Mipp2 Multiple inositol polyphosphate phosphatase 2
106630
47455
14163
108018
CG4733 PR72 PR72
107621
34894
107621
34894
Wing phenotype
34894
Wing phenotype
107621
Wing phenotype
34894
Wing phenotype
CG4827 veil veil
CG4965 twe twine
49359
100050
46064
104147

CG4993	PRL-1	PRL-2	107836																				
			45518																				
CG5026			105674																				
			34916																				
CG5150			102646																				
			15298																				
CG5171			108686																				
			5171R-3																				
CG5177			27367																				
			103024				Wing phenotype																
			5177R-1																				
			5177R-2																				
CG5276			47995																				
			106691																				
CG5361			51984				Wing phenotype																
			51985																				
CG5567			44319																				
			106981																				
CG5577			22163																				
			5577R-1																				
CG5643	wdb	widerborst	27470					27470			Wing phenotype							27470			Wing phenotype		
			101406					101406			Wing phenotype/ Lethal	101406						101406			Wing phenotype		
			5643R-3					5643R-3										5643R-3					
CG5650	Pp1-87B	Protein phosphatase 1 at 87B	35024				Wing phenotype/ Lethal											35024			Lethal		
			35025				Wing phenotype											35025					
			32414				Wing phenotype					32414			Wing phenotype			32414			Lethal		
CG5656			110733																				
			18119																				
CG5671	Pten	Pten	101475				Wing phenotype																
			35731																				
CG5784	Mapmoduli n	Mapmodulin	100283																				
			49385																				
CG5820	Gp150		36301				Wing phenotype					36301						36301			Wing phenotype		
			100134				Wing phenotype											100134			Wing phenotype		
CG5830			101539																				
			5830R-2																				
CG5851	sds22	sds22	42051				Lethal					42051						42051			Wing phenotype		
			5851R-1				Wing phenotype/Lethal											5851R-1			Wing phenotype		
CG6036			21023																				
			105568																				
CG6235	tws	twins	104167				Wing phenotype					104167			Wing phenotype			104167			Wing phenotype		
																		6235R-2			Wing phenotype		
			34340				Wing phenotype											34340			Wing phenotype		
CG6238	ssh	slingshot	107998																				
			30136				Wing phenotype																
CG6380			29950																				
			100121																				
CG6542	EDTP	Egg-derived tyrosine phosphatase	6542R-1				Wing phenotype																
			6542R-2																				
			46070					46070															
			34378					34378										34378			Wing phenotype		

CG6562	synj	synaptojanin	6562R-1		Wing phenotype/ Lethal	27489			6562R-1		Wing phenotype		6562R-1		Wing phenotype
			6562R-2		Wing phenotype/ Lethal										
CG6571	rdgC	retinal degeneration C	35105												
			6571R-2												
CG6593	Pp1α-96A	Protein phosphatase 1α at 96A	27673		Lethal				27673		Wing phenotype		27673		Lethal
			105525		Lethal								105525		Lethal
CG6656			104175												
			1633												
CG6746			46513		Wing phenotype										
			103625												
CG6805			107728												
			34615												
CG6896	MYPT-75D	MYPT-75D	109909		Wing phenotype				109909				109909		Wing phenotype
			6896R-1		Wing phenotype								6896R-1		Wing phenotype
			34688												
CG6899	Ptp4E	Protein tyrosine phosphatase 4E	1012												
			27232												
CG6939	Sbf	SET domain binding factor	22317												
			32419												
CG7067	NitFhit	Nitrilase and fragile histidine triad fusion protein	27831												
			108545												
CG7109	mts	microtubule star	41924		Wing phenotype/ Lethal	41924		Wing phenotype	41924		Lethal		41924		Lethal
			35171		Wing phenotype	35171							35171		Wing phenotype
			35172												
CG7115			9404		Wing phenotype	9404		Wing phenotype	9404				9404		Wing phenotype
			103354			103354							103354		
			39065			39065							39065		
CG7134	cdc14	cdc14	103627		Wing phenotype										
			7134R-6		Wing phenotype										
CG7180			102397												
			34369												
CG7378			106098												
			35226												
CG7615	fig	fos intronic gene	47314												
			47312												
CG7789			[redacted]												
			3019												
CG7850	puc	puckered	3018		Wing phenotype										
CG7899	Acph-1	Acid phosphatase 1	110470												
			3579		Wing phenotype										
CG7913	Pp2A-B'	Pp2A-B'	22614												
			107057												
CG7942	ldbr	lariat debranching	110582		Wing phenotype										

		enzyme	7942R-4			Wing phenotype																	
CG8105			104510																				
			8037																				
CG8147			2892			Wing phenotype																	
			100052																				
		Protein	107386																				
CG8402	PpD3	phosphatase D3	24308			Wing phenotype							24308						24308			Wing phenotype	
			8402R-1			Wing phenotype													8402R-1			Wing phenotype	
CG8455			47953																				
			8455R-2																				
			108802																				
CG8509			28915			Wing phenotype																	
			8509R-2																				
			107849						107849										107849				
CG8584			26987			Wing phenotype																	
			26988						26988			Wing phenotype	26988						26988			Wing phenotype	
CG8804	wun	wunen	6447																				
			51090																				
			103452						103452			Wing phenotype							103452			Wing phenotype	
			4176			Wing phenotype			4176			Lethal	4176			Wing phenotype							
CG8805	wun2	wunen-2	4177			Wing phenotype													4177			Wing phenotype	
			8805R-1			Wing phenotype			8805R-1			Lethal							8805R-1			Wing phenotype	
			32423						32423														
CG8822	PpD6	Protein phosphatase D6	104211																				
			36047																				
CG8889	Mppe	Metallophosphoesterase	108190			Wing phenotype																	
			8889R-2																				
CG8980	NiPp1	Nuclear inhibitor of Protein phosphatase 1	108859																				
			42175																				
			29032						29032										29032				
			31552																				
CG9115	mtm	myotubularin	9115R-1						9115R-1			Wing phenotype	9115R-1						9115R-1			Wing phenotype	
			9115R-2																				
CG9128	Sac1	Sac1	37216																				
			44376																				
CG9151	acj6	abnormal chemosensory jump 6	9827																				
			105292																				
		Protein	29057			Lethal							29057			Wing phenotype			29057			Wing phenotype	
CG9156	Pp1-13C	phosphatase 1 at 13C							9156R-1			Lethal							9156R-1			Wing phenotype	
			107770																107770				
		Protein	37436																				
CG9181	Ptp61F	tyrosine phosphatase 61F	108888																				
CG9236	Cib2	CIB2 ortholog	101474																				
			39162																				
CG9238			100593																				
			24150																				
CG9267			101546																				
			2879																				
			104860			Wing phenotype													104860			Wing phenotype	
CG9311	mop	myopic	14174			Wing phenotype/ Lethal							14174			Wing phenotype			14174			Wing phenotype	
																			9311R-3			Wing phenotype	

			9311R-4		Lethal														
CG9351	flfl	falafel	24143		Wing phenotype							24143		Wing phenotype				24143		Wing phenotype
			103793		Wing phenotype		103793		Wing phenotype									103793		Wing phenotype
CG9389			44663																	
CG9391			23723																	
			23725																	
CG9449			12895																	
			102991																	
CG9451			109721																	
			14344																	
CG9452			100887																	
			51202																	
CG9493	Pez	Pez	108301				108301											108301		Wing phenotype
			9493R-3		Wing phenotype													9493R-3		Wing phenotype
							9493R-2											9493R-2		Wing phenotype
			40743		Wing phenotype		40743		Lethal			40743		Wing phenotype				40743		Wing phenotype
CG9554	eya	eyes absent	43911		Wing phenotype							43911						43911		Wing phenotype
			108071				108071		Wing phenotype									108071		Wing phenotype
			28733				28733											28733		
CG9601			24070																	
			108251																	
CG9619	Gbs-76A	Glycogen binding subunit 76A	103044																	
			36121																	
CG9764	yrt	yurt	28674																	
			107016																	
CG9784			108075																	
			30098		Wing phenotype															
CG9801			104525																	
CG9819	CanA-14F	Calcineurin A at 14F	109858		Wing phenotype															
			30105																	
CG9842	Pp2B-14D	Protein phosphatase 2B at 14D	46873																	
			103144																	
CG9856	PTP-ER	Protein tyrosine phosphatase-ERK/Enhancer of Ras1	9856R-1																	
			9856R-2																	
			32359																	

Weak-Medium Decrease -0.2 to -0.5
Medium-Strong Decrease > -0.5
Weak-Medium Increase +0.2 to +0.5
Medium-Strong Increase > +0.5
No change

Transgenic RNAi Project strains are in blue font
National Institute of Genetics strains have the letter R in the stock number
All other strains are from Vienna Drosophila RNAi Center

Fig. S6

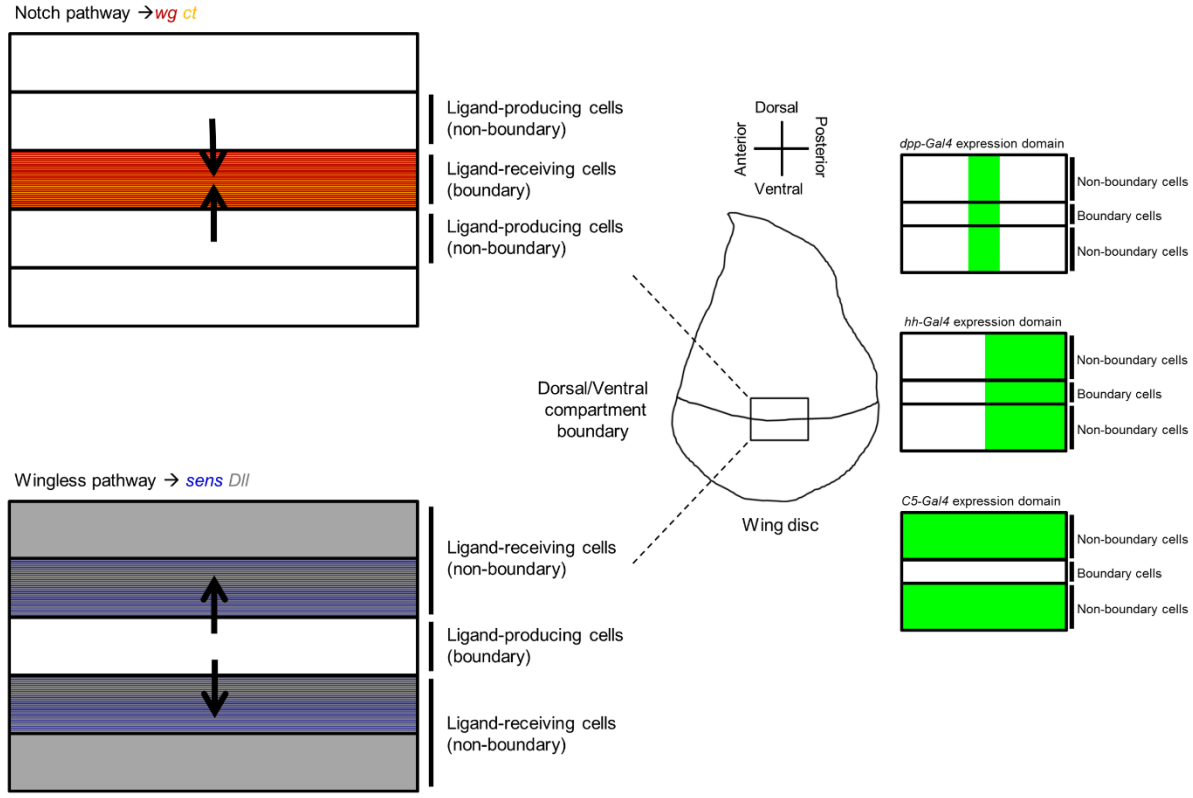

Notch pathway →wg ct
Ligand-producing cells (non-boundary)
Ligand-receiving cells (boundary)
Ligand-producing cells (non-boundary)
Wingless pathway → sens Dll
Ligand-receiving cells (non-boundary)
Ligand-producing cells (boundary)
Ligand-receiving cells (non-boundary)
Dorsal
Anterior
Posterior
Ventral
Dorsal/Ventral compartment boundary
Wing disc
dpp-Gal4 expression domain
Non-boundary cells
Boundary cells
Non-boundary cells
hh-Gal4 expression domain
Non-boundary cells
Boundary cells
Non-boundary cells
C5-Gal4 expression domain
Non-boundary cells
Boundary cells
Non-boundary cells

Fig. S7

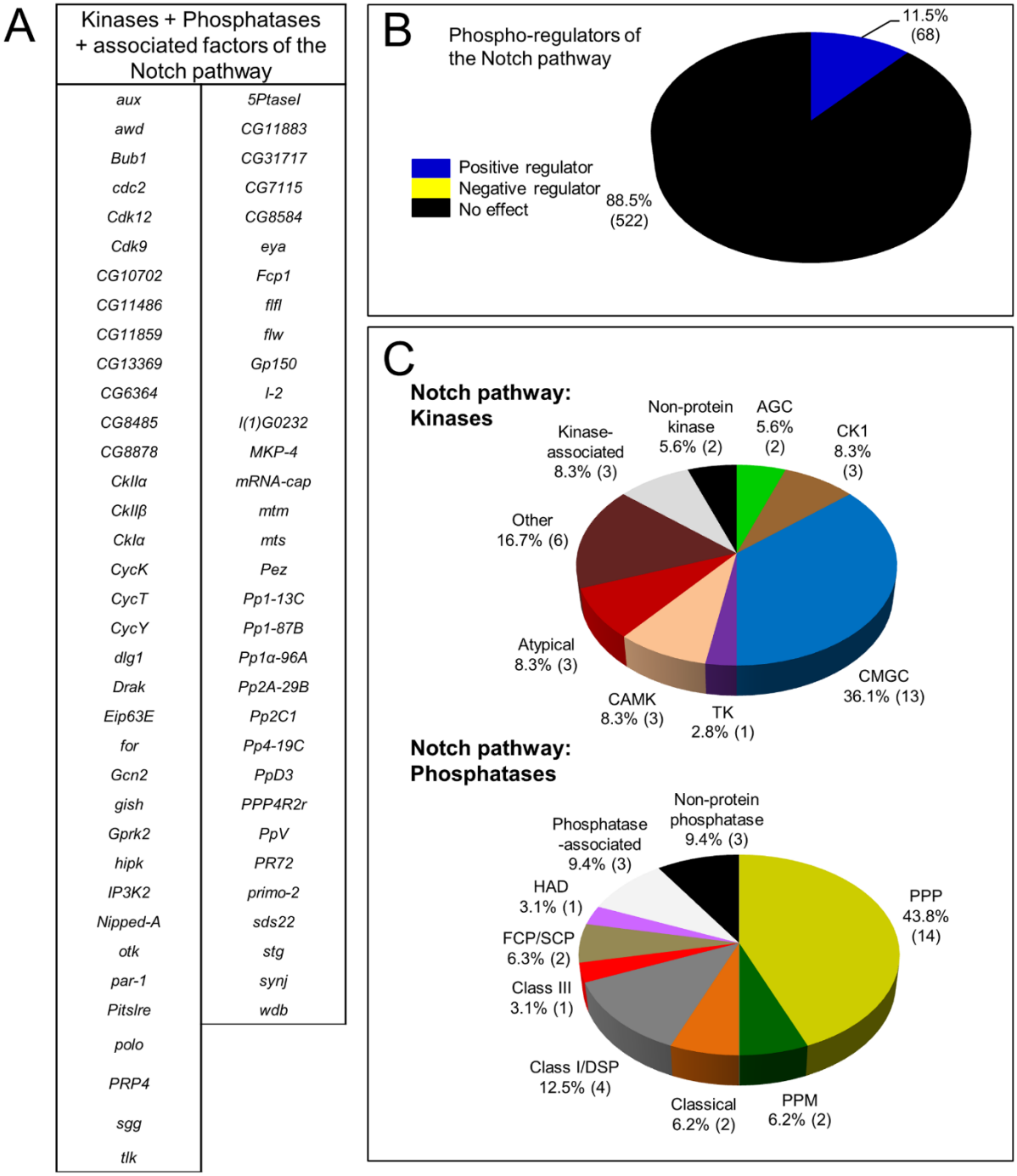

A
Kinases + Phosphatases + associated factors of the Notch pathway
aux
awd
Bub1
cdc2
Cdk12
Cdk9
CG10702
CG11486
CG11859
CG13369
CG6364
CG8485
CG8878
CkIIα
CkIIβ
CkIα
CycK
CycT
CycY
dlg1
Drak
Eip63E
for
Gcn2
gish
Gprk2
hipk
IP3K2
Nipped-A
otk
par-1
Pitslre
polo
PRP4
sgg
tlk
5Ptasel
CG11883
CG31717
CG7115
CG8584
eya
Fcp1
flfl
flw
Gp150
l-2
l(1)G0232
MKP-4
mRNA-cap
mtm
mts
Pez
Pp1-13C
Pp1-87B
Pp1α-96A
Pp2A-29B
Pp2C1
Pp4-19C
PpD3
PPP4R2r
PpV
PR72
primo-2
sds22
stg
synj
wdb
B
Phospho-regulators of the Notch pathway
Positive regulator
Negative regulator
No effect
11.5% (68)
88.5% (522)
C
Notch pathway: Kinases
Non-protein kinase 5.6% (2)
AGC 5.6% (2)
CK1 8.3% (3)
Kinase-associated 8.3% (3)
Other 16.7% (6)
Atypical 8.3% (3)
CAMK 8.3% (3)
TK 2.8% (1)
CMGC 36.1% (13)
Notch pathway: Phosphatases
Non-protein phosphatase 9.4% (3)
Phosphatase -associated 9.4% (3)
HAD 3.1% (1)
FCP/SCP 6.3% (2)
Class III 3.1% (1)
Class I/DSP 12.5% (4)
Classical 6.2% (2)
PPM 6.2% (2)
PPP 43.8% (14)

Fig. S8

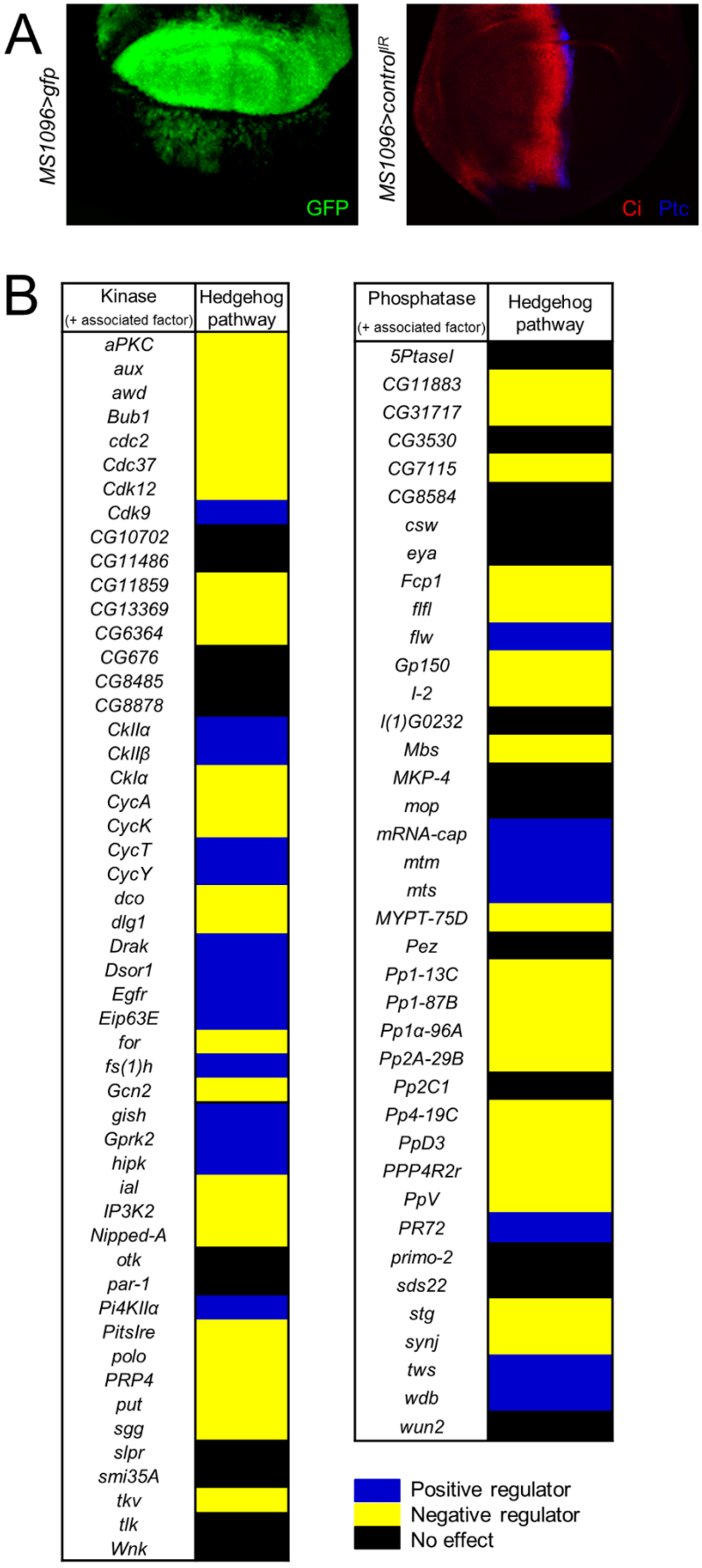
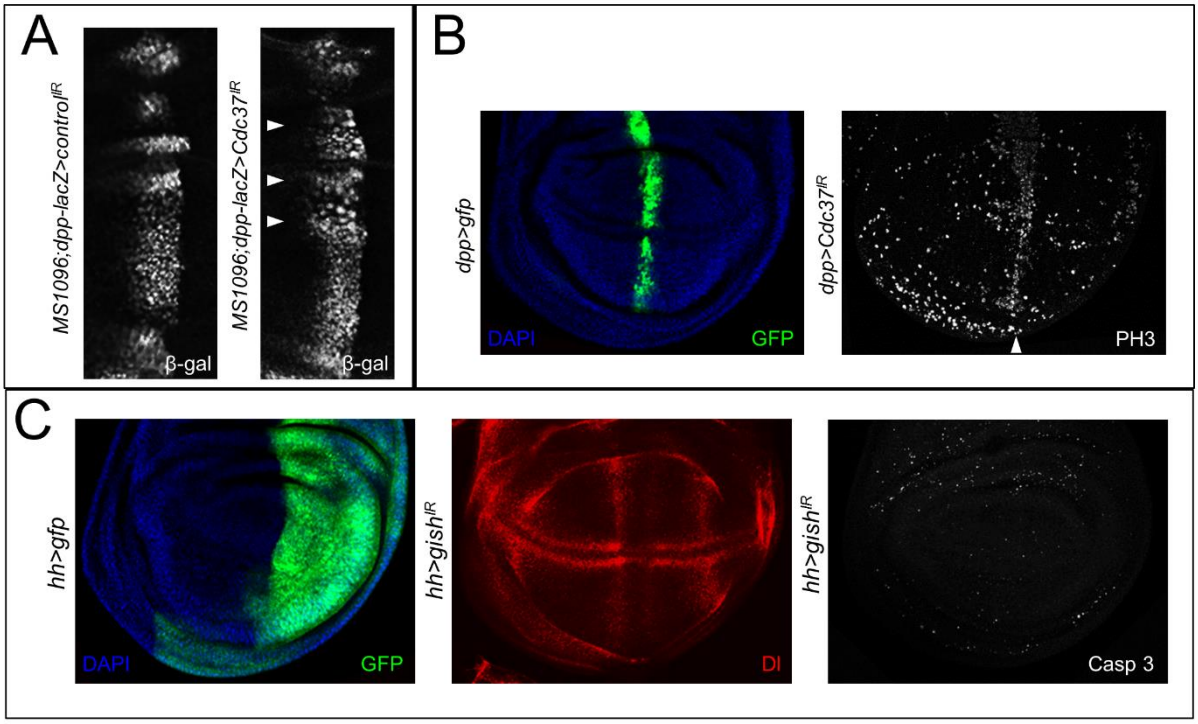

A
MS1096>gfp
GFP
MS1096>control^IR
Ci Ptc
B
Kinase (+ associated factor)
Hedgehog pathway
aPKC
aux
awd
Bub1
cdc2
Cdc37
Cdk12
Cdk9
CG10702
CG11486
CG11859
CG13369
CG6364
CG676
CG8485
CG8878
CkIIα
CkIIβ
CkIα
CycA
CycK
CycT
CycY
dco
dlg1
Drak
Dsor1
Egfr
Eip63E
for
fs(1)h
Gcn2
gish
Gprk2
hipk
ial
IP3K2
Nipped-A
otk
par-1
Pi4KIIα
Pitslre
polo
PRP4
put
sgg
slpr
smi35A
tkv
tlk
Wnk
Phosphatase (+ associated factor)
Hedgehog pathway
5Ptasel
CG11883
CG31717
CG3530
CG7115
CG8584
csw
eya
Fcp1
flfl
flw
Gp150
l-2
l(1)G0232
Mbs
MKP-4
mop
mRNA-cap
mtm
mts
MYPT-75D
Pez
Pp1-13C
Pp1-87B
Pp1α-96A
Pp2A-29B
Pp2C1
Pp4-19C
PpD3
PPP4R2r
PpV
PR72
primo-2
sds22
stg
synj
tws
wdb
wun2
Positive regulator
Negative regulator
No effect

Fig. S9


A
MS1096;dpp-lacZ>control^IR
β-gal
MS1096;dpp-lacZ>Cdc37^IR
β-gal
B
dpp>gfp
DAPI
GFP
dpp>Cdc37^IR
PH3
C
hh>gfp
DAPI
GFP
hh>gish^IR
Dl
hh>gish^IR
Casp 3