A Screen for X-Linked Mutations Affecting *Drosophila* Photoreceptor Differentiation Identifies *Casein Kinase 1α* as an Essential Negative Regulator of Wingless Signaling

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ABSTRACT The Wnt and Hedgehog signaling pathways are essential for normal development and are misregulated in cancer. The casein kinase family of serine/threonine kinases regulates both pathways at multiple levels. However, it has been difficult to determine whether individual members of this family have distinct functions *in vivo*, due to their overlapping substrate specificities. In *Drosophila melanogaster*, photoreceptor differentiation is induced by Hedgehog and inhibited by Wingless, providing a sensitive system in which to identify regulators of each pathway. We used a mosaic genetic screen in the *Drosophila* eye to identify mutations in genes on the X chromosome required for signal transduction. We recovered mutations affecting the transcriptional regulator CREB binding protein, the small GTPase dynamin, the cytoskeletal regulator Actin-related protein 2, and the protein kinase Casein kinase 1α. Consistent with its reported function in the β-Catenin degradation complex, *Casein Kinase 1α* mutant cells accumulate β-Catenin and ectopically induce Wingless target genes. In contrast to previous studies based on RNA interference, we could not detect any effect of the same *Casein Kinase 1α* mutation on Hedgehog signaling. We thus propose that Casein kinase 1α is essential to allow β-Catenin degradation and prevent inappropriate Wingless signaling, but its effects on the Hedgehog pathway are redundant with other Casein kinase 1 family members.

FORWARD genetic screens are a powerful method with which to uncover unanticipated molecular requirements for specific biological processes. Screens for developmental defects in model organisms have identified functions for numerous genes that are conserved throughout evolution and misregulated in human pathologies. Although large-scale RNAi transgenic collections have now made reverse genetic screens possible in *Drosophila* (Dietzl *et al.* 2007; Ni *et al.* 2008; Ni *et al.* 2011), it is difficult to achieve specific and complete loss of gene activity with this method, making it poorly suited to assigning functions to individual members of gene families. Because development of the complex, yet nonessential, *Drosophila* eye relies on most of the major signaling pathways (Doroquez and Rebay 2006; Roignant and Treisman 2009), it provides a sensitive system in which to screen for defects indicative of abnormal signaling.

The adult eye consists of an array of 800 ommatidia, each containing eight photoreceptor neurons (R1–R8), and develops from the larval eye imaginal disc. In the third larval instar, photoreceptor differentiation initiates at the posterior margin of the eye disc and propagates toward the anterior under the control of the morphogen Hedgehog (Hh) (Ready *et al.* 1976; Heberlein *et al.* 1993; Ma *et al.* 1993). Hh secreted by differentiating photoreceptors induces immediately anterior cells to form a transient indentation known as the morphogenetic furrow (MF) (Corrigall *et al.* 2007; Escudero *et al.* 2007), and to express the bone morphogenetic protein (BMP) family member Decapentaplegic (Dpp) (Heberlein *et al.* 1993; Ma *et al.* 1993) and the proneural transcription factor Atonal (Ato) (Jarman *et al.* 1994; Domínguez 1999). Notch (N)-mediated lateral inhibition then contributes to refining Ato expression into single cells that differentiate as R8 photoreceptors (Cagan and Ready 1989; Dokucu *et al.* 1996; Baker and Yu 1997). R8 cells

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secrete Spitz (Spi), a ligand for the epidermal growth factor receptor (EGFR) (Freeman 1994; Tio *et al.* 1994), which induces the stepwise differentiation of neighboring cells into the seven remaining photoreceptors (Tomlinson and Ready 1987; Freeman 1996; Freeman 1997; Dominguez *et al.* 1998). The EGFR-dependent ETS transcription factor Pointed (Pnt) directly activates *hh* expression in these newly recruited photoreceptors (O'Neill *et al.* 1994; Rogers *et al.* 2005), creating an indirect autoregulatory loop between Hh and EGFR signaling that drives the anterior propagation of photoreceptor differentiation (Rogers *et al.* 2005; Roignant and Treisman 2009).

The morphogen Wingless (Wg) promotes head cuticle formation by cells at the margins of the eye disc, excluding retinal differentiation from these regions (Legent and Treisman 2008). Wg prevents ectopic initiation and progression of the MF (Ma and Moses 1995; Treisman and Rubin 1995) by repressing *drumstick*, which encodes an activator of *hh* (Bras-Pereira *et al.* 2006); retinal determination genes such as *eyes absent* (*eya*) (Baonza and Freeman 2002; Kenyon *et al.* 2003); and *dpp* (Heslip *et al.* 1997). Mutations in *wg* or its effector *dishevelled* (*dsh*) result in expansion of the retinal field into head regions (Ma and Moses 1995; Heslip *et al.* 1997). Conversely, loss of negative regulators of the Wg-responsive transcription factor β-Catenin/Armadillo (Arm), such as Axin or glycogen synthase kinase 3 (GSK3) / Shaggy (Sgg), maximally activates the pathway and transforms retinal cells into head cuticle (Heslip *et al.* 1997; Lee and Treisman 2001; Baonza and Freeman 2002).

The Wg and Hh signaling pathways share several common elements, including members of the Casein kinase 1 (CK1) family of serine/threonine protein kinases. Cell culture and RNAi experiments have shown that CK1γ and CK1ε can phosphorylate the Wg co-receptor LRP6/Arrow (Arr) (Davidson *et al.* 2005; Swiatek *et al.* 2006; Zhang *et al.* 2006; Casagolda *et al.* 2010) and the downstream component Dsh (Klein *et al.* 2006; Bernatik *et al.* 2011). CK1α is thought to behave as a priming kinase for Arm, triggering its proteasomal degradation and inhibiting transcriptional output from the pathway (Liu *et al.* 2002; Yanagawa *et al.* 2002; Marin *et al.* 2003). Similarly, phosphorylation of Cubitus interruptus (Ci), the transcription factor downstream of Hh, promotes its processing into a repressor form; both CK1α and CK1ε have been reported to contribute to this (Jia *et al.* 2005). CK1 enzymes also phosphorylate and activate Smoothened (Smo) and Fused (Fu), components of the Hh pathway that act upstream of Ci (Jia *et al.* 2004; Zhou and Kalderon 2011). However, the roles of individual CK1 family members have been difficult to establish with certainty in the absence of specific point mutations (Zhang *et al.* 2006).

We describe here a mosaic genetic screen for X-linked mutations that affect the pattern of photoreceptor differentiation. We used the Flipase-Flipase recognition target (FLP-FRT) technique (Xu and Rubin 1993) with FLP driven by the eye-specific *eyeless* (*ey*) promoter (Newsome *et al.* 2000), as in our previous screen of the autosomes (Janody *et al.* 2004). In the screen, we recovered alleles of genes known to influence N, Wg, and EGFR signaling, including the genes that encode CREB binding protein (CBP) and dynamin. In addition, we identified the first mutant alleles of *Actin Related Protein 2* (*Arp2*) and *Casein Kinase 1α* (*CK1α*). Our results demonstrate that CK1α is an essential negative regulator of Wg signaling, which cannot be replaced by other CK1 family members. In contrast, our mutation in *CK1α* has no effect on the transduction of Hh signaling.

Materials and Methods

Fly stocks and genetics

For the screen, isogenic *yellow (y) white (w) FRT19A* males were mutagenized with 15 mM ethyl methanesulfonate (EMS, Sigma), a concentration determined in a pilot screen to induce one lethal mutation in every five X chromosomes, and mated to *ey-FLP1*, *P[w+, ubiquitin (ubi)-GFP]*, *FRT19A* females. F1 females with eye phenotypes were mated to *FM6 w/Y* males, and stocks were then established over the *FM7i pAct-GFP* balancer. For each mutant female, at least four F3 stocks were established and retested (Figure 1). In the secondary screen, mutant females were mated to *ey-FLP1*, *P[w+, arm-lacZ]*, *FRT19A* males to examine clones in the larval eye disc. Lethal mutations were roughly mapped by crossing to the Bloomington *Drosophila* Stock Center X chromosome duplication (Dp1) kit (2007), and subsequent fine-scale mapping was achieved by recombination with *P[w+]* insertions in the region (Figure 4E).

Other stocks used were *Df(1)EDF7161* P(*w+*) [11A1;11B14] / *Dp(1;Y)BSC1*, *y+* [10C1-2;11D3-8], *aos-lacZ*^{W11}, *E(spl)m8-lacZ*, *ds-lacZ*^{05124}, *Dll-lacZ*^{01092}, *dpp-lacZ*$^{BS3.0}$, *ptc-GAL4*$^{559.1}$, *ptc-lacZ*^{AT90}, *vgBE-lacZ* (Bloomington *Drosophila* Stock Center), *Y hs-hid* (a gift from Ruth Lehmann), *fz3-RFP* (Olson *et al.* 2011), *UAS-dTCF*^{DN} (van de Wetering *et al.* 1997), *UAS-CK1α::Flag*, *UAS-CG2577* (Zhang *et al.* 2006), *UAS-dco*^{KD} (Kinase Domain), *dco*^{3} (Cho *et al.* 2006). Stocks used to generate clones were: (1) *y,w, ey*FLP1, P[*w+, arm-lacZ*], *FRT19A*, (2) *w, hs*FLP^{122}, P[*w+, ubi-GFP*], *FRT19A*, (3) *y,w, hs*FLP^{122}, P[*w+, ubi-RFP*], *FRT19A*, (4) *y,w,* P[*w+, ubi-GFP*], *M(1) RpS5a*^{2}, *FRT19A*; *hs*FLP^{38}, (5) *w, ey*FLP1, *tub-GAL80*, *FRT19A;*; *tub-GAL4*, *UAS-CD8::GFP*, (6) *w, hs*FLP^{122}, *tub-GAL80*, *FRT19A;*; *tub-GAL4*, *UAS-CD8::GFP*.

Immunohistochemistry

Third instar eye and wing imaginal discs were dissected and stained according to (Legent and Treisman 2008). Antibodies used were: rabbit anti-β−galactosidase (1:5000, Cappel), chicken anti-GFP (1:500, Aves), mouse anti-Flag (1:500, Sigma), rabbit anti-Ato (1:5000; Jarman *et al.* 1994), guinea pig anti-Sens (1:1000; Nolo *et al.* 2000) mouse anti-N^{ECD} (1:10), mouse anti-Dl^{ECD} (1:10), mouse anti-Arm (1:10), mouse anti-Smo (1:1000), mouse anti-Ptc

(1:10), mouse anti-En (1:2), rat anti-Ci (1:2), rat anti-Elav (1:100) (Developmental Studies Hybridoma Bank, University of Iowa), rabbit anti-EGFR^{CTER} (1:500; Rodrigues *et al.* 2005), guinea pig anti-dMyc (1:100, a gift from Ginés Morata), and mouse anti-Omb (1:100; Shen *et al.* 2010).

Results

A mosaic screen for X-linked mutations that disrupt photoreceptor differentiation

To identify novel X-linked genes required for the normal pattern of photoreceptor differentiation, regardless of any earlier essential requirement, we conducted a mosaic screen of the X chromosome using the FLP-FRT technique (Figure 1A) (Xu and Rubin 1993). Mutations were induced by EMS treatment in the germline of isogenic males carrying an FRT insertion near the centromere (Figure 1B). In heterozygous daughters, FLP recombinase specifically expressed in the developing eye under the control of the *ey* promoter (Newsome *et al.* 2000) catalyzed chromatid exchange between homologous X chromosomes, resulting in clones of cells homozygous for the mutagenized chromosome arm. The presence of a *P* element marked with *white+* (*w+*) on the wild-type chromosome allowed mutant clones to be recognized in the adult eye by their lack of red pigmentation (Figure 1A). This method enabled us to generate retinal clones homozygous for novel X-linked mutations in the first generation (F1) with high efficiency (Figure 1B).

Adult flies were primarily screened for a lack of *white* clones in the retina and a reduction in eye size, indicating that mutant clones failed to contribute to the adult retina, but persisted long enough to prevent their replacement by wild-type cells through compensatory proliferation (Ryoo *et al.* 2004). This phenotype was often accompanied by disruption of the eye shape (Figure 1C), formation of cuticle scars (Figure 1D), or protrusion of cuticle outgrowths from the retina (Figure 1E). Although most such mutations were homozygous lethal, we also recovered a few hemizygous viable mutants. For instance, loss of the selectin encoded by *furrowed* (*fw*) (Leshko-Lindsay and Corces 1997) results in a reduced and irregularly shaped eye with depressions in its surface (Figure 1F), while mutants for *lozenge* (*lz*), which encodes a transcription factor required for normal differentiation of several retinal cell types (Flores *et al.* 1998), have smooth, glossy eyes (Figure 1G). In addition to mutations causing eye defects, we also recovered mutants with patterning defects of the head capsule, such as triplication of the antennae (Figure 1H).

Since meiotic recombination in the female germline could result in loss of the mutation from the *w* chromosome, we established balanced stocks from the progeny of four individual females lacking the P(w^+) element and identified those carrying the mutation by crossing them back to the *ey*–FLP stock (Figure 1B). To select mutations that disrupted photoreceptor differentiation during larval development, we conducted a secondary screen in third instar eye–antennal discs. Mutant clones lacking the marker *arm-lacZ* (Vincent *et al.* 1994) were examined for aberrations in the pattern of photoreceptors expressing the neuronal nuclear protein Elav (Robinow and White 1991). We did not pursue mutations such as *lz* in which photoreceptor differentiation appeared essentially normal at this stage (Figure 2A) despite their severe phenotype in adults (Figure 1G). We also discarded mutations that strongly impaired cell growth or viability, resulting in very small clones.

A total of 43414 F1 flies were screened, allowing us to identify 490 mutants, of which 79% survived and were fertile. Many mutations were not recovered from the four independent lines established, probably due to germline mosaicism in the mutant mother. A total of 139 mutant stocks were established, 119 of these were retained following the secondary screen (Table 1), and 95% of them were hemi-/homozygous lethal. Complementation tests for lethal mutations on the X chromosome require the presence of a duplication that restores male viability. We therefore screened a collection of duplications covering most of the X chromosome for their ability to rescue males hemizygous for each mutation. Sixty-seven mutant stocks were rescued by at least 1 of the 20 duplications of the Bloomington Dp1 kit (2007), allowing for subsequent fine mapping by meiotic recombination with a set of P(*w+*) markers (Zhai *et al.* 2003), followed by complementation tests with candidate genes in the region (see Figure 4E). These results are summarized in Table 2.

CBP is required for the recruitment of R1–R7

Several of our mutations were in genes already known to act in photoreceptor differentiation. We identified six alleles of *retina aberrant in pattern (rap)/Cdh1*, which encodes a component of the anaphase promoting complex/cyclosome, a multi-subunit E3 ubiquitin ligase (Sigrist and Lehner 1997), on the basis of a partial lack of photoreceptor differentiation in *rap* mutant clones (Figure 2B). Clones mutant for *discs large (dlg1)*, which encodes a neoplastic tumor suppressor and component of the Scribble polarity module (Woods and Bryant 1991), showed overgrowth as well as a complete lack of photoreceptors (Figure 2C). Components of the signaling pathways involved in eye patterning and photoreceptor differentiation were also recovered. We isolated alleles of *N*, *sgg*, and several positive regulators of EGFR/MAPK signaling: *Raf/pole hole*, *MEK/Downstream of raf1*, and *SHP2/corkscrew (csw)* (Table 2). In clones mutant for these components of the EGFR pathway, R8 differentiated but failed to recruit the full complement of photoreceptors (Figure 2D).

We observed a similar phenotype in clones homozygous for a mutation in *nejire* (*nej*); only one Elav-positive photoreceptor was present in most mutant ommatidia (Figure 2E), suggesting a defect in EGFR signaling. We used *argos* (*aos*)*-lacZ*, a direct transcriptional target of the EGFR pathway in R1–R7 (Schweitzer *et al.* 1995; Golembo *et al.* 1996),

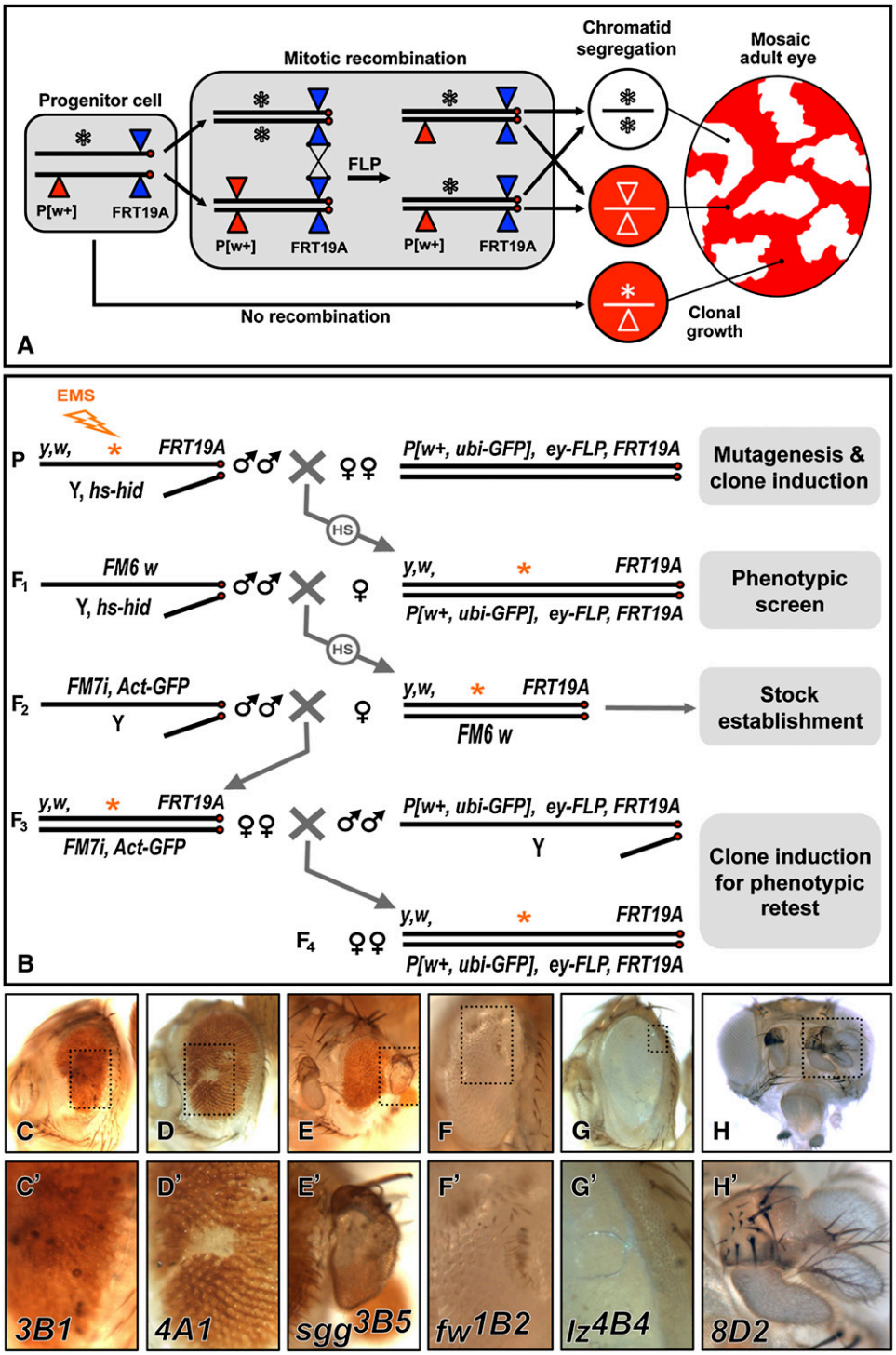


Figure 1 Design and results of the screen. (A) During larval development of flies heterozygous for an EMS-induced mutation (*), *ey*-FLP induces mitotic recombination between FRT sites on duplicated homologous chromosomes. Chromatid segregation at mitosis can produce daughter cells homozygous for the mutation (*/*). In the adult eye, a P*(w+)* element on the wild-type chromosome allows homozygous mutant clones to be recognized by their lack of red pigment. (B) EMS-mutagenized FRT19A males were crossed to *P[w+, ubi-GFP], ey-FLP, FRT19A* females. Induction of the cell death gene *head involution defective (hid)* present on the Y chromosome by heat shock (HS) ensured that the F1 daughters screened were virgins. Selected females were mated to *FM6*, *w*, and then *FM7i*, *pAct-GFP* marked balancers to establish F3 mutant stocks. Four independent stocks for each mutation were retested by crossing to the *ey*-FLP line (F4). (C–H) Adult eyes showing mutant phenotypes. Ommatidia fail to differentiate in *3B1* clones (C), and transform into scars in *4A1* clones (D) or cuticle outgrowths in sgg^{3B5} clones (E). Males hemizygous for fw^{1B2} display reduced, bumpy eyes (F), while the retina of lz^{4B4} males is smooth and glossy (G). Ectopic antennae form in *8D2* mutant clones (H).

as a reporter for EGFR signaling. *aos-lacZ* was not expressed in nej^{8B27} mutant clones (Figure 2F), despite the presence of cells expressing Senseless (Sens), a marker for R8 (Figure 2G). *nej* encodes the histone acetyltransferase CBP/p300 (Akimaru *et al.* 1997), a transcriptional coactivator and scaffolding protein that links DNA-binding factors and the basal transcriptional machinery to signaling cascades. Our findings are consistent with genetic interactions previously observed between EGFR components and *nej* in adult eyes (Anderson *et al.* 2005), the persistence of Ato expression in *nej* clones (Kumar *et al.* 2004), and the requirement for CBP in MAPK-dependent activation of Ets transcription factors in mouse cell culture (Foulds *et al.* 2004).

Loss of dynamin has opposite effects on Notch and EGFR signaling

We mapped the mutation *7C7* to *shibire* (*shi*), which encodes dynamin, a GTPase required to pinch off endocytic

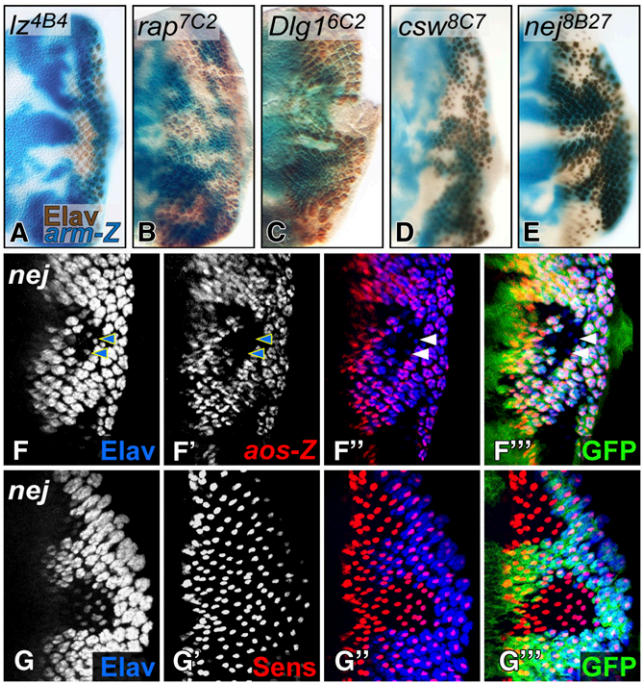


Figure 2 Isolation of mutations in genes known to affect photoreceptor differentiation. (A–E) Photoreceptors are stained with anti-Elav (brown) in third instar eye discs carrying mutant clones marked by the absence of *arm-lacZ* staining (blue). *lz*^{4B4} clones (A) show only minor defects in the pattern of photoreceptors; *rap*^{7C2} clones (B) show a partial loss of photoreceptors; *dlg1*^{6C2} clones (C) overgrow and fail to differentiate any photoreceptors; *csw*^{8C7} (D) and *nej*^{8B27} (E) clones contain many ommatidia consisting of only one Elav-positive cell. (F–G) *nej*^{8B27} clones are marked by the absence of GFP (green). (F) *aos-lacZ* (red), which marks R1–7 photoreceptors, is absent in the few remaining Elav-positive (blue) photoreceptors (arrowheads) in *nej*^{8B27} clones. (G) *nej*^{8B27} clones still differentiate R8 cells marked by Sens (red).

vesicles from the plasma membrane (Chen *et al.* 1991). *shi*^{7C7} mutant clones displayed a neurogenic phenotype in which virtually all mutant cells posterior to the furrow differentiated as Elav-positive photoreceptors (Figure 3A). This phenotype is reminiscent of mutations in components of the N pathway that affect its ability to resolve proneural cell clusters into single R8 cells through lateral inhibition (Li and Baker 2001). Several studies have shown that dynamin is required both for endocytosis and activation of the N ligand Delta (Dl) and for N signaling in the receiving cell (Seugnet *et al.* 1997; Vaccari *et al.* 2008; Windler and Bilder 2010). Consistent with a defect in N-mediated lateral inhibition, we found that *shi*^{7C7} clones downregulated the N transcriptional reporter *E(spl)m8-lacZ* (Figure 3A"), differentiated supernumerary Sens-positive R8 cells (Figure 3B), and failed to refine Ato expression to a single R8 cell per ommatidium (Figure 3C). Together with the apical accumulation of Dl and N in *shi*^{7C7} mutant clones (Figure 3, D and E), these results confirm that dynamin is required for endocytic trafficking of the ligand and its receptor and subsequent transduction of the signaling pathway. *N* and *Dl* themselves also have an earlier role in the activation of *ato* expression, and clones homozygous for these mutations thus fail to differentiate any photoreceptors (Baker and Yu 1997; Ligoxygakis *et al.* 1998). Our observations indicate that *shi* is not required for this proneural function of N.

Table 1 Outcome of the genetic screen

	Total	%
Flies screened	43,414	
Mutants identified in primary screen	490	1.13
Viable and fertile mutants	387	79
Recovered mutant stocks	139	28
Mutants kept after secondary screen	119	24

Percentages in the right column refer to the 490 mutants identified in our primary screen, except for 1.13%, which refers to the 43,414 flies screened.

Interestingly, not all the ectopic neurons in *shi*^{7C7} clones were R8 photoreceptors (Figure 3B). Since EGFR signaling induces the differentiation of R1–R7, the presence of extra R1–R7 cells in *shi*^{7C7} mutant clones raised the possibility that dynamin might inhibit EGFR signaling in addition to promoting N activity. Indeed, EGFR protein accumulated in subregions of *shi*^{7C7} clones, mostly just posterior to the MF (Figure 3, F and G), suggesting that a failure to internalize the receptor in the absence of dynamin may allow extended EGFR signaling. Consistently, we observed increased expression of *aos-lacZ*, a transcriptional reporter for EGFR signaling, in *shi*^{7C7} clones. This was not a downstream consequence of the increased number of photoreceptors, as *aos-lacZ* expression preceded Elav expression (Figure 3H). As a whole, these results suggest that *shi*^{7C7} cells differentiate as ectopic photoreceptors as a result of both an impairment in N-mediated lateral inhibition and an increase in EGFR signaling.

Arp2 mutations result in supernumerary R8 cells

A different kind of neurogenic phenotype was observed in clones homozygous for a mutation that we named *cassandra* (*casa*). Not all *casa* mutant cells differentiated as neurons, but most ommatidia contained extra photoreceptors (Figure 4A). In adult eyes, large *casa* clones generated in a *Minute* background displayed enlarged facets with craters of missing lens material (Figure 4B). Staining for Sens revealed that *casa* mutant ommatidia often contained two neighboring R8 cells (Figure 4C), or occasionally R8 triplets (Figure 4D). Using rescue by X chromosomal duplications followed by recombination with *P*-element markers, we mapped *casa* to the 14C–E region (Figure 4E). Sequencing of candidates in the region revealed a nonsense mutation at position 89 in the gene *Actin related protein 14D* (*Arp14D*) (Figure 4E) that encodes the Arp2 subunit of the Arp2/3 complex required for the polymerization of branched actin filaments (Pollard 2007). Mutations in other subunits and regulators of this complex cause a very similar adult eye phenotype (Zallen *et al.* 2002). The recruitment of multiple R8 cells in *Arp2*^{casa} mutant ommatidia might be explained by reduced N signaling; in sensory organ precursor lineages, Arp3 has been reported to be required for normal Dl presentation (Rajan

Table 2 Mutations recovered from the screen

Function	Fly gene name	Vertebrate homologs	CG no.	Cytology	No. of alleles
EGFR/MAPK pathway	*corkscrew (csw)*	SHP-2	3954	2D1–2	7
	pole hole (phl)	Raf	2845	3A1	6
	Downstream of raf1 (Dsor1)	MEK/MAPKK	15793	8D2–3	5
Wingless pathway	*shaggy (sgg)*	GSK3β	2621	3A8–3B1	9
	Casein Kinase 1α (CK1α)	CK1α	2028	11B7	1
Notch pathway	*Notch (N)*	N	3936	3C7–9	1
Endocytosis/cytoskeleton	*shibire (shi)*	Dynamin	18102	13F18	1
	Actin-related protein 14D (Arp14D)	Arp2	9901	14E1	1
Transcriptional regulation	*nejire (nej)*	CBP	15319	8F7–9	2
	lozenge (lz)	AML1	1689	8D5–6	3
Adhesion/polarity	*furrowed (fw)*	P-selectin	1500	11A1	3
	discs large 1 (dlg1)	DLG1	1725	10B6–10	3
Cell cycle regulation	*retina aberrant in pattern (rap)*	Cdh1/Fzr	3000	4C11–12	6
Unidentified genes	Complementation group A			2D1–2;3C5–6	4
	Complementation group B			2D1–2;3A1–2	2
	Complementation group C			5A1–6C	2
	Other hits				63

et al. 2009). Such an effect of the Arp2/3 complex on the N pathway must be subtle and/or tissue specific, because loss of *Arp2* had no visible effect on N-dependent differentiation of the wing margin (Figure 4F).

A novel mutation upregulates Wg target genes

In eye imaginal discs, Wg is expressed at the anterior lateral margins in regions that will differentiate as head cuticle. Activation of Wg signaling within the eye field (*e.g.*, in *sgg* clones; Figures 1E and 5C) transforms retinal tissue into ectopic head cuticle (Legent and Treisman 2008). Clones homozygous for our mutation *8B12* similarly resulted in cuticle outgrowths protruding from the retina of adult flies (Figure 5A). In third instar eye discs, *8B12* mutant clones completely lacked photoreceptors, had smooth borders, and overgrew (Figure 5B), again resembling *sgg* mutations (Figure 5C). Given this similarity, we tested whether *8B12* affected the expression of known Wg target genes in the eye disc. The unconventional cadherin Dachsous (Ds) and the transcription factor Optomotor-blind (Omb) are activated by Wg signaling at the anterior lateral margins of the eye disc (Figure 5, D and F) (Zecca *et al.* 1996). We observed strong autonomous upregulation of Omb and the transcriptional reporter *ds-lacZ* in *8B12* mutant clones (Figure 5, E and G), consistent with ectopic activation of the Wg pathway.

To determine whether the *8B12* mutation affects a general regulator of Wg signal transduction, we next investigated its effect on Wg functions in wing development. Wg is normally expressed along the dorsal/ventral (D/V) boundary of the wing pouch, where it induces the differentiation of sensory bristles at the adult wing margin (Figure 5H). Consistent with a gain of Wg function, *8B12* mutant clones within the wing blade differentiated ectopic bristles (Figure 5I). In wing discs, Wg elicits graded responses depending on its concentration. Sens is a high-threshold target expressed in cells abutting the source of Wg secretion (Figure 5J), while Distalless (Dll) is a mid-threshold target induced in a broader domain (Figure 5K)(Neumann and Cohen 1996; Neumann and Cohen 1997). Both targets were strongly and autonomously induced in *8B12* mutant clones in the wing pouch (Figure 5, J and K). Misexpression of Sens and Dll was confined to the wing pouch, suggesting that additional inputs required for their expression are lacking in other regions of the wing disc (Figure 5, J and K). *8B12* had no effect on the boundary enhancer of the *vestigial* gene (*vgBE*), a N target expressed at the D/V boundary of the wing pouch, demonstrating its specificity for the Wg pathway (Figure 5L). Another N target gene, *wg* itself, also never showed increased expression in *8B12* clones (data not shown).

CK1α is specifically required for Wg signaling

We mapped *8B12* to the cytological region 11A–C and found a missense mutation in the gene *CK1α*, but no sequence changes in the neighboring CK1 family member *CG2577*. The mutation transforms the conserved glycine 43 into an aspartic acid (Figure 6A) and falls within subdomain II of the serine/threonine kinase domain of *CK1α* (Santos *et al.* 1996). Expression of a FLAG-tagged wild-type *CK1α* cDNA in *8B12* clones, using the mosaic analysis with a repressible cell marker (MARCM) system (Lee and Luo 2001), rescued both photoreceptor differentiation in the eye and ectopic Sens in the wing (Figure 6, B and C). Additionally, the lethality of hemizygous *8B12* males was rescued by *CK1α* expression, but not by two closely related CK1 family members, *CG2577* or *discs overgrown* (*dco*) (Figure 6D). These results confirm that the phenotypes observed are due to the sequence change in *CK1α*. To our knowledge, *8B12* is the first mutation described in this gene in *Drosophila*. Homozygous $CK1\alpha^{8B12}$ animals died primarily during embryogenesis and early larval stages, but showed no obvious cuticle patterning defects (data not shown), perhaps due to a maternal contribution of *CK1α*. Trans heterozygotes over a deficiency died at the same stages (Figure 6E), suggesting that $CK1\alpha^{8B12}$ is a strong hypomorph or a null allele.

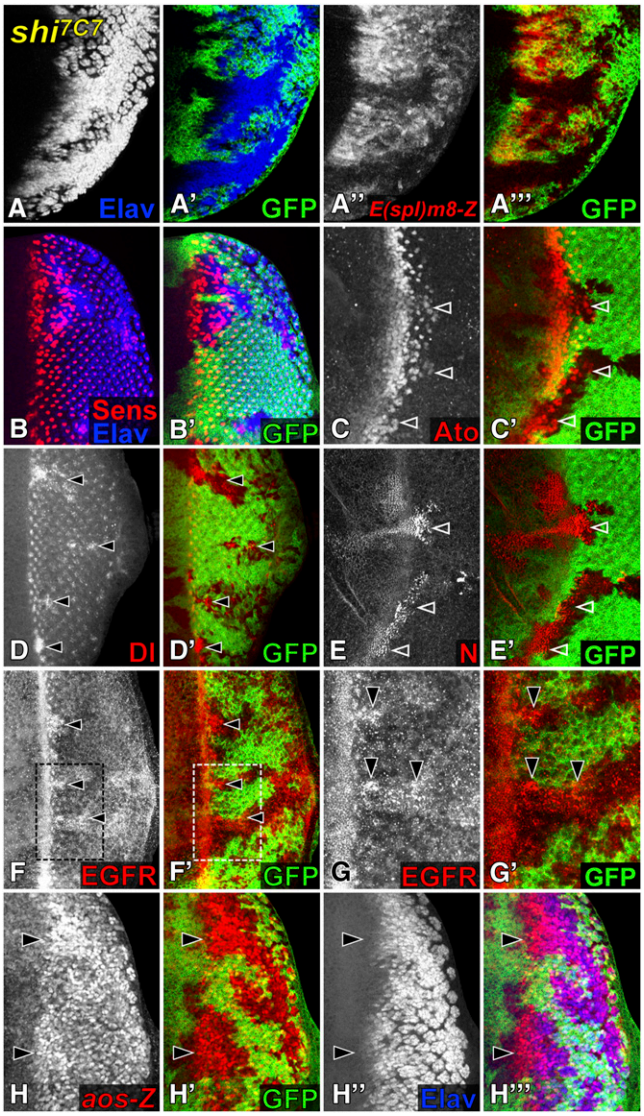


Figure 3 *shi* mutant cells alter N and EGFR signaling to induce excessive photoreceptor differentiation. (A–H) *shi*^{7C7} clones in third instar eye discs are marked by the absence of GFP (green). (A) Virtually all *shi*^{7C7} cells posterior to the MF differentiate as Elav-positive photoreceptors (blue). The N transcriptional reporter *E(spl)m8-lacZ* (red) is also downregulated. (B) Many but not all ectopic photoreceptors in *shi*^{7C7} clones (stained with Elav in blue) express the R8 marker Sens (red). (C) *shi*^{7C7} clones differentiate extra Ato-positive (red) R8 cells just posterior to the MF (arrowheads). In apical regions, *shi*^{7C7} clones accumulate increased levels of Dl (red in D), N (red in E), and EGFR (red in F, boxed region enlarged in G), especially just posterior to the MF (arrowheads). (H) *shi*^{7C7} clones show increased expression of the EGFR transcriptional reporter *aos-lacZ* (red), in cells that have not yet differentiated into Elav (blue)-expressing photoreceptors (arrowheads).

Biochemical and RNAi-based studies have implicated CK1α as a priming kinase for β-Catenin/Arm phosphorylation and subsequent proteolytic processing by the ubiquitin–proteasome pathway (Liu *et al.* 2002; Yanagawa *et al.* 2002). However, the presence of eight CK1 family members in *Drosophila* and the difficulty of achieving both efficient and specific loss of function by RNAi have left open the possibility that other CK1 family members contribute to Arm degradation *in vivo* (Jia *et al.* 2005; Zhang *et al.* 2006). We tested this using an enhancer from the *frizzled 3* (*fz3*) gene that is directly regulated by Wg through TCF binding sites (Olson *et al.* 2011). *fz3–RFP*, which is normally expressed in response to high Wg signaling in the notum and to a lesser extent at the D/V boundary in the wing disc (Figure 7A), was strongly induced in *CK1α*^{8B12} clones (Figure 7B), indicating robust pathway activation. If phosphorylation of Arm by CK1α is essential for its destruction, Arm levels should increase in the absence of CK1α. Indeed, *CK1α*^{8B12} clones in the wing pouch, like cells abutting the source of Wg expression at the D/V boundary, accumulated high levels of Arm (Figure 7C). Arm enrichment was particularly evident at the apical/lateral plasma membrane (Figure 7D), but was also visible more basally (Figure 7E). Thus no other CK1 family member can substitute for CK1α to restrict Arm accumulation.

To test whether the *CK1α* eye phenotype results from increased Wg signaling, we impaired signal transduction downstream of Arm accumulation by expressing a dominant negative form of the transcription factor dTCF (van de Wetering *et al.* 1997). Wg signaling is not required for normal photoreceptor differentiation, and MARCM-induced expression of *dTCF*^{DN} in the eye field had no effect (Figure 7H). It was nevertheless sufficient to restore photoreceptor differentiation in *CK1α*^{8B12} clones (Figure 7, F and G), indicating that this effect of *CK1α* mutations is due to increased transcriptional activity of Arm. As a whole, these results demonstrate the requirement for CK1α to restrict normal Wg signaling *in vivo*.

CK1α regulates Myc expression but not Hh signaling

CK1α is a Ser/Thr kinase predicted to phosphorylate multiple substrates. The *8B12* mutation might abolish all CK1α activity, or specifically affect Arm phosphorylation. Another target predicted from biochemical and RNAi studies is the growth regulator dMyc. Direct phosphorylation of dMyc by CK1α is thought to lead to its ubiquitin-mediated degradation (Galletti *et al.* 2009). In the wing disc, *dmyc* is expressed in the notum and wing pouch except near the D/V boundary, where its transcription is repressed by N signaling (Herranz *et al.* 2008). In *CK1α*^{8B12} mutant clones in the wing hinge, dMyc was strongly upregulated (Figure 8A), probably reflecting the absence of CK1α-induced dMyc protein degradation. As reported for *CK1α* RNAi (Galletti *et al.* 2009), *CK1α*^{8B12} clones in the wing pouch downregulated dMyc, potentially due to increased repression by N. However, we believe it is more likely to be an indirect effect, because loss of *CK1α* does not increase the ability of N to regulate other target genes (Figure 5L).

CK1α has also been predicted to affect components of the Hh pathway. In the wing disc, Hh is secreted by posterior (P) cells and diffuses into the anterior (A) compartment to elicit concentration-dependent responses. The seven-transmembrane domain protein Smo contains CK1 phosphorylation

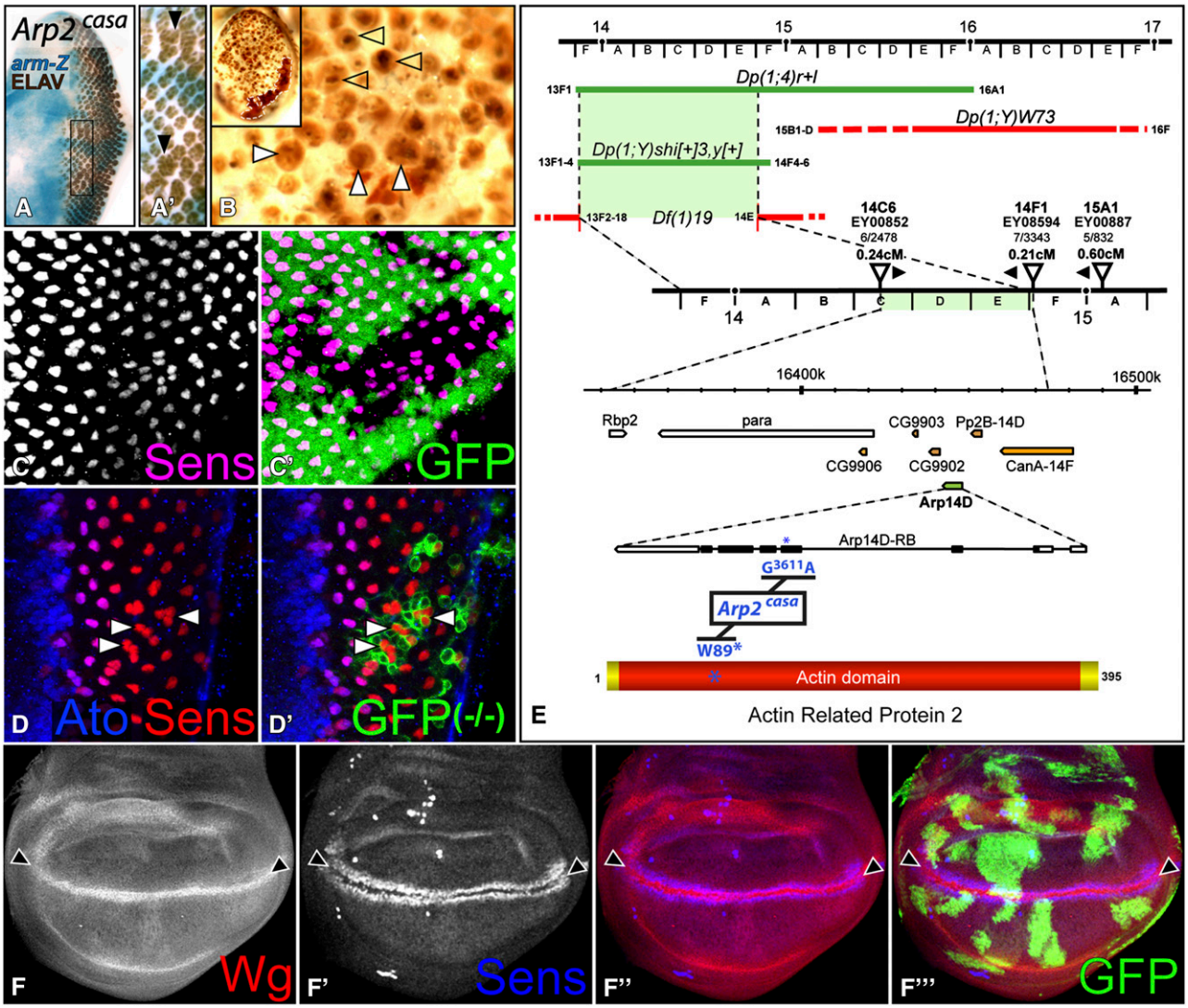


Figure 4 Arp2 restricts R8 differentiation. (A) *Arp2*^{casa} clones, marked by the absence of *arm-lacZ* (blue), exhibit enlarged ommatidia containing supernumerary photoreceptors (Elav in brown, arrowheads). (B) In adult eyes, large *Arp2*^{casa} clones in a *Minute* background exhibit enlarged ommatidia (solid arrowheads) with craters of missing lens material (open arrowheads). A dashed line surrounds *w+* heterozygous tissue in the inset. (C) GFP-negative *Arp2*^{casa} clones contain duplicated Sens-positive R8 cells (magenta). (D) Occasionally, R8 triplets (labeled with Sens in red and Ato in blue, arrowheads) are observed in *Arp2*^{casa} clones (positively marked by coexpression of GFP in green). (E) Diagram of the mapping of *Arp2*^{casa} indicating cytogenetic bands and aberration breakpoints. Duplications (Dp) in green rescue hemizygous males, whereas those in red do not. *Arp2*^{casa} does not complement the deficiency *Df(1)19*. The map distances and orientations of *Arp2*^{casa} with respect to three P elements in the region are indicated. The sequence change G3611A in the *Arp14D* gene (green) and corresponding W89* nonsense mutation in Arp2 are shown in blue. No sequence changes were detected in neighboring genes (orange). (F) In the wing pouch, GFP (green)-negative *Arp2*^{casa} clones, in a *Minute* background, show normal expression of the D/V boundary markers Wg (red) and Sens (blue) (arrowheads).

sites that are required for Hh-driven Smo accumulation at the cell surface and subsequent signal transduction (Jia *et al.* 2004). Smo is downregulated in clones lacking the catalytic subunit of *Protein Kinase A* (*PKAc*), which primes Smo for CK1 phosphorylation (Jia *et al.* 2004). In contrast, *CK1*α^{8B12} clones left Smo levels and localization unaffected (Figure 8B), suggesting that CK1α is not essential for Smo phosphorylation. The downstream transcription factor Ci also contains CK1 phosphorylation sites, which are required for proteolytic processing of the full-length activator form of Ci (Ci^{155}) into a C-terminally truncated repressor form (Ci^{75}). While CK1δ/ε/Dco is clearly required for the downregulation of Ci, CK1α was also thought to contribute (Jia *et al.* 2005). We found that Ci^{155} was unaffected in *CK1*α^{8B12} clones (Figure 8C). Consistently, neither the low-threshold transcriptional target *decapentaplegic* (*dpp*) (Figure 7G) nor the high-threshold targets *patched* (*ptc*) (Figure 8, D–F) and Engrailed (En) (Figure 8H) were modified by *CK1*α^{8B12}, although the same clones upregulated the Wg target Sens (Figure 8, B, D, H, and J). *CK1*α^{8B12} clones in a hypomorphic *dco*^{3} background similarly failed to affect the levels of Ci^{155} or its target Ptc (Figure 8, I and J), suggesting

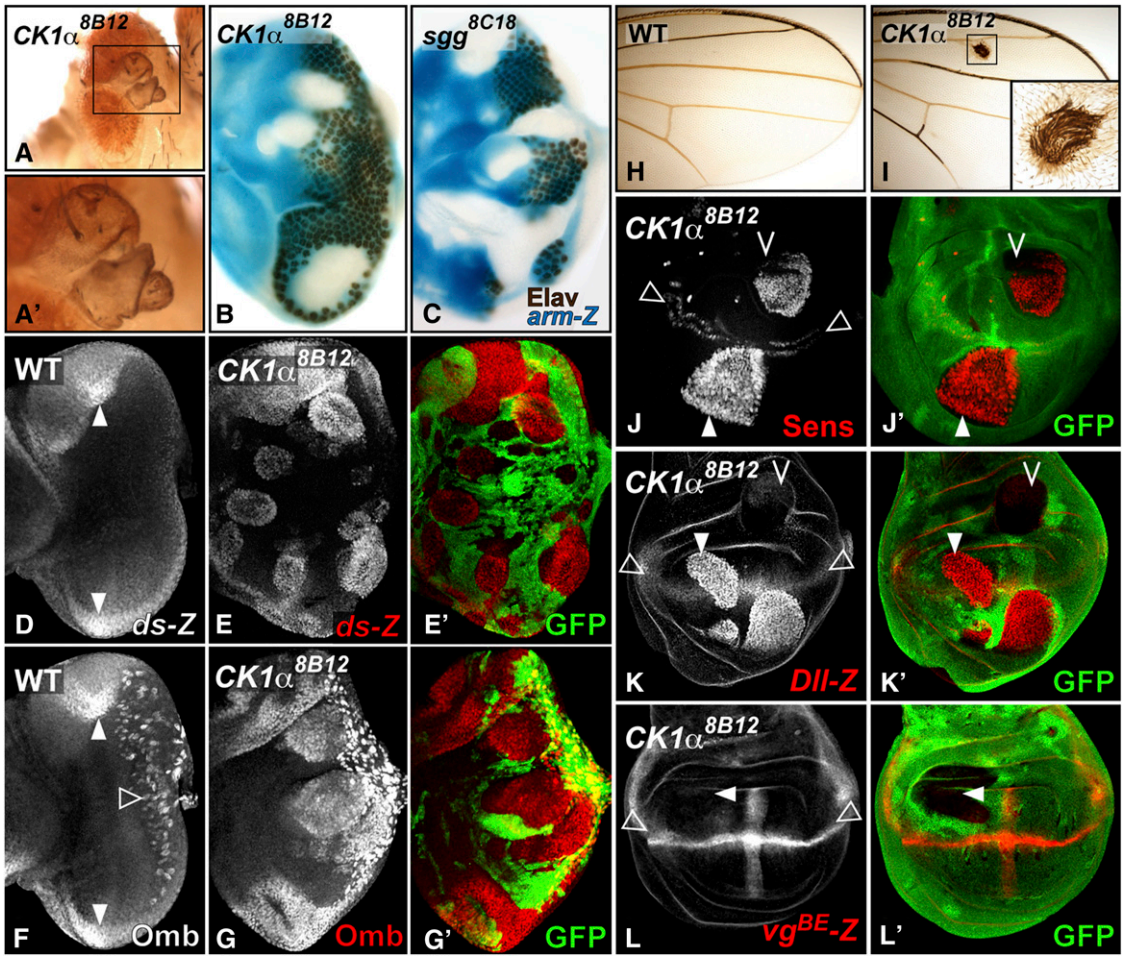
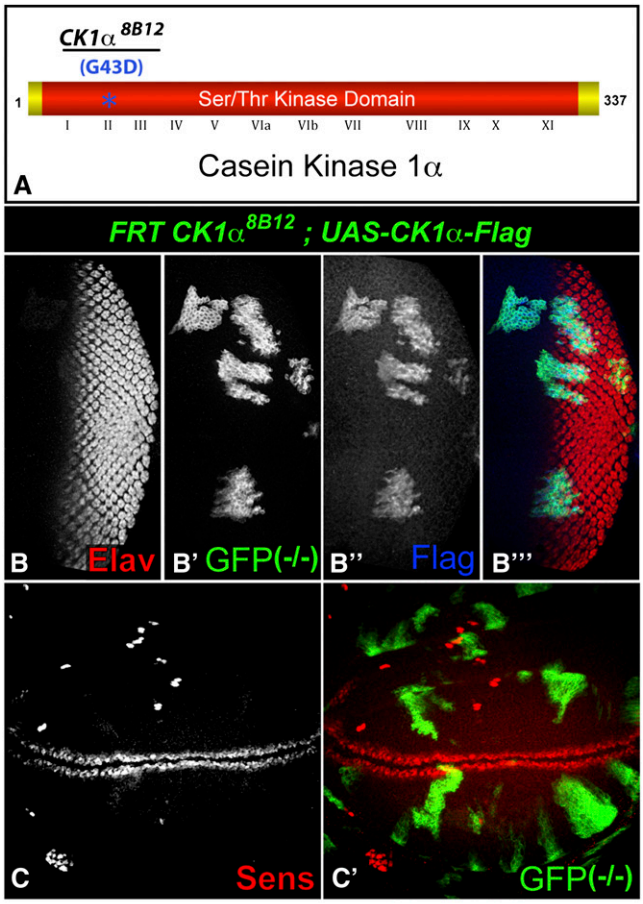


Figure 5 *CK1α*^{8B12} clones induce ectopic Wg target gene expression. (A) Adult eyes containing *CK1α*^{8B12} clones display cuticle protrusions in the retina (inset in A'). (B and C) *CK1α*^{8B12} (B) or *sgg*^{8C18} (C) mutant clones in the eye disc marked by the absence of *arm-lacZ* staining (blue) are overgrown, lack Elav staining (brown), and have smooth boundaries. (D–G) Eye discs stained for *ds-lacZ* (D,E) or Omb (F and G). *ds-lacZ* (D) and Omb (F) are normally expressed at the anterior lateral margins of the eye disc (solid arrowheads). Omb also marks retinal glial cells (open arrowhead). (E–G) GFP (green)-negative *CK1α*^{8B12} clones ectopically express *ds-lacZ* (red in E) and Omb (red in G). (H and I) Adult wing blades of wild type (H) or flies carrying *CK1α*^{8B12} clones (I). *CK1α*^{8B12} clones differentiate ectopic bristles (inset in I). (J–L) Third instar wing discs carrying GFP-negative *CK1α*^{8B12} clones. *CK1α*^{8B12} clones in the wing pouch (arrowheads) ectopically express high levels of Sens (red in J) and *Dll-lacZ* (red in K), but do not misexpress *vgBE-lacZ* (red in L). Cells outside the wing pouch fail to induce ectopic expression (chevrons in J and K). Normal expression of these genes at and/or adjacent to the D/V boundary of the wing pouch is shown by open arrowheads.

that a low level of CK1δ/ε activity can prevent ectopic Hh signaling in the absence of CK1α. In contrast to the strong upregulation of Ci^{155}, *ptc* and *dpp* observed in mutants for *sgg/GSK3*, a kinase required to phosphorylate and negatively regulate both Arm and Ci (Zecca *et al.* 1996; Jia *et al.* 2002; Price and Kalderon 2002), these results do not support an essential role for CK1α in either positive or negative regulation of Hh signal transduction.

Discussion

This study completes our genome-wide screen for mutations that affect photoreceptor differentiation in *Drosophila* by identifying mutations on the X chromosome. Using EMS as a mutagen allowed us to identify alleles of genes for which a recent screen of lethal transposon insertions on the X chromosome (Call *et al.* 2007) failed to detect eye phenotypes, such as *fw*, *nej*, *shi*, and *CK1α*. However, mapping lethal point mutations is much slower than mapping transposon insertion sites, and some of the mutations we generated are still unidentified. Saturation is difficult to achieve by either method. Insertional bias makes transposable elements inefficient at mutating many genes, while the probability of isolating mutations in a gene by chemical mutagenesis is proportional to the size of the essential protein domains. The mosaic screening strategy imposes further restrictions: pericentromeric regions proximal to the FRT19A insertion cannot be screened by this method, and genes may not be identified if they act nonautonomously and/or only in a small region of the eye disc.



D

Genotype	rescued males	Percentage of the expected number
$CK1\alpha^{8B12}$ / Y	0 / 500	–
$CK1\alpha^{WT}$ / Y; UAS-CK1α	64 / 188	**103**
$CK1\alpha^{8B12}$ / Y; UAS-CK1α	50 / 168	**119**
$CK1\alpha^{WT}$ / Y; UAS-cg2577	157 / 251	**125**
$CK1\alpha^{8B12}$ / Y; UAS-cg2577	0 / 72	**0**
$CK1\alpha^{WT}$ / Y; UAS-dco^{KD}	41 / 86	**95**
$CK1\alpha^{8B12}$ / Y; UAS-dco^{KD}	0 / 150	**0**

E

Genotype	eggs #	embryonic death	larval death
$CK1\alpha^{8B12}$ / $CK1\alpha^{8B12}$	458	208 (45.4 %)	250 (54.6 %)
$CK1\alpha^{8B12}$ / Df(CK1α)	254	114 (44.9 %)	140 (55.1 %)

Figure 6 *8B12* is a missense mutation in *CK1α*. (A) Diagram of the CK1α protein showing the G43D mutation in subdomain II of the Ser/Thr kinase domain in $CK1\alpha^{8B12}$. (B and C) third instar eye (B) and wing (C) discs expressing GFP (green) and FLAG-tagged *CK1α* (blue) within $CK1\alpha^{8B12}$ clones. (B) Differentiation of Elav-positive (red) photoreceptors is rescued. (C) Ectopic expression of Sens (red) is abolished. (D) Table showing the survival of hemizygous $CK1\alpha^{8B12}$ males expressing *UAS–CK1α*, but not *UAS–cg2577* or *UAS–*dco^{KD}, driven by *tub*–GAL4. The number and percentage of males of the expected genotype is displayed. (E) Table showing the rate of embryonic and larval death of $CK1\alpha^{8B12}$ homozygotes *vs.* $CK1\alpha^{8B12}$/*Df(CK1α)* transheterozygotes.

Eye phenotypes reveal specific functions of general regulators

Several of the mutations we identified showed specific effects on eye development despite a predicted general role of the protein affected. For example, our mutation in *nej* specifically disrupts R1–R7 differentiation, which depends on EGFR signaling. CBP has been shown to potentiate MAPK-enhanced transcriptional activation by the Ets-2 transcription factor (Foulds *et al.* 2004), but it is also important for Dpp, Hh, and Wg signaling in some contexts (Akimaru *et al.* 1997; Waltzer and Bienz 1998; Waltzer and Bienz 1999; Li *et al.* 2007). CBP functions as a histone acetyltransferase that acts on H3K27, H3K56, and trimethylated H3K4 (Das *et al.* 2009; Tie *et al.* 2009; Crump *et al.* 2011). The specific effects of nej^{8B27} suggest that acetylation of these histones is most critical for EGFR signaling during eye disc development.

Similarly, dynamin plays a general role in endocytosis, but shi^{7C7} mutant clones show defects characteristic of the N and EGFR signaling pathways. The neurogenic phenotype observed appears to result from both loss of N-mediated lateral inhibition, giving rise to extra R8 cells, and increased EGFR-mediated recruitment of non-R8 photoreceptors. In the *Drosophila* ovary, dynamin-dependent endocytosis is required for the activity of both the ligand Dl in the germline cells and its receptor N in the follicle cells (Vaccari *et al.* 2008; Windler and Bilder 2010). shi^{7C7} retinal clones likewise show apical accumulation of N and Dl, but reduced expression of a transcriptional target of N. Interestingly, the ability of N and Dl to induce the early expression of *ato* (Baker and Yu 1997; Ligoxygakis *et al.* 1998) is only slightly affected in *shi* clones. This proneural function of N uses a distinct transcriptional mechanism from its role in lateral inhibition (Li and Baker 2001), and our results suggest that it also differs in its requirement for dynamin-dependent endocytosis of N and Dl. EGFR also accumulates apically in *shi* mutant cells, but shows an increased ability to activate its transcriptional target *aos*. Dynamin may reduce EGFR signaling by internalizing it from the plasma membrane (Vieira *et al.* 1996) or may act in late endosomes to promote EGFR degradation (Schroeder *et al.* 2010). Our observations highlight the distinct effects of endocytic trafficking on signaling through the N and EGFR pathways.

The seven-protein Arp2/3 complex that regulates actin branching has been reported to be required in the *Drosophila* sensory organ precursor lineage for trafficking of endocytosed Dl-positive vesicles to the actin-rich apical microvilli of the signal-sending cell. *Arp3* mutants show impaired N signaling and fail to differentiate external sensory organs (Rajan *et al.* 2009). Our screen isolated the first reported *Arp2* mutation in *Drosophila*. The formation of extra R8 cells in $Arp2^{casa}$ clones could similarly result from reduced N signaling, although we have not been able to detect defects in Dl trafficking in these mutant cells at the light microscope level. The requirement for Arp2 in N signaling may be specific for its function in lateral inhibition, since specification of the wing margin by N-mediated inductive signaling proceeds normally in *Arp2* mutant cells.

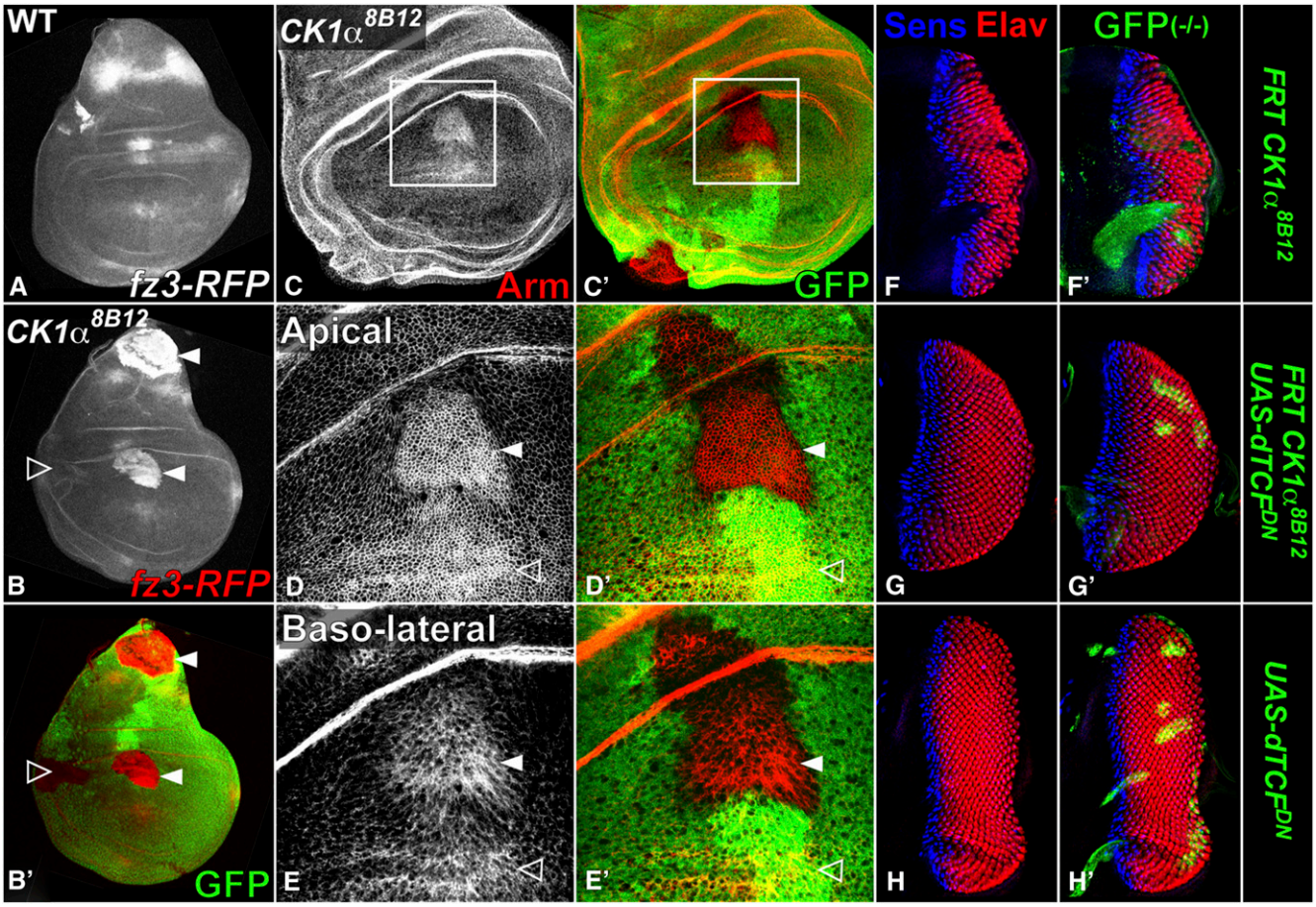


Figure 7 Loss of *CK1α* activates Wg signaling by promoting Arm accumulation. (A–E) Third instar wing discs; (A) wild type or (B–E) carrying *CK1α*^{8B12} clones marked by the absence of GFP (green). (A) *fz3–RFP* is expressed as a stripe in the notum and more weakly in the central hinge and wing pouch. (B) *CK1α*^{8B12} clones induce ectopic *fz3–RFP* (red) expression in the notum and pouch (arrowheads) but not in the lateral hinge (open arrowhead). (C–E) Arm (red) accumulates close to the D/V boundary (open arrowhead) and in *CK1α*^{8B12} clones within the wing pouch (solid arrowhead). Single confocal sections show prominent Arm enrichment at the apical/lateral plasma membrane (D) and also more basally (E). (F–H) Eye discs containing clones marked by coexpression of GFP (green) and stained for Elav (red) and Sens (blue). The lack of photoreceptors in *CK1α*^{8B12} clones (F) is rescued by expression of dominant negative dTCF (G). (H) *UAS–dTCF*^{DN} control clones do not affect photoreceptor differentiation.

A CK1α mutation affects signaling by Wg, but not Hh

Our screen also identified the first reported allele of *Drosophila CK1α*. Although RNAi experiments had implicated CK1α in the control of both Wg and Hh signaling, our mutation specifically affects the Wg pathway. Biochemical experiments have shown that in the absence of Wg, cytoplasmic Arm is destabilized by a "destruction complex" in which Axin and adenomatous polyposis coli facilitate Arm phosphorylation by the kinases CK1α and Sgg/GSK3. Phosphorylation of β-Catenin by CK1α is essential to priming it for GSK3 phosphorylation and subsequent degradation (Amit *et al.* 2002; Liu *et al.* 2002; Yanagawa *et al.* 2002). Our finding that *in vivo*, loss of *CK1α* strongly increases Arm levels and upregulates Wg target genes supports this model. Since *sgg* mutants also show Arm accumulation and increased Wg signaling (Peifer *et al.* 1994; Siegfried *et al.* 1994), phosphorylation of Arm by both CK1α and GSK3 is necessary to keep Wg signaling in check.

In *Drosophila*, the contribution of other CK1 family members (*CK1ε/dco*, *CK1γ/Gish*, *CG7094*, *CG2577*, *CG12147*, *CG9962*, and *Asator*) to Wg signaling *in vivo* has been difficult to assess because the only tools available for many of them were RNAi transgenes, which face a tradeoff between knock-down efficiency and specificity. CK1δ and CK1ε can interact with Axin and phosphorylate β-Catenin *in vitro* (Amit *et al.* 2002; Sakanaka 2002), and genetic evidence suggested that they played both positive and negative roles in Wg signaling (Klein *et al.* 2006; Zhang *et al.* 2006; Bernatik *et al.* 2011). However, our results demonstrate that no other paralog can compensate for *CK1α* to negatively regulate Wg signaling. Consistently, an RNAi screen in *Drosophila* cultured cells identified *CK1α*, but not *CK1ε*, as a negative regulator of Wg reporter activity (Dasgupta *et al.* 2005). The conditional ablation of mouse *CK1α* from intestinal epithelia similarly induces β-Catenin accumulation accompanied by robust activation of many Wnt target genes (Elyada *et al.* 2011). The role of CK1α as the primary family

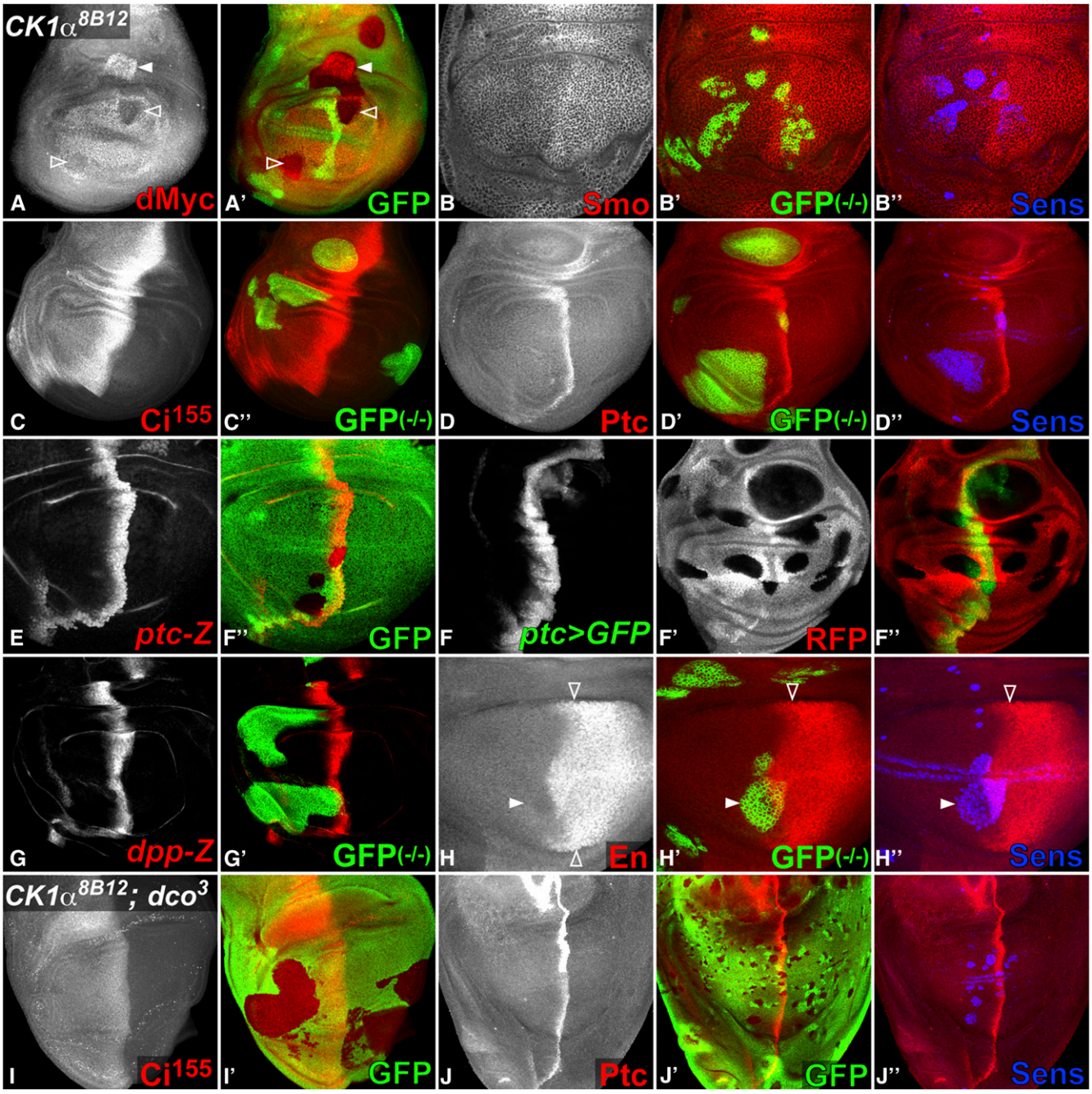


Figure 8 *CK1α*^{8B12} clones do not affect Hh signaling. (A–J) Wing discs carrying *CK1α*^{8B12} clones in a *dco*^{WT} background (A–H) or in *dco*^{3} homozygotes (I–J). Anterior is to the left and dorsal up. Mutant clones are marked by the absence of GFP (green in A, E, I, J) or RFP (red in F) or by coexpression of GFP (green in B–D and G–H). (B'', D'',H'', J'') *CK1α*^{8B12} clones in the wing pouch upregulate the Wg target Sens (blue). (A) dMyc (red) is upregulated in *CK1α*^{8B12} clones in the wing hinge (solid arrowhead) but downregulated in the wing pouch (open arrowheads). (B) Expression of the Hh effector Smo (red) in the posterior compartment and just anterior to the A/P boundary is not affected in *CK1α*^{8B12} clones. *CK1α*^{8B12} clones also do not alter the expression of Ci^{155}, normally observed in the A compartment with elevated levels along the A/P boundary (red in C), or the Hh targets Ptc (red in D), *ptc-lacZ* (red in E), *ptc-GAL4*, *UAS–GFP* (green in F), or *dpp-lacZ* (red in G). Amplification of the signal in *ptc-GAL4*, *UAS–GFP* should make it especially sensitive to changes in *ptc* transcription. (H) En (red) is expressed in the posterior compartment and activated by high levels of Hh in anterior cells abutting the A/P boundary (open arrowheads). *CK1α*^{8B12} clones do not affect En expression (solid arrowhead). *CK1α*^{8B12} clones in a homozygous *dco*^{3} background do not affect Ci^{155} (red in I) or Ptc (red in J).

member that negatively regulates Arm/β-catenin thus seems to be conserved across species.

Interestingly, many studies in different organisms indicate that upstream components of the pathway are also CK1-regulated. Phosphorylation of Dsh by CK1ε is a crucial step in the initiation and subsequent termination of signal transduction (Peters *et al.* 1999; Yanagawa *et al.* 2002; Matsubayashi *et al.* 2004; Klein *et al.* 2006; Bernatik *et al.*

2011; Del Valle-Perez *et al.* 2011). Phosphorylation of the Wg coreceptor Arr/LRP6 by the membrane associated CK1γ/Gish (Davidson *et al.* 2005; Zhang *et al.* 2006) or by CK1δ/α (Zeng *et al.* 2005; Zhang *et al.* 2006) promotes Wg signaling, while LRP6 phosphorylation by CK1ε may have both positive and negative effects (Swiatek *et al.* 2006; Casagolda *et al.* 2010), Although we have not assessed the effect of *CK1*α^{8B12} on either Arr or Dsh, our results establish that *in vivo* the negative regulation of Arm stability is epistatic to any positive contribution of CK1α in the Wg pathway.

In addition to Wnt transduction, the CK1 family has also been shown to regulate Hh signaling in *Drosophila*. In the absence of Hh, the transcription factor Ci is ubiquitinated following PKA-primed phosphorylation by GSK3 and CK1. Subsequent proteasomal processing cleaves full-length Ci^{155} into a repressor form, Ci^{75} (Jia *et al.* 2002; Price and Kalderon 2002; Jia *et al.* 2005; Smelkinson and Kalderon 2006). RNAi and overexpression experiments *in vivo* and in cultured cells suggested that both CK1α and CK1ε contribute to Ci phosphorylation and cleavage (Price and Kalderon 2002; Lum *et al.* 2003; Jia *et al.* 2005). Surprisingly, we found that Ci^{155}, as well as the Hh targets *ptc*, *en*, and *dpp*, were unaffected by *CK1*α^{8B12} clones in wing discs. In contrast, *GSK3/sgg* mutant clones strongly upregulate the three Hh readouts (Jia *et al.* 2002; Price and Kalderon 2002). Since cells double mutant for *CK1*α and the hypomorphic allele *dco*^{3} also did not upregulate Hh signaling, a low level of CK1ε activity may be sufficient to phosphorylate Ci *in vivo*.

CK1 enzymes have also been implicated in the positive regulation of Hh transduction at the level of the transmembrane protein Smo. Hh reception promotes PKA-primed phosphorylation of Smo by CK1, inducing its cell-surface accumulation and signaling activity (Jia *et al.* 2004; Apionishev *et al.* 2005). The kinase Gprk2 further phosphorylates Smo and promotes its optimal conformation, allowing for maximal signaling (Chen *et al.* 2010; Chen *et al.* 2011). CK1 also contributes to Hh signaling by phosphorylating the downstream kinase Fu (Zhou and Kalderon 2011). Although *CK1*α RNAi can attenuate Smo levels and reduce the expression of the Hh target genes *collier*, *ptc*, and *en* (Jia *et al.* 2004; Apionishev *et al.* 2005; Zhou and Kalderon 2011), we did not detect any change in Smo levels or Hh target gene expression in *CK1*α^{8B12} clones in the wing disc. We cannot rule out the possibility that *CK1*α^{8B12} is a hypomorph that retains sufficient activity to sustain normal Hh signaling. However, we note that it activated maximal levels of Wg signaling, comparable to mutations in *sgg* or *axin* (Heslip *et al.* 1997; Lee and Treisman 2001). In addition, the missense mutation is not specific for Arm phosphorylation, as another phosphorylation substrate, dMyc, was also upregulated. It seems likely that CK1α acts redundantly with other CK1 family members to phosphorylate targets in the Hh pathway.

The role of CK1α in Wnt signaling and growth regulation is conserved across species and relevant to human cancer. Conditional ablation of CK1α in the mouse intestine triggers massive Wnt activation associated with anti-tumorigenic activation of the p53 pathway. Knocking down both *CK1*α and *p53* allows for extensive proliferation of invasive carcinomas, establishing *CK1*α as a tumor suppressor (Elyada *et al.* 2011). Indeed, *CK1*α expression is reduced during tumor progression of human melanoma cell lines (Sinnberg *et al.* 2010). The generation of *CK1*α mutations in both flies and mice has clarified which of the previously predicted functions of *CK1*α do in fact specifically require this enzyme *in vivo*.

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Literature Cited

Akimaru, H., Y. Chen, P. Dai, D. X. Hou, M. Nonaka *et al.*, 1997 *Drosophila* CBP is a co-activator of Cubitus interruptus in Hedgehog signalling. Nature 386: 735–738.

Amit, S., A. Hatzubai, Y. Birman, J. S. Andersen, E. Ben-Shushan *et al.*, 2002 Axin-mediated CKI phosphorylation of beta-catenin at Ser 45: a molecular switch for the Wnt pathway. Genes Dev. 16: 1066–1076.

Anderson, J., R. Bhandari, and J. P. Kumar, 2005 A genetic screen identifies putative targets and binding partners of CREB-binding protein in the developing *Drosophila* eye. Genetics 171: 1655–1672.

Apionishev, S., N. M. Katanayeva, S. A. Marks, D. Kalderon, and A. Tomlinson, 2005 *Drosophila* Smoothened phosphorylation sites essential for Hedgehog signal transduction. Nat. Cell Biol. 7: 86–92.

Baker, N. E., and S. Y. Yu, 1997 Proneural function of neurogenic genes in the developing *Drosophila* eye. Curr. Biol. 7: 122–132.

Baonza, A., and M. Freeman, 2002 Control of *Drosophila* eye specification by Wingless signalling. Development 129: 5313–5322.

Bernatik, O., R. S. Ganji, J. P. Dijksterhuis, P. Konik, I. Cervenka *et al.*, 2011 Sequential activation and inactivation of Dishevelled in the Wnt/beta-catenin pathway by casein kinases. J. Biol. Chem. 286: 10396–10410.

Bras-Pereira, C., J. Bessa, and F. Casares, 2006 *odd-skipped* genes specify the signaling center that triggers retinogenesis in *Drosophila*. Development 133: 4145–4149.

Cagan, R. L., and D. F. Ready, 1989 Notch is required for successive cell decisions in the developing *Drosophila* retina. Genes Dev. 3: 1099–1112.

Call, G. B., J. M. Olson, J. Chen, N. Villarasa, K. T. Ngo *et al.*, 2007 Genomewide clonal analysis of lethal mutations in the

Drosophila melanogaster eye: comparison of the X chromosome and autosomes. Genetics 177: 689–697.
Casagolda, D., B. Del Valle-Perez, G. Valls, E. Lugilde, M. Vinyoles *et al.*, 2010 A p120-catenin-CK1epsilon complex regulates Wnt signaling. J. Cell Sci. 123: 2621–2631.
Chen, M. S., R. A. Obar, C. C. Schroeder, T. W. Austin, C. A. Poodry *et al.*, 1991 Multiple forms of dynamin are encoded by *shibire*, a *Drosophila* gene involved in endocytosis. Nature 351: 583–586.
Chen, Y., S. Li, C. Tong, Y. Zhao, B. Wang *et al.*, 2010 G protein-coupled receptor kinase 2 promotes high-level Hedgehog signaling by regulating the active state of Smo through kinase-dependent and kinase-independent mechanisms in *Drosophila*. Genes Dev. 24: 2054–2067.
Chen, Y., N. Sasai, G. Ma, T. Yue, J. Jia *et al.*, 2011 Sonic Hedgehog dependent phosphorylation by CK1alpha and GRK2 is required for ciliary accumulation and activation of Smoothened. PLoS Biol. 9: e1001083.
Cho, E., Y. Feng, C. Rauskolb, S. Maitra, R. Fehon *et al.*, 2006 Delineation of a Fat tumor suppressor pathway. Nat. Genet. 38: 1142–1150.
Corrigall, D., R. F. Walther, L. Rodriguez, P. Fichelson, and F. Pichaud, 2007 Hedgehog signaling is a principal inducer of Myosin-II-driven cell ingression in *Drosophila* epithelia. Dev. Cell 13: 730–742.
Crump, N. T., C. A. Hazzalin, E. M. Bowers, R. M. Alani, P. A. Cole *et al.*, 2011 Dynamic acetylation of all lysine-4 trimethylated histone H3 is evolutionarily conserved and mediated by p300/CBP. Proc. Natl. Acad. Sci. USA 108: 7814–7819.
Das, C., M. S. Lucia, K. C. Hansen, and J. K. Tyler, 2009 CBP/p300-mediated acetylation of histone H3 on lysine 56. Nature 459: 113–117.
DasGupta, R., A. Kaykas, R. T. Moon, and N. Perrimon, 2005 Functional genomic analysis of the Wnt-wingless signaling pathway. Science 308: 826–833.
Davidson, G., W. Wu, J. Shen, J. Bilic, U. Fenger *et al.*, 2005 Casein kinase 1 gamma couples Wnt receptor activation to cytoplasmic signal transduction. Nature 438: 867–872.
Del Valle-Perez, B., O. Arques, M. Vinyoles, A. G. de Herreros, and M. Dunach, 2011 Coordinated action of CK1 isoforms in canonical Wnt signaling. Mol. Cell. Biol. 31: 2877–2888.
Dietzl, G., D. Chen, F. Schnorrer, K.-C. Su, Y. Barinova *et al.*, 2007 A genome-wide transgenic RNAi library for conditional gene inactivation in *Drosophila*. Nature 448: 151–156.
Dokucu, M. E., S. L. Zipursky, and R. L. Cagan, 1996 *atonal*, *rough* and the resolution of proneural clusters in the developing *Drosophila* retina. Development 122: 4139–4147.
Domínguez, M., 1999 Dual role for Hedgehog in the regulation of the proneural gene *atonal* during ommatidia development. Development 126: 2345–2353.
Domínguez, M., J. D. Wasserman, and M. Freeman, 1998 Multiple functions of the EGF receptor in *Drosophila* eye development. Curr. Biol. 8: 1039–1048.
Doroquez, D. B., and I. Rebay, 2006 Signal integration during development: mechanisms of EGFR and Notch pathway function and cross-talk. Crit. Rev. Biochem. Mol. Biol. 41: 339–385.
Elyada, E., A. Pribluda, R. E. Goldstein, Y. Morgenstern, G. Brachya *et al.*, 2011 CKIalpha ablation highlights a critical role for p53 in invasiveness control. Nature 470: 409–413.
Escudero, L. M., M. Bischoff, and M. Freeman, 2007 Myosin II regulates complex cellular arrangement and epithelial architecture in *Drosophila*. Dev. Cell 13: 717–729.
Flores, G. V., A. Daga, H. R. Kalhor, and U. Banerjee, 1998 Lozenge is expressed in pluripotent precursor cells and patterns multiple cell types in the *Drosophila* eye through the control of cell-specific transcription factors. Development 125: 3681–3687.
Foulds, C. E., M. L. Nelson, A. G. Blaszczak, and B. J. Graves, 2004 Ras/mitogen-activated protein kinase signaling activates Ets-1 and Ets-2 by CBP/p300 recruitment. Mol. Cell. Biol. 24: 10954–10964.
Freeman, M., 1994 The *spitz* gene is required for photoreceptor determination in the *Drosophila* eye where it interacts with the EGF receptor. Mech. Dev. 48: 25–33.
Freeman, M., 1996 Reiterative use of the EGF receptor triggers differentiation of all cell types in the *Drosophila* eye. Cell 87: 651–660.
Freeman, M., 1997 Cell determination strategies in the *Drosophila* eye. Development 124: 261–270.
Galletti, M., S. Riccardo, F. Parisi, C. Lora, M. K. Saqcena *et al.*, 2009 Identification of domains responsible for ubiquitin-dependent degradation of dMyc by glycogen synthase kinase 3beta and casein kinase 1 kinases. Mol. Cell. Biol. 29: 3424–3434.
Golembo, M., R. Schweitzer, M. Freeman, and B. Z. Shilo, 1996 *argos* transcription is induced by the *Drosophila* EGF receptor pathway to form an inhibitory feedback loop. Development 122: 223–230.
Heberlein, U., T. Wolff, and G. M. Rubin, 1993 The TGF beta homolog *dpp* and the segment polarity gene *hedgehog* are required for propagation of a morphogenetic wave in the *Drosophila* retina. Cell 75: 913–926.
Herranz, H., L. Perez, F. A. Martin, and M. Milan, 2008 A Wingless and Notch double-repression mechanism regulates G1-S transition in the *Drosophila* wing. EMBO J. 27: 1633–1645.
Heslip, T. R., H. Theisen, H. Walker, and J. L. Marsh, 1997 Shaggy and dishevelled exert opposite effects on Wingless and Decapentaplegic expression and on positional identity in imaginal discs. Development 124: 1069–1078.
Janody, F., J. D. Lee, N. Jahren, D. J. Hazelett, A. Benlali *et al.*, 2004 A mosaic genetic screen reveals distinct roles for *trithorax* and *Polycomb* group genes in *Drosophila* eye development. Genetics 166: 187–200.
Jarman, A., E. Grell, L. Ackerman, L. Jan, and Y. Jan, 1994 *atonal* is the proneural gene for *Drosophila* photoreceptors. Nature 369: 398–400.
Jia, J., K. Amanai, G. Wang, J. Tang, B. Wang *et al.*, 2002 Shaggy/GSK3 antagonizes Hedgehog signalling by regulating Cubitus interruptus. Nature 416: 548–552.
Jia, J., C. Tong, B. Wang, L. Luo, and J. Jiang, 2004 Hedgehog signalling activity of Smoothened requires phosphorylation by protein kinase a and casein kinase I. Nature 432: 1045–1050.
Jia, J., L. Zhang, Q. Zhang, C. Tong, B. Wang *et al.*, 2005 Phosphorylation by double-time/CKIepsilon and CKIalpha targets Cubitus interruptus for Slimb/beta-TRCP-mediated proteolytic processing. Dev. Cell 9: 819–830.
Kenyon, K. L., S. S. Ranade, J. Curtiss, M. Mlodzik, and F. Pignoni, 2003 Coordinating proliferation and tissue specification to promote regional identity in the *Drosophila* head. Dev. Cell 5: 403–414.
Klein, T. J., A. Jenny, A. Djiane, and M. Mlodzik, 2006 CKIepsilon/discs overgrown promotes both Wnt-Fz/beta-catenin and Fz/PCP signaling in *Drosophila*. Curr. Biol. 16: 1337–1343.
Kumar, J. P., T. Jamal, A. Doetsch, F. R. Turner, and J. B. Duffy, 2004 CREB binding protein functions during successive stages of eye development in *Drosophila*. Genetics 168: 877–893.
Lee, J. D., and J. E. Treisman, 2001 The role of Wingless signaling in establishing the anteroposterior and dorsoventral axes of the eye disc. Development 128: 1519–1529.
Lee, T., and L. Luo, 2001 Mosaic analysis with a repressible cell marker (MARCM) for *Drosophila* neural development. Trends Neurosci. 24: 251–254.
Legent, K., and J. E. Treisman, 2008 Wingless signaling in *Drosophila* eye development. Methods Mol. Biol. 469: 141–161.

Leshko-Lindsay, L. A., and V. G. Corces, 1997 The role of selectins in *Drosophila* eye and bristle development. Development 124: 169–180.

Li, J., C. Sutter, D. S. Parker, T. Blauwkamp, M. Fang *et al.*, 2007 CBP/p300 are bimodal regulators of Wnt signaling. EMBO J. 26: 2284–2294.

Li, Y., and N. E. Baker, 2001 Proneural enhancement by Notch overcomes Suppressor-of-Hairless repressor function in the developing *Drosophila* eye. Curr. Biol. 11: 330–338.

Ligoxygakis, P., S. Y. Yu, C. Delidakis, and N. E. Baker, 1998 A subset of *Notch* functions during *Drosophila* eye development require *Su(H)* and the *E(spl)* gene complex. Development 125: 2893–2900.

Liu, C., Y. Li, M. Semenov, C. Han, G. H. Baeg *et al.*, 2002 Control of beta-catenin phosphorylation/degradation by a dual-kinase mechanism. Cell 108: 837–847.

Lum, L., S. Yao, B. Mozer, A. Rovescalli, D. Von Kessler *et al.*, 2003 Identification of Hedgehog pathway components by RNAi in *Drosophila* cultured cells. Science 299: 2039–2045.

Ma, C., and K. Moses, 1995 Wingless and Patched are negative regulators of the morphogenetic furrow and can affect tissue polarity in the developing *Drosophila* compound eye. Development 121: 2279–2289.

Ma, C., Y. Zhou, P. Beachy, and K. Moses, 1993 The segment polarity gene *hedgehog* is required for progression of the morphogenetic furrow in the developing *Drosophila* eye. Cell 75: 927–938.

Marin, O., V. H. Bustos, L. Cesaro, F. Meggio, M. A. Pagano *et al.*, 2003 A noncanonical sequence phosphorylated by casein kinase 1 in beta-catenin may play a role in casein kinase 1 targeting of important signaling proteins. Proc. Natl. Acad. Sci. USA 100: 10193–10200.

Matsubayashi, H., S. Sese, J. S. Lee, T. Shirakawa, T. Iwatsubo *et al.*, 2004 Biochemical characterization of the *Drosophila* Wingless signaling pathway based on RNA interference. Mol. Cell. Biol. 24: 2012–2024.

Neumann, C., and S. Cohen, 1996 A hierarchy of cross-regulation involving *Notch*, *wingless*, *vestigial* and *cut* organizes the dorsal/ventral axis of the *Drosophila* wing. Development 122: 3477–3485.

Neumann, C. J., and S. M. Cohen, 1997 Long-range action of Wingless organizes the dorsal-ventral axis of the *Drosophila* wing. Development 124: 871–880.

Newsome, T., B. Asling, and B. Dickson, 2000 Analysis of *Drosophila* photoreceptor axon guidance in eye-specific mosaics. Development 127: 851–860.

Ni, J.-Q., M. Markstein, R. Binari, B. Pfeiffer, L.-P. Liu *et al.*, 2008 Vector and parameters for targeted transgenic RNA interference in *Drosophila melanogaster*. Nat. Methods 5: 49–51.

Ni, J.-Q., R. Zhou, B. Czech, L.-P. Liu, L. Holderbaum *et al.*, 2011 A genome-scale shRNA resource for transgenic RNAi in *Drosophila*. Nat. Methods 8: 405–407.

Nolo, R., L. A. Abbott, and H. J. Bellen, 2000 Senseless, a Zn finger transcription factor, is necessary and sufficient for sensory organ development in *Drosophila*. Cell 102: 349–362.

Olson, E. R., R. Pancratov, S. S. Chatterjee, B. Changkakoty, Z. Pervaiz *et al.*, 2011 Yan, an ETS-domain transcription factor, negatively modulates the Wingless pathway in the *Drosophila* eye. EMBO Rep. 12: 1047–1054.

O'Neill, E. M., I. Rebay, R. Tjian, and G. M. Rubin, 1994 The activities of two Ets-related transcription factors required for *Drosophila* eye development are modulated by the Ras/MAPK pathway. Cell 78: 137–147.

Peifer, M., D. Sweeton, M. Casey, and E. Wieschaus, 1994 Wingless signal and Zeste-white 3 kinase trigger opposing changes in the intracellular distribution of Armadillo. Development 120: 369–380.

Peters, J. M., R. M. McKay, J. P. McKay, and J. M. Graff, 1999 Casein kinase I transduces Wnt signals. Nature 401: 345–350.

Pollard, T. D., 2007 Regulation of actin filament assembly by Arp2/3 complex and formins. Annu. Rev. Biophys. Biomol. Struct. 36: 451–477.

Price, M. A., and D. Kalderon, 2002 Proteolysis of the Hedgehog signaling effector Cubitus interruptus requires phosphorylation by Glycogen Synthase Kinase 3 and Casein Kinase 1. Cell 108: 823–835.

Rajan, A., A. C. Tien, C. M. Haueter, K. L. Schulze, and H. J. Bellen, 2009 The Arp2/3 complex and WASp are required for apical trafficking of Delta into microvilli during cell fate specification of sensory organ precursors. Nat. Cell Biol. 11: 815–824.

Ready, D., T. Hanson, and S. Benzer, 1976 Development of the *Drosophila* retina, a neurocrystalline lattice. Dev. Biol. 53: 217–240.

Robinow, S., and K. White, 1991 Characterization and spatial distribution of the ELAV protein during *Drosophila melanogaster* development. J. Neurobiol. 22: 443–461.

Rodrigues, A. B., E. Werner, and K. Moses, 2005 Genetic and biochemical analysis of the role of Egfr in the morphogenetic furrow of the developing *Drosophila* eye. Development 132: 4697–4707.

Rogers, E. M., C. A. Brennan, N. T. Mortimer, S. Cook, A. R. Morris *et al.*, 2005 Pointed regulates an eye-specific transcriptional enhancer in the *Drosophila hedgehog* gene, which is required for the movement of the morphogenetic furrow. Development 132: 4833–4843.

Roignant, J.-Y., and J. E. Treisman, 2009 Pattern formation in the *Drosophila* eye disc. Int. J. Dev. Biol. 53: 795–804.

Ryoo, H. D., T. Gorenc, and H. Steller, 2004 Apoptotic cells can induce compensatory cell proliferation through the JNK and the Wingless signaling pathways. Dev. Cell 7: 491–501.

Sakanaka, C., 2002 Phosphorylation and regulation of beta-catenin by casein kinase I epsilon. J. Biochem. 132: 697–703.

Santos, J. A., E. Logarinho, C. Tapia, C. C. Allende, J. E. Allende *et al.*, 1996 The *casein kinase 1 alpha* gene of *Drosophila melanogaster* is developmentally regulated and the kinase activity of the protein induced by DNA damage. J. Cell Sci. 109: 1847–1856.

Schroeder, B., S. G. Weller, J. Chen, D. Billadeau, and M. A. McNiven, 2010 A Dyn2–CIN85 complex mediates degradative traffic of the EGFR by regulation of late endosomal budding. EMBO J. 29: 3039–3053.

Schweitzer, R., R. Howes, R. Smith, B. Z. Shilo, and M. Freeman, 1995 Inhibition of *Drosophila* EGF receptor activation by the secreted protein Argos. Nature 376: 699–702.

Seugnet, L., P. Simpson, and M. Haenlin, 1997 Requirement for Dynamin during Notch signaling in *Drosophila* neurogenesis. Dev. Biol. 192: 585–598.

Shen, J., C. Dahmann, and G. O. Pflugfelder, 2010 Spatial discontinuity of *optomotor-blind* expression in the *Drosophila* wing imaginal disc disrupts epithelial architecture and promotes cell sorting. BMC Dev. Biol. 10: 23.

Siegfried, E., E. L. Wilder, and N. Perrimon, 1994 Components of Wingless signalling in *Drosophila*. Nature 367: 76–80.

Sigrist, S. J., and C. F. Lehner, 1997 *Drosophila fizzy-related* down-regulates mitotic cyclins and is required for cell proliferation arrest and entry into endocycles. Cell 90: 671–681.

Sinnberg, T., M. Menzel, S. Kaesler, T. Biedermann, B. Sauer *et al.*, 2010 Suppression of casein kinase 1alpha in melanoma cells induces a switch in beta-catenin signaling to promote metastasis. Cancer Res. 70: 6999–7009.

Smelkinson, M. G., and D. Kalderon, 2006 Processing of the *Drosophila* Hedgehog signaling effector Ci-155 to the repressor

Ci-75 is mediated by direct binding to the SCF component Slimb. Curr. Biol. 16: 110–116.
Swiatek, W., H. Kang, B. A. Garcia, J. Shabanowitz, G. S. Coombs *et al.*, 2006 Negative regulation of LRP6 function by Casein kinase I epsilon phosphorylation. J. Biol. Chem. 281: 12233–12241.
Tie, F., R. Banerjee, C. A. Stratton, J. Prasad-Sinha, V. Stepanik *et al.*, 2009 CBP-mediated acetylation of histone H3 lysine 27 antagonizes *Drosophila* Polycomb silencing. Development 136: 3131–3141.
Tio, M., C. Ma, and K. Moses, 1994 Spitz, a *Drosophila* homolog of Transforming growth factor-alpha, is required in the founding photoreceptor cells of the compound eye facets. Mech. Dev. 48: 13–23.
Tomlinson, A., and D. F. Ready, 1987 Neuronal differentiation in the *Drosophila* ommatidium. Dev. Biol. 120: 366–376.
Treisman, J. E., and G. M. Rubin, 1995 *wingless* inhibits morphogenetic furrow movement in the *Drosophila* eye disc. Development 121: 3519–3527.
Vaccari, T., H. Lu, R. Kanwar, M. E. Fortini, and D. Bilder, 2008 Endosomal entry regulates Notch receptor activation in *Drosophila melanogaster*. J. Cell Biol. 180: 755–762.
van de Wetering, M., R. Cavallo, D. Dooijes, M. van Beest, J. van Es *et al.*, 1997 Armadillo coactivates transcription driven by the product of the *Drosophila* segment polarity gene *dTCF*. Cell 88: 789–799.
Vieira, A. V., C. Lamaze, and S. L. Schmid, 1996 Control of EGF receptor signaling by clathrin-mediated endocytosis. Science 274: 2086–2089.
Vincent, J., and C. Girdham, and P. O'Farrell, 1994 A cell-autonomous, ubiquitous marker for the analysis of *Drosophila* genetic mosaics. Dev. Biol. 164: 328–331.
Waltzer, L., and M. Bienz, 1998 *Drosophila* CBP represses the transcription factor TCF to antagonize Wingless signalling. Nature 395: 521–525.
Waltzer, L., and M. Bienz, 1999 A function of CBP as a transcriptional co-activator during Dpp signalling. EMBO J. 18: 1630–1641.
Windler, S. L., and D. Bilder, 2010 Endocytic internalization routes required for Delta/Notch signaling. Curr. Biol. 20: 538–543.
Woods, D. F., and P. J. Bryant, 1991 The *discs-large* tumor suppressor gene of *Drosophila* encodes a guanylate kinase homolog localized at septate junctions. Cell 66: 451–464.
Xu, T., and G. Rubin, 1993 Analysis of genetic mosaics in developing and adult *Drosophila* tissues. Development 117: 1223–1237.
Yanagawa, S.-i., Y. Matsuda, J.-S. Lee, H. Matsubayashi, S. Sese *et al.*, 2002 Casein kinase I phosphorylates the Armadillo protein and induces its degradation in *Drosophila*. EMBO J. 21: 1733–1742.
Zallen, J. A., Y. Cohen, A. M. Hudson, L. Cooley, E. Wieschaus *et al.*, 2002 SCAR is a primary regulator of Arp2/3-dependent morphological events in *Drosophila*. J. Cell Biol. 156: 689–701.
Zecca, M., K. Basler, and G. Struhl, 1996 Direct and long-range action of a Wingless morphogen gradient. Cell 87: 833–844.
Zeng, X., K. Tamai, B. Doble, S. Li, H. Huang *et al.*, 2005 A dual-kinase mechanism for Wnt co-receptor phosphorylation and activation. Nature 438: 873–877.
Zhai, R., P. Hiesinger, T. Koh, P. Verstreken, K. Schulze *et al.*, 2003 Mapping *Drosophila* mutations with molecularly defined P element insertions. Proc. Natl. Acad. Sci. USA 100: 10860–10865.
Zhang, L., J. Jia, B. Wang, K. Amanai, K. A. Wharton *et al.*, 2006 Regulation of Wingless signaling by the CKI family in *Drosophila* limb development. Dev. Biol. 299: 221–237.
Zhou, Q., and D. Kalderon, 2011 Hedgehog activates fused through phosphorylation to elicit a full spectrum of pathway responses. Dev. Cell 20: 802–814.

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