**Chapter 15. Overlapping PCP functions and Pk family proteins.**

Cytoskeletal remodelling is driven by the self-assembly of cytoskeletal sub-units. In particular, the components of the mitotic spindle and contractile ring must be delivered to specific locations at precise stages of the cell-cycle. Active transport mechanisms also regulate the distribution of nuclei, Golgi, centrioles and mitochondria. Thus, cell shape and the cell-cycle progression are coupled throughout morphogenesis, with many conserved genetic functions.

The PCP phenotype of the *dsh*$^{1}$ mutant is similar to *fz*$^{1}$, or *pk-sple*$^{13}$; however, embryonic lethal alleles of *dsh* give segmentation and D/V patterning defects [1 2]. Similarly, Fz function is deployed during terminal PCP signalling; while Fz2, Fz3 and Fz4 have overlapping, and partially complimentary, functions during earlier developmental stages [3 4 5]. Most mutant alleles of the *stan* cadherin are recessive lethal, although the viable alleles have an associated PCP phenotype [6]. Thus, although PCP genes are deployed during terminal differentiation, related functions may be required during earlier developmental stages. As with other morphogenetic functions, LOF mutants of *pk* are associated with multiple pleiotropic phenotypes in (apparently) unrelated processes. The wild-type function of *pk* has remained enigmatic, largely due to the complex complementation patterns between different mutant alleles. The initial genetic analysis identified three classes of allele: The *pk*$^{1}$ mutant (later designated as *pk*$^{pk1}$) shows extreme alterations in bristle and hair orientations in the notum, wing and haltere. In contrast, *sple*$^{1}$ (later designated *pk*$^{sple1}$) gives extreme polarity alterations in the abdomen and legs, but wild-type polarity in the notum and wing; while double-mutant (*pk*$^{pk\text{-}sple}$) alleles show a moderate phenotype over the whole body with rough eyes, like the *fz*$^{1}$ or *dsh*$^{1}$ mutants [7]. The original *pk* and *sple* mutants were described as separate genes and, indeed, heterozygous *pk*$^{1}$/*sple*$^{1}$ flies are completely wild-type [8]. During gastrulation, the Pk protein is expressed between the parasegmental En stripes and in the CNS; while *pk* transcripts are expressed one or two rows of cells to either side of the ventral mid-line and segmental infoldings (Fig. 20).

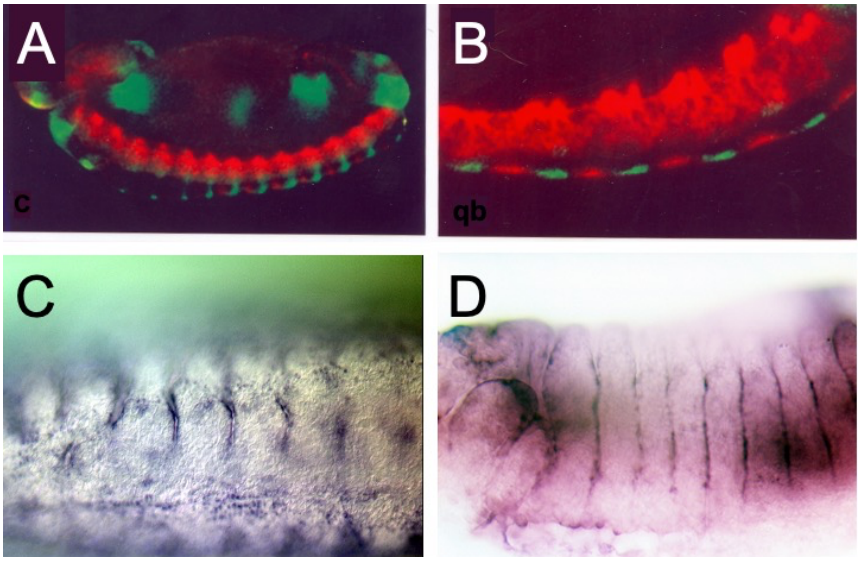


**Fig. 20. Embryonic Pk expression**, stage 13. **A.** and **B**. Pk antibody (red) Wg (green), David Tree, D. Phil thesis, University of Cambridge, 1999. Pk antibody against common peptide segment. **C.** *in situ* probe to *pk* common exons, showing *pk* transcripts preferentially localized to Ap surface of enfolded cells along segmental boundaries and cells on either side of the V mid-line. **D.** *in situ* probe to *pk*$^{B}$ 5' exon. D. Gubb, unpublished.

In the third larval (L3) leg discs, Pk is expressed in concentric rings (Fig. 21), while the phenocritical period for the *pk*$^{sple}$ tarsal phenotype is during the second larval (L2) stage [9] [10]. *pk* transcripts are uniformly expressed across the pre-pupal wing blade and leg discs, but absent from the presumptive wing veins and tarsal segment boundaries [11].

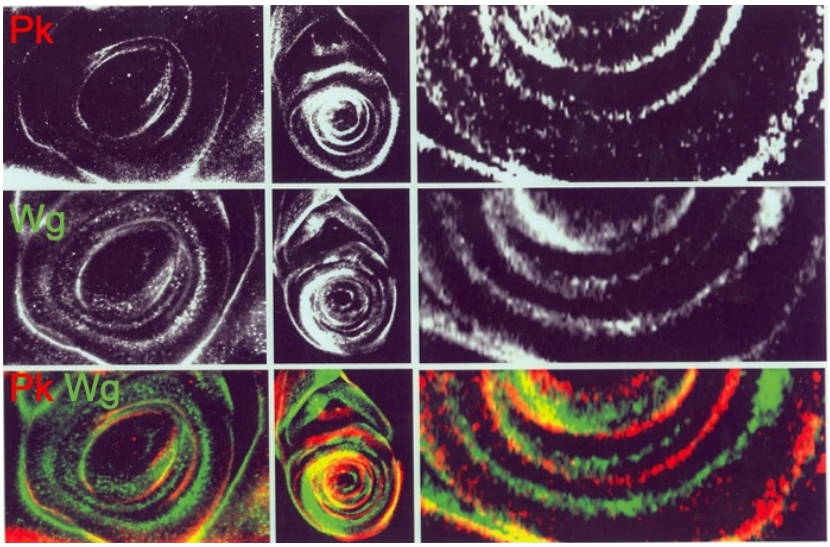


**Fig. 21. Pk and Wg expression in leg disc**. Pk antibody (red) Wg antibody (green). Rings of Pk expression tend to be displaced from the rings of Wg expression, near segmental boundaries. However, Pk and Wg distribution overlaps in some cells. David Tree, D. Phil thesis, university of Cambridge, 1999. Pk antibody against 3' common peptide segment.

Adult *pk*$^{pk}$ wings show reversed bristles and hairs along the anterior D/V margin, proximal to the junction of the first and second (V1 and V2) and a cruciform disclination near the tip of V5 (Fig. 21). Other PCP mutants, also show slightly elevated A marginal bristles, but their rotation is much less than in *pk*$^{pk}$ [12]. The *pk*$^{pk}$ wing phenotype is completely supressed by *mwh* (in *pk*$^{pk}$*; mwh* double-mutants), except along the anterior D/V margin, which retains *pk* polarity (Gubb and Garcia-Bellido 1982) [13]. By implication, cytoskeletal remodelling may be differentially regulated along the anterior D/V margin, rather than via the Mwh/Rho1 pathway within the wing blade [14] [15] [16]. Notably, *pk*$^{pk}$ mutant clones are elongated along the anterior D/V margin, and only a few cells in width, re-examination of preparations from [17]. The overexpression of Pk$^{sple}$ in the D wing disc reverses the orientation of marginal bristles and hairs, but the polarity adjacent bristles and hairs on V wing surface remains wild-type (Fig. 22).

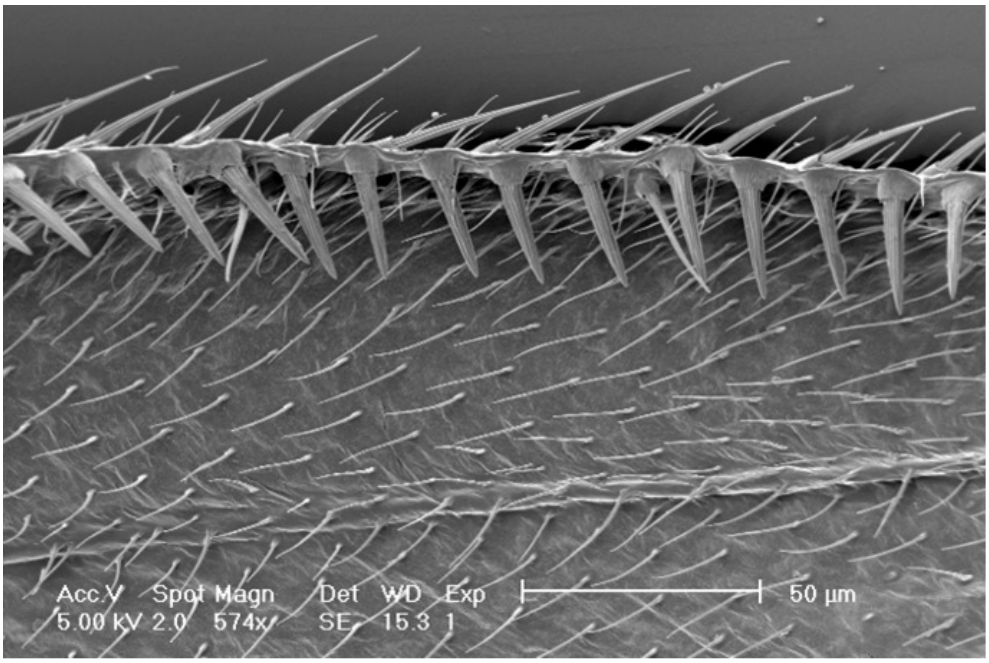


**Fig. 22. Overexpression of Pk$^{sple}$ in the D wing.** Marginal bristles and hairs are rotated but adjacent bristles and hairs on the V wing surface retain wild-type polarity. In this strain, a slight contraction of the D surface causes the margin to fold inwards.

Meanwhile, the *pk* cognate gene, *espinas* (*esn*), partially complements the multiple wing hair phenotype associated with other PCP genes. Thus, chromosomal deletions that include *esn* and the *pk$^{pk}$* 5' exon, show *pk* mutant polarity with multiple wing hairs (Fig. 23).

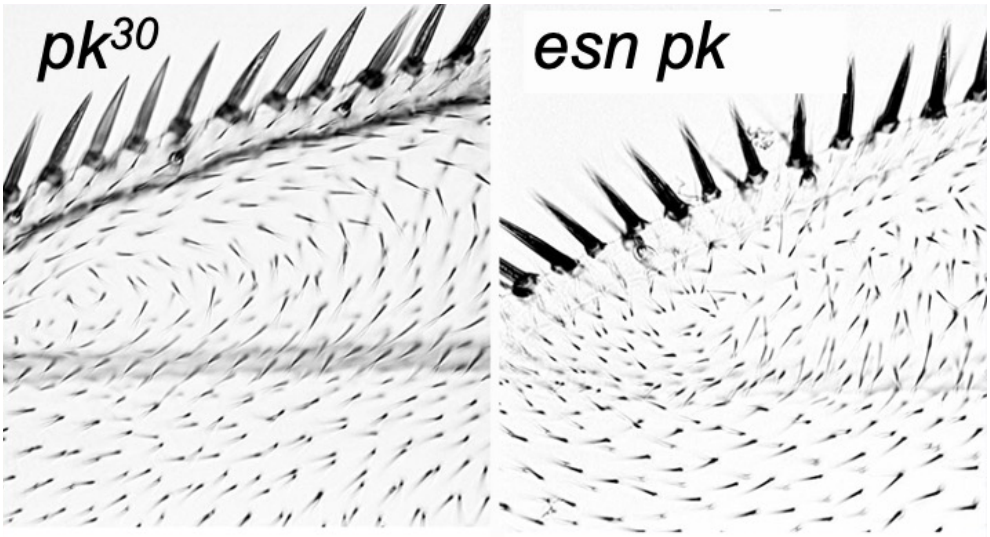


**Fig. 23. Partial complementation of *pk* by *esn*. A.** Wing blade near tip of V2, in *pk$^{pk30}$*, a homozygous deletion (1.1 kb) of the 5' *pk* exon, phase contrast. Hairs on the V wing surface give shadows from below the plane of focus. **B.** A *esn pk* trans-heterozygous deletion of esn and 5' *pk* exon*: Df(2R)pk-sple 51/Df(2R) nap2* shows a *pk$^{pk}$* polarity pattern, with additional hairs. Similarly, deletions removing *esn* and the entire *pk* gene show the *pk$^{pk\text{-}sple}$* polarity pattern with additional hairs, unpublished observations.

In addition, the $pk^{pk\text{-}sple13}$ double mutant shows a bald patch lacking bristles in the anterior notum, as does transgenic overexpression of *esn* and $pk^{pk}$ (Fig. 24).

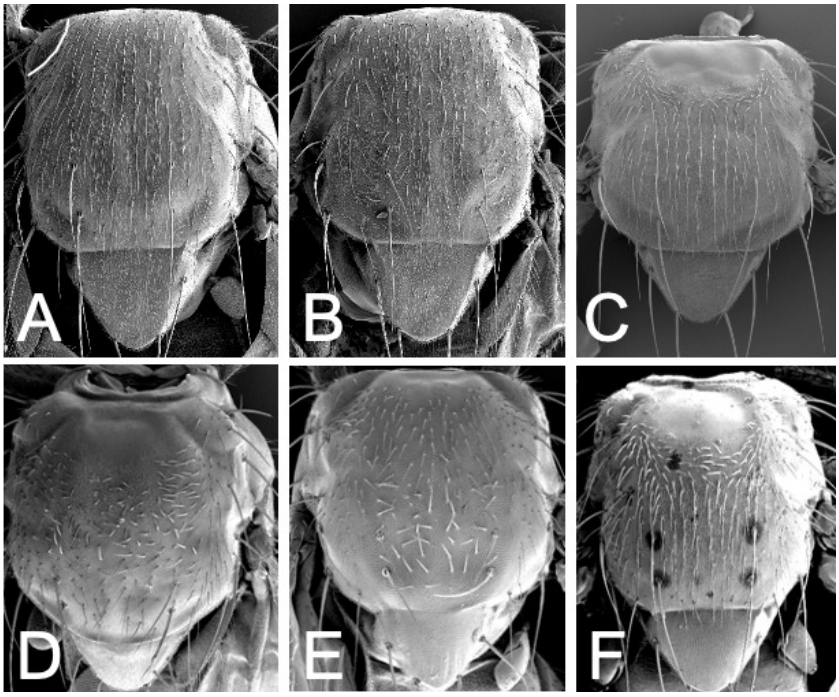


**Fig. 24. Notal bristle patterns, pk LOF and GOF.** SEM images of mesothorcic surface. **A.** wild-type, white line indicates fused prothorax (humeral plate). **B.** $pk^{pk30}$ **C.** $pk^{pk\text{-}sple13}$. **D.** *pnr-Gal4; UAS-*$pk^{pk}$. **E.** *pnr-Gal4; UAS-*$pk^{sple}$. **F.** *pnr-Gal4; UAS-esn*. A bald patch (C, D, F) is consistent with reduced in Ap trafficking of the cytoskeletal components required for bristle formation.

Over-expression of Esn in *da-Gal4; UAS-esn* flies is semi-lethal, with partial larval paralysis and upturned terminal segments (Fig. 25).

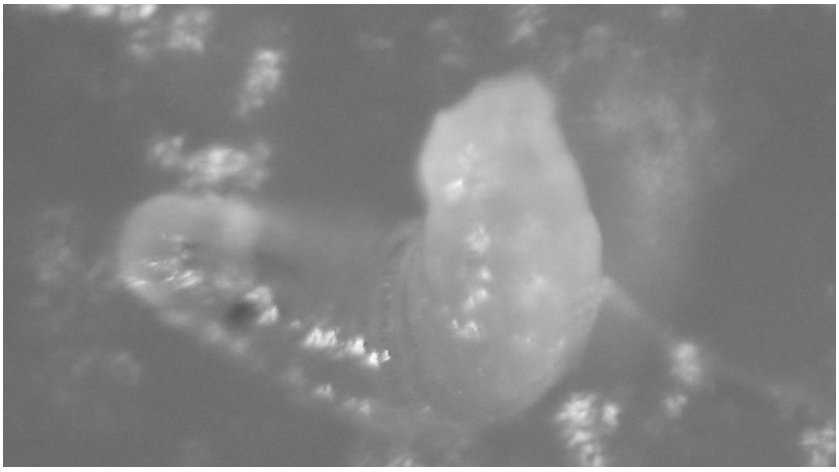

**Fig. 25. Larval overexpression of *esn*.** Movements are disco-ordinated and larvae fail to burrow through the medium, in *da-Gal4/UAS-esn*. Occasional larvae survive to L3 stage, with upturned posterior segments.

By contrast, the *pk*$^{MI07065}$ gene-trap transposon insertion is associated with a gain of function (GOF) female sterile phenotype, which can be suppressed by over-expression of E-cadherin (see below, Chapter **17**). These additional phenotypes indicate perturbations in multiple morphogenetic functions.

At the molecular level, the *pk* gene encodes three protein isoforms with different N-terminal peptides and a single 3' UTR. The common exons of Pk encode a PET (Prickle, Espinas, Testin) domain and triple LIM domains [11]. It is lack of the Pk$^{sple}$ isoform that leads to chiral patterning defects and myoclonous epilepsy [18]; while loss of the Pk$^{pk}$ isoform alters bristle and hair orientations in the wing and notum. No mutant phenotype has been associated with second isoform, Pk$^{PB}$, which is expressed during embryogenesis. The number of Pk-family genes in vertebrates is increased compared to *Drosophila*, but these orthologues encode single protein isoforms, with the exception of murine Pk2 (J. Sutherland, personal communication) and human Pk4 [19]. Human Pk1 mutations are associated with foetal agenesis of the *corpus callosum*, altered facial nerve migration, autism spectrum disorders, cancer cell metastasis and focal adhesion disassembly in migrating cells [20 21 22] (Yang et al., 2014) [24 25 23]; while myoclonous epilepsy is associated with LOF of either Pk1 or Pk2 [26 27]. As in *Drosophila*, the vertebrate *pk* mutants are viable and recessive, except for associated late-onset neuronal defects [26 27].

In addition to *pk* and *esn*, the PET family of *Drosophila* includes *tes* and *limpet* (*lmpt*). All four genes are expressed during embryogenesis, although null mutants lack embryonic phenotypes in the fly, apart from occasional segmental pathfinding defects with *esn* [11 28 29 30]. Pk and Esn carry a C-terminal CAAX box motif, which may allow reversible lipidation of membrane-associated proteins [31], while Tes and Lmpt lack the CAAX motif. Notably, the uptake of Gram-positive bacteria and fungi by haemocytes is blocked in *lmpt* mutants [29 32]. Like Pk, Lmpt interacts with Dsh and the actin cytoskeletal remodelling factors RhoGEF2 and Drk (Downstream receptor kinase) [33]. *Tes* null mutants show a PCP phenotype in the inner ear of the mouse and defective female reproductive tract development [34]. By contrast, morpholino knockdown of *tes* in *Xenopus* gives embryonic neural crest and axial elongation defects, which are enhanced by the double knockdown of *tes* and *pk* [35 36]. In mice and humans, *tes* null mutations are dominant, haplo-insufficient tumour suppressors, despite being homozygous viable [34 37 38 39].

**Summary:**

**Cytoskeletal remodelling requires precise sub-cellular localisation and assembly of cytoskeletal components in stoichiometric ratios. In consequence, complex, dose-sensitive phenotypes may be associated with the morphogenetic functions that regulate cytoskeletal remodelling and intracellular signal transmission. In particular, the alternative Pk isoforms of *Drosophila*, are mutually antagonistic and partially complemented by Esn. The *pk* mutants of both flies and vertebrates are viable, despite which heterozygous null mutants may show defects in neuronal function and oncogenesis. Thus, PCP defects are associated with complex developmental alterations and adult-onset disease syndromes.**

**References:**


1. Gubb, D. Genes controlling cellular polarity in Drosophila. *Dev. Suppl* 269–277 (1993).

2. Boutros, M, Patricio, N, Strutt, D, & Mlodzik, M. Dishevelled activates JNK and discriminates between JNK Pathways in planar polarity and wingless signaling. *Cell* **94**, (1998).
3. Zhang, J. & Carthew, R. W. Interactions between wingless and DFz2 during Drosophila wing development. *Development* **125**, 3075–3085 (1998).
4. Moline, M. M., Dierick, H. A., Southern, C. & Bejsovec, A. Non-equivalent roles of Drosophila frizzled and dfrizzled2 in embryonic wingless signal transduction. *Curr. Biol.* **10**, 1127–1130 (2000).
5. Sivasankaran, R., Calleja, M., Morata, G. & Basler, K. The Wingless target gene Dfz3 encodes a new member of the Drosophila Frizzled family. *Mech. Dev.* **91**, 427–431 (2000).
6. Chae, J. *et al.* The Drosophila tissue polarity gene starry night encodes a member of the protocadherin family. *Development* **126**, 5421–5429 (1999).
7. Gubb, D. & Garcia-Bellido, A. A genetic analysis of the determination of cuticular polarity during development in Drosophila melanogaster. *J. Embryol. Exp. Morphol.* **68**, 37–57 (1982).
8. Lindsley, D. L & Zimm, G. G. *The Genome of Drosophila Melanogaster.* (Academic press, 1992).
9. Held, L. I., Duarte, C. M. & Derakhshanian, K. Extra tarsal joints and abnormal cuticular polarities in various mutants of Drosophila melanogaster. *Rouxs Arch. Dev. Biol.* **195**, 145–157 (1986).
10. Held, L. I. When does the spiny-legs[1] allele of the prickle gene cause extra joints? *D.I.S.* **94**, 47–52 (2011).
11. Gubb, D. *et al.* The balance between isoforms of the prickle LIM domain protein is critical for planar polarity in Drosophila imaginal discs. *Genes Dev.* **13**, 2315–2327 (1999).
12. Gubb, D. Genes controlling cellular polarity in Drosophila. *Dev. Suppl* 269–277 (1993).
13. Lu, Q., Yan, J. & Adler, P. N. The Drosophila planar polarity proteins inturned and multiple wing hairs interact physically and function together. *Genetics* **185**, 549–558 (2010).
14. Rogers, S. L., Wiedemann, U., Hacker, U., Turck, C. & Vale, R. D. Drosophila RhoGEF2 associates with microtubule plus ends in an EB1-dependent manner. *Curr. Biol.* **14**, 1827–1833 (2004).
15. Yan, J., Lu, Q., Fang, X. & Adler, P. N. Rho1 has multiple functions in Drosophila wing planar polarity. *Dev. Biol.* **333**, 186–199 (2009).
16. Lu, Q., Yan, J. & Adler, P. N. The Drosophila planar polarity proteins inturned and multiple wing hairs interact physically and function together. *Genetics* **185**, 549–558 (2010).
17. Gubb, D. & Garcia-Bellido, A. A genetic analysis of the determination of cuticular polarity during development in Drosophila melanogaster. *J. Embryol. Exp. Morphol.* **68**, 37–57 (1982).
18. Ehaideb, S. N. *et al.* prickle modulates microtubule polarity and axonal transport to ameliorate seizures in flies. *Proc Natl Acad Sci U A* **111**, 11187–92 (2014).
19. Asad, M. *et al.* FZD7 drives in vitro aggressiveness in Stem-A subtype of ovarian cancer via regulation of non-canonical Wnt/PCP pathway. *Cell Death Dis.* **5**, e1346 (2014).
20. Carreira-Barbosa, F. *et al.* Prickle 1 regulates cell movements during gastrulation and neuronal migration in zebrafish. *Development* **130**, 4037–46 (2003).

21. Fujimura, L., Watanabe-Takano, H., Sato, Y., Tokuhisa, T. & Hatano, M. Prickle promotes neurite outgrowth via the Dishevelled dependent pathway in C1300 cells. *Neurosci Lett* **467**, 6–10 (2009).
22. Paemka, L. *et al.* Prickle1 interaction with Synapsin1 reveals a role in autism spectrum disorders. *PLoS ONE* **8**, e80737 (2013).
23. Lim, B. C. *et al.* Prickle1 promotes focal adhesion disassembly in cooperation with the CLASP–LL5β complex in migrating cells. *J. Cell Sci.* **129**, 3115 (2016).
24. Bassuk, A. G. & Sherr, E. H. A de novo mutation in Prickle1 in fetal agenesis of the corpus callosum and polymicrogyria. *J. Neurogenet.* **29**, 174–177 (2015).
25. Daulat, A. M. *et al.* Prickle1 Contributes to cancer cell dissemination through its interaction with mTORC2. *Dev. Cell* **37**, 311–325 (2016).
26. Bassuk, A. G. *et al.* A homozygous mutation in human Prickle1 causes an autosomal-recessive progressive myoclonus epilepsy-ataxia syndrome. *Am. J. Hum. Genet.* **83**, 572–581 (2008).
27. Manak, J. R. *et al.* Mutations in Prickle orthologs cause seizures in flies, mice, and humans. *Am. J. Hum. Genet.* **88**, 138–149 (2011).
28. Coumailleau, F. & Schweisguth, F. Insensible is a novel nuclear inhibitor of notch activity in Drosophila. *PLoS ONE* **9**, e98213 (2014).
29. Jin, L. H. *et al.* Identification and functional analysis of antifungal immune response genes in Drosophila. *PLoS Pathog.* **4**, e1000168 (2008).
30. Matsubara, D., Horiuchi, S. Y., Shimono, K., Usui, T. & Uemura, T. The seven-pass transmembrane cadherin Flamingo controls dendritic self-avoidance via its binding to a LIM domain protein, Espinas, in Drosophila sensory neurons. *Genes Dev.* **25**, 1982–1996 (2011).
31. Clarke, S. Protein isoprenylation and methylation at carboxyl-terminal cysteine residues. *Annu. Rev. Biochem.* **61**, 355–386 (1992).
32. Nedelsky, N. B. *et al.* Native functions of the androgen receptor are essential to pathogenesis in a Drosophila model of spinobulbar muscular atrophy. *Neuron* **67**, 936–952 (2010).
33. Rhee, D. Y. *et al.* Transcription Factor Networks in Drosophila melanogaster. *Cell Rep.* **8**, 2031–2043 (2014).
34. Ren, D.-D. *et al.* Testin interacts with Vangl2 genetically to regulate inner ear sensory cell orientation and the normal development of the female reproductive tract in mice. *Dev. Dyn. Off. Publ. Am. Assoc. Anat.* **242**, 1454–1465 (2013).
35. Takeuchi, M. *et al.* The prickle-related gene in vertebrates is essential for gastrulation cell movements. *Curr Biol* **13**, 674–9 (2003).
36. Dingwell, K. S. & Smith, J. C. Tes regulates neural crest migration and axial elongation in Xenopus. *Dev. Biol.* **293**, 252–267 (2006).
37. Tobias, E. S., Hurlstone, A. F., MacKenzie, E., McFarlane, R. & Black, D. M. The TES gene at 7q31.1 is methylated in tumours and encodes a novel growth-suppressing LIM domain protein. *Oncogene* **20**, 2844–2853 (2001).
38. Drusco, A. *et al.* Knockout mice reveal a tumor suppressor function for Testin. *Proc. Natl. Acad. Sci. U. S. A.* **102**, 10947–10951 (2005).
39. Zhu, J. *et al.* Testin is a tumor suppressor and prognostic marker in breast cancer. *Cancer Sci.* **103**, 2092–2101 (2012).