

A novel planar polarity gene *pepsinogen-like* regulates *wingless* expression in a posttranscriptional manner

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Abstract

Background: Planar cell polarity (PCP) originally referred to the coordination of global organ axes and individual cell polarity within the plane of the epithelium. More recently, it has been accepted that pertinent PCP regulators play essential roles not only in epithelial sheets, but also in various rearranging cells.

Results: We identified *pepsinogen-like* (*pcl*) as a new planar polarity gene, using *Drosophila* wing epidermis as a model. Pcl protein is predicted to belong to a family of aspartic proteases. When *pcl* mutant clones were observed in pupal wings, PCP was disturbed in both mutant and wild-type cells that were juxtaposed to the clone border. We examined levels of known PCP proteins in wing imaginal discs. The amount of the seven-pass transmembrane cadherin Flamingo (Fmi), one of the PCP 'core group' members, was significantly decreased in mutant clones, whereas neither the amount of nor the polarized localization of Dachshous (Ds) at cell boundaries was affected. In addition to the PCP phenotype, the *pcl* mutation caused loss of wing margins. Intriguingly, this was most likely due to a dramatic decrease in the level of Wingless (Wg) protein, but not due to a decrease in the level of *wg* transcripts.

Conclusions: Our results raise the possibility that Pcl regulates Wg expression post-transcriptionally, and PCP, by proteolytic cleavages.

Introduction

In epithelia, cells are polarized along a fixed axis within the plane, which is critical for many organ functions. Underlying mechanisms of this planar cell polarity (PCP) have been best studied in the *Drosophila* wing, where epidermal cells somehow sense an organ axis, localize an assembly of actin filaments at the distal cell vertexes, and produce single wing hairs in pupae (Adler 2002). It has been shown that evolutionary conserved regulators of PCP orchestrate a variety of collective cell behaviors, such as polarized protrusive cell activity, directional cell movement, and oriented cell division, so they are crucial for the normal development of both epithelial and non-epithelial tissues (Seifert & Mlodzik 2007; Gray et al. 2011; Vichas and Zallen, 2011).

In spite of a number of molecular players identified, a long-standing question is how exactly individual cell polarity is coordinated with global organ axes. At the molecular level, this coordination is visible in the localization of the 'core group' of the PCP regulators at selective plasma membrane domains, such as proximodistal cell boundaries in the *Drosophila* wing epidermis; and this 'core pathway' plays an instructive role in the polarity establishment (Goodrich & Strutt 2011). The core group includes the seven-pass transmembrane cadherin Flamingo / Starry night (Fmi / Stan) and Frizzled (Fz) (Usui et al. 1999; Chae et al. 1999; Strutt 2001). The outstanding question above can now be rephrased as how the polarized core protein localization becomes aligned with organ axes, and what is the molecular

identity of the polarizing cue.

Without the function of a distinct group of PCP regulators, the localization of the core proteins is misaligned with the proximodistal axis of the wing. This group includes atypical cadherins Fat (Ft) and Dachshous (Ds), and the Golgi kinase Four-jointed (Fj), which we refer to as the "Ft/Ds group" (Adler et al. 1998; Strutt & Strutt 2002; Matakatsu & Blair 2004; Ishikawa et al. 2008; Sharma and McNeill 2013). The Ft/Ds group can influence core protein localization, for example, by affecting the cell division axis and cell rearrangement (Ma et al., 2003; Aigouy et al., 2010), or by controlling the polarity of planar microtubules that are proposed to contribute to directionally biased transport of Fz (Shimada et al. 2006; Harumoto et al. 2010). The relationship between the Ft/Ds group and the core group has been a target of intense investigations (Casal et al., 2006; Thomas & Strutt 2012; Brittle et al., 2012; Sagner et al., 2012; Blair, 2012).

Aside from the Ft/Ds group, there has been a persistent candidate for the polarizing cue: the Wnt family, which acts in regulating development and also impacts diseases such as cancer (Sugimura and Li, 2010; Rao and Kühl, 2010; Clevers and Nusse, 2012; Nusse and Varmus, 2012). Vertebrate Wnts could serve an instructive role, linking both cellular and organ polarity (Gao et al., 2011; Gray et al., 2011). In insects, Wingless (Wg), the *Drosophila* orthologue of Wnt1, was shown to be a morphogen that governs the dorsal-ventral patterning of the wing (Herranz and Milán, 2008); but it has been controversial whether Wg and other *Drosophila* Wnts provide the cue across the entire wing or not (Chen et al., 2008; Sagner et al., 2012; Wu et al., 2013).

To identify additional components that mediate PCP establishment, we conducted a mosaic screen of the X chromosome and isolated mutations that provoked drastic misorientation of wing hairs (Mouri et al., 2012). In this study, we focused on one intriguing mutation, which mislocalized Fmi and in addition down-regulated Wg protein. The causative gene is *pepsinogen-like (pcl)*, whose product is highly homologous to members of the aspartic protease family including cathepsin D and E, pepsin, and beta-site APP-cleaving enzyme (BACE) (Dunn, 2002). These proteases show broad substrate specificities, and their activities are kept tightly in check to prevent uncontrolled proteolysis (Conus and Simon, 2010). Compared to the established roles of aspartic proteases in digestion and immunity, less is known about their contributions to developmental events. We discuss how the PCP phenotype and the down-regulation of Wg in the *pcl* mutant clones are related to each other.

Results and Discussion

A mutation in *pepsinogen-like* (*pcl*) results in a planar polarity phenotype and loss of the wing margin

To identify novel planar polarity genes, we performed a mosaic screen for X-chromosome mutations. We generated mosaic clones of about 3000 lethal chromosomes and searched for the polarity phenotype in adult wings (see details in Mouri et al., 2012). We isolated 30 chromosomes that caused severe misorientation of wing hairs, and focused on one of them, #11166 (Fig. 1). In addition to the polarity defect, #11166 clones showed loss of wing margins (arrowheads in Fig. 1B). As described below, #11166 was mutated in *pepsinogen-like* (*pcl*)/CG13374 (McQuilton et al., 2012); thus, we designated this allele as *pcl^l* and hereafter refer to it as such.

Next, we observed *pcl^l* homozygous clones in pupal wings 32 hr after puparium formation (APF). A subpopulation of mutant cells along the distal clone border showed misorientation of prehair (Fig. 2A-2C; see left arrowhead in Fig. 2A); in contrast, mutant cells further inside the clone (e.g., near the left edge of Fig. 2A) did not. Intriguingly, neighboring wild-type cells that were located distal to the mutant clone also showed the misorientation (right arrowhead in Fig. 2A). These local cell autonomous and non-cell autonomous phenotypes were also revealed by mislocalization of Fmi at anterior-posterior cell boundaries in the clone (Fig. 2B and arrowheads in 2E) and in the adjacent wild-type cells (arrowheads in Fig. 2F), in contrast to the normal localization at distal cell boundaries (arrowheads in Fig. 2D). This

mislocalization of Fmi was reminiscent of that in Ft/Ds group mutant clones (Ma et al., 2003; Strutt and Strutt, 2002). Clones with mutations in Ft/Ds group genes mislocalize Fmi proteins at the wrong cell boundaries (anterior-posterior boundaries) both cell autonomously and non-cell autonomously. This phenotype contrasts with mutant clones of core group genes, where Fmi no longer localizes tightly to particular cell boundaries (Usui et al., 1999).

***pcl*¹ is required for normal planar polarity and wing-margin formation**

Through genetic mapping and sequencing, we found mutations that resulted in two adjoining amino-acid substitutions in the *pcl* coding region, raising the possibility that *pcl* was the gene responsible for the planar polarity defect (Fig. 3A, see also Experimental Procedures). The Pcl protein is predicted to belong to a family of aspartic proteases, and the substituted amino acids were located just N-terminal to a sequence, SSTY, which is well conserved among aspartic proteases.

To verify that *pcl* was the responsible gene for the #11166 phenotypes, we performed a rescue experiment using two duplications (Venken et al., 2010) that partially overlapped each other. Dp(1; 3)DC007, which includes *pcl*, rescued the lethality and restored the wing phenotypes to normal (Fig. 3B and 3C), whereas Dp(1; 3)DC098 did not (data not shown). Because *pcl* is the only annotated gene that is inside DC007 and not contained on DC098, we concluded that *pcl* is most likely the responsible gene. We also performed a rescue experiment by expressing the *pcl* cDNA under the control of the *armadillo-GAL4* or *daughterless-GAL4*. Both of the GAL4 lines rescued both

lethality and the margin phenotype. We note, however, the PCP phenotype was only partially rescued, and a moderate polarity defect was still observed in the rescued animals (Fig. 3D and E).

***pcl* regulates *wg* expression in a posttranscriptional manner**

It is known that the Wingless (Wg) and Notch pathways are required for formation of the wing margin (Neumann and Cohen, 1996; Herranz and Milán, 2008). The loss of wing margins elicited by the *pcl* mutation implied that *pcl* was necessary for either the Wg or the Notch pathway. To address which pathway and which step in either pathway was primarily affected by *pcl¹*, we examined the expression levels of various proteins or markers in wing imaginal discs in late 3rd instar larvae that were populated by *pcl¹* mutant clones. As previously established, in the wild-type disc, Wg is expressed in the future wing margin (Couso et al., 1994; Micchelli et al., 1997). By contrast, the Wg signal was significantly decreased in the *pcl* mutant clones (Fig. 4A and 4A'). In some smaller mutant clones, the reduction of Wg signal was less obvious than in larger clones (Fig. 4B and 4B'), possibly due to a perdurance effect. The level of Cut protein was also lower in the mutant clones (Fig. 4C and 4C'). Because the *cut* gene is one of the direct targets in Wg-responsive cells and is essential for the margin formation, these results imply that the *pcl¹* mutation reduced the amount of Wg protein, which resulted in less *cut* expression in the signal-receiving cells, and ultimately the loss of the wing margin (Couso et al., 1994; Micchelli et al., 1997).

How, then, does the *pcl* mutation abrogate expression of *wg*? In the wing

disc, transcription of the *wg* gene is induced by Notch signaling (Rulifson and Blair, 1995). Thus, we examined the effect of the *pcl* mutation on Notch signaling by using *vg-lacZ* (Neumann and Cohen, 1996) and *wg-lacZ* (Kassis et al., 1992) reporter genes as readouts. Intriguingly, we could not detect any change in either the *vg-lacZ* or the *wg-lacZ* signal in *pcl* mutant clones (Fig. 4D-4E'). These results suggested that Notch signaling and its downstream events, including transcription of *wg*, were not affected by the *pcl* mutation. Therefore, the reduction of Wg protein was possibly due to a defect(s) in post-transcriptional regulation, such as at the level of translation, intracellular or extracellular degradation, and/or secretion.

In contrast to the large number of studies on the signaling pathway in Wnt signal-responsive cells, our knowledge is still limited about how Wnt proteins such as Wg are processed and secreted. Enzymes and secretory proteins that are dedicated to Wnt signals have been identified (Herr et al., 2012). Nonetheless, only a few proteins are known to regulate the secretion of Wnt protein: Porcupine (Porc) palmitoylates Wnt at the ER, whereas Wntless/Evenness Interrupted (Wls/Evi) facilitates the secretion of Wnt protein from the Golgi to the extracellular space (Kadowaki et al., 1996; Bänziger et al., 2006; Bartscherer et al., 2006). Wg proteins accumulate in Wg-producing cells that are mutant for *porc* or *wls* (van den Heuvel et al., 1993; Bänziger et al., 2006; Bartscherer et al., 2006), which contrasts with the disappearance of Wg protein in *pcl* clones. We suspected that *pcl* might regulate Wg proteins by modulating the level of Porc or Wls. However, when we immunostained Wls protein, we saw no difference between *pcl* mutant

and wild-type cells (Fig. 4F and 4F').

The *pcl* mutation does not affect the expression of *four-jointed* and *dachsous*

Because the mislocalization of Fmi in *pcl¹* mutant clones resembled that in *fj* or *ds* mutant clones as described above, it could be that *pcl* was functionally associated with these genes. To test this possibility, we observed the signal of a *fj-lacZ* reporter gene in *pcl¹* mutant clones. We did not detect significant changes in the signal between *pcl* mutant and wild-type cells (Fig. 5A-5A"). We also immunostained pupal wings with anti-Dachsous (Ds) antibody, and did not detect any alteration of the Ds level between mutant clones and adjacent WT clones in the wing pouch (Fig. 5B-5B').

We further examined quantitatively whether the amount of Ds and its polarized localization at cell boundaries were affected or not. For this purpose, we focused on mutant clones that reached the dorsal hinge region where Ds is localized in a polarized fashion along the proximal-distal axis (Brittle et al., 2012), and compared "PCP nematic" (Aigouy et al., 2010) in the wild-type cells with that of the mutant cells (Fig. 5C-F, see also experimental procedures). We could detect significant differences in none of the amount (Fig. 5D) or the magnitude (Fig. 5E) of and the axis (Fig. 5F) of the PCP nematic between the wild-type and mutant cells.

These results suggest that *pcl* did not regulate the expression level of *fj* or the amount and the polarized localization of Ds; however, it does not necessarily exclude possible functional interactions between Pcl and these

proteins. For example, the Ds ectodomain is subject to endoproteolytic processing and this is modulated by Ft (Ambegaonkar et al., 2012), implying a possible involvement of the hypothetical molecular activity of Pcl.

Fmi protein is less abundant in *pcl* mutant clones in larval imaginal discs

Although the loss of wing margins was most likely a direct consequence of the decrease in Wg as described above, how did the *pcl* mutation give rise to the defect in PCP? It has been recently reported that Wg, together with DWnt4, plays a role in PCP by providing a long-range directional cue to cells (Wu et al., 2013). However, mutant clones that remove *wg*, *dWnt4*, and two other *dWnts* genes cause misorientation of wing hairs along the margin (Wu et al., 2013), and adult wings comprised predominantly of *wg* mutant cells show a mild hair misorientation only at the distal margin (Sagner et al., 2012). In contrast, the aberrant PCP phenotype along *pcl* mutant clone borders was seen when the clones were generated either along the margin or inside the wing blade (Figure 1B and 1D, and Figure 2A-2C). So a decrease in the amount of Wg in the *pcl* clones may not be a sole cause of the PCP phenotype.

To explore the basis of the *pcl* PCP phenotype, we examined levels of PCP core proteins in *pcl* mutant clones in both pupal wings and larval discs. We found that Fmi at cell boundaries was significantly decreased, but not totally eliminated, in *pcl* mutant clones in 3rd instar larval discs (Fig. 6A-6C) and this decrease in the apical Fmi was not associated with relocalization of

Fmi at the basal level in the same cells (data not shown). In some small clones, down-regulation of Fmi was less obvious. In contrast, expression levels of a basolateral marker Discs large (Dlg), the *Drosophila* β -catenin Armadillo (Arm), one of the PCP core-group members Dishevelled (Dsh), or DE-cadherin was not altered in mutant clones (Fig. 6D-6E'; data of Dsh and DE-cadherin were not shown). This specific reduction of Fmi in *pcl* mutant clones in larval imaginal discs is puzzling, because the amount of Fmi was not apparently decreased (although it was mislocalized) in pupal wings as described above (Fig. 2B and 2E). It has been shown that cell-boundary localization of Fmi and other core-group proteins is already polarized at a late larval stage (Classen et al., 2005; Sagner et al., 2012). It remains to be studied whether the decrease in the Fmi amount in discs is at least one cause of the clone-border selective PCP phenotype in pupal wings.

Conclusion: Pcl acts in both PCP and Wg signaling

In this study, we reported that both of the two well-known developmental mechanisms, PCP formation and Wnt signaling, require *pcl* encoding a putative aspartic protease. Aspartic protease family proteins play extracellular and intracellular roles; for example, pepsinogen digests foods in the stomach lumen, whereas cathepsins function in adaptive immunity in lysosomes (Conus and Simon, 2010). Further studies will clarify where in the *Drosophila* wing epidermis and where in the cell Pcl is required, whether Pcl indeed possesses an aspartic protease activity or not, and if so, what are its endogenous substrates. Considering that aspartic proteases have broad

substrate specificity (Conus and Simon, 2010), Pcl may indirectly control the activity and/or stability of Wg and Fmi through proteolytic cleavages of distinct substrates.

Experimental procedures

Mutant screening

Mutation screening was done as described previously (Mouri et al., 2012). Briefly, mutations were induced in *w FRT19A/Y* males, and the mutagenized chromosomes were balanced. Mutant clones were induced and phenotypes were analyzed in the adult wing. *pcl¹/pcl¹* was early larval lethal.

Fly strains and genetics

Control strains used were *y w*. Transgene UAS-*pcl* was expressed by using the GAL4/UAS system (Brand and Perrimon, 1993). Driver strains employed were *armadillo (arm)-GAL4* and *daughterless (ds)-GAL4*, both of which were obtained from the Bloomington Drosophila Stock Center.

Duplication and deficiency strains used in mapping (described below) were provided by the Drosophila Genetic Resource Center (DGRC). Other stocks were *vg-lacZ* (Neumann and Cohen, 1996), *wg-lacZ* (Kassis et al., 1992), *fj-lacZ* (Brodsky and Steller, 1996), *Dp(1; 3)DC007* and *Dp(1; 3)DC098* (Bloomington Drosophila Stock Center). All fly embryos, larvae, pupae, and adults were reared at 25 °C unless described otherwise. Exact genotypes of individual animals used in Figures are as follows:

Fig. 1

(A and C) *y w*

(B and D) *w pcl¹ FRT19A/w FRT19A; vg-GAL4 UAS-FLP/+*

Fig. 2 and Fig. 6

w pcl^l FRT19A/y w ubi-GFP FRT19A; vg-GAL4 UAS-FLP/+

Fig. 3

(B and C) *w pcl^l FRT19A/Y; Dp(1; 3)DC007/+*

(D and E) *w pcl^l FRT19A/Y; UAS-pcl/arm-GAL4*

Fig. 4

(A–C', F, and F') *w pcl^l FRT19A/y w ubi-GFP FRT19A; vg-GAL4 UAS-FLP/+*

(D and D') *w pcl^l FRT19A/y w ubi-GFP FRT19A; vg-GAL4 UAS-FLP/vg-lacZ*

(E and E') *w pcl^l FRT19A/y w ubi-GFP FRT19A; vg-GAL4 UAS-FLP/wg-lacZ*

Fig. 5

(A) *w pcl^l FRT19A/y w ubi-GFP FRT19A; vg-GAL4 UAS-FLP/fj-lacZ*

(B–C') *w pcl^l FRT19A/y w ubi-GFP FRT19A; vg-GAL4 UAS-FLP/+*

Mapping

To identify the affected genes in isolated mutants, #11166/FM7 flies were mated with four deficiency and duplication stocks (DGRC stock number 108921, 108145, 108138 and 106068). Taking together the information of lethality and deficiency/duplication points in individual lines, we narrowed the genomic region of the responsible gene of stock #11166 to 200 kb, which included 3 genes (*CG32816*, *l(1)sc*, and *pcl*). We sequenced coding regions of these 3 genes in the female genome of #11166, and found two substitution mutations in the *pcl* gene.

Molecular biology

A cDNA fragment encoding the *pcl* gene was cloned into a pUAST-based plasmid containing a UAS promoter. To generate transgenic flies, this construct, pUAST-*pcl*, was microinjected into fly embryos carrying the attP2-site, and integrated into the site by phiC31-mediated site-specific recombination (Bateman et al., 2006; Bischof et al., 2007).

Immunohistochemistry

Wing imaginal discs in wandering 3rd instar larvae or pupal wings were fixed and used for immunohistochemistry. Primary antibodies used were rabbit anti-GFP (Molecular Probes), mouse anti-Fmi #74 and rat anti-Fmi (Usui et al., 1999), rabbit anti-Dlg (Woods and Bryant, 1991), rabbit anti-Ds (Strutt and Strutt, 2002), and rabbit anti-Wls (Port et al., 2008). Mouse anti-Arm N2 7A1, mouse anti-Wg 4D4, and mouse anti-Cut 2B10 were obtained from the Developmental Studies Hybridoma Bank (DSHB) at the University of Iowa. Secondary antibodies were purchased from Molecular Probes and Jackson ImmunoResearch. Immunostaining and acquisition of confocal images were done as described previously (Mouri et al., 2012), and fluorescent intensity was measured by using Image J software (NIH).

Quantification of Ds distribution

We followed the quantification method (nematic order), which is essentially described by Aigouy et al. (2010), to determine the magnitude and axis of

nematic order for a single cell as the “PCP nematic” for that cell. We calculated the average value of the intensity for $I(r, \theta)$ for each θ section with a 5° range (e.g., 2.5° – 7.5° , 7.5° – 12.5° , etc.), where r is the distance between the pixel and the center of the cell and θ is the angle indicating the position of the pixel.

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Figure Legends

Fig. 1 A mutation in *pci* results in a planar cell polarity phenotype and loss of the margin in the wing.

(A, B) Wild-type (A) and *pci*¹ mosaic (B) adult wings. Clones of *pci*¹ in the adult wing caused loss of the wing margin (arrowheads). In this and all subsequent figures, distal is to the right and anterior is at the top. (C, D) Higher-power images of wild-type (C) and *pci*¹ mosaic wings (D) that are marked by red boxes in (A) and (B), respectively. (D) Wing hairs were misdirected posteriorly.

Fig. 2 The *pci*¹ mutation produces a non-cell autonomous effect.

(A-C) The 32 h APF (after puparium formation) wing was stained with phalloidin (A), for Fmi (B), and for a clone marker GFP (blue in C). A homozygous mutant clone was recognized by the absence of the GFP marker, and wild-type cells that border the clone are indicated with yellow dots (A and B). Magenta arrowheads in 'A' indicate misoriented prehairsts of the wild-type cells (right) and in the mutant clone (left). Scale bar: 10µm. (D-F) Higher-power images of wild-type cells deep inside the clone (D), mutant cells close to the clone border (E) and wild-type cells close to the border (F) in 'A'. Both the mutant and wild-type cells close to the border mislocalized Fmi at anterior-posterior cell boundaries (arrowheads in 'D' and

'F'), in contrast to normal localization at proximal-distal boundaries of the wild-type cells (arrowheads in 'D').

Fig. 3 *pcl* is the responsible gene. (A) Representation of the domain structure of Pcl protein. The thick red bar indicates a well-conserved domain among aspartic proteases. Partial amino acid sequences of Pcl and three other aspartic proteases, human cathepsin E (hCathE), human pepsinogen 5 (hPGA5), and human cathepsin D (hCathD), are aligned below. Asterisks indicate conserved amino acids among these proteases. *pcl^l* had two mutations in the coding sequence of this domain (ACTCAA instead of AATAAA), which substitute amino acids NK with TQ.

(B and C) The misorientation phenotype of wing hairs in the *pcl^l* mutant was rescued by duplication Dp(1; 3)DC007. (C) Higher-power image of a region that is marked by red box in (B).

(D and E) The misorientation phenotype was partially rescued by *UAS-pcl* that was expressed by *armadillo-GAL4*. (E) Higher-power image of a region that is marked by red box in (D).

Fig. 4 *pcl* regulates Wingless (Wg) expression.

(A-B') Wing imaginal discs of 3rd instar larvae were stained for Wg (magenta in the merged images in A and B), Cut (magenta in C), and a clone marker GFP (green in A-F). Mutant clones of *pcl* were marked by the loss of GFP. Expression of Wg or Cut was reduced in the mutant clones (arrowheads in A' and C'), but it was less obvious in clones in B'. (D - F')

Expression of *vestigial-lacZ* (*vg-lacZ*) (magenta in D), *wg-lacZ* (magenta in E), and Wntless (Wls; magenta in F) along the dorsal-ventral boundary was not altered in the mutant clones. Scale bar: 20µm.

Fig. 5 The *pcl*^Δ mutation does not significantly affect *fj* and *ds* expression.

(A-A'') Mutant clones of *pcl* in the wing disc were marked by the loss of GFP (green in A, and A''). Expression of *fj-lacZ* (magenta in A, and A') was not significantly altered in the clones (arrowhead).

(B-C') *pcl* mosaic clones were stained for Dachshous (Ds) (magenta in B and C, and B' and C') and for the clone marker GFP (green in B and C). A *pcl* mosaic clone in a 24 h APF wing (B-B') and those in the dorsal hinge region in the larval wing disc (C and C'). The nematic orders in the individual cells are overlaid on the image of Ds signals (yellow bars in C'). Distal is to the bottom of the panel. Scale bar: 20µm in A-B', 5µm in C and C'.

(D-F) Quantifications of the amount of Ds (D) and its polarized localization (E and F) in the wild-type cells (WT; N=140) and *pcl* mutant cells (*pcl*; N=96). (D and E) Box-and-whisker plots depicting the signal intensity of Ds at cell boundaries (D) and the magnitudes of nematic order for individual cells (E). The box plots show median (line), top, bottom (whiskers), after removing outliers, and 25th and 75th percentile (boxes). The signal intensity of Ds is indicated by artificial unit per pixel (D) and the magnitude of nematic order (length of each yellow bar in C') is indicated by artificial unit (E). $p > 0.05$, Wilcoxon rank sum test. NS: not significant. (F) The axis distributions of the

PCP nematic are shown by rose diagrams in a point symmetry manner. Each diagram is composed of 24 bins of 15° each, with an approximate direction of the dorsal/ventral compartment boundary (the presumptive wing margin) set to 0°, and concentric circles are drawn with 5% increments between them. The distributions in the two genotyped cells are not significantly different ($p > 0.05$, Mardia-Watson-Wheeler test). We performed the quantifications of total four discs that had mutant clones in the dorsal hinge regions, and the data of one of the four are shown in “C-F”, which is similar to that of the remaining three discs (data not shown).

Fig. 6 Fmi is less abundant in *pcl* mutant clones.

pcl mutant clones in wing discs were stained for Fmi (magenta in A and B, and A' and B'), Discs large (Dlg; magenta in D, and D'), or Armadillo (Arm; magenta in E, and E'). The mutant clone in E and E' was located in the future notum where the reduction of Wg signaling had less of an effect on the expression of Arm, compared to the wing margin. *pcl* mutant clones were recognized by the absence of the GFP marker (A-E). (B and B') Higher-power image of A and A', respectively. (C) A plot of fluorescent intensities of Fmi (magenta) and GFP (green) along the line in A that spans a *pcl* mutant clone and its adjacent wild-type clone. Scale bar: 10µm.

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