Planar cell polarity: the *prickle* gene acts independently on both the Ds/Ft and the Stan Systems

José Casal[1], Beatriz Ibáñez-Jiménez and Peter A. Lawrence[2]

Department of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ, United Kingdom

[1]jec85@cam.ac.uk

[2]pal38@cam.ac.uk

ABSTRACT

Epithelial cells are polarised within the plane of the epithelium, forming oriented structures whose coordinated and consistent polarity (planar cell polarity, PCP) relates to the principal axes of the body or organ. In Drosophila at least two separate molecular systems generate and interpret intercellular polarity signals: Dachsous/Fat, and the "core" or Stan system. Here we study the *prickle* gene and its protein products Prickle and Spiny leg. Much research on PCP has focused on the asymmetric localisation of proteins in the cell and as a result *prickle* was placed in the heart of the Stan system. Here we ask if this view is correct and how the *prickle* gene relates to the two systems. We find that *prickle* can affect, separately, both systems — however, neither Pk nor Sple are essential components of the Ds/Ft or the Stan system, nor do they act as a functional link between the two systems.

INTRODUCTION

Planar cell polarity (PCP) refers to a property that all, or most, epithelial cells have — they are coordinately oriented in the plane of the epithelial sheet and, sometimes, they demonstrate this by forming oriented structures. These oriented structures can be cell organelles such as cilia, or multicellular organs such as mammalian hairs (Tree et al., 2002a; Wang and Nathans, 2007; Goodrich and Strutt, 2011; Butler and Wallingford, 2017). *Drosophila* has been used to identify most of the genes involved in PCP and has proved the most amenable of all animals for elucidating its mechanisms. Most studies have asked where PCP gene products are localised in the cell and asked how these localisations relate to the propagation of polarity from cell to cell. Here we study one PCP gene, *prickle* (*pk*) which has been investigated intensively over the last 20 years. We design genetic experiments aimed at clarifying its function in the wildtype, particularly in relation to the two best defined PCP systems, the Dachsous/Fat (Ds/Ft) system and the Starry night (Stan) or core system.

A brief history of *pk*

The *pk* mutant was discovered in 1938 by Ives who described the thoracic bristles as disoriented: "irregularly erected and whorled, giving a prickle effect" (Ives, 1947). Later, a similar and closely linked mutation *spiny legs* (*sple*) was found (Gubb and Garcia-Bellido, 1982). Each mutation affects one of two homologous transcripts of the *pk* gene that encode the Pk and Sple proteins; both proteins contain protein-protein binding LIM domains, they differ in the N terminus (Gubb et al., 1999) and have sequence elements conserved to vertebrates. In vertebrates, many syndromes due to

pk mutations have been classified, perhaps prematurely, as planar polarity phenotypes (Tissir and Goffinet, 2013).

(i) *pk*: a founding member of the "core" PCP pathway

In the 1990s, the *pk* gene was grouped with a few other genes that affected polarity; their proteins constituted the "core system", all being asymmetrically but briefly localised in the wing cell just prior to formation of an oriented hair; for example, Pk is enriched on or near the proximal membrane of each cell (Tree et al., 2002b), while Frizzled (Fz) is localised distally (Strutt, 2001). Other core proteins include Vang Gogh (Vang), Dishevelled (Dsh) and Stan, also known as Flamingo (for a review see Goodrich and Strutt, 2011; Adler, 2012; Butler and Wallingford, 2017). The localisation of these proteins is mutually dependent; when one protein is removed, the others become evenly distributed around the cell periphery (reviewed in Strutt and Strutt, 2009). These observations led to a hypothesis that sets of core proteins associate asymmetrically on the wing cell membrane, proximally or distally, as a response to the direction of slope of a tissue-wide polarising gradient (Tree et al., 2002a; Strutt and Strutt, 2008; Strutt, 2009). Tree et al (2002b) then built a model in which Dsh, Pk and Fz interact with each other to amplify their asymmetric localisation within the cell to consolidate its polarity. They argued that "*planar cell polarity signaling in Drosophila requires the receptor Frizzled and the cytoplasmic proteins Dishevelled and Prickle.*" Perhaps most significantly they proposed that Pk in one cell interacts or "*is linked*" to the localisation of Fz and Dsh in the adjacent cell, ie there would be an intercellular bridge consisting of two different complexes facing each other across the cell membrane. This model is still widely accepted and Pk is considered to be an essential component of this link.

(ii) Pk is not needed for PCP signalling by the Stan system

Nevertheless, genetic experiments argue that, functionally, Pk and Sple are not required for polarity signalling from cell to cell. Adler et al. (2000) found that a weak allele of *pk* actually increased those local changes in cell polarity that are induced by clones mutant for other core genes. Further, Lawrence et al. (2004) showed that complete loss of Pk and Sple also increases polarisation by the core system genes; they proposed that the key molecules in the core system do not include Pk but are Stan, Fz and Vang. By contrast with the behaviour of *pk*, removal of each of these genes either blocks (*stan* or *fz*) or severely impairs (*Vang*) intercellular propagation of PCP by the Stan system (Taylor et al., 1998; Lawrence et al., 2004; Strutt and Strutt, 2007; Strutt and Warrington, 2008; Struhl et al., 2012). We renamed the core system the "Stan system" to emphasise the unique and central role of Stan; we use this name from now

on. This conclusion was later supported by Strutt and Strutt (2007) who presented further evidence that "*Dishevelled, Prickle and Diego are not needed for intercellular communication*" —they are not required for the propagation of polarity from cell to cell.

(iii) What are the functions of Pk and Sple?

When only the *pk* isoform is overexpressed everywhere, polarity of the A compartment is almost entirely reversed while the P compartment is normal. By contrast, when the *sple* isoform is overexpressed everywhere, polarity of the P compartment is completely reversed while the A compartment is normal (Lawrence et al., 2004). These results allow the hypothesis that Pk and Sple have similar basic functions (to turn around polarity): the local outcome depending on the distribution of both proteins and varied regional responses to them (Gubb and Garcia-Bellido, 1982). For example, in the wildtype, Pk is found to be most effective in the wing, in the P compartment of the abdomen and the posterior part of the thorax while Sple is thought to predominate in the A compartment of the abdomen and anterior region of the thorax (Gubb et al., 1999; Ayukawa et al., 2014; Merkel et al., 2014; Ambegaonkar and Irvine, 2015).

(iv) Current views of the *pk* gene

It is widely thought that PCP is produced by three tiers of gene activity (Tree et al., 2002a; Tree et al., 2002b; Yang et al., 2002; Klein and Mlodzik, 2005; Strutt and Strutt, 2005; Strutt and Strutt, 2007; Axelrod, 2009) in which gradients of activity of the protocadherins Dachsous (Ds) and Fat (Ft) orient the Stan system which in turn orients effector functions. Recently it has been posited that the *pk* gene intervenes between the polarising information specified by the direction of the gradients of Ds/Ft activity and its interpretation by the Stan system. These articles (Hogan et al., 2011; Ayukawa et al., 2014; Olofsson et al., 2014; Ambegaonkar and Irvine, 2015) also support earlier conclusions that Pk and Sple act discordantly on polarity output in different tissues and have improved the evidence that changes in the levels of Pk and/or Sple can turn around the orientation of polarised structures (Ayukawa et al., 2014). Moreover, they highlight Sple as the main component of a molecular link between Ds/Ft and the Stan systems. Here we provide genetic evidence that questions these hypotheses: we conclude that Pk and Sple are not essential components of either system nor do they function as components of a link between the two systems. We add to evidence that the Ds/Ft system acts independently of the Stan system and provide data that Pk and Sple can rectify the output of the Ds/Ft system. Pk and Sple

also, separately, affect the output of the Stan system; they do not change the sign of polarisation but alter how far the polarising signal can spread.

RESULTS

Explaining terms and methods

We make genetically marked clones of cells of different genotypes to ask how the two different genetic systems, the Ds/Ft system and the Stan system, define cell polarity in the anterior (A) and posterior compartments (P) of the adult abdomen. We assay function of the Ds/Ft system by the ability of "sending cells" in clones that, say, overexpress *ft*, to change the polarity of "receiving cells" nearby the clone. As a result, hairs and bristles around the clones may point "inwards" or "outwards", that is, in or away from the clone. For the Ds/Ft system, Ds, Ft and Dachs (D) are each essential; however removal of only Ds or Ft causes a misdistribution of the D protein in each cell (Ambegaonkar et al., 2012; Pan et al., 2013), leading to an adventitious phenotype of whorly polarity (Ambegaonkar et al., 2012; Lawrence and Casal, 2013). Therefore the cleanest way to break the Ds/Ft system completely and persuasively is to remove D as well as Ds or Ft. To break the Stan system we remove Stan; *stan*$^-$ cells cannot send or receive signals, for example *stan*$^-$ receiving cells cannot respond to cells that overexpress *fz* (Genotype 1) even when those sending cells also express *stan* (Lawrence et al., 2004; Casal et al., 2006). Using these functional assays we ask whether and how Pk and Sple cooperate with the Ds/Ft and the Stan systems.

Figure 1 acts as a summary of, and a guide to, all the experiments and results.

Do the Ds/Ft and Stan systems act independently in both A and P compartments?

There is a school of thought that upstream polarity information —given by the direction of slopes of gradients of Ds and Four-jointed (Fj) activity— is interpreted by the Stan system (Yang et al., 2002; Ma et al., 2003; Goodrich and Strutt, 2011; reviewed in Butler and Wallingford, 2017). Experiments in the adult abdomen showed that the non-autonomous effects on neighbouring cells by clones, for example, lacking *ft,* depended on the compartment. *ft*$^-$ clones in the A compartment made the surrounding cells point inwards towards the clone, while the same clones in the P compartment caused the surrounding cells to point outwards. We therefore argued that the gradient slopes of Ds and Fj activities might have different signs in the anterior (A) and the posterior (P) compartments (Casal et al., 2002). But if that were true then, because all the hairs point the same way (backwards) in the wildtype, hair polarity cannot be a direct readout of the gradient slope of the Ds/Ft system.

Experimental evidence provided a solution to this conundrum: perhaps Pk or Sple rectify the reading of a gradient in either the A or the P compartment so that all hairs point in the same direction (Lawrence et al., 2004). But, later experiments argued that the Stan system and the Ds/Ft system act independently of each other (Casal et al., 2006; Lawrence et al., 2007; Brittle et al., 2012) —implying that rectification due to Pk and/or Sple does not alter a direct input from the Ds/Ft system into the Stan system but avoids dissonance between their independent inputs into PCP.

(i) clones affecting the Ds/Ft system function when the Stan system is broken

Clones overexpressing *ft* polarise both wildtype cells (Genotype 2) and cells in which the Stan system is broken —we used flies lacking *stan*, (Genotype 3) or, in the case of A clones, both *stan* and *fz* (Casal et al., 2006). In both cases the receiving cells tend to point hairs outwards from the clone in the A compartments (Casal et al., 2006) and inwards in the P compartments (Figure 2 and Figure 3). Consistent with these results, clones overexpressing the extracellular domain of Ds also polarise both wildtype cells (Genotype 4) and cells in which the Stan system is broken (*stan*$^{-}$ Genotype 5) inwards in the anterior portion of A compartments (Casal et al., 2002; Casal et al., 2006). These clones are ineffective in the posterior parts of A compartments and in P compartments (Figure S3), probably because the activity of Ds is normally high in these places (Casal et al., 2002).

(ii) clones affecting the Stan system function when the Ds/Ft system is broken

Clones that overexpress *fz*, in either the A or P compartments, normally turn the polarity of receiving cells to point outwards from the clone in A (Casal et al., 2006) and also in P (Genotype 6, Figure S2). They do the same in *ds*$^{-}$ flies but with a longer range (Genotype 7; Adler et al., 1998; Ma et al., 2003; Casal et al., 2006; Figure S1).

These experiments have established that the two systems act independently; we now ask are Pk and Sple are essential components of either the Ds/Ft or the Stan systems?

How do Pk and Sple interact with each of the two systems?

(i) evidence from epistasis

ds^{-} and *pk-sple*$^{-}$ flies differ in phenotype in the dorsal abdomen: the most useful difference is seen in the P compartment, where, in *ds*$^{-}$ flies, hairs in the anterior region of the P compartment are in whorls (probably due to the misdistribution of Dachs) but in its posterior part the hairs point directly anteriorward. By contrast, in *pk-sple*$^{-}$ flies, the entire P compartment has normal polarity (Lawrence et al., 2004). We find

that, in the abdomen, *ds*⁻ *pk-sple*⁻ flies (Genotype 8) are little different from *ds*⁻ flies (Figure 1). It follows that the *ds* mutation is epistatic to a mutation that removes both *pk* and *sple* functions; a finding suggesting that the Pk gene acts entirely through the Ds/Ft system. However other results argue that Pk and Sple can act independently of the Ds/Ft system (see below). By contrast, when *pk-sple*⁻ *stan*⁻ flies are compared to each single mutant, they differ from both, having an additive phenotype (Genotype 9 and Genotype 10, Figure 1). These results suggest that Pk and Sple act separately but differently on each of the two systems.

(ii) The Stan system functions well, both in cells that lack *pk* and *sple* and in cells that have *pk* or *sple* overexpressed

1. In *pk-sple*⁻ flies. In the abdomen of *pk-sple*⁻ flies (Genotype 11), polarity of most of the A compartment is reversed, but the P compartment is normal. Clones of cells that overexpress *fz* (Genotype 12 or, alternatively, lack *fz,* Genotype 13) in such *pk-sple*⁻ flies strongly polarise receiving cells in both A and P compartments; in both compartments the clones affect mutant receiving cells with the same sign as in wildtype receiving cells, that is outwards from the clones that overexpress *fz* and inwards towards clones that lack *fz*, independently of the prevailing polarity of the receiving cells (Figure S2). Thus, the Stan system does not need Pk or Sple to send polarity signals or to repolarise receiving cells (cf Lawrence et al., 2004).

2. When *pk* or *sple* are overexpressed. In flies in which either *sple* (Genotype 14) or *pk* (Genotype 15) are overexpressed, large areas of each abdominal segment show abnormal polarity. Nevertheless *fz*⁻ clones polarise receiving cells of both compartments inwards —as they do in wildtype flies—, independently of the prevailing polarity of those receiving cells (Figure 6). All these results are mutually consistent: they show that polarity changes induced by the Stan system do not require products of the *pk* gene, showing that Pk and Sple are not essential components of the Stan system in the wildtype.

3. However, the *fz*⁻ clones do not behave exactly as they would in a wildtype background: absence or excess of Pk and Sple change the amount of polarisation caused by clones with altered amounts of Fz. In A compartments of the abdomen, clones of cells that lack *fz* alter polarity of surrounding wildtype cells. The number of rows of receiving cells affected, the range, varies with the amount of Pk and/or Sple protein: in *pk-sple*⁻ flies (Genotype 13) the range of polarisation due to *fz*⁻ clones or excess *fz* (Figure 4 in Lawrence et al., 2004) is increased, resembling the increase in range observed when *fz*⁻ clones are induced in *ds*⁻ flies (Genotype 16). Raising the level of Pk ubiquitously does not change that range (Genotype 15), while when Sple

levels are raised (Genotype 14), polarisation is reduced (Figure S5). In the P compartments, we detected no effects on range; either in *pk-sple*$^{-}$ flies or when the levels of either Pk or Sple were increased (Figure S2 and Figure S5). These results add to evidence that the Stan system can function independently of Pk and Sple.

(iii) Pk and Sple alter polarity even when the Stan system is broken

Uniform overexpression of *pk* causes large changes of polarity in the abdomen of flies with a broken Stan system (*stan*$^{-}$, Genotype 17) in the A compartment, without affecting the P compartment (Figure 4). While generalised overexpression of *sple* also affects the polarity of *stan*$^{-}$ flies, but altering the polarity of the P compartment of the abdomen, without much affecting the A compartment (Figure 5).

(iv) Pk and Sple affect PCP even when the Ds/Ft system is broken

1. General overexpression of *pk* or *sple* in a broken Ds/Ft system. If Pk and Sple acted exclusively on the Ds/Ft system, one would expect Pk and Sple proteins not to affect PCP if the Ds/Ft system were broken. But we find that ubiquitous overexpression of *pk* alters polarity of the A compartment (and part of the P compartment) of *ds*$^{-}$ *pk-sple*$^{-}$ (Genotype 19, Figure 1), *d*$^{-}$ (Genotype 20, Figure S4) and *ft*$^{-}$ *d*$^{-}$ flies (Genotype 21, Figure 4). Similarly, general overexpression of *sple* affects the polarity of the P compartment of the abdomen of *ds*$^{-}$ *pk-sple*$^{-}$ (Genotype 22, Figure 1), *d*$^{-}$ (Genotype 23, Figure S4) and *ft*$^{-}$ *d*$^{-}$ flies (Genotype 24, Figure 5).

In *d*$^{-}$ flies, the A and P compartments are largely normal but a section of the P compartment is reversed, as in *ds*$^{-}$ (or *ft*$^{-}$) flies. When ubiquitous Pk is added to *d*$^{-}$ or *ft*$^{-}$ *d*$^{-}$ flies, the anterior part of the A compartment is altered to point forwards and the reversed rear section of the P compartment is "rescued" so that it points backwards, as in the wildtype. Thus Pk affects both the A and the P compartment in these flies. However, unlike Pk, ubiquitous Sple affects *d*$^{-}$ and *ft*$^{-}$ *d*$^{-}$ flies differentially: in a *d*$^{-}$ background there is no change to the A compartment, but the whole P compartment is largely reversed. But, in a *ft*$^{-}$ *d*$^{-}$ background the anterior region of the A compartment points laterally and, as noted by Sharp and Axelrod (2016) the P compartment is rescued, having a normal orientation — thus Pk and Sple have similar effects on *ft*$^{-}$ *d*$^{-}$ but very different effects on *d*$^{-}$ flies. It follows from these findings that Ft has outputs that are independent of D and that these outputs are altered by Sple but not by Pk. Note that both Sple and Pk can rescue the reversed polarity in the P compartment in a completely broken Ds/Ft system (*ft*$^{-}$ *d*$^{-}$) perhaps through their effects on the Stan system or, maybe, through other contributors to PCP (Figure 4, Figure 5 and Figure S4).

2. Clones that overexpress *pk* or *sple* in a broken Ds/Ft system. We find that clones of cells overexpressing *sple* (Genotype 25; Lawrence et al., 2004) or *pk* (Genotype 26; data not shown), have small nonautonomous effects in the wildtype and, more so, in ds^- flies (Genotype 27 and Genotype 28) where they polarise receiving cells to point strongly inwards (Figure 7). Perhaps these clones act via the Stan system? It is pertinent that both wing and abdominal cells that overexpress the *pk* gene accumulate Vang uniformly on the cell membrane (Bastock et al., 2003; Olofsson et al., 2014). If this were to happen in our experiments, then the clone could behave as if it were overexpressing *Vang* and should polarise surrounding cells inwards, as observed; this effect should be stronger in ds^- than in ds^+ cells, also as observed. To test this hypothesis further we made $Vang^-$ clones that overexpressed *pk* (Genotype 29), as well as control $Vang^-$ clones (Genotype 30), in ds^- flies. Both these types of clones behaved like $Vang^-$ clones in wildtype flies (Genotype 31), and could not be distinguished from each other, ie they polarise ds^- receiving cells strongly outwards (Figure 7), confirming the hypothesis that cells overexpressing *pk* polarise cells because they accumulate Vang, a Stan system protein. Thus, overexpressing Pk interferes with the Stan system. These results show that Pk and Sple do have functions that are independent of the Ds/Ft system.

(v) The Ds/Ft system functions well but abnormally, both in cells that lack Pk and Sple and in cells that have *pk* or *sple* overexpressed

1. In *pk-sple*$^-$ flies. Clones of cells overexpressing *ft* repolarise receiving cells strongly, even if they lack Pk and Sple (Genotype 32). However it surprised us that in the largely reversed A compartment of the *pk-sple*$^-$ abdomen, the hairs around the clones point inwards (the opposite sign induced by such clones in the wildtype) and also inwards in the P compartment (the same sign as in wildtype, Figure 2). Clones overexpressing *ds* in *pk-sple*$^-$ flies (Genotype 33) act comparably, the hairs around such clones point outwards in A (the opposite sign induced by such clones in the wildtype) and outwards, but weakly, in the P compartment (the same sign as in wildtype, see Figure S3). Thus in clones of both genotypes, in the A compartments, the sign of the effect is the opposite from when such clones are made in the wildtype (Genotype 2 and Genotype 4). Nevertheless, in both these genotypes, in the P compartments, the sign of the polarising effect is the same as wildtype. Quantitation of overexpressing *ft* and *ds* clones confirms these results and also shows that these clones (in the A compartment) affect the polarity of both wildtype and $stan^-$ receiving cells (Genotype 5 and Genotype 3) to the same extent. They also affect *pk-sple*$^-$ $stan^+$ and *pk-sple*$^-$ $stan^-$ (Genotype 34 and Genotype 35) receiving cells with the same strength (Figure 3 and Figure S3). These results show that neither Pk, Sple nor Stan

are required for polarity signalling by the Ds Ft system, although Pk and Sple can change the sign of the response. They also show that Pk and Sple do not act as the link between the Ds/Ft system and the Stan system, because if they were an essential link, the removal of Pk and Sple would block effects on polarity caused by overexpressing *ft*.

2. In flies in which *pk* or *sple* are overexpressed. Clones that lack *ft* made in flies in which *pk* is generally overexpressed (Genotype 36) behave as follows: where the polarity of much of the surrounding background is reversed from normal, with the hairs pointing forwards (ie in the A compartment), ft^- clones act with the opposite sign to that in the wildtype (Genotype 37) and hairs around the clone tend to point outwards (Figure 6). In the P compartment, where overexpression of *pk* produces no change to polarity, the ft^- clones behave as they do in the wildtype, that is the hairs point outwards from the clone (Figure 6).

Clones that lack *ft* made in flies in which *sple* is generally overexpressed (Genotype 38) behave as follows: in the A compartment, which has normal polarity, these clones affect these receiving cells as they affect wildtype cells; hairs around the clone point inwards (Figure 6). In the P compartment, where the polarity of the surrounding background is reversed from normal with the hairs pointing forwards, the ft^- clones now polarise receiving cells with the opposite sign to that in the wildtype, that is the hairs point inwards into the clone (Figure 6).

In the A compartment of the abdomen, clones that lack *ds* have effects of the opposite sign to ft^- clones in both classes (see previous points 1 and 2) of experiments above —as would be expected. However ds^- clones have little or no effect in the P compartment in all genotypes tested (data not shown, Genotype 39, Genotype 40 and Genotype 41).

3. These results show that the Ds/Ft system can function independently of Pk and Sple but that Pk and Sple can modulate the sign of its output. This dramatic effect could, in principle, be due to Pk and/or Sple affecting the patterns of expression of *ds*, and/or *fj* and thereby changing the orientation of the Ds/Ft system gradients. To test we studied the expression of enhancer traps for *ds* and *fj* loci in *pk-sple*$^-$ flies and saw no departure from the wildtype patterns (Genotype 43, Genotype 44, Genotype 45 and Genotype 46; Figure S6). It follows that Pk and Sple determine whether polarised structures in the cell, the hairs and bristles, point up or down the gradients of Ds and Fj.

Discussion

Our aim is to understand the contribution of Pk and Sple to building planar cell polarity in the wildtype fly. The main results and conclusions are listed below.

The Ds/Ft system and the Stan system act independently and are not linked via Sple and/or Pk

ft-overexpressing clones reorient wildtype receiving cells, outwards in the A compartment (Casal et al., 2006) and inwards in the P (this paper). These clones have the same effects on cells in which the Stan system of PCP is broken (for example in *stan*$^{-}$ flies; Figure 2, Figure 3 and Figure S3). It follows that polarisation cannot be due to any intracellular interaction between Stan and any component of the Ds/Ft system within the sending cells. However it could be argued that extra Ft in the sending cell, attracting Ds in the receiving cell, would, non-autonomously, influence some residual capability of the Stan system in the receiving *stan*^{3}/*stan*^{E59} cells to receive and propagate polarity to neighbouring cells. Yet, clones that overexpress both *fz* and *stan* (ie cells that have a fully functional Stan system) fail to repolarise *stan*^{3}/*stan*^{E59} cells (Casal et al., 2006). Thus the propagation of polarity change observed around cells that overexpress *ft* cannot be due to any non-autonomous effect on the Stan system. These results show that for both compartments of the abdomen, the Ds/Ft system acts independently of the Stan system (Casal et al., 2006; Lawrence et al., 2007; Lawrence, 2011).

Here we make *stan*^{E59} clones (*stan*^{E59} introduces a premature stop codon in the ectodomain Usui et al., 1999) that overexpress *ft* or *ds* in *pk-sple*$^{-}$ *stan*^{3}/ *pk-sple*$^{-}$ *stan*^{E59} flies; and show that these clones repolarise the receiving cells. This polarisation cannot depend on Pk and Sple intervening, inside the cells of the clone, between the Ds/Ft and the Stan systems because these sending cells lack the *stan* and *prickle* genes completely while the host flies lack the *prickle* gene and any functional Stan (see previous paragraph). Our finding conflicts with current models in which the *pk* gene products are proposed to link the two systems of PCP (Hogan et al., 2011; Ayukawa et al., 2014; Merkel et al., 2014; Olofsson et al., 2014; Ambegaonkar and Irvine, 2015).

Another argument is relevant here: if polarisation induced by clones affecting the Ds/Ft system were to act through and depend on the Stan system via a molecular link of Pk and or Sple, then we would expect the polarising output from Stan system clones (eg from *fz*$^{-}$ clones) to be different in sign, depending on the presence or absence of that Pk/Sple link (cf Figure 7 in Ayukawa et al., 2014). However, this is not the case (Figure S2E and Figure S2F).

Pk/Sple act independently of the Stan system

Loss of the *pk* gene or overexpressing the Pk isoform reverses polarity of most of the A compartment, having strong effects even in flies with a broken Stan system (*stan*$^{-}$) Similarly, overexpressing Sple reverses polarity in the P compartment in *stan*$^{-}$ flies; it follows that Pk and Sple can act independently of the Stan system. This does not fit easily with the current view that Pk functions as part of the Stan system, for example the lack of requirement for the *pk* gene contrasts with a strong requirement for the other key Stan system genes (eg Fz, Stan or Vang) in the receiving cells (Taylor et al., 1998; Lawrence et al., 2004; Strutt and Strutt, 2007; Strutt and Warrington, 2008; Struhl et al., 2012).

Remember that the Stan system proteins Stan, Fz, Vang and Pk are all preferentially localised to specific regions of the cell membrane and this is considered to be important for their functions in PCP. Nevertheless, *pk-sple*$^{-}$ receiving cells, in which Stan, Fz and Vang are now not visibly localised (reviewed in Strutt, 2009), can respond at least as well to such sending cells as **wild type** ones (Adler et al., 2000; Lawrence et al., 2004). This dilemma might resolve if the observed asymmetry were not so directly related to function as has been assumed and were more a consequence than a cause of polarity (Lawrence et al., 2004).

Pk and Sple modulate the Ds/Ft system, determining the polarity of its output

Sending cells that overexpress *ds* or *ft*, or lack *ds* or *ft*, change the polarity of receiving cells, even in the absence of Pk and Sple— these proteins cannot be necessary for the Ds/Ft system to function and propagate polarity from cell to cell. However the sign of this change depends on whether the receiving cells contain, lack or overexpress products of the *pk* gene. These results show that the Pk and Sple can alter the sign of polarisation that is produced by the Ds/Ft system. But, how do Pk and Sple have their effects on polarity? It appears that the sign of polarisation depends on the relative amounts of Pk and Sple in a particular region of the fly (Gubb et al., 1999; Ayukawa et al., 2014). One model is that the Ds/Ft proteins might act through Pk and Sple to bias the orientation of microtubules and these might affect PCP by transporting Stan system components preferentially to one side of the cell (Harumoto et al., 2010; Matis et al., 2014; Olofsson et al., 2014). But, the correlation between microtubule orientation and PCP is inconsistent (Harumoto et al., 2010; Sharp and Axelrod, 2016) leading to doubts about the validity of the hypothesis (Ambegaonkar and Irvine, 2015). Also, this model is now contradicted by our results, which show that abnormal amounts of Ds, Ft, Sple or Pk can all affect PCP even when the Stan system is broken.

A diagram suggesting how the *pk* gene might fit into the organisation of PCP is given in Figure 8.

The functions of Pk and Sple

It has been suggested that Pk and Sple do fundamentally different things (Ayukawa et al., 2014; Ambegaonkar and Irvine, 2015); however our findings fit better with the view that the two isoforms have similar molecular functions and the differences between them are due to their expression in different patterns (Gubb et al., 1999). Indeed, in the results (section **v**) where we study the behaviour of *ft*⁻ or *ft*-expressing clones we found that removal of Pk and Sple or ubiquitous expression of either can eliminate any differences in responses between the A and the P compartment cells.

It might appear that Pk can act in only the A compartment and Sple in the P, but it cannot be so simple, for the universal expression of either Pk or Sple can rescue the reverted polarity at the back of the P compartment in *ft*⁻ *d*⁻ flies (Figure 4). Also, when *sple* is generally overexpressed in *ft*⁻ *d*⁻ flies, polarity of the anterior region of the A compartment is considerably altered (Figure 5). Looking at the P compartment the action of Pk appears to be independent of an intact Ds/Ft system, but the effects of Sple in the P compartment depend on whether the background genotype is *d*⁻ or *ft*⁻ *d*⁻ (Figure S4). Part of this difference could be due to specific binding occurring between Sple and Ds but not between Pk and Ds (Ambegaonkar and Irvine, 2015). To explain: in *d*⁻, Ds protein will be localised and able to interact with both Ft and Sple, while, in *ft*⁻ *d*⁻, any interaction between Ds and Sple cannot affect binding between Ds and Ft. However Ayukawa et al. (2014) find that both Sple and Pk bind to each other and to D (but not to Ds), suggesting the situation is more complex.

But why are the Pk and Sple proteins asymmetrically localised in the cell? Part of the answer could be that Pk and Sple work with and/or bind to components of the Ds/Ft system which are themselves asymmetrically localised. (Ayukawa et al., 2014; Ambegaonkar and Irvine, 2015). But this cannot be all of the answer as Pk is not properly localised in *stan*⁻ cells (Tree et al., 2002b), in which Ds and Ft are, presumably, normally localised.

How can we understand the effect of Pk and Sple on the Stan system, particularly on range? In the A compartment, a high level of Sple reduces polarity changes induced by *fz*⁻ clones, while the loss of the *pk* gene increases their range. One explanation could depend on Sple and Pk (or the lack of these proteins) acting on the Ds/Ft system —if they made the polarity induced by Ds/Ft in the cells more (or less) robust it would make it more difficult (or easier) for clones affecting the Stan system to alter PCP. Another explanation could relate to some direct effect of Pk (and Sple)

on Vang (Bastock et al., 2003) which fits our observations with clones overexpressing Pk (Figure 7). The function of Vang in the Stan system is somewhat unclear; like Pk, Vang is present in larger than stoichiometric amounts in relation to the two molecules that form the intercellular bridge, Stan and Fz (Strutt et al., 2016), yet affects bridge function (Struhl et al., 2012). The abdominal phenotypes of *Vang*$^{-}$ and *pk-sple*$^{-}$ are somewhat similar, both having areas of reversed polarity (Lawrence et al., 2004), suggesting a commonality of function. Indeed there is a recent model proposing that Pk acts on the stability of Fz intracellularly (via Dsh) and in the adjacent cell (via Vang); the former effect may involve endocytosis (Warrington et al., 2017). Our experiments argue that the function of Pk is not limited to the Stan system but includes, independently, the Ds/Ft system. In any case we have no explanation for the lack of apparent effects of Pk and Sple on the range of *fz*$^{-}$ clones in the P compartment.

What could be the purpose of such complexity? In *Drosophila* the consistent orientation of the wing hairs may have led to an oversimplified and idealised picture. Elsewhere, the presentation of PCP is more complex: consider the mixed orientation of rows of hairs and denticles on the Drosophila larva, differing dorsally and ventrally, or, in mammals, the startlingly diverse orientation of stereocilia in the vestibular system, or the complex patterns of hair orientation on the skin. Two separate genetic systems which generate polarity by reading the slopes of morphogen gradients, plus Pk and Sple to modulate output in different parts of the body, could generate much of this flexibility in PCP.

Conclusion

Our experiments argue that Pk and Sple are not essential components of either the Ds/Ft or the Stan systems. We have shown that they do not function as a link between the two systems. Instead, Pk and Sple appear to modulate the polarity outputs of both the Ds/Ft system and the Stan system with the most conspicuous effects on the former. Both these systems are different in their components but similar in their logic; both utilise intercellular molecular bridges that become distributed asymmetrically within each cell. Pk and Sple could help produce this asymmetry— perhaps via a generic function in cell biology whose mechanism is still undescribed.

Materials and Methods

Mutations and transgenes

The FlyBase (Gramates et al., 2017) entries for relevant mutations and transgenes are the following: *tub.Gal4: Scer\GAL4*$^{alphaTub84B.PL}$. *tub.Gal80: Scer\GAL80*$^{alphaTub84B.P}$.

475 *UAS.ectoDs: $ds^{ecto.Scer\UAS}$. UAS.ft: $ft^{Scer\UAS.cMa}$. UAS.fz : $fz^{Scer\UAS.cSa}$. UAS.pk: $pk^{Scer\UAS.cGa}$.*
476 *UAS.sple: $pk^{sple.Scer\UAS}$. ck^{UAH21}. d^{GC13}. ds^{UA071} and ds^{2D60b}. fj^{p1}. ft^{8} and $ft^{G\text{-}rv}$. fz^{15}. $pk^{pk\text{-}sple\text{-}13}$.*
477 *pwn^{1}. sha^{1}. $stan^{3}$ and $stan^{E59}$. trc^{1}.*

478 ## Experimental Genotypes

479 Genotype 1: **UAS.*fz* clones in *stan*⁻ flies:** *y w hs.FLP; FRT42D tub.Gal80 $stan^{3}$*
480 *hs.CD2, y^{+}/ FRT42D pwn $stan^{E59}$; UAS.fz/ tub.Gal4*

481 Genotype 2: **UAS.*ft* clones in wild type flies:** *y w hs.FLP tub.Gal4 UAS.nls-GFP/ y*
482 *w hs.FLP; d^{GC13} FRT42D pwn sha/ d^{GC13} FRT42D tub.Gal80, y^{+}; UAS.ft/+*

483 Genotype 3: **UAS.*ft* clones in *stan*⁻ flies:** *y w hs.FLP; FRT42D pwn $stan^{E59}$ sha/*
484 *FRT42D tub.Gal80 $stan^{3}$ hs.CD2, y^{+}; UAS.ft/ tub.Gal4*

485 Genotype 4: **UAS.*ectoDs* clones in wild type flies:** *y w hs.FLP tub.Gal4 UAS.nls-*
486 *GFP/ y w hs.FLP; FRT42D pwn $stan^{E59}$ sha/ FRT42D tub.Gal80;*
487 *UAS.ectoDs/ +*

488 Genotype 5: **UAS.*ectoDs* clones in *stan*⁻ flies:** *y w hs.FLP; FRT42D pwn $stan^{E59}$ sha/*
489 *FRT42D tub.Gal80 $stan^{3}$ hs.CD2y^{+}; UAS.ectoDs/ tub.Gal4*

490 Genotype 6: **UAS.*fz* clones in wild type flies:** *y w hs.FLP; FRT42D pwn/ FRt42D*
491 *tub.G80, y^{+}; tub.Gal4/ UAS.fz*

492 Genotype 7: **UAS.*fz* clones in *ds*⁻ flies:** *y w hs.FLP tub.Gal4 UAS.nls-GFP/ y w*
493 *hs.FLP; ds^{UA071} FRT42D pwn/ ds^{UA071} FRT42D tub.Gal80; UAS.fz*
494 *hs.CD2, y^{+}/ +*

495 Genotype 8: ***ds*⁻ *pk*⁻*sple*⁻ flies:** *y w hs.FLP; ds^{UA071} $pk^{pk\text{-}sple\text{-}13}$; UAS.sple/ TM2*

496 Genotype 9: ***pk-sple*⁻ *stan*⁻ flies:** *y w hs.FLP tub.Gal4 UAS.nls-GFP; FRT42D*
497 *$pk^{pk\text{-}sple\text{-}13}$ $stan^{3}$ tub.Gal80/ FRT42D $pk^{pk\text{-}sple\text{-}13}$ $stan^{E59}$ sha; MRS/ TM2*

498 Genotype 10: ***stan*⁻ flies:** *y w hs.FLP; FRT42D tub.Gal80 $stan^{3}$ hs.CD2, y^{+}/ FRT42D*
499 *pwn $stan^{E59}$; MRS/ TM2*

500 Genotype 11: ***pk-sple*⁻ flies:** *$pk^{pk\text{-}sple\text{-}13}$*

501 Genotype 12: **UAS.*fz* clones in *pk*⁻*sple*⁻ flies:** *y w hs.FLP tub.Gal4 UAS.nls-GFP/ y w*
502 *hs.FLP; FRT42D $pk^{pk\text{-}sple\text{-}13}$ sha/ FRT42D $pk^{pk\text{-}sple\text{-}13}$ tub.Gal80; UAS.fz fz^{15}*
503 *$fz2^{C1}$ FRT2A/ +*

504 Genotype 13: ***fz*⁻ clones in *pk-sple*⁻ flies:** *y w hs.FLP122; FRT42 $pk^{pk\text{-}sple\text{-}13}$/ CyO;*
505 *fz[P21] trc FRT2A/ tub.Gal80 FRT2A*

Genotype 14: ***fz*⁻ clones in *tub.Gal4 UAS.sple* flies:** *y w hs.FLP tub.Gal4 UAS.nls-GFP/ w; UAS.sple/ +; UAS.sple; fz^{15} trc^{C1} FRT2A / hs.GFPw^{+} hs.CD2, y^{+} ri FRT2A*

Genotype 15: ***fz*⁻ clones in *tub.Gal4 UAS.pk* flies:** *y w hs.FLP tub.Gal4 UAS.nls-GFP/ w; UAS.pk/ +; UAS.sple; fz^{15} trc^{C1} FRT2A / hs.GFPw^{+} hs.CD2, y^{+} ri FRT2A*

Genotype 16: ***fz*⁻ clones in *ds*⁻ flies:** *ds^{UA071} FRT39/ ds^{33k} bw^{V1}; fz^{H51} trc^{C1} ri FRT2A/ hs.CD2, y^{+} hs.GFP ri FRT2A/ TM3*

Genotype 17: ***stan*⁻ *tub.Gal4 UAS.pk* flies:** *y w hs.FLP tub.Gal4 UAS.nls-GFP/ y w hs.FLP; FRT42D pwn $stan^{E59}$ sha/ FRT42D $stan^{3}$; UAS.pk/ TM2*

Genotype 18: ***stan*⁻ *tub.Gal4 UAS.sple* flies:** *y w hs.FLP tub.Gal4 UAS.nls-GFP/ y w hs.FLP; FRT42D pwn $stan^{E59}$ sha/ FRT42D $stan^{3}$; UAS.sple/ TM2*

Genotype 19: ***ds*⁻ *pk*⁻*sple*⁻ *tub.Gal4 UAS.pk* flies:** *y w hs.FLP tub.Gal4 UAS.nls-GFP/ y w hs.FLP; ds^{UA071} $pk^{pk\text{-}sple\text{-}13}$; UAS.pk/ TM2*

Genotype 20: ***d*⁻ *tub.Gal4 UAS.pk* flies:** *y w hs.FLP tub.Gal4 UAS.nls-GFP/ y w hs.FLP; d^{GC13} pr cn/ $ft^{G\text{-}rv}$ d^{GC13} FRT40; UAS.pk/ +*

Genotype 21: ***ft*⁻ *d*⁻ *tub.Gal4 UAS.pk* flies:** *y w hs.FLP tub.Gal4 UAS.nls-GFP/ y w hs.FLP; ft^{8} d^{GC13} FRT40A/ $ft^{G\text{-}rv}$ d^{GC13} FRT40A; UAS.sple/ +*

Genotype 22: ***ds*⁻ *pk*⁻*sple*⁻ *tub.Gal4 UAS.sple* flies:** *y w hs.FLP tub.Gal4 UAS.nls-GFP/ y w hs.FLP; ds^{UA071} $pk^{pk\text{-}sple\text{-}13}$; UAS.sple/ TM2*

Genotype 23: ***d*⁻ *tub.Gal4 UAS.sple* flies:** *y w hs.FLP tub.Gal4 UAS.nls-GFP/ w; $ft^{G\text{-}rv}$ d^{GC13}/ d^{GC13} pr cn; UAS.sple/ +*

Genotype 24: ***ft*⁻ *d*⁻ *tub.Gal4 UAS.sple* flies:** *y w hs.FLP tub.Gal4 UAS.nls-GFP/ y w hs.FLP; ft^{8} d^{GC13} FRT40A/ $ft^{G\text{-}rv}$ d^{GC13} FRT40A; UAS.sple/ +*

Genotype 25: ***UAS.sple* clones in wild type flies:** *y w hs.FLP tub.Gal4 UAS.nls-GFP/ y w hs.FLP;FRT42D pwn/ FRT42D tub.Gal80; UAS.sple/ +*

Genotype 26: ***UAS.pk* clones in wild type flies:** *y w hs.FLP tub.Gal4 UAS.nls-GFP/ y w hs.FLP;FRT42D pwn/ FRT42D tub.Gal80; UAS.pk/ +*

Genotype 27: ***UAS.sple* clones in *ds*⁻ flies:** *y w hs.FLP tub.Gal4 UAS.nls-GFP/ y w hs.FLP; ds^{UA071} ck^{UAh21} FRT40A/ ds^{UA071} tub.Gal80 FRT40A; UAS.sple/ MRS*

Genotype 28: ***UAS.pk* clones in *ds*⁻ flies:** *y w hs.FLP tub.Gal4 UAS.nls-GFP/ y w hs.FLP; ds^{UA071} ck^{UAh21} FRT40A/ ds^{UA071} tub.Gal80 FRT40A; UAS.pk/ MRS*

Genotype 29: ***Vang*⁻ *UAS.pk* clones in *ds*⁻ flies:** *y w hs.FLP tub.Gal4 UAS.nls-GFP/ y w hs.FLP; ds^{UA071} FRT42D tub.Gal80/ ds^{UA071} hs.CD2, y^{+} FRT42D pwn $Vang^{stbm-6}$ sha; UAS.pk/ +*

Genotype 30: ***Vang*⁻ clones in *ds*⁻ flies:** *y w hs.FLP; ds^{UA071} FRT42D tub.Gal80/ ds^{UA071} hs.CD2, y^{+} FRT42D pwn $Vang^{stbm-6}$ sha; UAS.pk/ +*

Genotype 31: ***Vang*⁻ clones in wild type flies:** *y/ y hs.FLP; FRT42D pwn $Vang^{stbm-6}$ FRT42D hs.CD2, y^{+}*

Genotype 32: ***UAS.ft* clones in *pk*⁻*sple*⁻ flies:** *y w hs.FLP122 tub.gal4 UAS.nls-GFP/ y w hs.FLP; FRT42D $pk^{pk\text{-}sple\text{-}13}$ sha/ FRT42 $pk^{pk\text{-}sple\text{-}13}$ tub.Gal80/ UAS.ft/ +*

Genotype 33: ***UAS.ectoDs* clones in *pk*⁻*sple*⁻ flies:** *y w hs.FLP122 tub.gal4 UAS.nls-GFP/ y w hs.FLP; FRT42D $pk^{pk\text{-}sple\text{-}13}$ sha/ FRT42 $pk^{pk\text{-}sple\text{-}13}$ tub.Gal80/ UAS.ectoDs/ +*

Genotype 34: ***UAS.ectoDs* clones in *pk*⁻*sple*⁻ *stan*⁻ flies:** *y w hs.FLP tub.Gal4 UAS.nls-GFP/ y w hs.FLP; FRT42D $pk^{pk\text{-}sple\text{-}13}$ $stan^{3}$ tub.Gal80, y^{+}/ FRT42 $pk^{pk\text{-}sple\text{-}13}$ $stan^{E59}$ sha ; UAS.ectoDs/ +*

Genotype 35: ***UAS.ft* clones in *pk*⁻*sple*⁻ *stan*⁻ flies:** *y w hs.FLP tub.Gal4 UAS.nls-GFP/ y w hs.FLP122; FRT42D $pk^{pk\text{-}sple\text{-}13}$ $stan^{3}$ tub.Gal80, y^{+}/ FRT42D $pk^{pk\text{-}sple\text{-}13}$ $stan^{E59}$ sha ; UAS.ft/ +*

Genotype 36: ***ft*⁻ clones in *tub.Gal4 UAS.pk* flies:** *y w hs.FLP tub.Gal4 UAS.GFP-nls/ y; ft^{15} stc FRT39/ FRT39; UAS.pk/ +*

Genotype 37: ***ft*⁻ clones in wild type flies:** *y w hs.FLP; ft^{15} stc FRT39/ FRT39*

Genotype 38: ***ft*⁻ clones in *tub.Gal4 UAS.sple* flies:** *y w hs.FLP tub.Gal4 UAS.GFP-nls/ y; ft^{15} stc FRT39/ FRT39; UAS.sple/ +*

Genotype 39: ***ds*⁻ clones in *tub.Gal4 UAS.sple* flies:** *w hs.FLP tub.Gal4 UAS.nls-GFP; ds^{UA071} ck^{UAh21} FRT40A/ FRT40A; UAS.sple/ +*

Genotype 40: ***ds*⁻ clones in *tub.Gal4 UAS.pk* flies:** *w hs.FLP tub.Gal4 UAS.nls-GFP; ds^{UA071} ck^{UAh21} FRT40A/ FRT40A; UAS.pk/ +*

Genotype 41: ***ds*⁻ clones in wild type flies:** *y w hs.FLP/ +; ds^{UA071} ck^{UAh21} FRT40A/ Dp(1;2)sc^{19} w^{+30c} FRT40A*

Genotype 42: **Control clones in *pk⁻sple⁻ stan⁻* flies:** *y w hs.FLP tub.Gal4 UAS.nls-GFP/ y w hs.FLP; FRT42D $pk^{pk\text{-}sple\text{-}13}$ $stan^{E59}$ sha/ FRT42D$pk^{pk\text{-}sple\text{-}13}$ $stan^{3}$ tub.Gal80, y^{+}; MRS/ +*

Genotype 43: ***ds.lacZ* flies:** *y hs.FLP/ +; ds^{2D60b} FRT42D $pk^{pk\text{-}sple\text{-}13}$/ +*

Genotype 44: ***fj.lacZ* flies:** *y hs.FLP/ +; FRT42D $pk^{pk\text{-}sple\text{-}13}$ fj^{P1}/ +*

Genotype 45: ***ds.lacZ pk-sple⁻* flies:** *y hs.FLP/ +; ds^{2D60b} FRT42D $pk^{pk\text{-}sple\text{-}13}$/ FRT42D $pk^{pk\text{-}sple\text{-}13}$*

Genotype 46: ***pk-sple⁻ fj.lacZ* flies:** *y hs.FLP/ +; FRT42D $pk^{pk\text{-}sple\text{-}13}$ fj^{P1}/ FRT42D FRT42D $pk^{pk\text{-}sple\text{-}13}$*

Clone induction and microscopy

Clones were induced by heat shocking third instar larvae for 1 hr at 34ºC. Adult abdominal cuticles were studied as before (e.g., Lawrence et al., 2004; Casal et al., 2006).

Quantitation

Individual hairs along the entire perimeter of each clone (about 60-100 hairs per clone) were each scored as pointing largely into, outwards or parallel to the clone. Parallel hairs, which averaged 8% of the hairs, were counted; half was added equally to the inwards and outwards sets. The average orientation is then found for each clone (between 10 and 20 clones per genotype).

For range measurments, for each clone (n=20) the maximum extent in cell rows of the induced polarity changes was measured. The observer was blinded as to genotypes; he chose clones located in the middle of the A compartment and the middle or rear of the hairy region of the P compartment; small clones were avoided. Statistical analysis and graphics were performed in R using standard packages (R Core Team, 2016) and the *reshape* and *ggplot* packages (Wickham, 2007, 2009).

ACKNOWLEDGEMENTS

We thank the Department of Zoology, the Wellcome (WT096645MA and WT107060MA) for generous support, Malcolm Burrows for encouragement, and Gary Struhl and David Strutt for stocks and constructive criticisms.

References

Adler, P.N. (2012). The f*rizzled/stan* pathway and planar cell polarity in the *Drosophila* wing. Curr. Top. Dev. Biol. *101*, 1-31.

Adler, P.N., Charlton, J., and Liu, J. (1998). Mutations in the cadherin superfamily member gene *dachsous* cause a tissue polarity phenotype by altering *frizzled* signaling. Development *125*, 959-968.

Adler, P.N., Taylor, J., and Charlton, J. (2000). The domineering non-autonomy of *frizzled* and *van Gogh* clones in the *Drosophila* wing is a consequence of a disruption in local signaling. Mech. Dev. *96*, 197-207.

Ambegaonkar, A.A., and Irvine, K.D. (2015). Coordination of planar cell polarity pathways through Spiny-legs. eLife *4*.

Ambegaonkar, A.A., Pan, G., Mani, M., Feng, Y., and Irvine, K.D. (2012). Propagation of Dachsous-Fat planar cell polarity. Curr. Biol. *22*, 1302-1308.

Axelrod, J.D. (2009). Progress and challenges in understanding planar cell polarity signaling. Semin. Cell Dev. Biol. *20*, 964-971.

Ayukawa, T., Akiyama, M., Mummery-Widmer, J.L., Stoeger, T., Sasaki, J., Knoblich, J.A., Senoo, H., Sasaki, T., and Yamazaki, M. (2014). Dachsous-dependent asymmetric localization of spiny-legs determines planar cell polarity orientation in *Drosophila*. Cell Rep. *8*, 610-621.

Bastock, R., Strutt, H., and Strutt, D. (2003). Strabismus is asymmetrically localised and binds to Prickle and Dishevelled during *Drosophila* planar polarity patterning. Development *130*, 3007-3014.

Brittle, A.L., Thomas, C., and Strutt, D. (2012). Planar polarity specification through asymmetric subcellular localization of Fat and Dachsous. Curr. Biol. *22*, 907-914.

Butler, M.T., and Wallingford, J.B. (2017). Planar cell polarity in development and disease. Nat. Rev. Mol. Cell Biol. *18*, 375-388.

Casal, J., Lawrence, P.A., and Struhl, G. (2006). Two separate molecular systems, Dachsous/Fat and Starry night/Frizzled, act independently to confer planar cell polarity. Development *133*, 4561-4572.

627 Casal, J., Struhl, G., and Lawrence, P.A. (2002). Developmental compartments and
628 planar polarity in *Drosophila*. Curr. Biol. *12*, 1189-1198.

629 Goodrich, L.V., and Strutt, D. (2011). Principles of planar polarity in animal
630 development. Development *138*, 1877-1892.

631 Gramates, L., Marygold, S., dos Santos, G., Urbano, J.-M., Antonazzo, G., Matthews,
632 B., Rey, A., Tabone, C., Crosby, M., Emmert, D., *et al.* (2017). FlyBase at 25: looking to
633 the future. Nucleic Acids Res. *45*, D663-D671.

634 Gubb, D., and Garcia-Bellido, A. (1982). A genetic analysis of the determination of
635 cuticular polarity during development in *Drosophila melanogaster*. J. Embryol. Exp.
636 Morph. *68*, 37-57.

637 Gubb, D., Green, C., Huen, D., Coulson, D., Johnson, G., Tree, D., Collier, S., and
638 Roote, J. (1999). The balance between isoforms of the prickle LIM domain protein is
639 critical for planar polarity in *Drosophila* imaginal discs. Genes Dev. *13*, 2315-2327.

640 Harumoto, T., Ito, M., Shimada, Y., Kobayashi, T.J., Ueda, H.R., Lu, B., and Uemura,
641 T. (2010). Atypical cadherins Dachsous and Fat control dynamics of noncentrosomal
642 microtubules in planar cell polarity. Dev. Cell *19*, 389-401.

643 Hogan, J., Valentine, M., Cox, C., Doyle, K., and Collier, S. (2011). Two *frizzled* planar
644 cell polarity signals in the *Drosophila* wing are differentially organized by the
645 *fat*/*dachsous* pathway. PLoS Genet *7*, e1001305.

646 Ives, P. (1947). [New mutants report.]. Drosophila Information Service *21*, 68-69.

647 Klein, T.J., and Mlodzik, M. (2005). Planar Cell Polarization: An Emerging Model
648 Points in the Right Direction. Ann. Rev. Cell Dev. Biol. *23*, 23.

649 Lawrence, P.A. (2011). Planar cell polarity Fashioning solutions. Fly *5*, 126-128.

650 Lawrence, P.A., and Casal, J. (2013). The mechanisms of planar cell polarity, growth
651 and the Hippo pathway: Some known unknowns. Dev. Biol. *377*, 1-8.

652 Lawrence, P.A., Casal, J., and Struhl, G. (2004). Cell interactions and planar polarity in
653 the abdominal epidermis of *Drosophila*. Development *131*, 4651-4664.

654 Lawrence, P.A., Struhl, G., and Casal, J. (2007). Planar cell polarity: one or two
655 pathways? Nature Reviews. Genetics *8*, 555-563.

656 Ma, D., Yang, C.H., McNeill, H., Simon, M.A., and Axelrod, J.D. (2003). Fidelity in
657 planar cell polarity signalling. Nature *421*, 543-547.

658 Matis, M., Russler-Germain, D.A., Hu, Q., Tomlin, C.J., and Axelrod, J.D. (2014).
659 Microtubules provide directional information for core PCP function. eLife *3*.

660 Merkel, M., Sagner, A., Gruber, F.S., Etournay, R., Blasse, C., Myers, E., Eaton, S., and
661 Julicher, F. (2014). The balance of prickle/spiny-legs isoforms controls the amount of
662 coupling between core and *fat* PCP systems. Curr. Biol. *24*, 2111-2123.

663 Olofsson, J., Sharp, K.A., Matis, M., Cho, B., and Axelrod, J.D. (2014). Prickle/spiny-
664 legs isoforms control the polarity of the apical microtubule network in planar cell
665 polarity. Development *141*, 2866-2874.

666 Pan, G., Feng, Y., Ambegaonkar, A.A., Sun, G., Huff, M., Rauskolb, C., and Irvine,
667 K.D. (2013). Signal transduction by the Fat cytoplasmic domain. Development *140*,
668 831-842.

669 R Core Team (2016). R: A Language and Environment for Statistical Computing
670 (Vienna, Austria.: R Foundation for Statistical Computing).

671 Sharp, K.A., and Axelrod, J.D. (2016). Prickle isoforms control the direction of tissue
672 polarity by microtubule independent and dependent mechanisms. Biol Open *5*, 229-
673 236.

674 Struhl, G., Casal, J., and Lawrence, P.A. (2012). Dissecting the molecular bridges that
675 mediate the function of Frizzled in planar cell polarity. Development *139*, 3665-3674.

676 Strutt, D. (2009). Gradients and the specification of planar polarity in the insect
677 cuticle. Cold Spring Harb Perspect Biol *1*, a000489.

678 Strutt, D., and Strutt, H. (2007). Differential activities of the core planar polarity
679 proteins during *Drosophila* wing patterning. Dev. Biol. *302*, 181-194.

680 Strutt, D., and Warrington, S.J. (2008). Planar polarity genes in the Drosophila wing
681 regulate the localisation of the FH3-domain protein Multiple Wing Hairs to control
682 the site of hair production. Development *135*, 3103-3111.

683 Strutt, D.I. (2001). Asymmetric localization of Frizzled and the establishment of cell
684 polarity in the *Drosophila* wing. Mol. Cell *7*, 367-375.

685 Strutt, H., Gamage, J., and Strutt, D. (2016). Robust asymmetric localization of planar
686 polarity proteins is associated with organization into signalosome-like domains of
687 variable stoichiometry. Cell Rep. *17*, 2660-2671.

688 Strutt, H., and Strutt, D. (2005). Long-range coordination of planar polarity in
689 Drosophila. BioEssays *27*, 1218-1227.

690 Strutt, H., and Strutt, D. (2008). Differential stability of flamingo protein complexes
691 underlies the establishment of planar polarity. Curr. Biol. *18*, 1555-1564.

692 Strutt, H., and Strutt, D. (2009). Asymmetric localisation of planar polarity proteins:
693 Mechanisms and consequences. Semin. Cell Dev. Biol. *20*, 957-963.

694 Taylor, J., Abramova, N., Charlton, J., and Adler, P.N. (1998). *Van Gogh*: a new
695 *Drosophila* tissue polarity gene. Genetics *150*, 199-210.

696 Tissir, F., and Goffinet, A.M. (2013). Shaping the nervous system: role of the core
697 planar cell polarity genes. Nat. Rev. Neurosci. *14*, 525-535.

698 Tree, D.R., Ma, D., and Axelrod, J.D. (2002a). A three-tiered mechanism for
699 regulation of planar cell polarity. Semin. Cell Dev. Biol. *13*, 217-224.

700 Tree, D.R., Shulman, J.M., Rousset, R., Scott, M.P., Gubb, D., and Axelrod, J.D.
701 (2002b). Prickle mediates feedback amplification to generate asymmetric planar cell
702 polarity signaling. Cell *109*, 371-381.

703 Usui, T., Shima, Y., Shimada, Y., Hirano, S., Burgess, R.W., Schwarz, T.L., Takeichi,
704 M., and Uemura, T. (1999). Flamingo, a seven-pass transmembrane cadherin,
705 regulates planar cell polarity under the control of Frizzled. Cell *98*, 585-595.

706 Wang, Y., and Nathans, J. (2007). Tissue/planar cell polarity in vertebrates: new
707 insights and new questions. Development *134*, 647-658.

708 Warrington, S.J., Strutt, H., Fisher, K.H., and Strutt, D. (2017). A dual function for
709 prickle in regulating Frizzled stability during feedback-dependent amplification of
710 planar polarity. Curr. Biol. *27*, 2784-2797.

711 Wickham, H. (2007). Reshaping Data with the reshape Package. J. Stat. Softw. *21*, 1--
712 20.

713 Wickham, H. (2009). ggplot2: Elegant Graphics for Data Analysis (Springer-Verlag
714 New York).

715 Yang, C., Axelrod, J.D., and Simon, M.A. (2002). Regulation of Frizzled by Fat-like
716 cadherins during planar polarity signaling in the *Drosophila* compound eye. Cell *108*,
717 675-688.

718

719

FIGURE LEGENDS

Figure 1. A baedeker of the experiments.
A summary of all experiments showing the polarities of hairs in the two abdominal compartments plus the effects of clones on polarity. UAS indicates overexpression of the said gene in the clones, tub.Gal4 UAS.x indicates generalised expression of x.

Figure 2. Clones that overexpress *ft* in various backgrounds.
The receiving cells point outwards in the A compartments (A-B), inwards in P compartments (D-E) of *stan*$^-$ and wildtype cells. The response of *pk-sple*$^-$ cells is inwards in both the A and P compartments (C,F). For all figures, clones are outlined in red dots, blue boxes delimit the areas detailed at higher magnification, blue arrows indicate orientation of hairs. For images of clones expressing *fz* in the same backgrounds, see Figure S2.

Figure 3. Effects of the *ft*-overexpressing clones in A and P compartments (cf Figure 2).
The orientations of hairs immediately adjacent to each clone are counted and displayed in box plots, each dot represents the data from one clone. The responses range from all pointing inwards (top of the graph) to all pointing outwards (bottom). Breaking the Stan system (*stan*$^-$) did not much affect any outcome, confirming that the Ft/Ds system does not act through the Stan system. However removing *pk* and *sple* changed the sign of response in the A compartment. (Control clones Genotype 42).

Figure 4. Effects of overexpressing *pk* on polarity of cells in which either the Stan system (*stan*$^-$) or the Ds/Ft system is broken (*ft*$^-$ *d*$^-$).
Background phenotypes (**B**-**D**). In the A compartments, generalised overexpression of *pk* changes the polarity of the anterior region of wildtype, *stan*$^-$ (Genotype 10) and *ft*$^-$ *d*$^-$ cells (**A**, **E** and **F**). In the P compartments, the region that normally points anteriorly in *ft*$^-$ *d*$^-$ points posteriorly (as in the wildtype) when *pk* is overexpressed (**G**). Compare Figure S4 for expression of *pk* in *d*$^-$ flies.

Figure 5. Effects of overexpressing *sple* on polarity of cells in which either the Stan system (*stan*$^-$) or the Ds/Ft system is broken (*ft*$^-$ *d*$^-$).
Overexpression of *sple* in *stan*$^-$ and the wildtype reverses all or most of the P compartment to point forwards (**A** and **E**) but overexpression of *sple* in a *ft*$^-$ *d*$^-$ background produces a P compartment of normal polarity (**G**) and even the rear of the P region, which points forward in *ft*$^-$ *d*$^-$ (**D**) is now "rescued" to normal polarity. Overexpression of *sple* in *ft*$^-$ *d*$^-$ flies also alters the polarity at the front of the A

compartment (**C** and **F**) turning the hairs laterally, while overexpressing *pk* turns the hairs to point anteriorly (Figure 4). Compare Figure S4 for expression of *sple* in d^- flies

Figure 6. Behaviour of fz^- and ft^- clones in flies overexpressing isoforms of the *pk* gene. fz^- clones behave normally, polarising receiving cells inwards in both A and P either in *tub.Gal4 UAS.pk* or *tub.Gal4 UAS.sple* flies, independently of the polarity of their surrounds (**A** and **B**). The effects of ft^- clones, but only in territories with reversed polarity, are the opposite of normal: in the wildtype these effects are inwards in A, outwards in P while in *tub.Gal4 UAS.pk* the cells close to the anterior clones point outwards (**C**) and in *tub.Gal4 UAS.sple* the cells nearby the posterior clones point inwards (**D**). See Figure S5 for analysis of maximum range of effects of fz^- clones.

Figure 7. Effects of *pk*-expressing clones in flies broken for the Ds/Ft system. Clones that overexpress *pk* polarise ds^- cells strongly inwards (**A**). Clones lacking *Vang* (**B**) as well as clones that, lacking *Vang*, also overexpress *pk* (**C**), polarise ds^- receiving cells strongly outwards.

Figure 8. Pk and Sple functions in the context of PCP.
PCP depends on molecular bridges between cells: for the Stan system the key bridge consists of a complex of Stan and Fz in one cell and Stan in the other; Vang promotes function of the Stan pillar of this bridge (Struhl et al., 2012). For the Ds/Ft system, Ds in one cell is linked to Ft in another, the activity of both is modulated by Fj (reviewed in Butler and Wallingford, 2017). Pk and/or Sple bind to Vang and promote asymmetrical distribution of Vang and other PCP molecules. Yet in the absence of Pk and Sple, the Stan system can still receive and send polarity information, implying that it is the asymmetric activation of protein complexes that polarise a cell rather than asymmetric localisation. Pk and Sple alter the sign of the polarity output of the Ds/Ft system, but by an unknown mechanism. Yet, Pk and Sple can alter polarity output even when the Ds/Ft system is broken. The results show that Pk and Sple can act separately on both systems, implying some general function of Pk and Sple in cell polarity. The indispensable elements of the two systems are shown in bold.

SUPPLEMENTARY FIGURE LEGENDS

Figure S1. *fz*-overexpressing clone in the P compartment of a *ds*$^{-}$ fly.
Hairs point outwards from the clone with range of 2-7 cells. Cells of the clone are marked with *pawn*, and outlined in red dots. Blue arrows indicate orientation of hairs.

Figure S2. The effects of *fz*-overexpressing clones on various genetic backgrounds in the A and P compartments —compare with Figure 2.
The clones polarise responding wildtype cells outwards in both compartments (**A** and **B**). This effect is blocked when the Stan system is broken (*stan*$^{-}$) (**C** and **D**). In a *pk-sple*$^{-}$ background the sign is also outwards but the range of repolarisation is strongly reduced in the A compartment. Clones are variously marked, see Genotypes in **Materials and Methods**.

Figure S3. Results of similar experiments to those in Figure 3, but here the clones were overexpressing the ectodomain of Ds.
The results are comparable with those of Figure 3 in the A compartments (although of the opposite sign to *ft*-overexpressing clones, as expected (Casal et al., 2006). None of the clones had significant effects in the P compartment — this lack of response is most simply explained by high ambient level of Ds in P, which is suggested by *ds.LacZ* expression (Casal et al., 2002). A response was visible in flies that lack *four-jointed* (*fj*) (data not shown), which increases the range of signalling by the Ds/Ft system (Casal et al., 2006). One-way Anova with post-hoc Tukey HSD analysis showing levels of significance for Figure 3 and S3, below (vertical lines are the 95% confidence intervals).

Figure S4. The effects of overexpression of *pk* and *sple* in *d*$^{-}$ flies.
In this background the effects of extra Pk are as in *ft*$^{-}$ *d*$^{-}$ flies: the anterior part of the A compartment points forward and the polarity of the P compartment is “rescued” (compare **C** and **D** with **A** and **B**; see Figure 4). However extra Sple increases the area of anteriorwards polarity in the P compartment (compare **E** with **B**; see Figure 5).

Figure S5. Range measurements for *fz*-expressing clones in wildtype and flies with a broken Ds/Ft system (*ds*$^{-}$).
For each clonal perimeter the maximum number of cell rows showing an induced polarity change was measured. Below are the results of one-way Anova with post-hoc Tukey HSD analysis.

Figure S6. Ventral cuticle of the abdominal segments stained for lacZ; **A**, *ds.lacZ* expression; **B**, *ds.lacZ* expression in *pk-sple*$^{-}$; **C**, *fj.lacZ* expression; **D**, *fj.lacZ*

817 expression in *pk-sple*$^{-}$. Red dots delineate the approximate boundaries between the A
818 and the P compartments. Arrows indicate the orientation of cell hairs in the pleura.

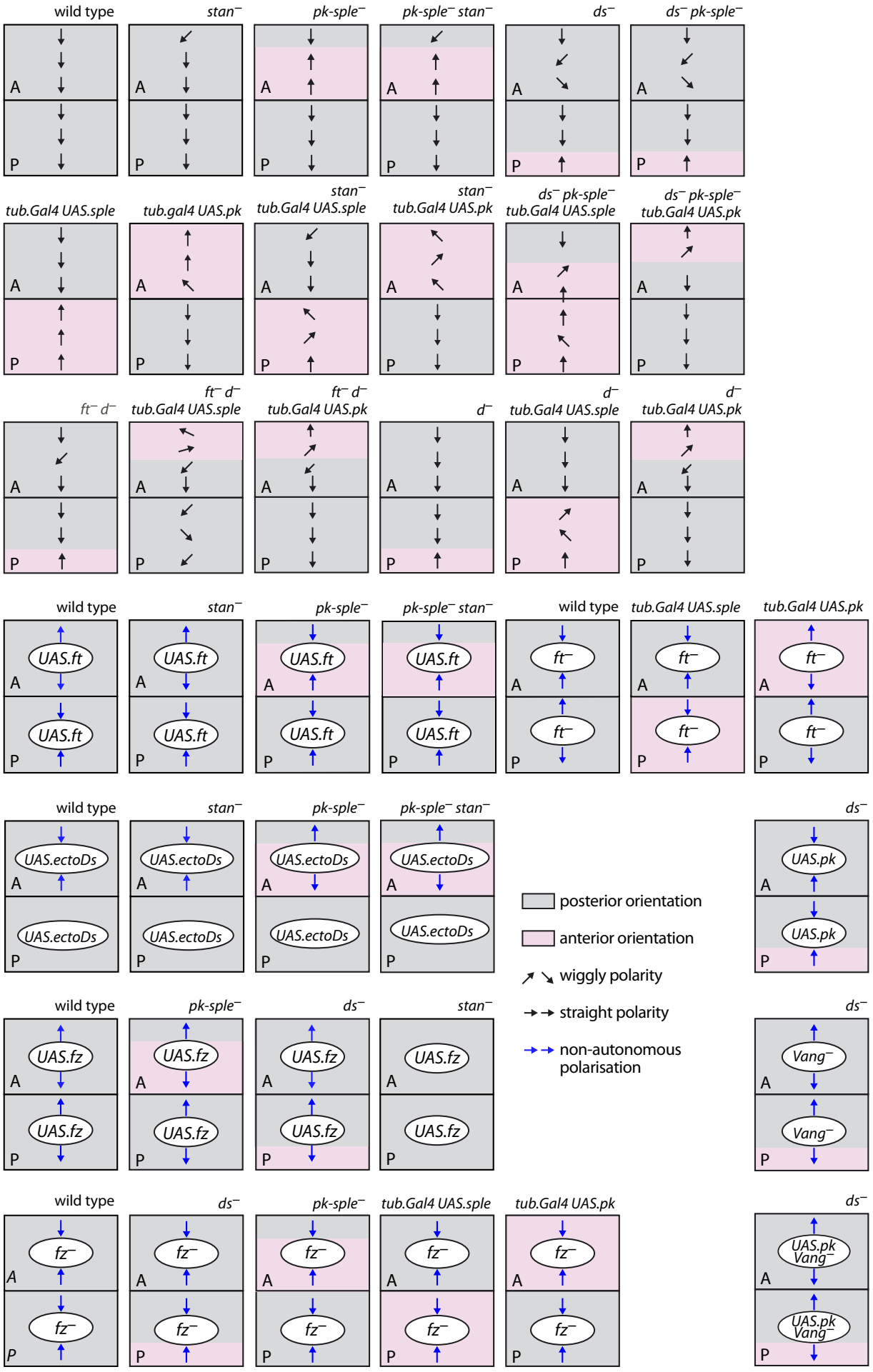


figure 1

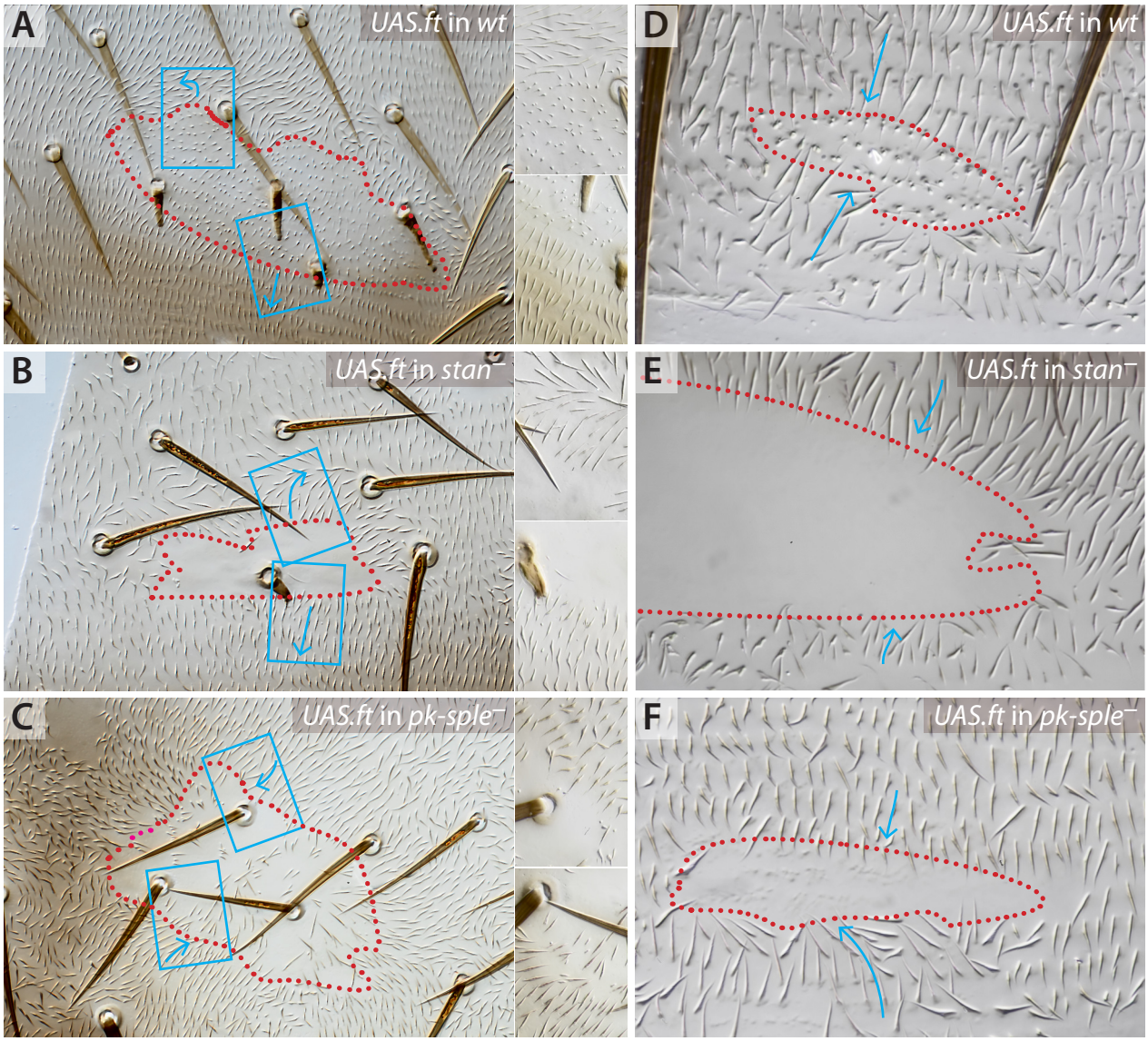


figure 2

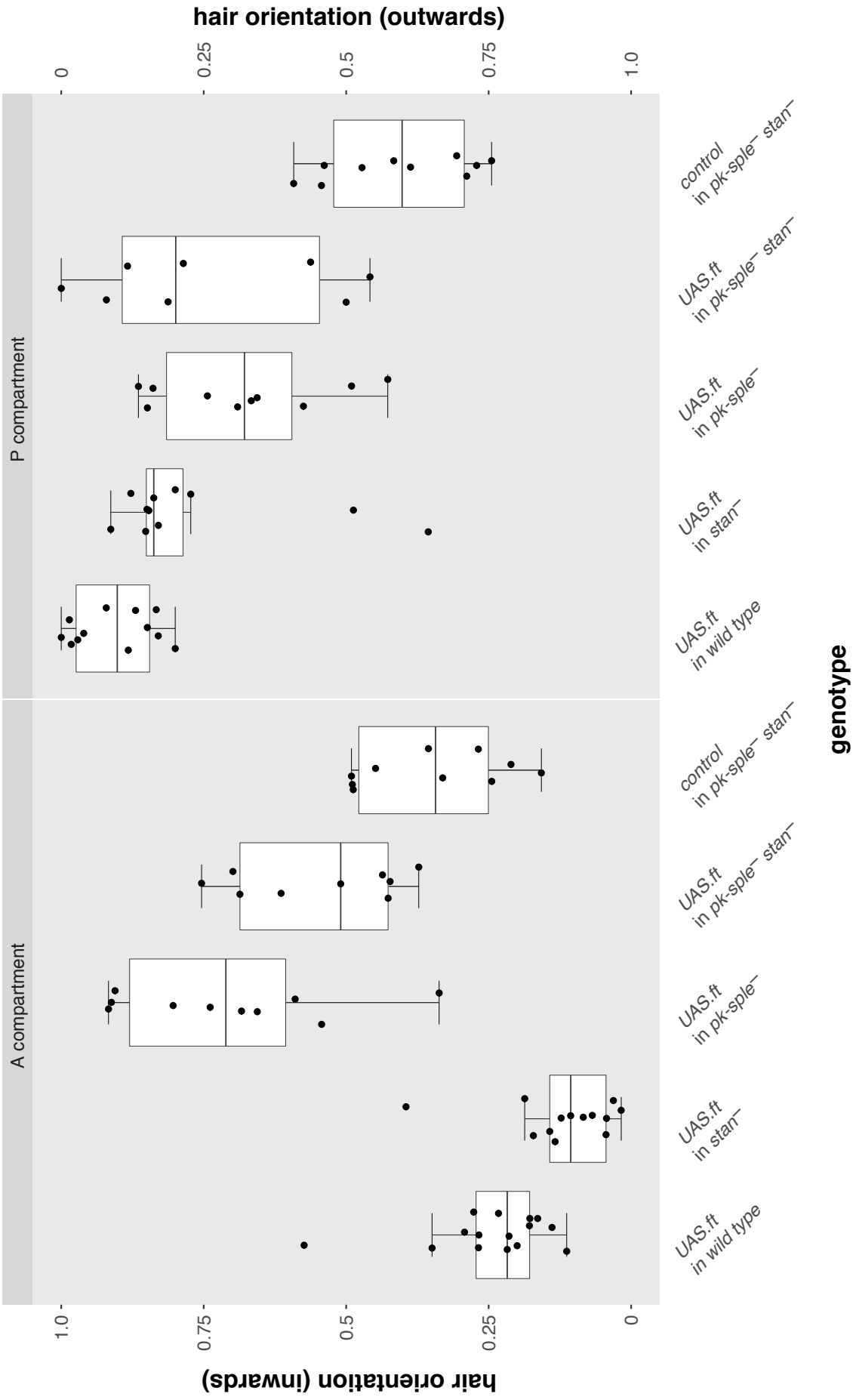


figure 3

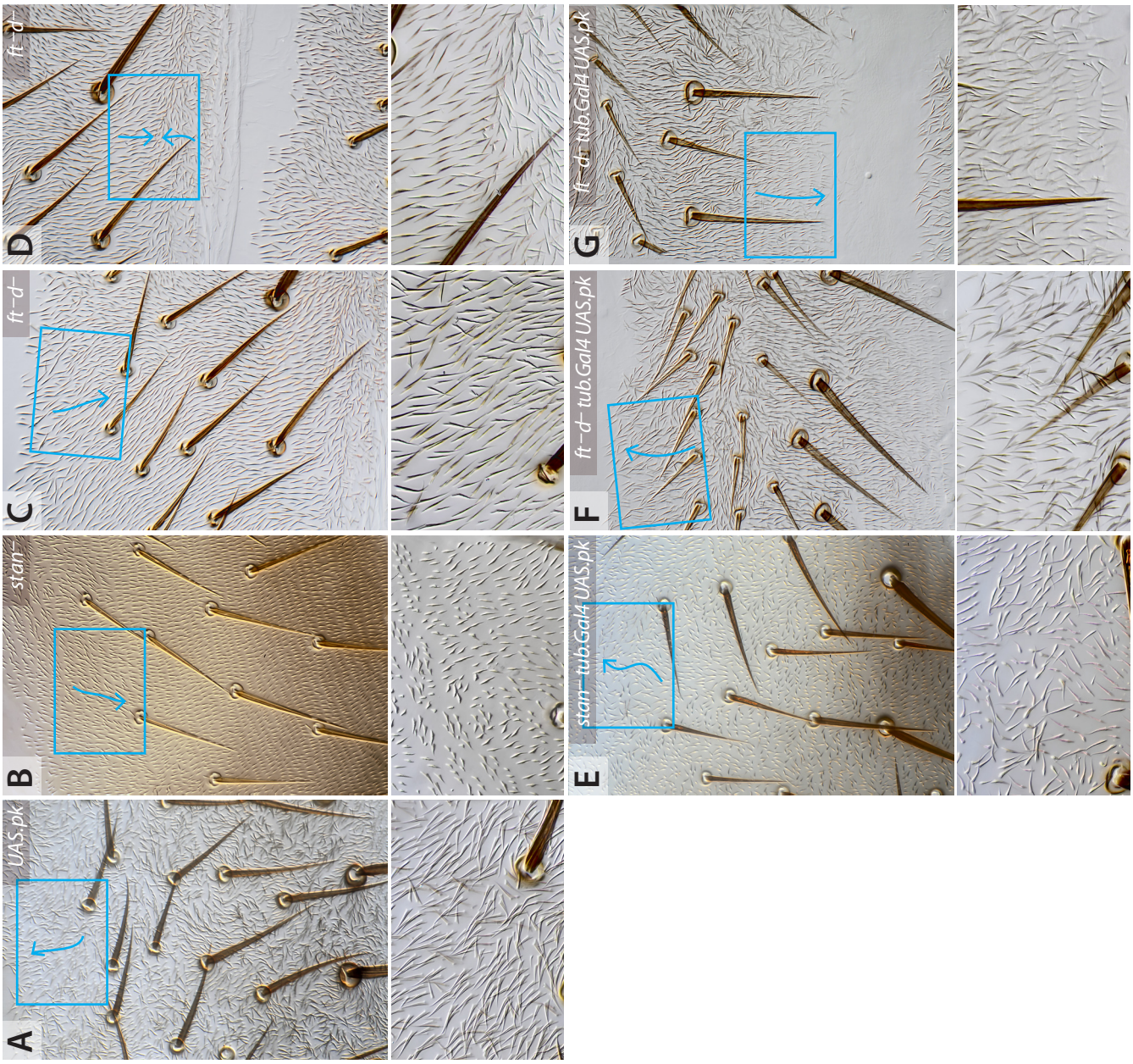


figure 4

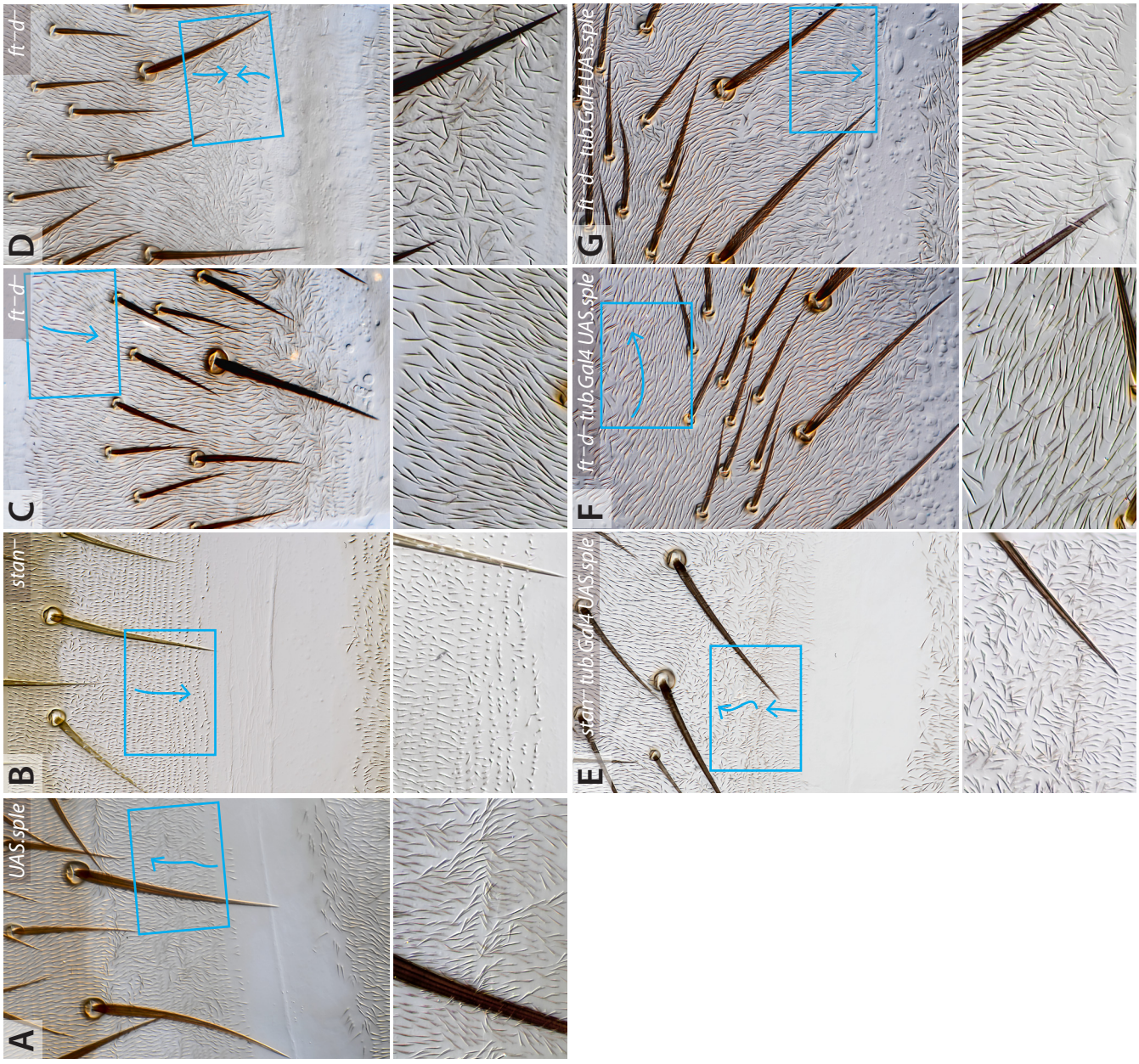


figure 5

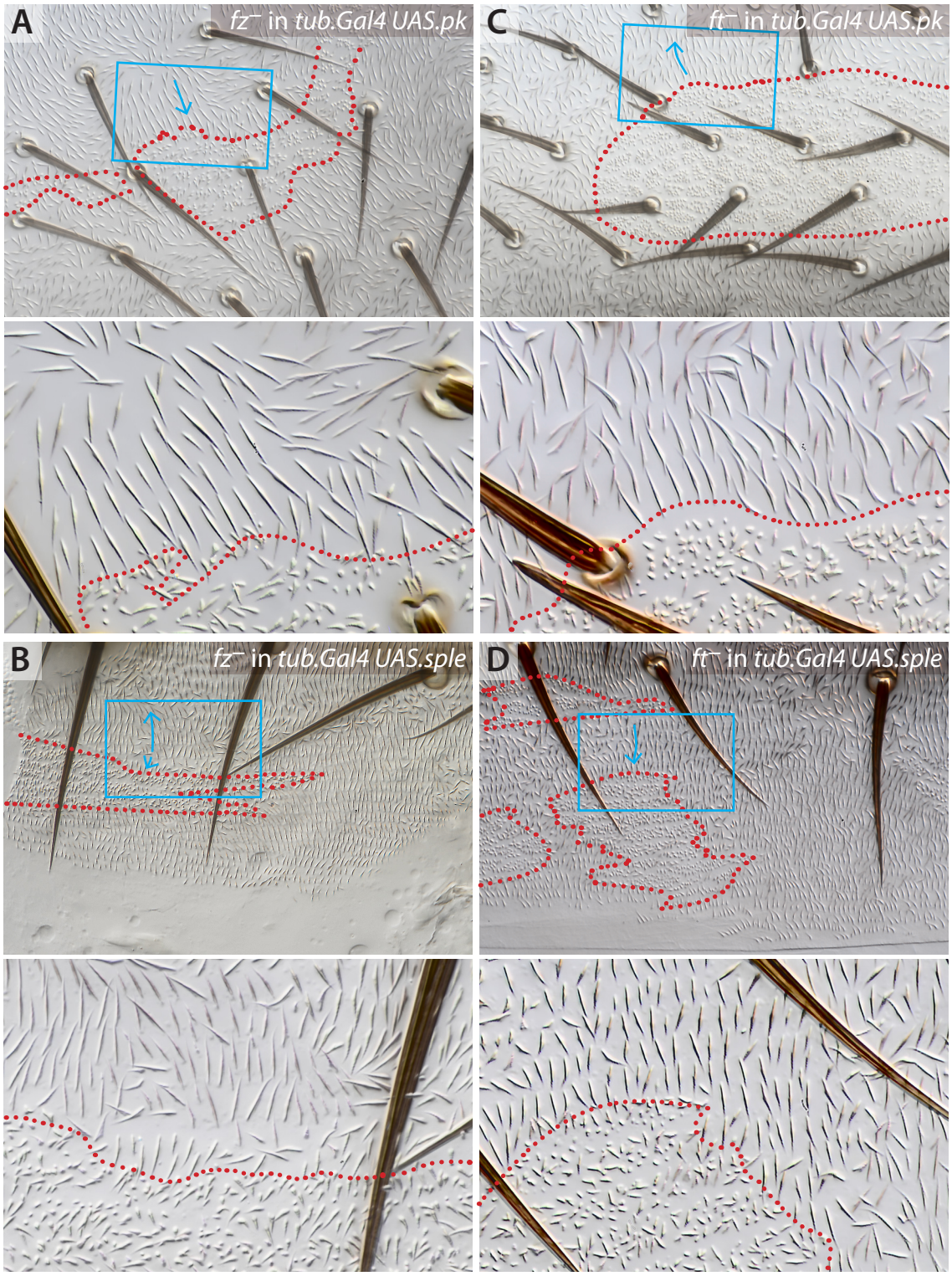


figure 6

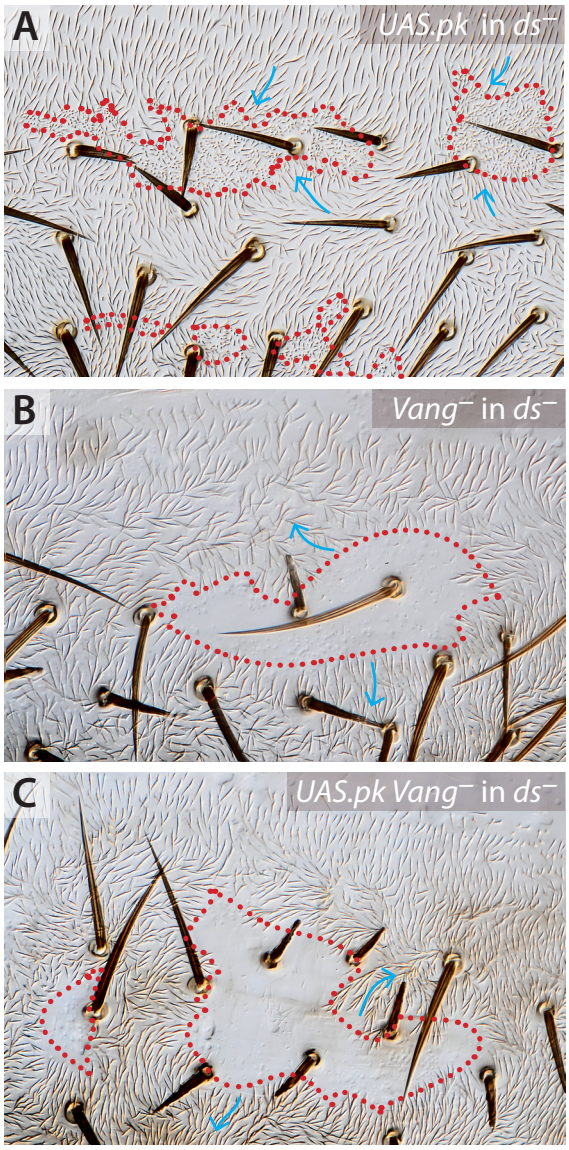


figure 7

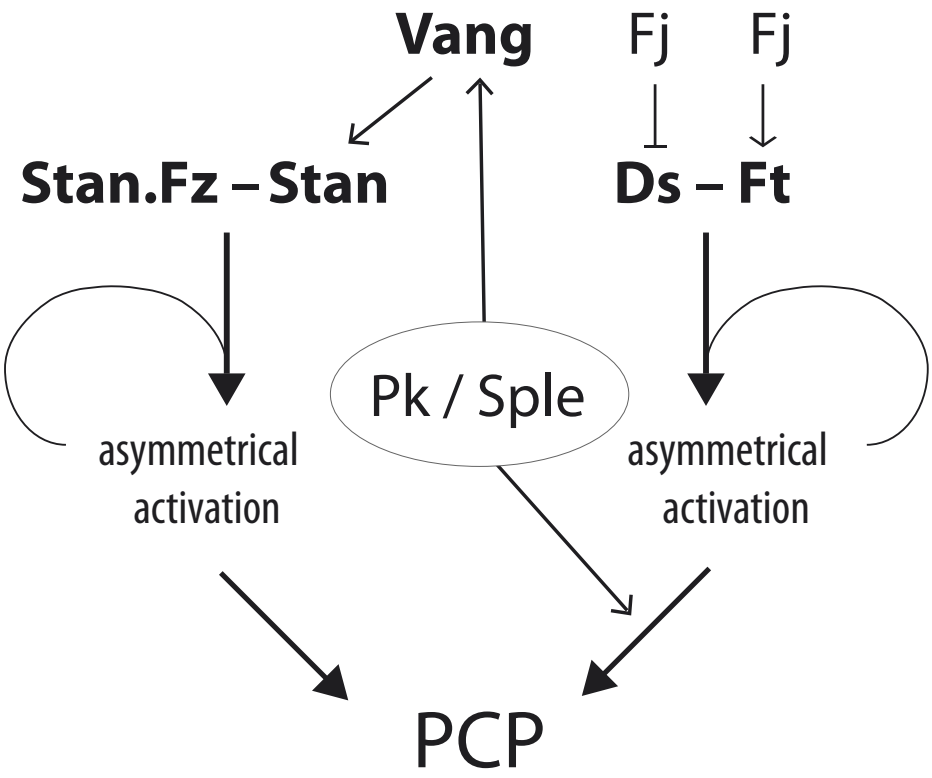


figure 8

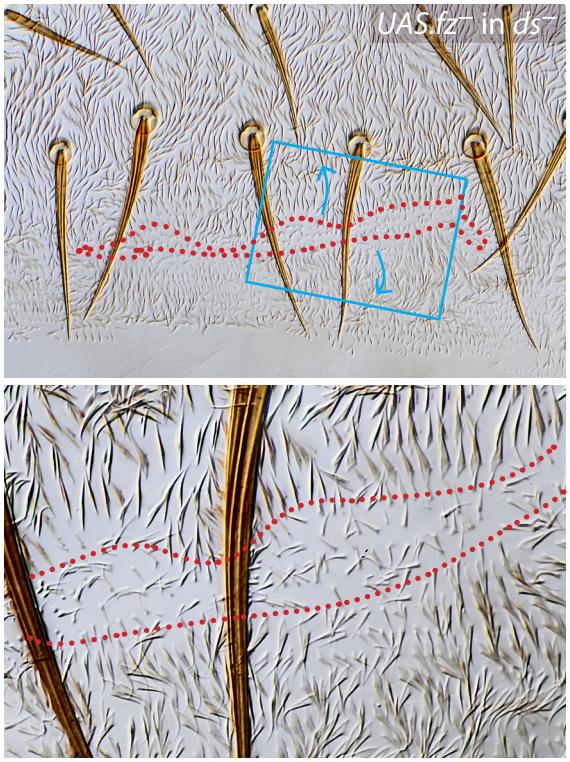


figure S1

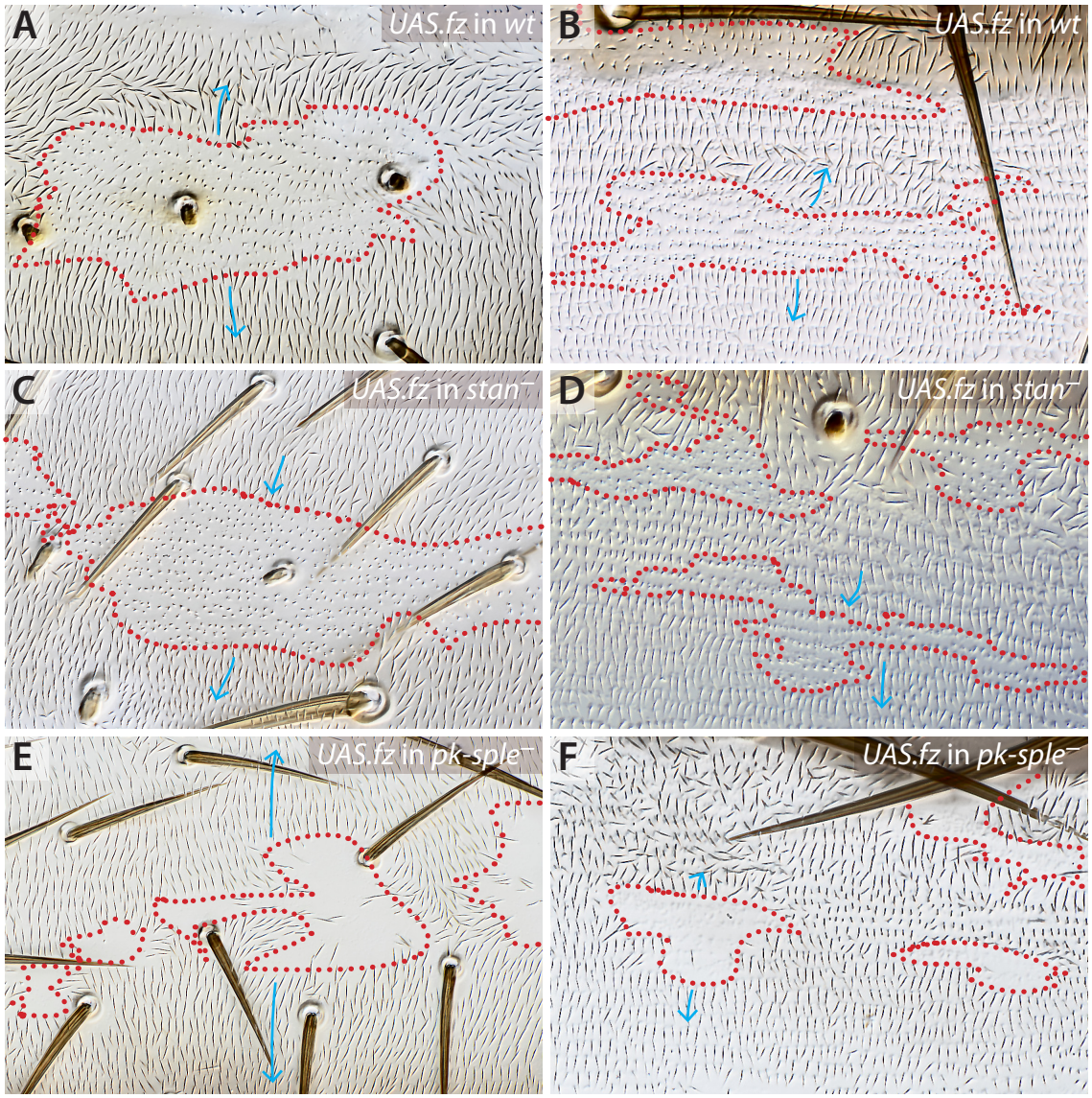
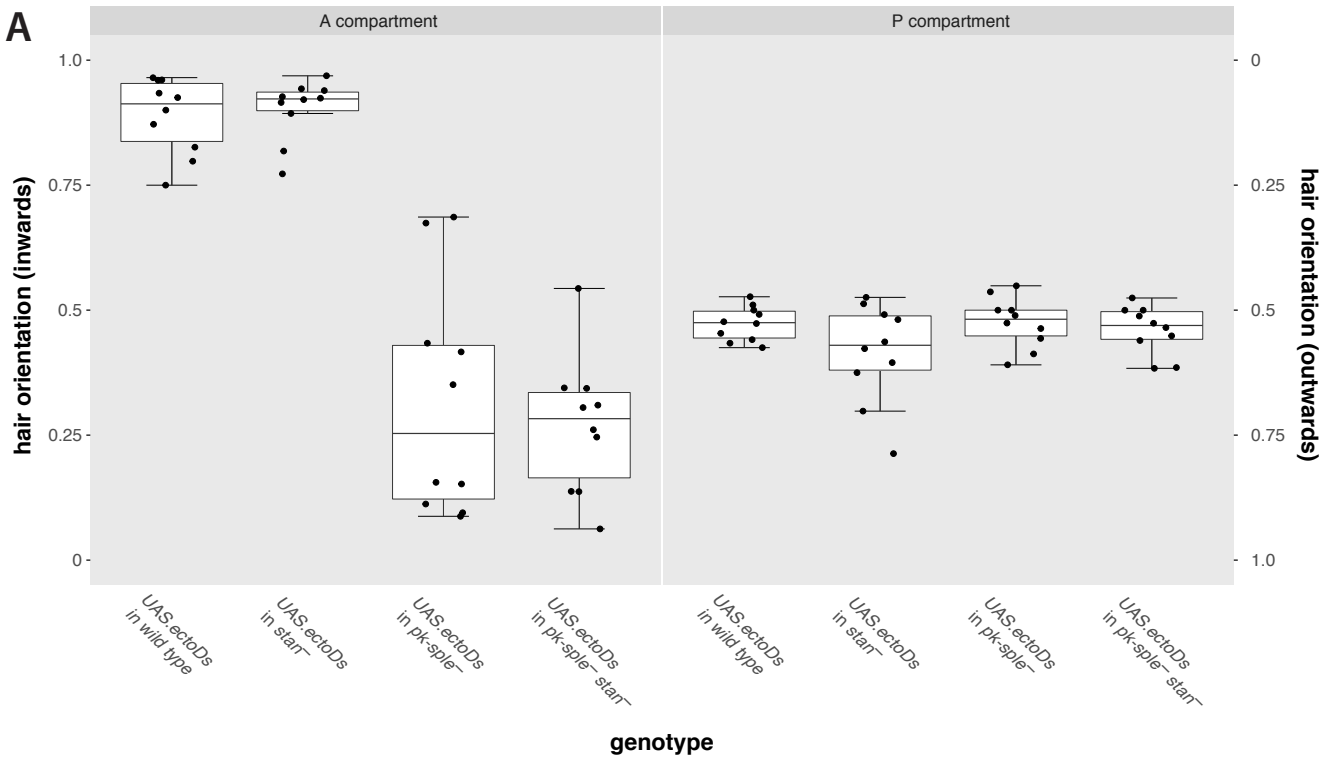


figure S2



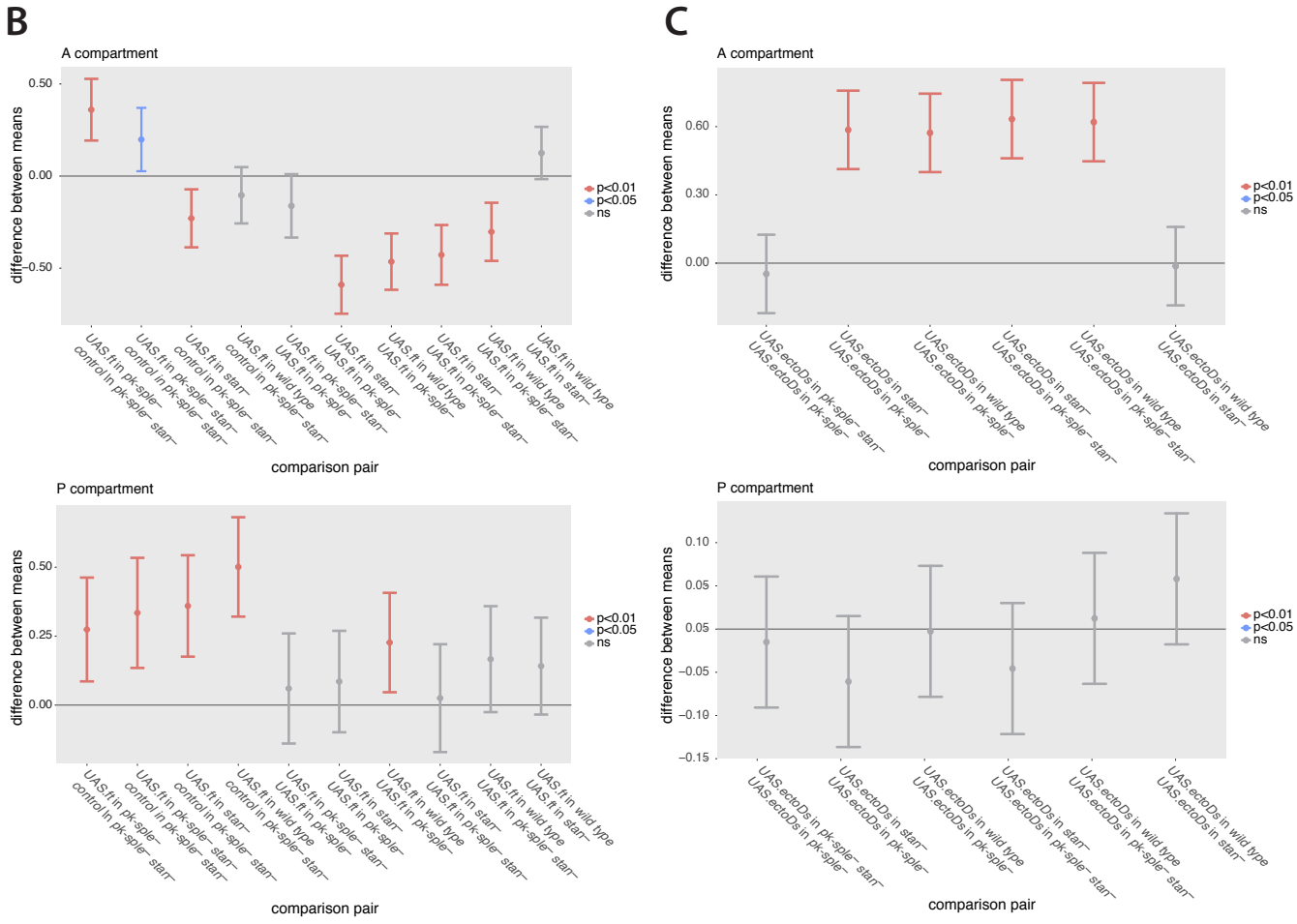
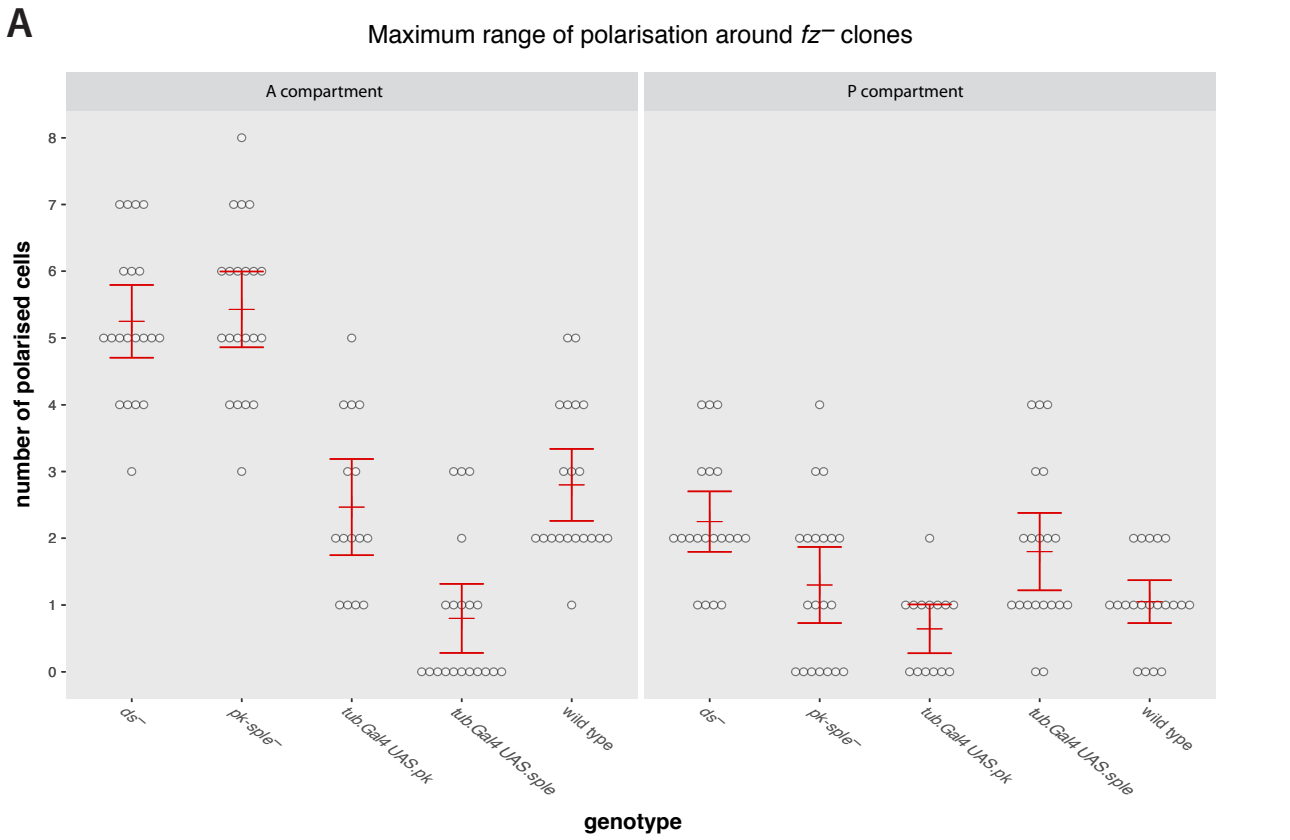


figure S3



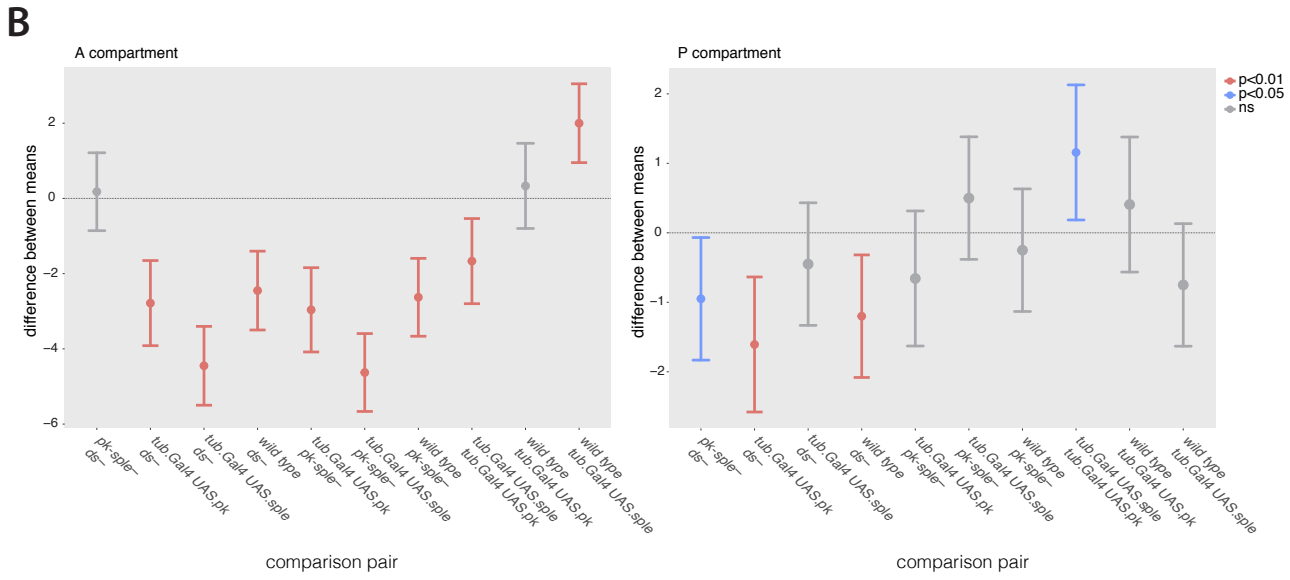


figure S5

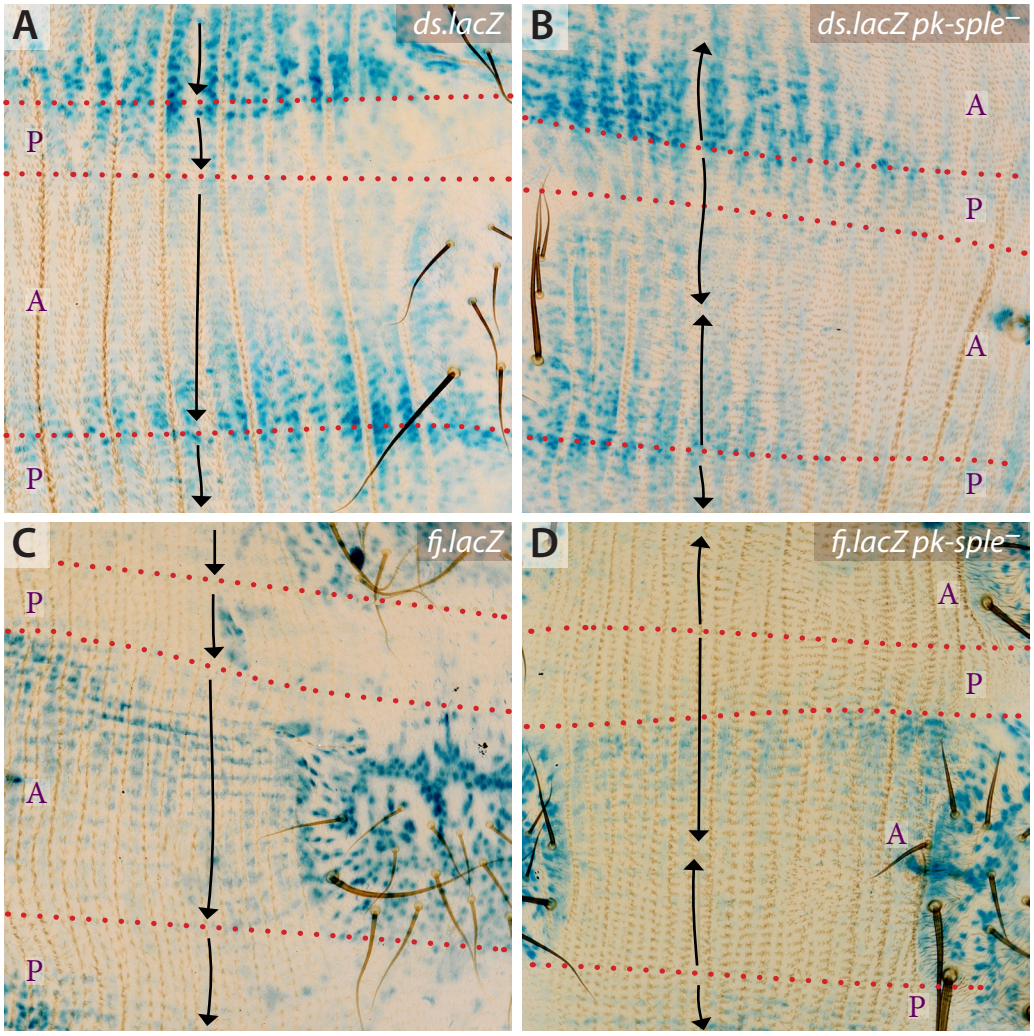


figure S6

Supplementary figure 6