Planar Cell Polarity: What Does The *prickle* Gene Do?

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ABSTRACT

Many, if not all, epithelial cells are polarised within the plane of the epithelium and some form oriented structures whose coordinated and consistent polarity (planar cell polarity, PCP) relates to the principal axes of the body or organ. PCP depends on intercellular communication of polarity signals; in Drosophila at least two separate molecular systems generate these signals: Dachsous/Fat, Ds/Ft and the core or Stan system and both are conserved widely (reviewed in Butler and Wallingford, 2017). Here we make a new attempt to understand the PCP gene *prickle* (*pk*) and its products Pk and Sple. Much research on PCP has asked how and why many PCP proteins, including Pk and Sple, are asymmetrically localised in the cell (Strutt and Strutt, 2009). This question led to the *pk* gene being placed at the heart of the core or Stan system (Tree et al., 2002b). Here we use direct genetic tests to ask if this view is correct and if and how the *pk* gene relates to the Stan and the Ds/Ft systems. We conclude that Pk and Sple have been widely misunderstood: we find they can affect, separately, the Ds/Ft system (reversing, or rectifying the polarity of its output) and the core or Stan system (being required for the asymmetrical distribution of its proteins). In the Stan system they appear to work via binding to Vang. Neither Pk nor Sple are essential components of either the Ds/Ft or the Stan systems nor do they act as a bridge 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, demonstrate this by forming oriented structures. These 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 PCP genes and is the most amenable of all animals for elucidating the mechanisms of PCP. PCP genes coordinate the polarity of a group of cells, a polarity that is related to the principal axes of the body or organ. During the last 20 years, researchers have investigated how gene products required for PCP are asymmetrically localised in the cell and studied the propagation of polarity from cell to cell. One PCP gene, *prickle* (*pk*), has a particularly chequered history. Here we make another experimental attempt to understand it. Figure 1 is a Baedeker of all the experiments and results.

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 encoding the Pk and Sple proteins; both proteins contain protein-protein

binding LIM domains, differ in the N terminus, (Gubb et al., 1999) and have sequence elements conserved to vertebrates. In vertebrates, syndromes due to *pk* mutations have been classified, somewhat impetuously, 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 cell; for example, Pk is enriched on or near the proximal membrane of each wing cell (Tree et al., 2002b), while Frizzled (Fz) is localised distally (Strutt, 2001). Other core proteins include Vang Gogh (Vang), Dishevelled (Dsh) and Starry Night (Stan), also known as Flamingo (for a review see 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. 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 is an intercellular bridge consisting of two different complexes facing each other across the cell membrane. Pk was thought to be an essential factor in this bridge and therefore proposed to have a central role in intercellular "*signalling*". This view of Pk has been repeated so many times in the literature that it has come to be accepted as true.

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

Nevertheless, the functional relevance of Pk to intercellular signalling by the core system suffered two severe blows when, first, Adler et al (2000) found that a weak allele of *pk* did not reduce polarisation caused by clones mutant for other core genes. Then, second, Lawrence et al. (2004) reported that the complete loss of Pk and Sple increases polarisation by the core system genes; they proposed that the key molecules in the core system are Stan, Fz and Vang. They 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 (Strutt and Strutt, 2007) who presented further evidence that "*Dishevelled, Prickle and Diego are not needed for intercellular communication*".

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

In the dorsal abdomen, when *pk* is overexpressed everywhere, polarity of the A compartment is almost entirely reversed while the P compartment is normal. By contrast, when *sple* 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 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 thought to be most effective in the wing, in the P compartment of the abdomen and the posterior part of the thorax while Sple predominates 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).

It was widely 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; reviewed in Butler and Wallingford, 2017). However, no clear evidence was offered as to how the two systems might be linked. Some experiments in the adult abdomen had argued that gradient slopes of Ds and Fj have different signs in the anterior (A) and the posterior (P) compartments (Casal et al., 2002). If these slopes were to be read by the Stan system, since all the hairs point backwards in the wildtype, at least one set of hairs (in either the A or the P compartment) must point in the opposite way, with respect to the Ds gradient, to the other set. Based on experimental evidence it was proposed that Pk or Sple might rectify the reading of one of these gradients to ensure that that the Stan system points all hairs in the same direction (Lawrence et al., 2004). However, 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; and more recently Brittle et al., 2012) — implying that rectification is not to alter a direct input from the Ds/Ft system into the Stan system but to avoid dissonance between their independent inputs into PCP.

(iv) Current views of the *pk* gene

Recent papers (Olofsson et al., 2014; Ambegaonkar and Irvine, 2015; Strutt et al., 2016) still judge *pk* to be a key member of the Stan system. They show that Pk and Sple act discordantly on polarity output in different tissues and have improved the evidence that Pk and Sple can turn around the orientation of polarised structures (Ayukawa et al., 2014). Some still hold to the earlier hypothesis that the Ds/Ft system functions via the Stan system; these authors envisage Pk and Sple as components of a Stan system that interprets the Ds/Ft gradients of activity (see discussion). Here we provide experimental evidence that much of this view is wrong: we conclude that Pk and Sple are neither essential nor active components of either system nor do they function as a bridge between the two systems. Instead, Pk and Sple rectify the polarity outputs of the Ds/Ft system and, separately, modulate the output of the Stan system.

Results

Explaining terms and methods

We use genetic mosaics 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* (Lawrence et al., 2004). Using these functional assays we ask whether and how Pk and Sple cooperate with the Ds/Ft and the Stan systems.

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

(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 (Figures 2 and 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 A compartments (Casal et al., 2002; Casal et al., 2006); however these clones are ineffective in P compartments (Figure S3).

(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; and Casal et al., 2006; Figure S1).

These experiments establish that the two systems act independently; we now ask are Pk and Sple part 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 whirls (probably due to the misdistribution of Dachs) but in its posterior part the hairs point 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 identical to *ds*⁻ flies (Figure 1). It follows that the *ds* mutation is epistatic to the *pk* and *sple* mutations. By contrast, when *pk-sple*⁻ *stan*⁻ flies are compared to each single mutant, they differ from both, having an additive phenotype (Figure 1). These results suggest that Pk and Sple may act separately 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 9), polarity of most of the A compartment is reversed, but the P compartment is normal. Clones of cells that overexpress *fz* (Genotype 10 or, alternatively, lack *fz*, Genotype 11) 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 12) or *pk* (Genotype 13) are overexpressed, *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, suggesting that Pk and Sple do not act as integral parts of the Stan system in the wildtype.

However, the *fz*⁻ clones do not behave exactly as they would in a wildtype background:

3. 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 11) the range of polarisation due to *fz*⁻ clones or excess *fz* (Lawrence et al., 2004 Figure 4) is increased, resembling the increase in range observed when *fz*⁻ clones are induced in *ds*⁻ flies (Genotype 14). Raising the level of Pk

ubiquitously does not change that range (Genotype 13), while when Sple levels are raised (Genotype 12), 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 (Figures S2 and S5). These results show 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 of *stan*$^-$ (Genotype 15) cells in the A compartment, without affecting the P compartment (Figure 4). Likewise, generalised overexpression of *sple* in flies with a broken Stan system (*stan*$^-$, Genotype 16) affects polarity of the P compartment of the abdomen, without affecting the A compartment: there is disturbance of polarity, with much reversal, although less than in *stan*$^+$ flies (Figure 5). These results show that Pk and Sple can function independently of the Stan system.

(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) in either *ds*$^-$ *pk-sple*$^-$ (Genotype 17, not shown), *d*$^-$ (Genotype 18, Figure S4) or in *ft*$^-$ *d*$^-$ flies (Genotype 19, Figure 4). Similarly, general overexpression of *sple* affects the polarity of the P compartment of the abdomen both in *ds*$^-$ *pk-sple*$^-$ (Genotype 20, not shown), *d*$^-$ (Genotype 21, Figure S4) and *ft*$^-$ *d*$^-$ flies (Genotype 22, 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". 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 and the P compartment is "rescued", having a normal orientation — indeed Pk and Sple have similar effects on *ft*$^-$ *d*$^-$ but very different effects on *d*$^-$ flies. It follows 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 (Figures 4, 5 and S4).

2. Clones that overexpress *pk* or *sple* in a broken Ds/Ft system. We find that clones of cells overexpressing *sple* (Genotype Z, Lawrence et al., 2004) or *pk* (Genotype 24; data

not shown), have small nonautonomous effects in the wildtype and, more so, in *ds*$^-$ flies (Genotype 25 and Genotype 26) in which they polarise receiving cells to point strongly inwards (Figure 7). Perhaps these clones act via the Stan system? It is pertinent that cells overexpressing 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 27), as well as control *Vang*$^-$ clones (Genotype 28), in *ds*$^-$ flies. Both these types of clones behaved like *Vang*$^-$ clones in wildtype flies (Genotype 29), and could not be distinguished from each other, ie they polarise *ds*$^-$ receiving cells 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 can function independently 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 30). 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 31) 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 confirms these results and shows that clones (in the A compartment) overexpressing either *ds* or *ft* affect the polarity of both wildtype and *stan*$^-$ (Genotype 5 and Genotype 3) cells in the same way and both *pk-sple*$^-$ *stan*$^+$ and *pk-sple*$^-$ *stan*$^-$ (Genotype 32 and Genotype 33) receiving cells in the opposite way (Figures 3 and S3). Results that underline again that the Ds/Ft system acts independently of the Stan system. They also show that Pk and Sple do not act as a functional link between the Ds/Ft system and the Stan system, because if they were a link, the removal of Pk and Sple would block effects on polarity caused by *ft*$^-$ cells.

2. In flies in which *pk* or *sple* are overexpressed. Clones that lack *ft* made in flies in which *pk* is generally overexpressed (Genotype 34) 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 35) 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 36) 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 (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 37, Genotype 38 and Genotype 39).

3. These results show that the Ds/Ft system can function independently of Pk and Sple but Pk and Sple modulate the sign of its output. This dramatic effect could, in principle, be due to Pk and/or Sple affecting the pattern of expression of *ds* or *ft* and changing the orientation of the 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 (data not shown). 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 and several speak against the currently prevailing opinions of the function of the *pk* gene in PCP. We discuss these issues one by one.

The Ds/Ft system and the Stan system are independent

ft-overexpressing clones reorient wildtype receiving cells, outwards in the A compartment (Casal et al., 2006) and inwards in the P. These clones have the same effects on cells in which the Stan system of PCP is broken (for example in *stan*⁻ flies; Figures 2 and 3). This result confirms that for both compartments, the Ds/Ft system acts independently of the Stan system (Casal et al., 2006; Lawrence et al., 2007; Lawrence, 2011). Nevertheless, there remains a long-standing conviction held by some that the Ds/Ft system does not act independently but directs the Stan system, raising an issue that is still being described as "*unresolved*" (Matis and Axelrod, 2013), "*controversial*" (Ambegaonkar and Irvine, 2015) and "*contentious*" (Butler and

Wallingford, 2017). It has been argued that the combination *stan*^{3}/*stan*^{E59} used in our key experiments (Casal et al., 2006) does not break the Stan system (Axelrod, 2009). However in those experiments, and in the present experiments, we deployed clones that overexpress *ft* that are also *stan*^{E59} homozygous (stanE59 introduces a premature stop codon in the ectodomain Usui et al., 1999). Yet these clones, as we pointed out in 2006 (Casal et al., 2006), which incontrovertibly lack any functional Stan protein, propagate the effects on polarity over several rows of *stan*^{3}/*stan*^{E59} receiving cells. 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 thereby influence some residual capability of the Stan system in the receiving 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 effect on the Stan system. Similarly, in our present experiments we deploy homozygous *stan*^{E59} clones in *stan*^{3}/*stan*^{E59} flies that are also *pk-sple*$^{-}$ homozygotes. When such clones also overexpress *ft* or *ds* they do repolarise the receiving cells. It follows that the observed polarisation cannot depend on Pk and Sple intervening between the Ds/Ft and the Stan systems. A conclusion that conflicts with current models of the *pk* gene (Hogan et al., 2011; Ayukawa et al., 2014; Merkel et al., 2014; Olofsson et al., 2014; Ambegaonkar and Irvine, 2015).

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. These results add to earlier findings that, in *pk-sple*$^{-}$ flies, the mechanisms of propagation due to the Stan system are preserved and even enhanced, raising doubt about the function of the asymmetric localisation per se (Adler et al., 2000; Lawrence et al., 2004). Clearly Pk and Sple do not function simply as elements of the Stan system.

Many will find this view heretic, but let us ask why was Pk originally classified as a core protein? The arguments that connected Pk and Sple to the Stan system were, firstly that these proteins and others of the Stan system (Fz and Vang) are themselves asymmetrically localised at the membrane and, secondly, in *pk-sple*$^{-}$ flies, the normal asymmetric localisation of Fz and Vang is not seen (reviewed in Strutt, 2009). Yet, as we have seen the connection between asymmetric localisation and propagation of polarity is tenuous; it can still be that this asymmetry is more a consequence than a cause of polarity. Other results argue that Pk and Sple are not standard members of the Stan system: the loss of key genes (eg Fz or Stan) in the receiving cells cripples or eliminates responses to abnormal amounts of other Stan system proteins in the

sending cells —yet receiving cells that are *pk-sple*⁻ respond at least as well to such sending cells (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 propagate polarity. 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 caused the Ds/Ft system. Also, the stark finding that clones with altered amounts of Ds or Ft have strong effects on polarity both in *pk-sple*⁻ and in *pk-sple*⁻ *stan*⁻ flies argues again against the current view that, in the wildtype, Ds/Ft output goes through Pk/Sple to the Stan system. Even advocates of this view encounter problems: Ayuwaka and colleagues have difficulties in demonstrating how Ds/Ft might deploy Pk and Sple to act on the Stan system : "*our experiments do not reveal.. how…polarized Sple complexes regulate the core proteins*" (Ayukawa et al., 2014). Others have difficulties in understanding what Pk does in the Stan system: "*the core function of Pk-Sple is not well defined*" (Olofsson et al., 2014). However if our conclusion that Pk and Sple can act independently on both the Stan and the Ds/Ft system is correct, then all these difficulties disappear. A diagram suggesting how the *pk* gene might fit into the organisation of PCP is given in Figure 8.

Some unanswered questions

1. 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 they might transport 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) and unconvincing: "*the inference that Ds/Ft orients PCP in wing by… microtubules is incorrect*" (Ambegaonkar and Irvine, 2015). Also, this model is 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.

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 related molecular functions but interact with the two PCP systems differently (Olofsson et al., 2014). 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 Pk can alter 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). In both compartments 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).

2. 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. Indeed, there is already evidence that Pk and Sple bind to Ds/Ft system components such as D (Ayukawa et al., 2014) and Ds (Ayukawa et al., 2014; Ambegaonkar and Irvine, 2015). Ayuwaka et al concluded "*the localization and function of Sple is regulated through its interaction with Ds group proteins*". 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.

3. 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 main bridge molecules, 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 suggest that any action of Pk might not be limited to the Stan system and include, also, 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.

4. 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 the startlingly diverse orientation of stereocilia in the vestibular system, or the complex patterns of hair orientation. Two separate genetic systems each generating polarity based on 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 bridge linking the two systems. Instead, Pk and Sple appear to modulate the polarity outputs of both the Ds/Ft system and the Stan system. Both these systems are different in their components but similar in their logic; both utilise intercellular bridges that are distributed asymmetrically within each cell. Pk and Sple could help produce this asymmetry— perhaps via a generic function in cell biology whose contribution to PCP is still undiscovered.

Materials and Methods

Mutations and transgenes

The FlyBase (Gramates et al., 2017) entries for mutations and transgenes are the following: *tub.Gal4*: *Scer\GAL4*$^{alphaTub84B.PL}$. *tub.Gal80*: *Scer\GAL80*$^{alphaTub84B.P}$. *UAS.ectoDs*: *ds*$^{ecto.Scer\UAS}$. *UAS.ft*: *ft*$^{Scer\UAS.cMa}$. *UAS.fz* : *fz*$^{Scer\UAS.cSa}$. *UAS.pk*: *pk*$^{Scer\UAS.cGa}$. *UAS.sple*: *pk*$^{sple.Scer\UAS}$. *ck*^{UAH21}. *d*^{GC13}. *ds*^{UA071}. *ft*^{8} and *ft*$^{G-rv}$. *fz*^{15}. *pk*$^{pk-sple-13}$. *pwn*^{1}. *sha*^{1}. *stan*^{3} and *stan*^{E59}. *trc*^{1}.

Experimental Genotypes

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

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

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

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

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

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

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

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

Genotype 9: ***pk-sple*⁻ flies**: *pk*$^{pk-sple-13}$

Genotype 10: ***UAS.fz* clones in *pk⁻sple⁻* flies**: *y w hs.FLP tub.Gal4 UAS.nls-GFP/ y w hs.FLP; FRT42D* $pk^{pk\text{-}sple\text{-}13}$ *sha/ FRT42D* $pk^{pk\text{-}sple\text{-}13}$ *tub.Gal80; UAS.fz* fz^{15} $fz2^{C1}$ *FRT2A/ +*

Genotype 11: ***fz⁻* clones in *pk-sple⁻* flies**: *y w hs.FLP122; FRT42* $pk^{pk\text{-}sple\text{-}13}$*/ CyO; fz[P21] trc FRT2A/ tub.Gal80 FRT2A*

Genotype 12: ***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.GFP*w^{+} *hs.CD2,* y^{+} *ri FRT2A*

Genotype 13: ***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.GFP*w^{+} *hs.CD2,* y^{+} *ri FRT2A*

Genotype 14: ***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 15: ***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 16: ***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 17: ***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 18: ***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 19: ***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 20: ***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 21: ***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 22: ***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 23: ***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 24: ***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 25: ***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 26: ***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 27: ***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 28: ***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 29: ***Vang*⁻ clones**: *y/ y hs.FLP; FRT42D pwn $Vang^{stbm-6}$FRT42D hs.CD2, y^+*

Genotype 30: ***UAS.ft* clones in *pk⁻sple⁻* flies**: *y w hs.FLP122 tub.gal4 UAS.nls-GFP/ y w hs.FLP; FRT42D $pk^{pk-sple-13}$ sha/ FRT42 $pk^{pk-sple-13}$ tub.Gal80/ UAS.ft/ +*

Genotype 31: ***UAS.ectoDs* clones in *pk⁻sple⁻* flies**: *y w hs.FLP122 tub.gal4 UAS.nls-GFP/ y w hs.FLP; FRT42D $pk^{pk-sple-13}$ sha/ FRT42 $pk^{pk-sple-13}$ tub.Gal80/ UAS.ectoDs/ +*

Genotype 32: ***UAS.ectoDs* clones in *pk⁻sple⁻ stan⁻* flies**: *y w hs.FLP tub.Gal4 UAS.nls-GFP/ y w hs.FLP; FRT42D $pk^{pk-sple-13}$ $stan^3$ tub.Gal80, y^+/ FRT42 $pk^{pk-sple-13}$ $stan^{E59}$ sha ; UAS.ectoDs/ +*

Genotype 33: ***UAS.ft* clones in *pk⁻sple⁻ stan⁻* flies**: *y w hs.FLP tub.Gal4 UAS.nls-GFP/ y w hs.FLP122; FRT42D $pk^{pk-sple-13}$ $stan^3$ tub.Gal80, y^+/ FRT42D $pk^{pk-sple-13}$ $stan^{E59}$ sha ; UAS.ft/ +*

Genotype 34: ***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 35: ***ft⁻* clones**: *y w hs.FLP; ft^{15} stc FRT39/ FRT39*

Genotype 36: ***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 37: ***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 38: ***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 39: ***ds⁻* clones**: *y w hs.FLP/ +; ds^{UA071} ck^{UAh21} FRT40A/ Dp(1;2)sc^{19} w^{+30c} FRT40A*

Genotype 40: **Control clones in *pk⁻sple⁻ stan⁻* flies**: *y w hs.FLP tub.Gal4 UAS.nls-GFP/ y w hs.FLP; FRT42D $pk^{pk-sple-13}$ $stan^{E59}$ sha/ FRT42D$pk^{pk-sple-13}$ $stan^3$ tub.Gal80, y^+; MRS/ +*

Genotype 41: ***stan⁻* flies**: *y w hs.FLP; FRT42D pwn $stan^{E59}$ sha/ FRT42D $stan^3$; UAS.sple/ +*

Clone induction and microscopy

Clones were induced by heat shocking third instar larvae for 1 hr at 34°C. 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 measurements, 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).

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672

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 40).

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 41) 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.

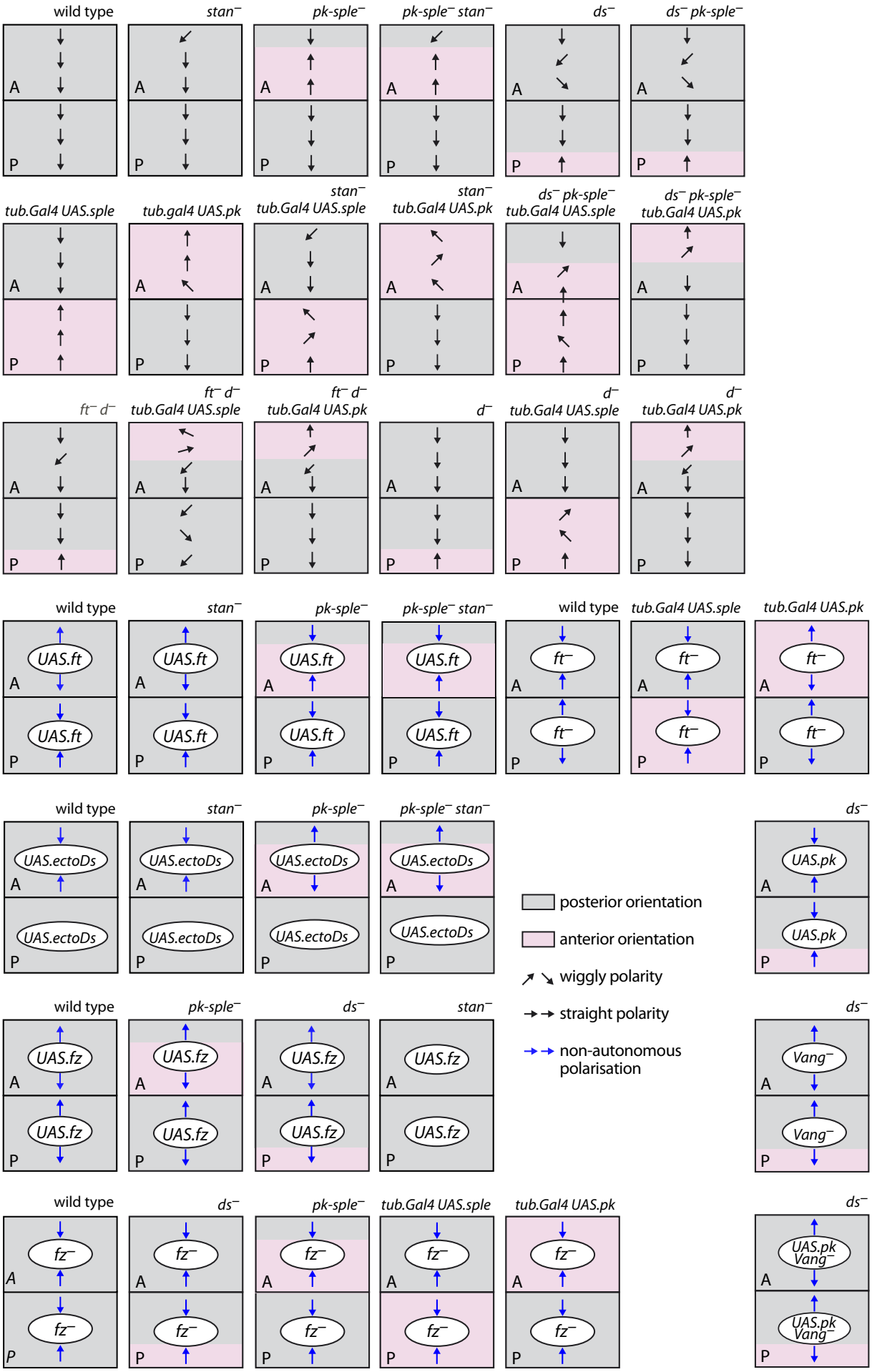

wild type
stan⁻
pk-sple⁻
pk-sple⁻ stan⁻
ds⁻
ds⁻ pk-sple⁻
tub.Gal4 UAS.sple
tub.gal4 UAS.pk
stan⁻ tub.Gal4 UAS.sple
stan⁻ tub.Gal4 UAS.pk
ds⁻ pk-sple⁻ tub.Gal4 UAS.sple
ds⁻ pk-sple⁻ tub.Gal4 UAS.pk
ft⁻ d⁻
ft⁻ d⁻ tub.Gal4 UAS.sple
ft⁻ d⁻ tub.Gal4 UAS.pk
d⁻
d⁻ tub.Gal4 UAS.sple
d⁻ tub.Gal4 UAS.pk
UAS.ft
ft⁻
UAS.ectoDs
UAS.pk
UAS.fz
Vang⁻
fz⁻
UAS.pk Vang⁻
A
P
posterior orientation
anterior orientation
wiggly polarity
straight polarity
non-autonomous polarisation

figure 1

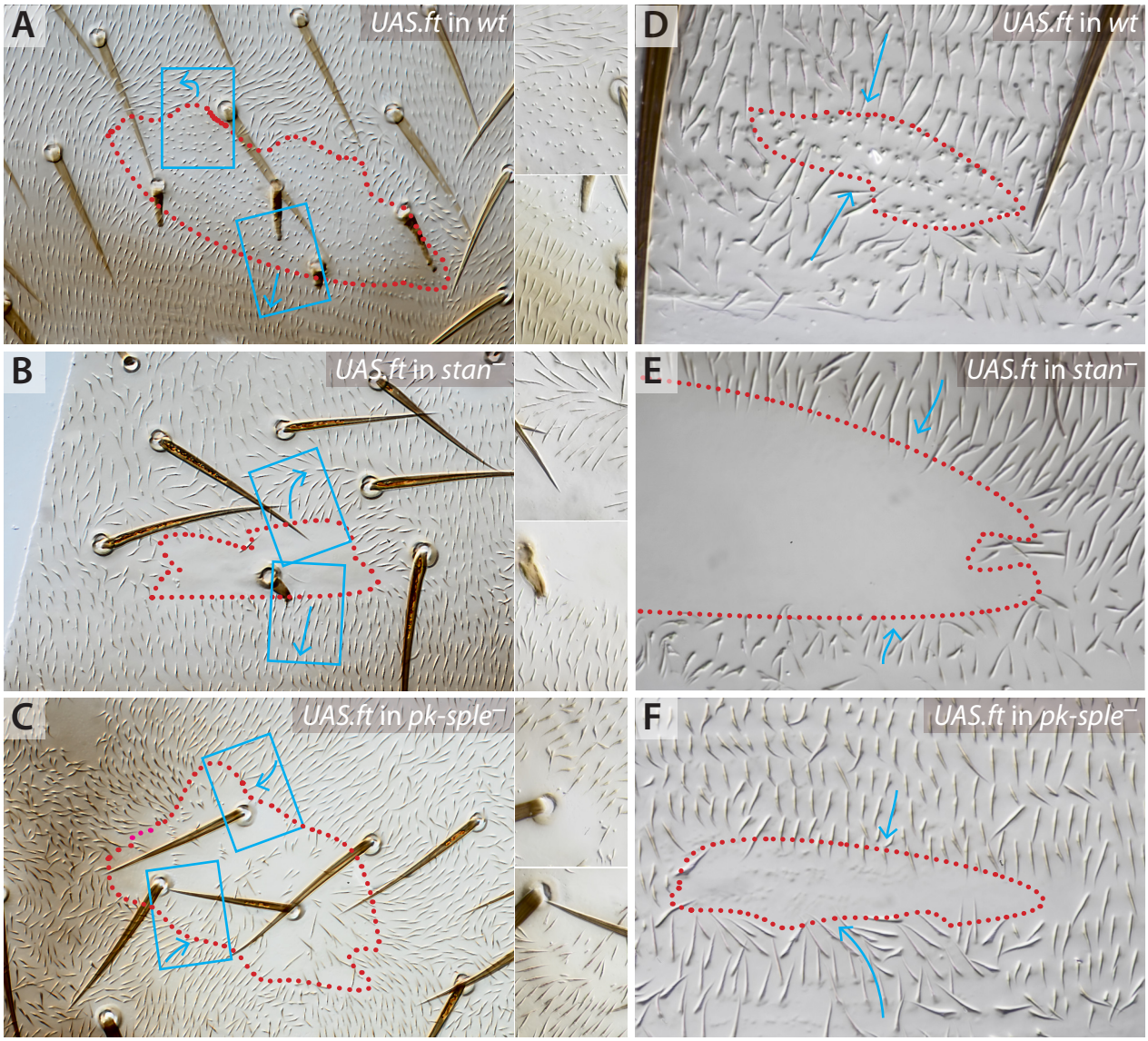

A
UAS.ft in wt
D
UAS.ft in wt
B
UAS.ft in stan⁻
E
UAS.ft in stan⁻
C
UAS.ft in pk-sple⁻
F
UAS.ft in pk-sple⁻

figure 2

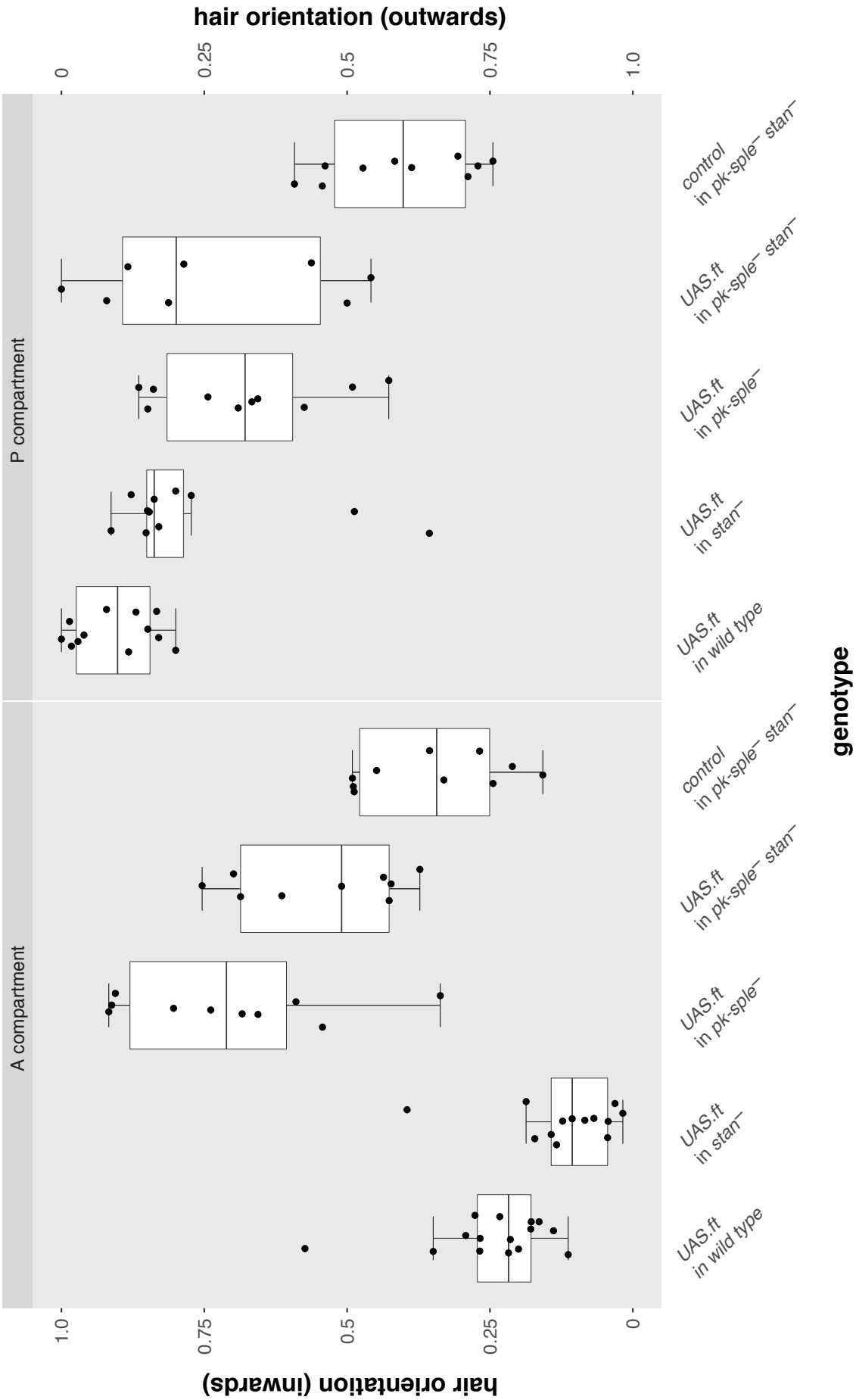

hair orientation (outwards)
0
0.25
0.5
0.75
1.0
P compartment
A compartment
control in pk-sple⁻ stan⁻
UAS.ft in pk-sple⁻ stan⁻
UAS.ft in pk-sple⁻
UAS.ft in stan⁻
UAS.ft in wild type
genotype
hair orientation (inwards)

figure 3

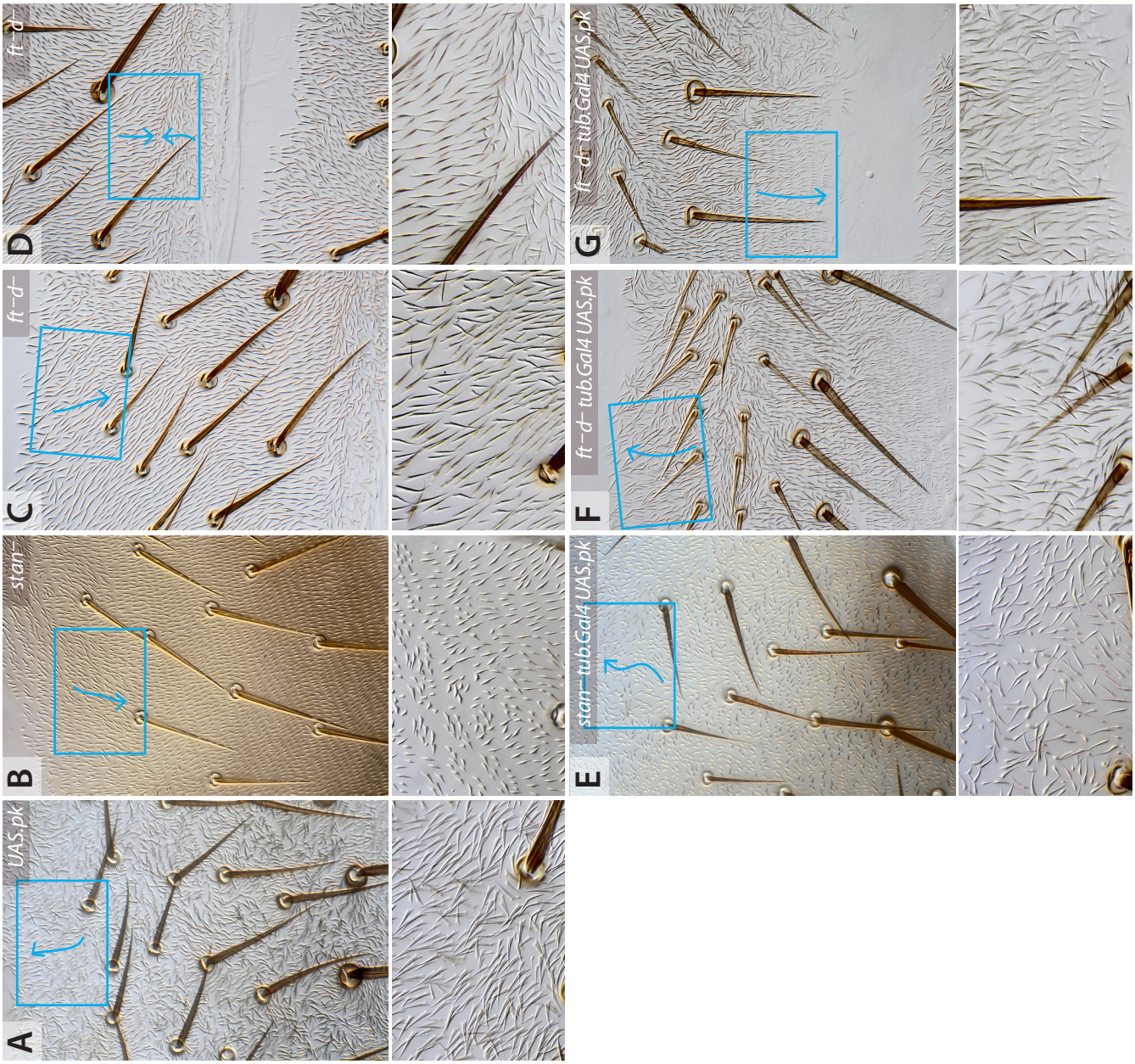

A
UAS.pk
B
stan−
C
ft−d−
D
ft−d−
E
stan− tub.Gal4 UAS.pk
F
ft−d− tub.Gal4 UAS.pk
G
ft−d− tub.Gal4 UAS.pk

figure 4

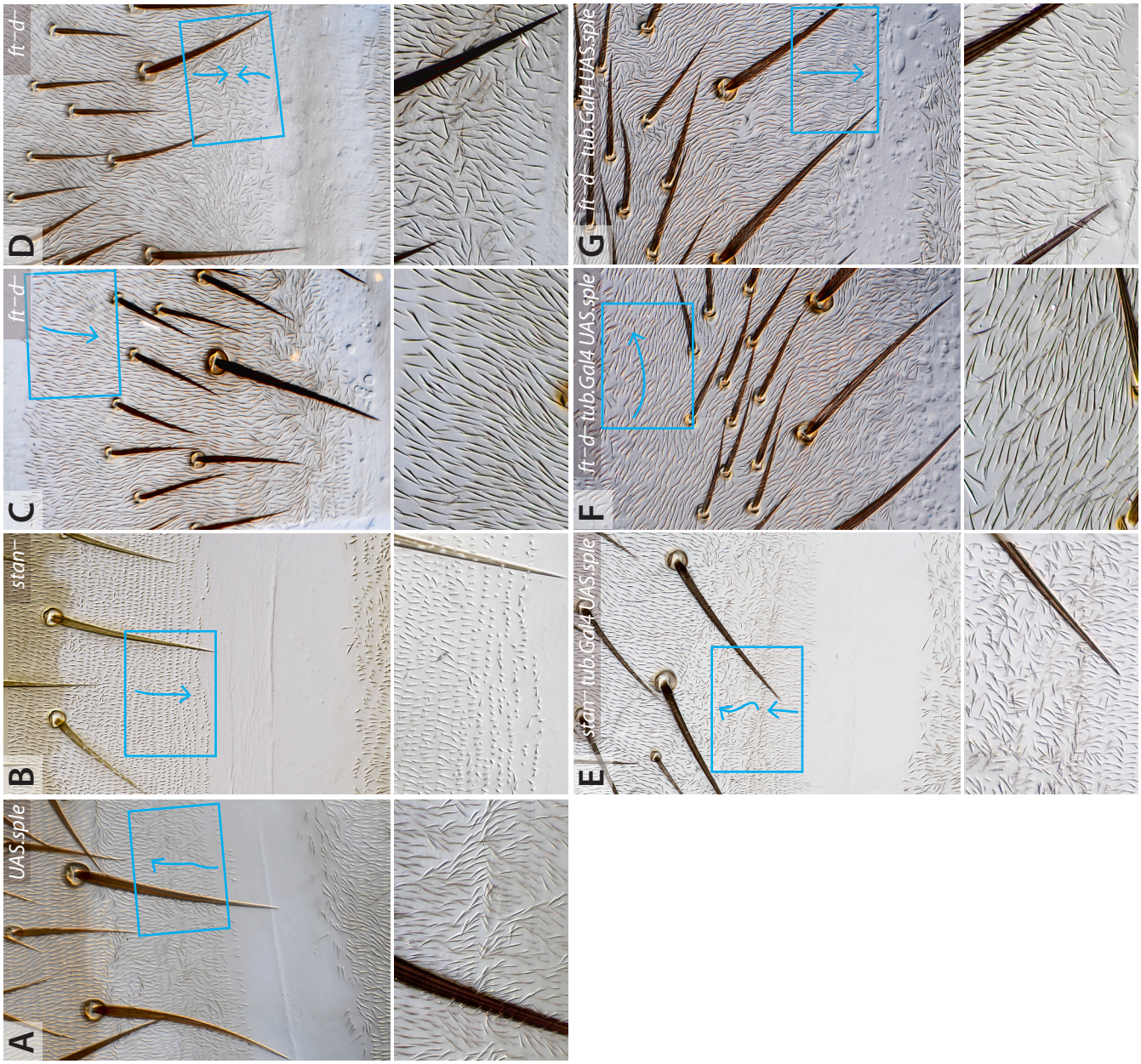

A
UAS.sple
B
stan
C
ft −d
D
ft −d
E
stan tub.Gal4 UAS.sple
F
ft −d tub.Gal4 UAS.sple
G
ft −d tub.Gal4 UAS.sple

figure 5

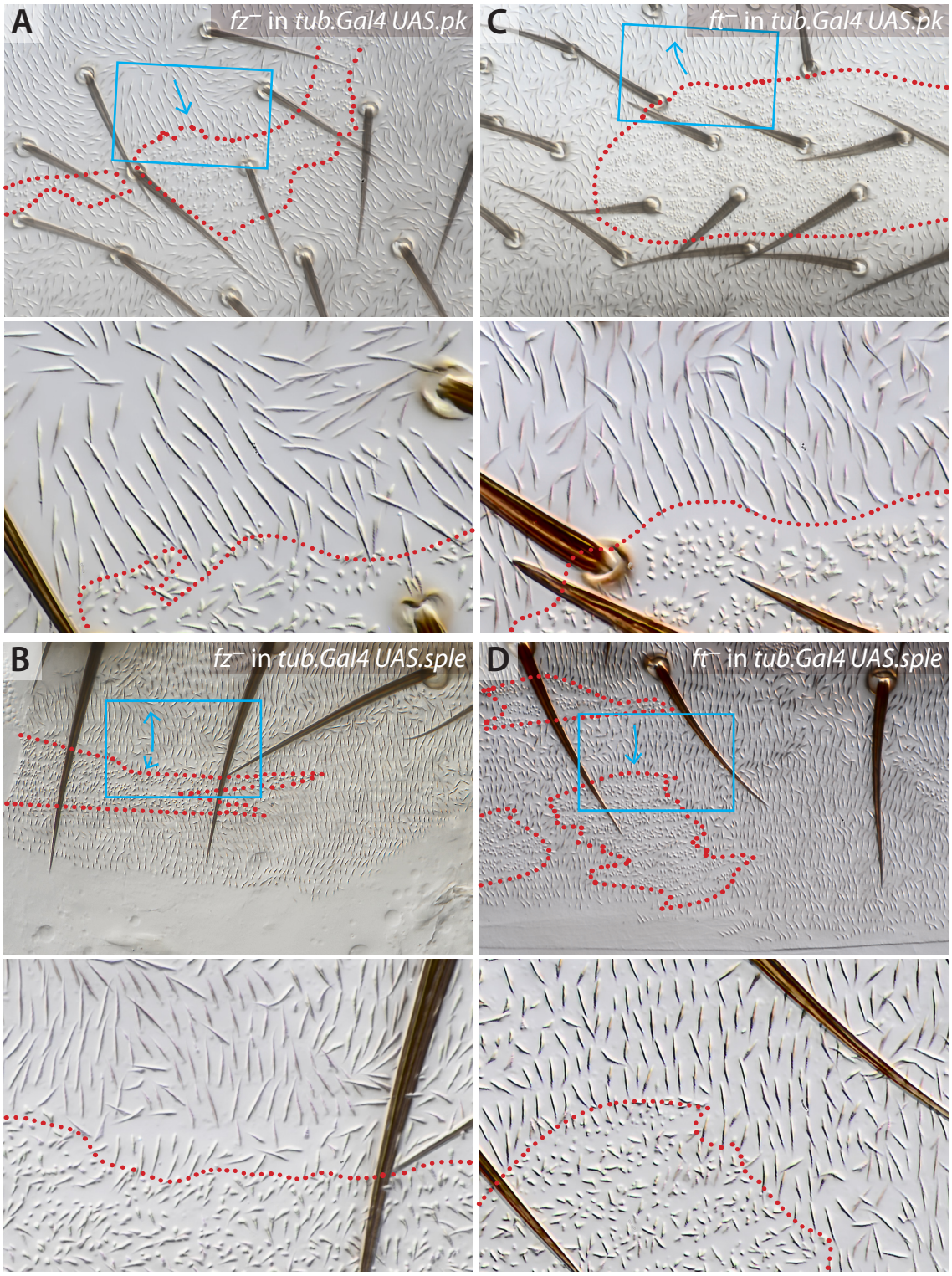

A
fz⁻ in tub.Gal4 UAS.pk
C
ft⁻ in tub.Gal4 UAS.pk
B
fz⁻ in tub.Gal4 UAS.sple
D
ft⁻ in tub.Gal4 UAS.sple

figure 6

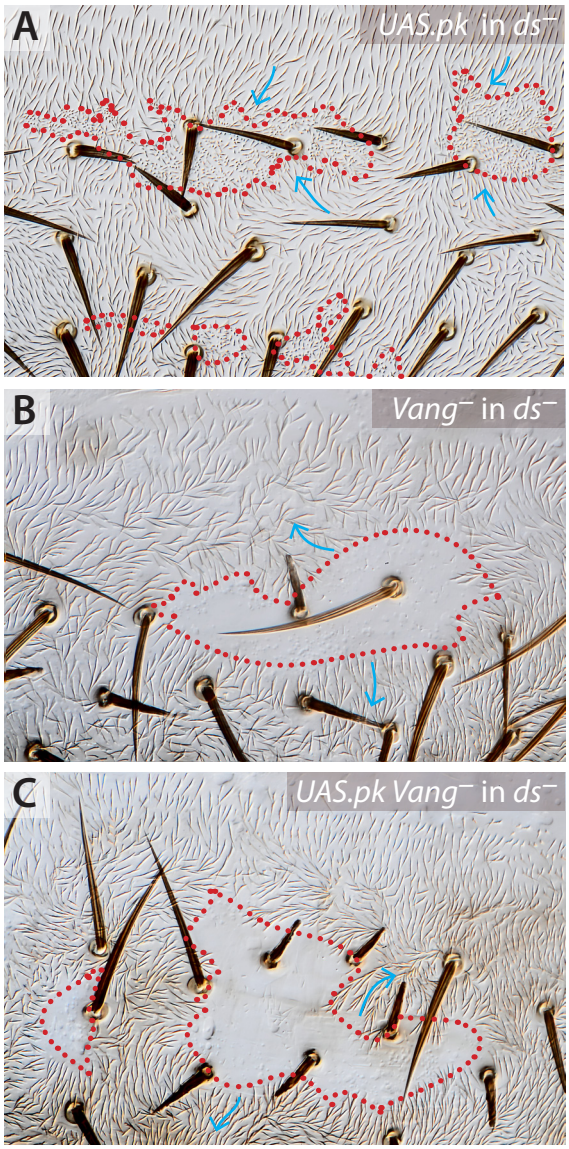


figure 7

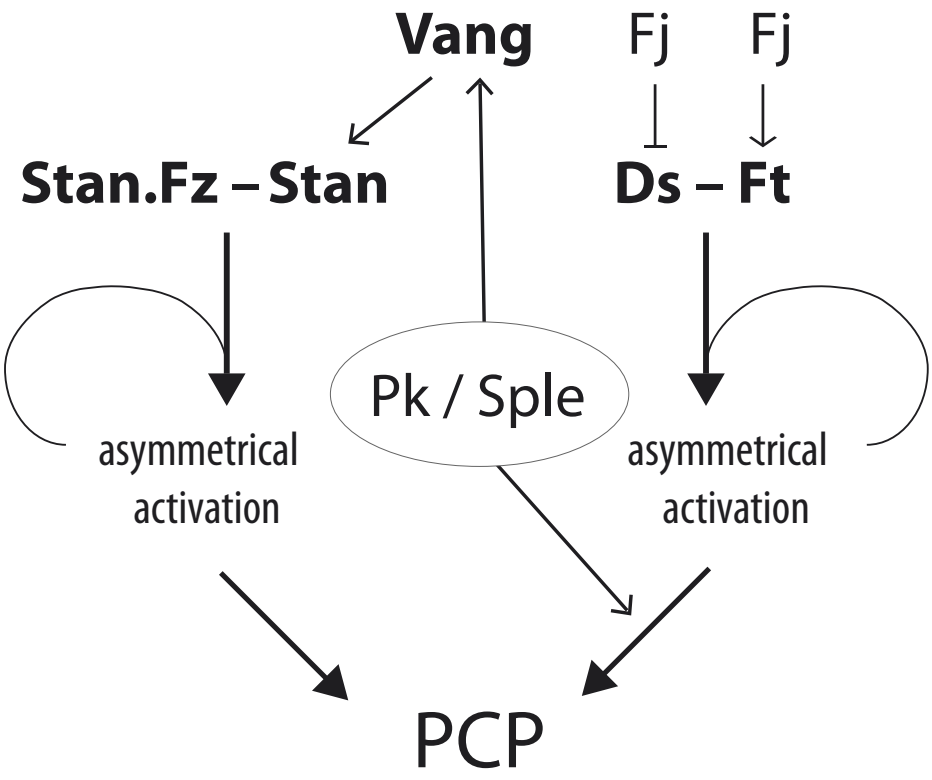

Vang
Fj
Fj
Stan.Fz – Stan
Ds – Ft
Pk / Sple
asymmetrical activation
asymmetrical activation
PCP

figure 8

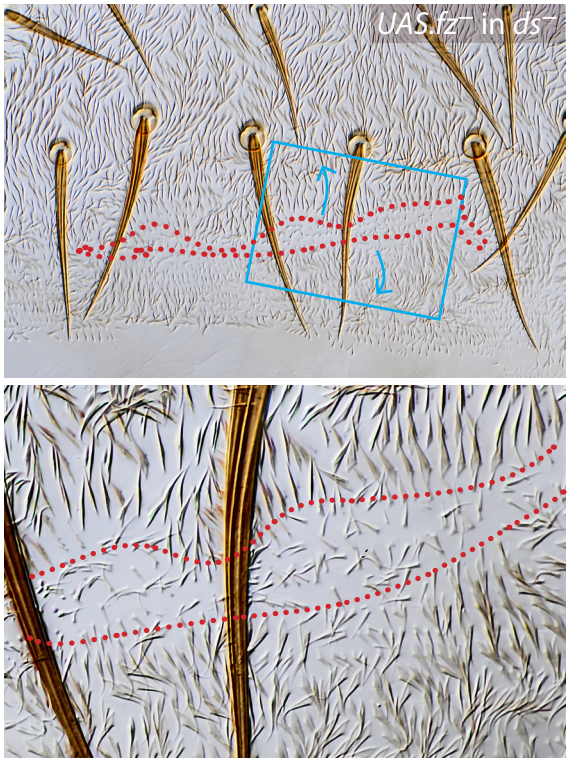


figure S1

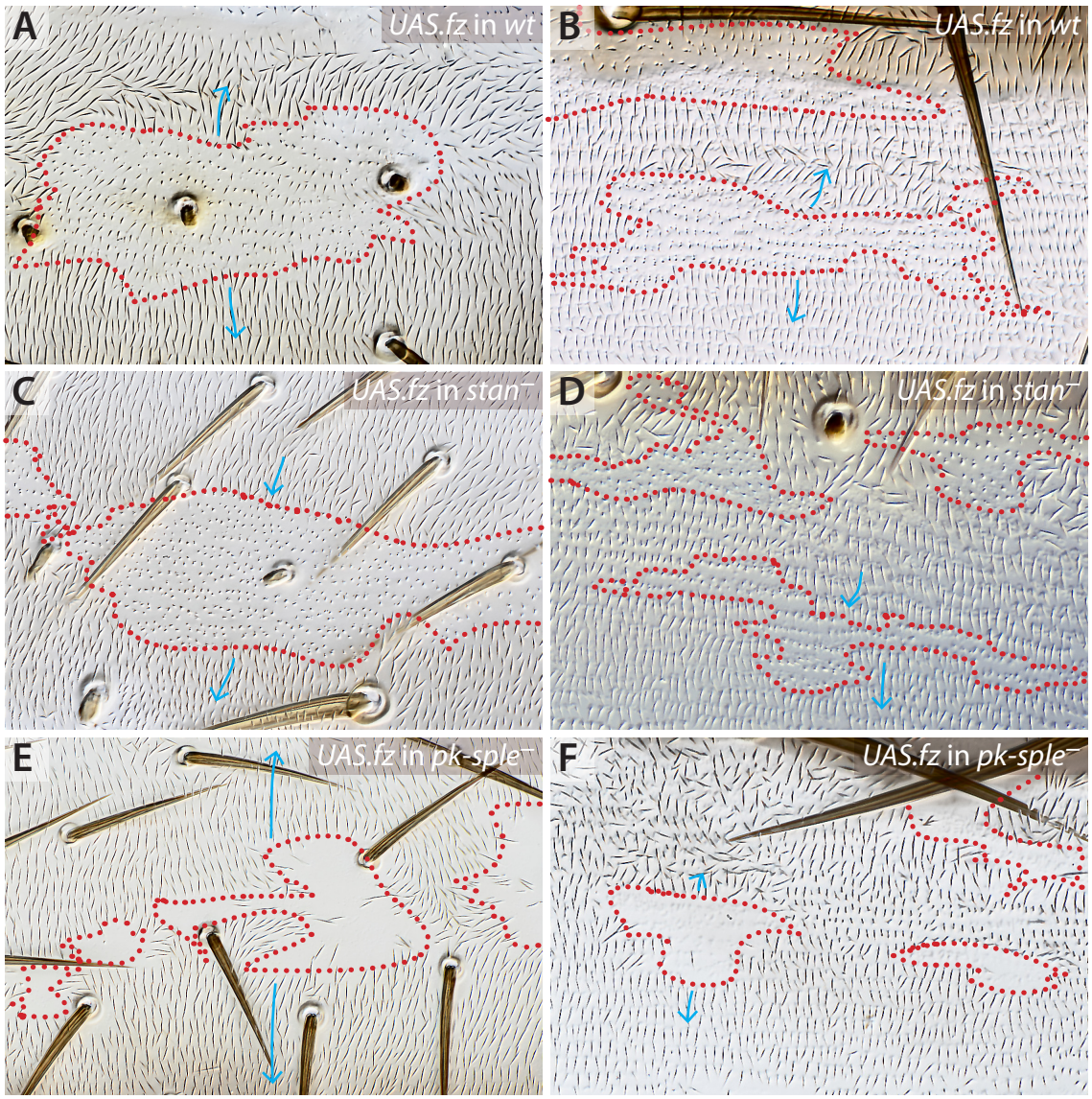
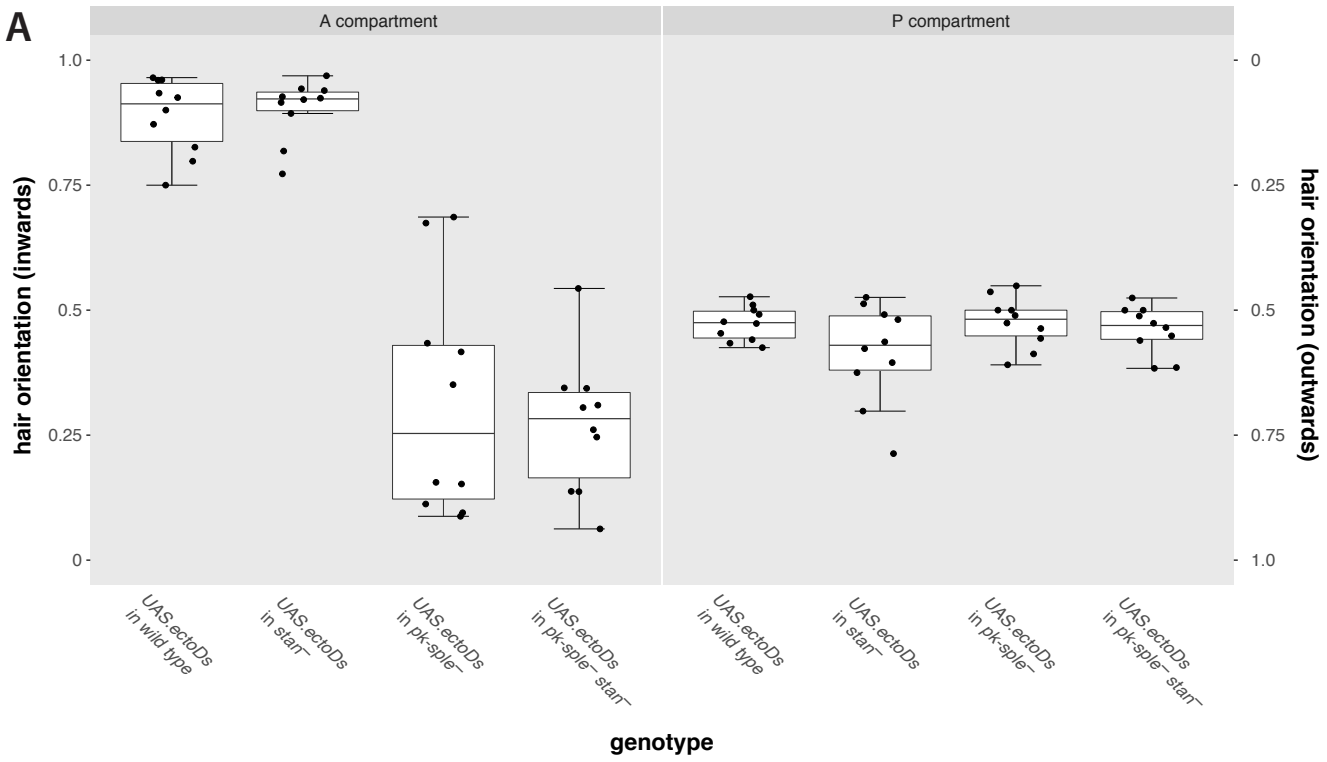
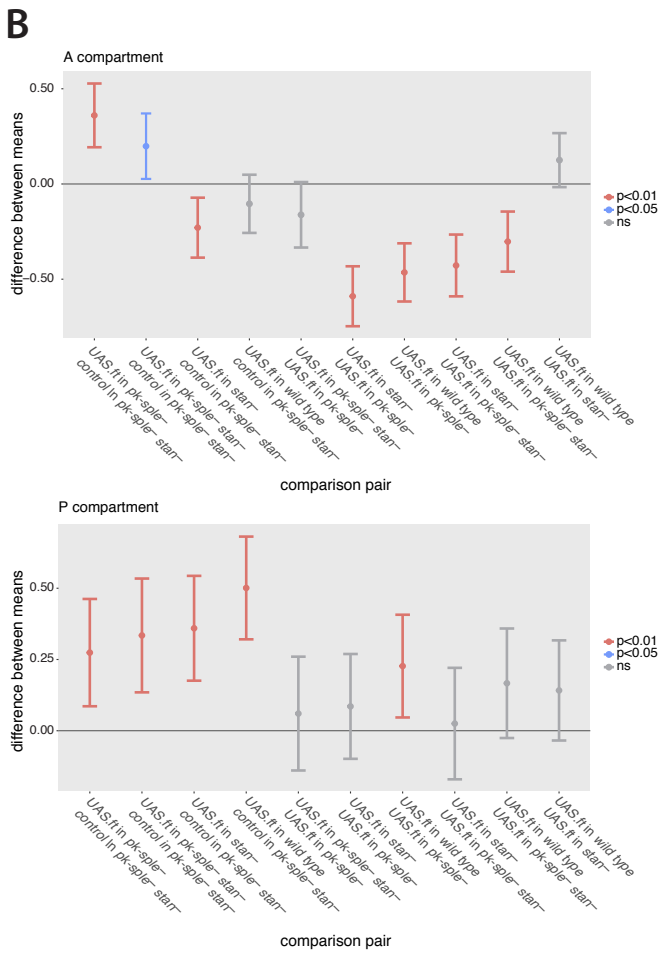

A
UAS.fz in wt
B
UAS.fz in wt
C
UAS.fz in stan⁻
D
UAS.fz in stan⁻
E
UAS.fz in pk-sple⁻
F
UAS.fz in pk-sple⁻

figure S2


A
A compartment
P compartment
hair orientation (inwards)
hair orientation (outwards)
1.0
0.75
0.5
0.25
0
UAS.ectoDs in wild type
UAS.ectoDs in stan¯
UAS.ectoDs in pk-sple¯
UAS.ectoDs in pk-sple¯ stan¯
genotype


B
A compartment
P compartment
difference between means
comparison pair
p<0.01
p<0.05
ns

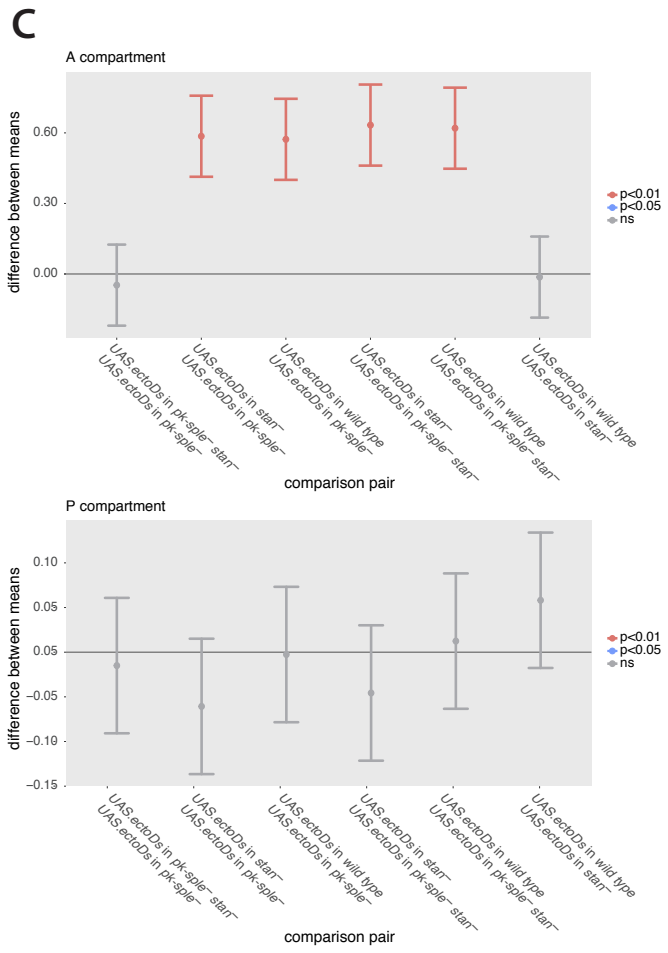

C
A compartment
P compartment
difference between means
comparison pair
p<0.01
p<0.05
ns

figure S3

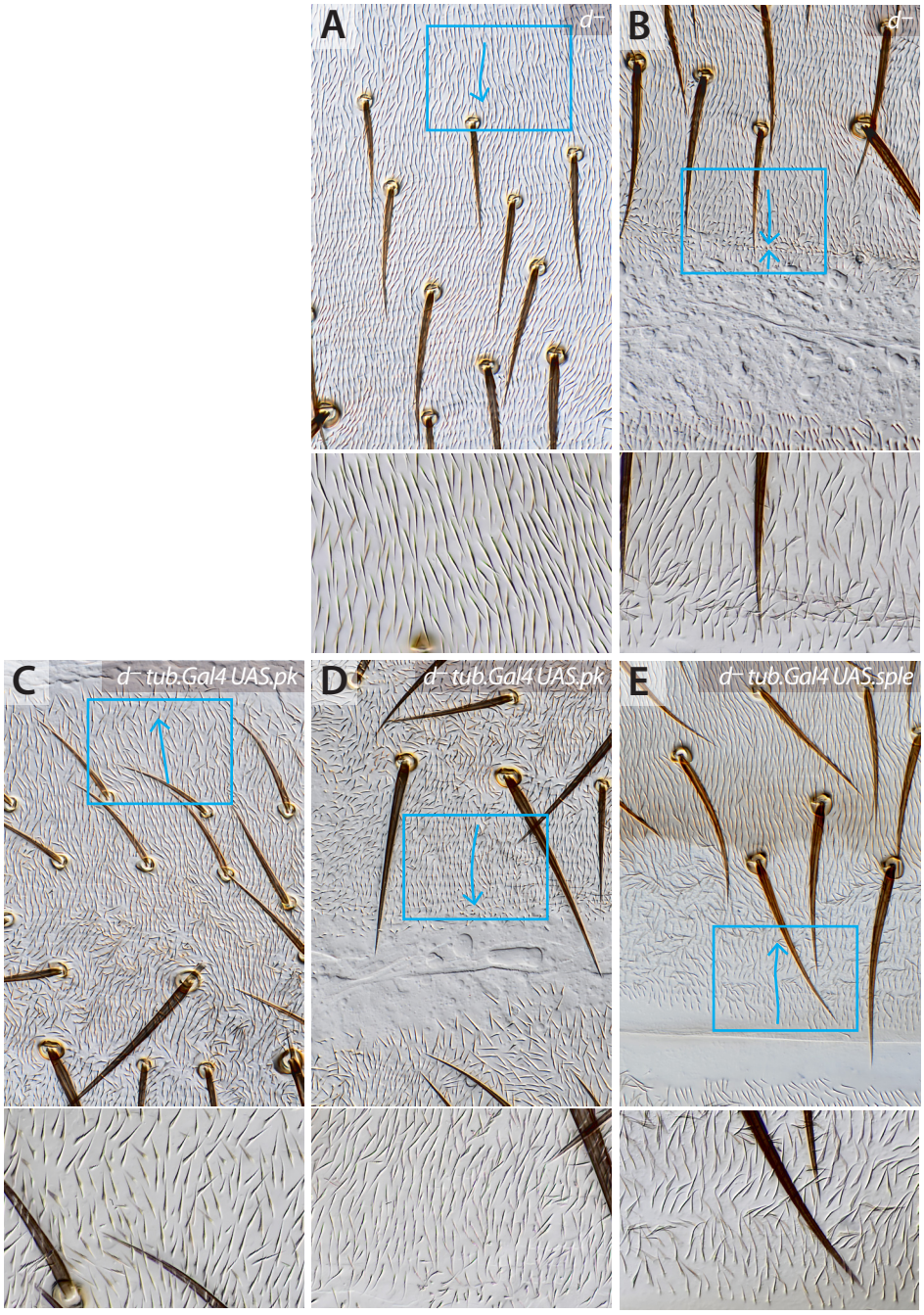


figure S4

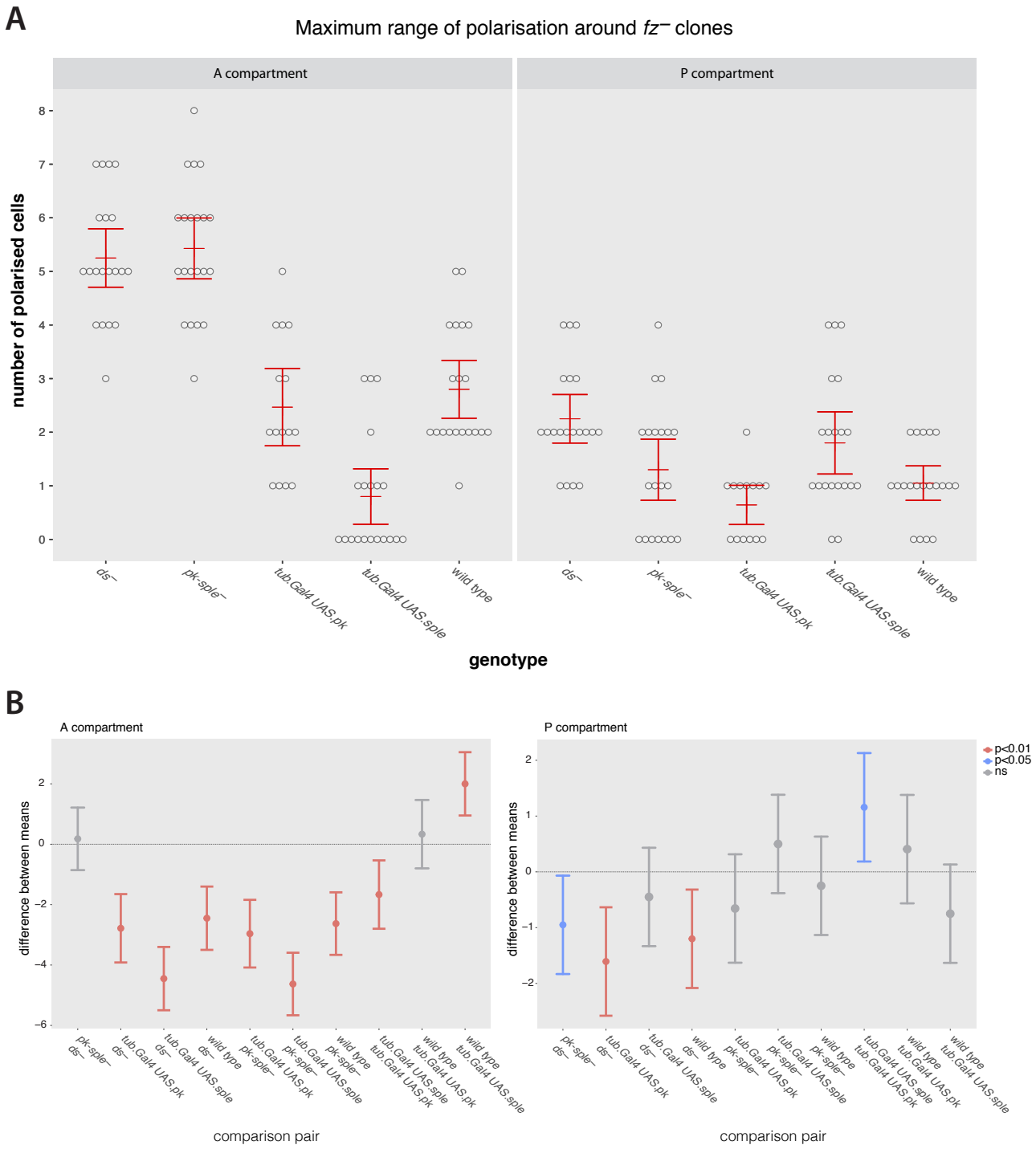

A
Maximum range of polarisation around fz⁻ clones
A compartment
P compartment
number of polarised cells
ds⁻
pk-sple⁻
tub.Gal4 UAS.pk
tub.Gal4 UAS.sple
wild type
genotype
B
A compartment
P compartment
difference between means
p<0.01
p<0.05
ns
comparison pair

figure S5