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 core 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 use genetic methods to investigate the function of the *prickle* (*pk*), a PCP gene.

The *pk* gene produces two homologous transcripts 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, syndromes due to *pk* mutations have been identified but it is not clear if they reveal genuine PCP functions of the *pk* genes (Tissir and Goffinet, 2013). In flies, in the absence of the *pk* gene, the polarities of bristles and hairs are altered and/or disordered over large areas of the cuticle. In the abdomen, when the *pk*

isoform is overexpressed everywhere, polarity of the anterior (A) compartment is almost entirely reversed while the posterior (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. These findings led to the hypothesis that the *pk* gene functions in the wildtype to turn around or "rectify" polarity in order to coordinate PCP over regions of the fly (Lawrence et al., 2004). These results are consistent with the hypothesis that Pk and Sple have similar functions in PCP, 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, of the two proteins, Pk is found to be the most effective agent 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).

One set of proteins constitute the "core system" of PCP. Because of the central importance of Starry night (Stan) to function of this system we renamed it the "Stan system" (Lawrence et al., 2004). Proteins classified as members of the Stan system include Stan, Fz, Vang Gogh (Vang), Dishevelled (Dsh), Diego (Dgo) and Pk/Sple (for a review see Goodrich and Strutt, 2011; Adler, 2012; Butler and Wallingford, 2017). Some of these proteins are asymmetrically distributed: in the wing, Pk is enriched on or near the proximal membrane of each cell (Tree et al., 2002b), while Frizzled (Fz) is localised distally (Strutt, 2001). 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). The current view is that Dsh, Pk and Fz interact with each other to amplify their asymmetric localisation within the cell and thereby consolidate its polarity. Groups of proteins associate in separate complexes on different sides of the wing cell and are associated with each other across the cell membrane, proximally or distally, as a response to an upstream signal (reviewed in Klein and Mlodzik, 2005).

Some argue 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;

79 Strutt and Strutt, 2007; Axelrod, 2009) in which asymmetrical distribution of the
80 protocadherins Dachsous (Ds) and Fat (Ft) provides an initial cue to orient the Stan
81 system which amplifies the signal to polarise effector functions. Recently it has been
82 posited that the *pk* gene intervenes between the polarising information specified by
83 the direction of the gradients of Ds/Ft activity and its interpretation by the Stan
84 system. These articles (Hogan et al., 2011; Ayukawa et al., 2014; Olofsson et al., 2014;
85 Ambegaonkar and Irvine, 2015) also support earlier conclusions that Pk and Sple act
86 discordantly on polarity output in different tissues and have improved the evidence
87 that changes in the levels of Pk and/or Sple can turn around the orientation of
88 polarised structures (Ayukawa et al., 2014). Moreover, they present arguments that
89 Sple is the main component of a molecular link between the Ds/Ft and Stan systems.

90 But, some earlier findings are inconsistent with the hypothesis that Pk/Sple acts as a
91 link:

92 1. The two systems can work independently of each other, at least in the
93 abdomen (Casal et al., 2006), meaning that neither the Ds/Ft system nor the
94 Stan system need each other to polarise cells. If so, there would be no need for
95 a general link between the two systems.
96 2. Genetic experiments argue that, functionally, Pk and Sple are not required for
97 polarity signalling from cell to cell, a central aspect of PCP. Adler et al. (2000)
98 found that a weak allele of *pk* did not block or inhibit but actually increased
99 those local changes in cell polarity that are induced by clones mutant for other
100 Stan system genes. Adler's finding was confirmed when Lawrence et al. (2004)
101 showed that complete loss of Pk and Sple increases polarisation by the Stan
102 system genes; they proposed that the key molecules in the Stan system do not
103 include Pk but are Stan, Fz and Vang. This conclusion was later supported by
104 Strutt and Strutt (2007) who presented further evidence that Dsh, Pk and Dgo
105 are not needed for the propagation of polarity from cell to cell.

106 Here we undertake genetic experiments aimed at clarifying the function of Pk/Sple in
107 the wildtype abdomen, particularly in relation to the two systems. We conclude that
108 Pk and Sple are not essential components of either system nor are they essential
109 components of a link between the two systems. We add to evidence that the Ds/Ft

system acts independently of the Stan system and, surprisingly, provide data that Pk and Sple can rectify the output of the Ds/Ft system, even in the absence of a functioning Stan system. Pk and Sple also, separately, affect the output of the Stan system; they 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". Such responses that are induced in cells nearby the clone are defined as "non-autonomous". 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 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?

It has been proposed that upstream polarity information specified by the Ds/Ft system 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 gradients of Ds and Fj activities might have opposite slopes in the anterior (A) and the posterior (P) compartments (Casal et al., 2002). But if that were true then, because the hairs in both compartments point the same way 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 can act independently of each other (Casal et al., 2006; Lawrence et al., 2007; and more recently 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

Previously we showed that clones affecting the Ds/Ft system could polarise cells non-autonomously and do so very well in the absence of a functioning Stan system. These studies were limited to the A compartments. Here we show that, in both the A and the P compartments, clones overexpressing *ft* polarise both wildtype cells (Genotype 2) and cells in which the Stan system is broken —we have 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 regions (Casal et al., 2002).

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

171 We can also look at the independence of the two systems by interfering with the Stan
172 system in clones in a background in which the Ds/Ft system is broken. Clones that
173 overexpress *fz*, in either the A or P compartments, normally turn the polarity of
174 receiving cells to point outwards from the clone in A (Casal et al., 2006) and also in P
175 (Genotype 6, Figure S2). They do the same in *ds*⁻ flies but with a longer range
176 (Genotype 7; Adler et al., 1998; Ma et al., 2003; Casal et al., 2006; Figure S1).

177 These and previous experiments have established that the two systems can act
178 independently in both compartments of the abdomen; we now ask are Pk and Sple
179 essential components of either the Ds/Ft or the Stan systems?

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

181 **(i) evidence from epistasis**

182 *ds*⁻ and *pk-sple*⁻ flies differ in phenotype in the dorsal abdomen: the most useful
183 difference is seen in the P compartment, where, in *ds*⁻ flies, hairs in the anterior region
184 of the P compartment are in whorls (probably due to the misdistribution of Dachs)
185 but in the posterior region of the P compartment the hairs point directly
186 anteriorward. By contrast, in *pk-sple*⁻ flies, the entire P compartment has normal
187 polarity (Lawrence et al., 2004). If Ds/Ft were to provide an upstream cue that is
188 interpreted by the Stan system via Pk/Sple one would expect the double mutant *ds*⁻ *pk-*
189 *sple*⁻ to have a phenotype more similar to the single *pk-sple*⁻ mutant than to the *ds*⁻
190 mutant phenotype. However we find that, in the abdomen, *ds*⁻ *pk-sple*⁻ flies (Genotype
191 8) are little different from *ds*⁻ flies (Figure 1). It follows that the *ds* mutation is epistatic
192 to a mutation that removes both *pk* and *sple* functions. Turning to interaction between
193 the Stan system and Pk/Sple. When *pk-sple*⁻ *stan*⁻ flies are compared to each single
194 mutant, they differ from both, having an additive phenotype (Genotype 9, Genotype
195 10, and Genotype 11, Figure 1), implying that the two genes act independently. Taken
196 together, these findings suggest that the *pk* gene acts entirely through the Ds/Ft
197 system. However other results argue that Pk and Sple can also act independently of
198 the Ds/Ft system (see below), and suggest instead that Pk and Sple act separately but
199 differently on each of the two systems.

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

202 It has been proposed that Pk/Sple act as a link between Ds/Ft and the Stan system,
203 rectifying the polarity output to ensure that the A and the P compartments have the
204 same polarity. Here we test this link by making clones that alter the Stan system in *pk-*
205 *sple*⁻ mutant flies or in flies with ubiquitous overexpression of *pk* or *sple*.

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

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

223 3. However, the *fz*⁻ clones do not behave exactly as they would in a wildtype
224 background: absence or excess of Pk and Sple change the amount of polarisation
225 caused by clones with altered amounts of Fz. In A compartments of the abdomen,
226 clones of cells that lack *fz* alter polarity of surrounding wildtype cells. The number of
227 rows of receiving cells affected, the range, varies with the amount of Pk and/or Sple
228 protein: in *pk-sple*⁻ flies (Genotype 13) the range of polarisation around the *fz*⁻ clones
229 or clones that contain 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 the Pk isoform ubiquitously does not alter the range of polarisation surrounding *fz*⁻ clones(Genotype 15), while when Sple levels are raised (Genotype 14), this range of polarisation is reduced (Figure S5). In the P compartments, we detected no effects on the range of repolarisation surrounding *fz*⁻ clones; 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) The Ds/Ft system functions well, both in cells that lack Pk and Sple and in cells that have *pk* or *sple* overexpressed

If Pk/Sple act as an essential link between Ds/Ft and the Stan system one may expect that clones with an altered Ds/Ft system not to have any effect on hair polarity in clones lacking both Pk and Sple. However the results were striking and did not support the link hypothesis.

1. In *pk-sple*⁻ flies. Clones of cells overexpressing *ft* repolarise receiving cells strongly, even if they lack Pk and Sple (Genotype 17). 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 18) 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 (Genotype 2 and Genotype 4) and *stan*⁻ receiving cells (Genotype 3 and Genotype 5) to the same extent. They also affect *pk-sple*⁻ *stan*⁺ (Genotype 17 and Genotype 18) and *pk-sple*⁻ *stan*⁻ (Genotype 19 and Genotype 20) receiving cells with the same strength (Figure 3 and

261 Figure S3). These results show that neither Pk, Sple nor Stan are required for polarity
262 signalling by the Ds/Ft system, although Pk and Sple can change the sign of the
263 response; suggesting that the formation of Ds-Ft bridges works well even the absence
264 of Pk/Sple and/or the Stan system. Any effect seen has to be downstream of these
265 bridges. They also show that Pk and Sple do not act as an essential link between the
266 Ds/Ft system and the Stan system, because if they were such a link, removal of Pk and
267 Sple would block effects on polarity caused by overexpressing *ft*.

268 Results supporting these conclusions came from clones that altered the Ds/Ft system
269 in flies whose polarity was altered by excess Pk or Sple:

270 2. In flies in which *pk* or *sple* are overexpressed. Clones that lack *ft* made in flies in
271 which *pk* is generally overexpressed (Genotype 21) behave as follows: where the
272 polarity of much of the surrounding background is reversed from normal, with the
273 hairs pointing forwards (ie in the A compartment), ft^- clones act with the opposite
274 sign to that in the wildtype (Genotype 22) and hairs around the clone tend to point
275 outwards (Figure 6). In the P compartment, where overexpression of *pk* produces no
276 change to polarity, the ft^- clones behave as they do in the wildtype, that is the hairs
277 point outwards from the clone (Figure 6).

278 Clones that lack *ft* made in flies in which *sple* is generally overexpressed
279 (Genotype 23) behave as follows: in the A compartment, which has normal polarity,
280 these clones affect these receiving cells as they affect wildtype cells; hairs around the
281 clone point inwards (Figure 6). In the P compartment, where the polarity of the
282 surrounding background is reversed from normal with the hairs pointing forwards,
283 the ft^- clones now polarise receiving cells with the opposite sign to that in the
284 wildtype, that is the hairs point inwards into the clone (Figure 6).

285 In the A compartment of the abdomen, clones that lack *ds* have effects of the
286 opposite sign to ft^- clones in both classes (see previous points 1 and 2) of experiments
287 above —as would be expected. However ds^- clones have little or no effect in the P
288 compartment in all genotypes tested (data not shown, Genotype 24, Genotype 25 and
289 Genotype 26).

290 3. These results show that the Ds/Ft system can function independently of Pk and Sple
291 but that Pk and Sple can modulate the sign of its output. This dramatic effect could, in
292 principle, be due to Pk and/or Sple affecting the patterns of expression of *ds*, and/or *fj*
293 and thereby changing the orientation of the Ds/Ft system gradients. To test we studied
294 the expression of enhancer traps for *ds* and *fj* loci in *pk-sple*$^{-}$ flies and saw no
295 departure from the wildtype patterns (Genotype 28, Genotype 29, Genotype 30 and
296 Genotype 31; Figure S6). It follows that Pk and Sple determine whether polarised
297 structures in the cell, the hairs and bristles, point up or down the gradients of Ds and
298 Fj.

299 **(iv) Pk and Sple alter polarity even when the Stan system is broken**

300 Were Pk/Sple to act only upstream of the Stan system one would not expect
301 overexpression of *pk* or *sple* to have an effect on the polarity of *stan*$^{-}$flies. However
302 uniform overexpression of *pk* causes large changes of polarity in the abdomen of
303 wildtype flies and flies with a broken Stan system (Genotype 32) in the A
304 compartment, without affecting the P compartment (Figure 4). While generalised
305 overexpression of *sple* also affects the polarity of both wildtype and *stan*$^{-}$ flies —
306 altering the polarity of the P compartment of the abdomen, without much affecting
307 the A compartment (Genotype 33, Figure 5).

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

309 If Pk and Sple acted exclusively on the Ds/Ft system, one would not expect Pk and
310 Sple proteins to affect PCP if the Ds/Ft system were broken.

311 1. General overexpression of *pk* or *sple* in a broken Ds/Ft system. But we find that
312 ubiquitous overexpression of *pk* alters polarity of the A compartment (and part of the
313 P compartment) of *ds*$^{-}$ *pk-sple*$^{-}$ (Genotype 34, Figure 1), *d*$^{-}$ (Genotype 35, Figure S4)
314 and *ft*$^{-}$ *d*$^{-}$ flies (Genotype 36, Figure 4). Similarly, general overexpression of *sple* affects
315 the polarity of the P compartment of the abdomen of *ds*$^{-}$ *pk-sple*$^{-}$ (Genotype 37, Figure
316 1), *d*$^{-}$ (Genotype 38, Figure S4) and *ft*$^{-}$ *d*$^{-}$ flies (Genotype 39, Figure 5).

317 In *d*$^{-}$ flies, the A and P compartments are largely normal but a section of the P
318 compartment is reversed, as in *ds*$^{-}$ (or *ft*$^{-}$) flies. When ubiquitous Pk is added to *d*$^{-}$ or
319 *ft*$^{-}$ *d*$^{-}$ flies, the anterior part of the A compartment is altered to point forwards and the

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

333 2. Clones that overexpress *pk* or *sple* in a broken Ds/Ft system. We find that clones of
334 cells overexpressing *sple* (Genotype 40; Lawrence et al., 2004) or *pk* (Genotype 41; data
335 not shown), have small non-autonomous effects in the wildtype and, more so, in ds^-
336 flies (Genotype 42 and Genotype 43) where they polarise receiving cells to point
337 strongly inwards (Figure 7). Perhaps these clones act via the Stan system? It is
338 pertinent that both wing and abdominal cells that overexpress the *pk* gene accumulate
339 Vang uniformly on the cell membrane (Bastock et al., 2003; Olofsson et al., 2014). If
340 this were to happen in our experiments, then the clone could behave as if it were
341 overexpressing *Vang* and should polarise surrounding cells inwards, as observed; this
342 effect should be stronger in ds^- than in ds^+ cells, also as observed. To test this
343 hypothesis further we made $Vang^-$ clones that overexpressed *pk* (Genotype 44), as well
344 as control $Vang^-$ clones (Genotype 45), in ds^- flies. Both these types of clones behaved
345 like $Vang^-$ clones in wildtype flies (Genotype 46), and could not be distinguished
346 from each other, ie they polarise ds^- receiving cells strongly outwards (Figure 7),
347 confirming the hypothesis that cells overexpressing *pk* polarise cells because they
348 accumulate Vang, a Stan system protein. Thus, overexpressing Pk interferes with the
349 Stan system. These results show that Pk and Sple do have functions that are
350 independent of the Ds/Ft system.

351 **(vi) Can Pk function independently of both systems of PCP?**

352 We have presented evidence that the Pk and Sple modulate, in different ways, both the
353 Ds/Ft and the Stan systems. We wondered if Pk and Sple proteins could have outputs
354 independently of these systems. To test, we have overexpressed *pk* in a genetic
355 background in which both systems are broken, viz: ft^{-} d^{-} $stan^{-}$. We found, using blind
356 screening, that could not distinguish the phenotypes of these flies with and without
357 the overexpression of *pk* (Genotype 47 and Genotype 48, Figure 4), arguing that the
358 polarity effects of an excess of Pk are due to Pk acting through the two systems and by
359 no other route.

360 Discussion

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

363 The Ds/Ft system and the Stan system act independently and are not 364 linked via Sple and/or Pk

365 *ft*-overexpressing clones reorient wildtype receiving cells, outwards in the A
366 compartment (Casal et al., 2006) and inwards in the P (this paper). These clones have
367 the same effects on cells in which the Stan system of PCP is broken (for example in
368 $stan^{-}$ flies; Figure 2, Figure 3 and Figure S3). It follows that this reorientation of
369 polarity cannot be due to any intracellular interaction between Stan and any
370 component of the Ds/Ft system within the sending cells. However it could be argued
371 that extra Ft in the sending cell, attracting Ds in the receiving cell, would, non-
372 autonomously, influence some residual capability of the Stan system in the receiving
373 $stan^{3}/stan^{E59}$ cells to respond and propagate polarity to neighbouring cells. Yet, even
374 clones that overexpress both *fz* and *stan* (ie cells that have a fully functional Stan
375 system) fail to repolarise $stan^{3}/stan^{E59}$ cells (Casal et al., 2006). Thus the propagation of
376 a polarity change observed around cells that overexpress *ft* cannot be due to any non-
377 autonomous effect on the Stan system. These results show that, at least for both
378 compartments of the abdomen, the Ds/Ft system acts independently of the Stan
379 system (Casal et al., 2006; Lawrence et al., 2007; Lawrence, 2011).

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

389 Another argument is relevant here: clones affecting the Ds/Ft system have
390 outputs of different sign in the A and P compartments (Casal et al., 2002). If this
391 divergent polarisation were to act through and depend on the Stan system via a
392 molecular link of Pk and/or Sple, then we would expect the polarising output from
393 Stan system clones (eg from *fz*$^{-}$ clones) to be also of different sign in the two
394 compartments and to be dependent on that link (cf Figure 7 in Ayukawa et al., 2014).
395 However, this is not the case (Figure S2E and Figure S2F).

396 Pk/Sple act independently of the Stan system

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

406 The Stan system proteins Stan, Fz, Vang and Pk are all preferentially localised to
407 specific regions of the cell membrane and this is considered to be important for their
408 functions in PCP. Nevertheless, *pk-sple*$^{-}$ receiving cells, in which Stan, Fz and Vang no
409 longer appear to be localised (reviewed in Strutt, 2009), can respond at least as well to

410 such sending cells as wildtype ones (Adler et al., 2000; Lawrence et al., 2004). This
411 dilemma could be resolved if the observed asymmetry were not so directly related to
412 function as has been assumed and were more a consequence than a cause of polarity
413 (Lawrence et al., 2004).

414 Pk and Sple modulate the Ds/Ft system, determining the polarity of its 415 output

416 Sending cells that overexpress *ds* or *ft*, or lack *ds* or *ft*, change the polarity of receiving
417 cells, even in the absence of Pk and Sple— it follows that these proteins cannot be
418 necessary for the Ds/Ft system to function and propagate polarity from cell to cell.
419 However the sign of this change depends on whether the receiving cells contain, lack
420 or overexpress products of the *pk* gene. These results show that the Pk and Sple can
421 alter the sign of polarisation that is produced by the Ds/Ft system. But, how do Pk and
422 Sple have their various effects on polarity? It appears that the sign of polarisation
423 depends on the relative amounts of Pk and Sple in a particular region of the fly (Gubb
424 et al., 1999; Ayukawa et al., 2014). One model is that the Ds/Ft proteins might act
425 through Pk and Sple to bias the orientation of microtubules and thus PCP —because
426 oriented microtubules could transport Stan system components preferentially to one
427 side of the cell (Shimada et al., 2006; Harumoto et al., 2010; Matis et al., 2014;
428 Olofsson et al., 2014). But, correlation between microtubule orientation, Pk/Sple
429 function, and PCP is inconsistent or lacking, clearly so in the distal half of the wing
430 and the P compartment of the adult abdomen (Harumoto et al., 2010; Sharp and
431 Axelrod, 2016), leading to doubts about the validity of the hypothesis (Ambegaonkar
432 and Irvine, 2015). Also, this model is now contradicted by our results, which show
433 that abnormal amounts of Ds, Ft, Sple or Pk can all affect PCP even when the Stan
434 system is broken.

435 A diagram suggesting how the *pk* gene might fit into the organisation of PCP is
436 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 ectopic 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 reversed polarity at the back of the P compartment in *ft*$^-$ *d*$^-$ flies (Figure 4, and Sharp and Axelrod, 2016). 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 direct interaction between Sple and Ds but not between Pk and Ds (Ambegaonkar and Irvine, 2015). However Ayukawa et al. (2014) find that both Sple and Pk bind to each other and to D (but not to Ds), suggesting the difference may have other causes.

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)

467 robust it would make it more difficult (or easier) for clones affecting the Stan system
468 to alter PCP. Another explanation could relate to some direct effect of Pk (and Sple)
469 on Vang (Bastock et al., 2003) which fits our observations with clones overexpressing
470 Pk (Figure 7). The function of Vang in the Stan system is somewhat unclear; like Pk,
471 Vang is present in larger than stoichiometric amounts in relation to the two molecules
472 that form the intercellular bridge, Stan and Fz (Strutt et al., 2016), yet affects bridge
473 function (Struhl et al., 2012). The abdominal phenotypes of *Vang*$^{-}$ and *pk-sple*$^{-}$ are
474 somewhat similar, both having areas of reversed polarity (Lawrence et al., 2004),
475 suggesting a commonality of function. Indeed there is a recent model proposing that
476 Pk acts on the stability of Fz intracellularly (via Dsh) and in the adjacent cell (via
477 Vang); the former effect may involve endocytosis (Warrington et al., 2017). Our
478 experiments argue that the function of Pk is not limited to the Stan system but
479 includes, independently, the Ds/Ft system. In any case we have no explanation for the
480 lack of apparent effects of Pk and Sple on the range of *fz*$^{-}$ clones in the P
481 compartment.

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

491 Conclusion

492 We have found for the abdomen that Pk and Sple are not essential components of
493 either the Ds/Ft or the Stan systems. We have shown that they do not function as a
494 link between the two systems. Instead, Pk and Sple appear to modulate the polarity
495 outputs of both the Ds/Ft system and the Stan system with the most conspicuous
496 effects on the former. Both these systems differ in their components but are similar in
497 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. Our genetic experiments on the abdomen point to conclusions that differ from the prevailing view that the *pk* gene mediates between the Ds/Ft and the Stan systems. We do not know if our conclusions apply to other organs in the fly, but if we adopt the hypothesis that they do, they suggest that current views of the wildtype functions of the *pk* gene should be reconsidered.

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*$^{alphaTub84B.P}$. *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} and *ds*^{2D60b}. *fj*^{p1}. *ft*^{8} and *ft*$^{G\text{-}rv}$. *fz*^{15}. *pk*$^{pk\text{-}sple\text{-}13}$. *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} $pk^{pk\text{-}sple\text{-}13}$; UAS.sple/ TM2*

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

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

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

Genotype 12: ***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 13: ***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 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: ***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 18: ***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 19: ***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 20: ***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 21: ***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 22: ***ft⁻* clones in wild type flies:** *y w hs.FLP;* ft^{15} *stc FRT39/ FRT39*

Genotype 23: ***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 24: ***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 25: ***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 26: ***ds⁻* clones in wild type flies:** *y w hs.FLP/ +;* ds^{UA071} ck^{UAh21} *FRT40A/ Dp(1;2)*sc^{19} w^{+30c} *FRT40A*

Genotype 27: **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 28: ***ds.lacZ* flies:** *y hs.FLP/ +;* ds^{2D60b} *FRT42D* $pk^{pk\text{-}sple\text{-}13}$*/ +*

Genotype 29: ***fj.lacZ* flies:** *y hs.FLP/ +; FRT42D* $pk^{pk\text{-}sple\text{-}13}$ fj^{P1}*/ +*

Genotype 30: ***ds.lacZ pk-sple⁻* flies:** *y hs.FLP/ +;* ds^{2D60b} *FRT42D* $pk^{pk\text{-}sple\text{-}13}$*/ FRT42D* $pk^{pk\text{-}sple\text{-}13}$

577 Genotype 31: ***pk-sple⁻ fj.lacZ* flies:** *y hs.FLP/ +; FRT42D pk$^{pk\text{-}sple\text{-}13}$ fj^{P1}/ FRT42D*
578 *FRT42D pk$^{pk\text{-}sple\text{-}13}$*

579 Genotype 32: ***stan⁻ tub.Gal4 UAS.pk* flies:** *y w hs.FLP tub.Gal4 UAS.nls-GFP/ y w*
580 *hs.FLP; FRT42D pwn stan^{E59} sha/ FRT42D stan^{3}; UAS.pk/ TM2*

581 Genotype 33: ***stan⁻ tub.Gal4 UAS.sple* flies:** *y w hs.FLP tub.Gal4 UAS.nls-GFP/ y w*
582 *hs.FLP; FRT42D pwn stan^{E59} sha/ FRT42D stan^{3}; UAS.sple/ TM2*

583 Genotype 34: ***ds⁻ pk⁻sple⁻ tub.Gal4 UAS.pk* flies:** *y w hs.FLP tub.Gal4 UAS.nls-GFP/*
584 *y w hs.FLP; ds^{UA071} pk$^{pk\text{-}sple\text{-}13}$; UAS.pk/ TM2*

585 Genotype 35: ***d⁻ tub.Gal4 UAS.pk* flies:** *y w hs.FLP tub.Gal4 UAS.nls-GFP/ y w*
586 *hs.FLP; d^{GC13} pr cn/ ft$^{G\text{-}rv}$ d^{GC13} FRT40; UAS.pk/ +*

587 Genotype 36: ***ft⁻ d⁻ tub.Gal4 UAS.pk* flies:** *y w hs.FLP tub.Gal4 UAS.nls-GFP/ y w*
588 *hs.FLP; ft^{8} d^{GC13} FRT40A/ ft$^{G\text{-}rv}$ d^{GC13} FRT40A; UAS.sple/ +*

589 Genotype 37: ***ds⁻ pk⁻sple⁻ tub.Gal4 UAS.sple* flies:** *y w hs.FLP tub.Gal4 UAS.nls-*
590 *GFP/ y w hs.FLP; ds^{UA071} pk$^{pk\text{-}sple\text{-}13}$; UAS.sple/ TM2*

591 Genotype 38: ***d⁻ tub.Gal4 UAS.sple* flies:** *y w hs.FLP tub.Gal4 UAS.nls-GFP/ w; ft$^{G\text{-}rv}$*
592 *d^{GC13}/ d^{GC13} pr cn; UAS.sple/ +*

593 Genotype 39: ***ft⁻ d⁻ tub.Gal4 UAS.sple* flies:** *y w hs.FLP tub.Gal4 UAS.nls-GFP/ y w*
594 *hs.FLP; ft^{8} d^{GC13} FRT40A/ ft$^{G\text{-}rv}$ d^{GC13} FRT40A; UAS.sple/ +*

595 Genotype 40: ***UAS.sple* clones in wild type flies:** *y w hs.FLP tub.Gal4 UAS.nls-GFP/*
596 *y w hs.FLP;FRT42D pwn/ FRT42D tub.Gal80; UAS.sple/ +*

597 Genotype 41: ***UAS.pk* clones in wild type flies:** *y w hs.FLP tub.Gal4 UAS.nls-GFP/ y*
598 *w hs.FLP;FRT42D pwn/ FRT42D tub.Gal80; UAS.pk/ +*

599 Genotype 42: ***UAS.sple* clones in *ds⁻* flies:** *y w hs.FLP tub.Gal4 UAS.nls-GFP/ y w*
600 *hs.FLP; ds^{UA071} ck^{UAh21} FRT40A/ ds^{UA071} tub.Gal80 FRT40A; UAS.sple/*
601 *MRS*

602 Genotype 43: ***UAS.pk* clones in *ds*⁻ flies:** *y w hs.FLP tub.Gal4 UAS.nls-GFP/ y w*
603 *hs.FLP; ds^{UA071} ck^{UAh21} FRT40A/ ds^{UA071} tub.Gal80 FRT40A; UAS.pk/*
604 *MRS*

605 Genotype 44: ***Vang*⁻ *UAS.pk* clones in *ds*⁻ flies:** *y w hs.FLP tub.Gal4 UAS.nls-GFP/ y*
606 *w hs.FLP; ds^{UA071} FRT42D tub.Gal80/ ds^{UA071} hs.CD2, y^{+} FRT42D pwn*
607 *$Vang^{stbm-6}$ sha; UAS.pk/ +*

608 Genotype 45: ***Vang*⁻ clones in *ds*⁻ flies:** *y w hs.FLP; ds^{UA071} FRT42D tub.Gal80/*
609 *ds^{UA071} hs.CD2, y^{+} FRT42D pwn $Vang^{stbm-6}$ sha; UAS.pk/ +*

610 Genotype 46: ***Vang*⁻ clones in wild type flies:** *y/ y hs.FLP; FRT42D pwn $Vang^{stbm-6}$*
611 *FRT42D hs.CD2, y^{+}*

612 Genotype 47: ***ft*⁻ *d*⁻ *stan*⁻ *tub.Gal4 UAS.pk* flies:** *y w hs.FLP tub.Gal4 UAS.nls-GFP/ y*
613 *w hs.FLP ; ft^{Gv-r} d^{GC13} $stan^{3}$/ ft^{8} d^{GC13} $stan^{E59}$; TM2/ UAS.pk*

614 Genotype 48: ***ft*⁻ *d*⁻ *stan*⁻ flies:** *y w hs.FLP ; ft^{Gv-r} d^{GC13} $stan^{3}$/ ft^{8} d^{GC13} $stan^{E59}$; TM2/*
615 *UAS.pk*

616 ## Clone induction and microscopy

617 Clones were induced by heat shocking third instar larvae for 1 hr at 34ºC. Adult
618 abdominal cuticles were studied as before (e.g., Lawrence et al., 2004; Casal et al.,
619 2006).

620 ## Quantitation

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

626 For range measurments, for each clone (n=20) the maximum extent in cell rows
627 of the induced polarity changes was measured. The observer was blinded as to
628 genotypes; he chose clones located in the middle of the A compartment and the

629 middle or rear of the hairy region of the P compartment; small clones were avoided.
630 Statistical analysis and graphics were performed in R using standard packages (R Core
631 Team, 2016) and the *reshape* and *ggplot* packages (Wickham, 2007; Wickham, 2009).

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FIGURE LEGENDS

Figure 1. A guide to all the experiments.

A summary of the results 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*. Grey areas show hairs pointing generally posteriorly; pink areas indicate mainly reversed polarity as is also indicated by the arrows.

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, see Figure S3 for statistical analysis, 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 27).

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*$^-$), or both are broken (*ft*$^-$ *d*$^-$ *stan*$^-$). Background phenotypes for the A compartments (**A, B, C** and **E**) and for the P compartments (**D**, **F**). In the A compartments, generalised overexpression of *pk* changes the polarity of the anterior region of wildtype, *stan*$^-$ (Genotype 32) 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 (**I**).

801 Ubiquitous Pk appears to have no effect on *ft*⁻ *d*⁻ *stan*⁻ in either A or P compartments
802 (**J** and **K**). Compare Figure S4 for expression of *pk* in *d*⁻ flies.

803 Figure 5. Effects of overexpressing *sple* on polarity of cells in which either the Stan
804 system (*stan*⁻) or the Ds/Ft system is broken (*ft*⁻ *d*⁻).
805 Overexpression of *sple* in *stan*⁻ and the wildtype reverses all or most of the P
806 compartment to point forwards (compare **A, B** and **E**) but overexpression of *sple* in a
807 *ft*⁻ *d*⁻ background produces a P compartment of normal polarity (**G**) and even the rear
808 of the P region, which points forward in *ft*⁻ *d*⁻ (**D**) is now "rescued" to normal polarity.
809 Overexpression of *sple* in *ft*⁻ *d*⁻ flies also alters the polarity at the front of the A
810 compartment (**C** and **F**) turning the hairs laterally, while overexpressing *pk* turns the
811 hairs to point anteriorly (Figure 4). Compare Figure S4 for expression of *sple* in *d*⁻ flies

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

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

824 Figure 8. Pk and Sple functions in the context of PCP.
825 PCP depends on molecular bridges between cells: for the Stan system the key bridge
826 consists of a complex of Stan and Fz in one cell and Stan in the other; Vang promotes
827 function of the Stan pillar of this bridge (Struhl et al., 2012). For the Ds/Ft system, Ds
828 in one cell is linked to Ft in another, the activity of both is modulated by Fj (reviewed
829 in Butler and Wallingford, 2017). Pk and/or Sple bind to Vang and promote
830 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.

839 ## Supplementary Figure Legends

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

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

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

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

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

873 Figure S6. Ventral cuticle of the abdominal segments stained for lacZ; **A**, *ds.lacZ*
874 expression; **B**, *ds.lacZ* expression in *pk-sple*$^{-}$; **C**, *fj.lacZ* expression; **D**, *fj.lacZ*
875 expression in *pk-sple*$^{-}$. Red dots delineate the approximate boundaries between the A
876 and the P compartments. Arrows indicate the orientation of cell hairs in the pleura.

877

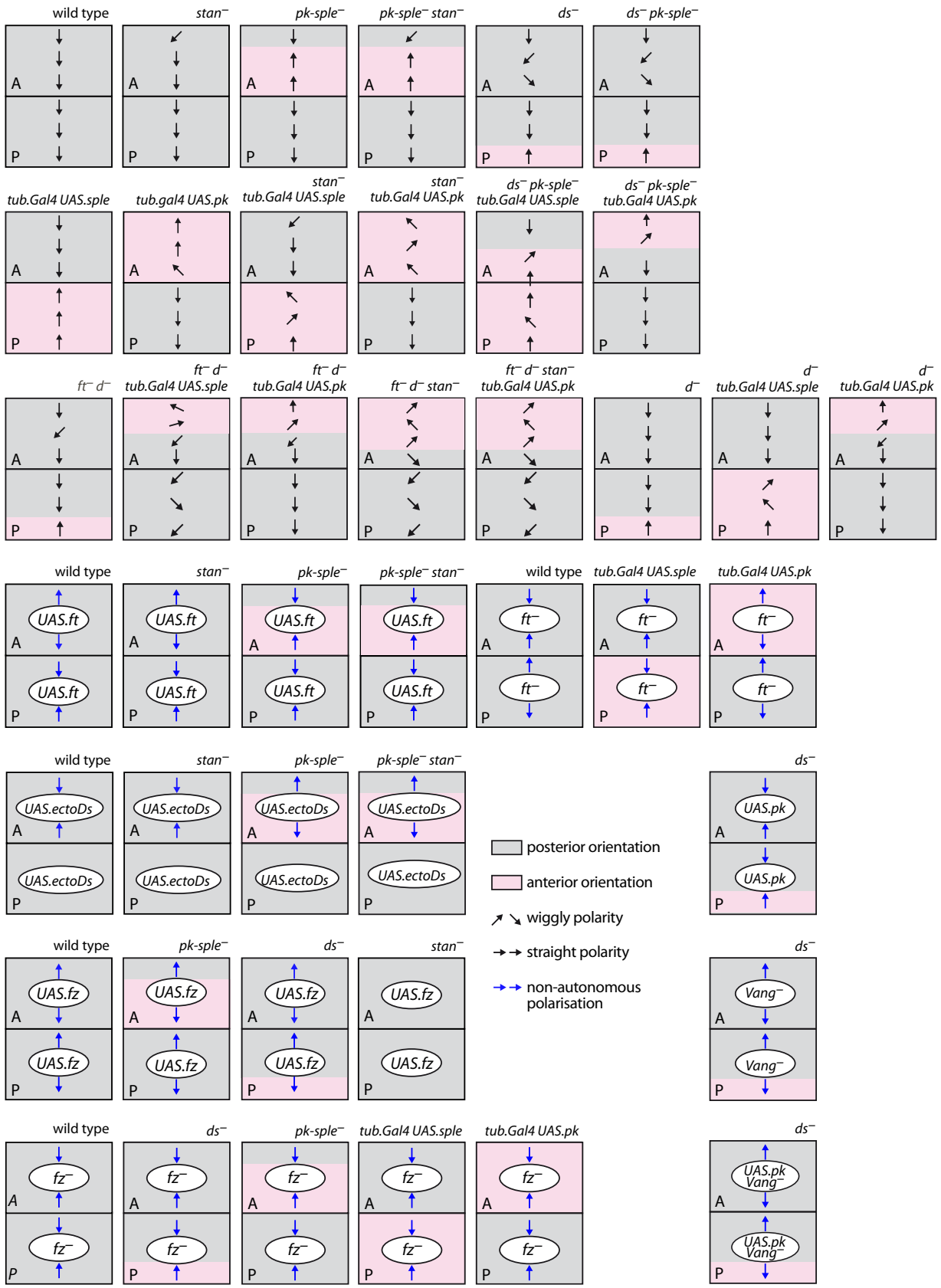
wild type
stan⁻
pk-sple⁻
pk-sple⁻ stan⁻
ds⁻
ds⁻ pk-sple⁻
tub.Gal4 UAS.sple
tub.gal4 UAS.pk
stan⁻ tub.Gal4 UAS.sple
stan⁻ tub.Gal4 UAS.pk
ds⁻ pk-sple⁻ tub.Gal4 UAS.sple
ds⁻ pk-sple⁻ tub.Gal4 UAS.pk
ft⁻ d⁻
ft⁻ d⁻ tub.Gal4 UAS.sple
ft⁻ d⁻ tub.Gal4 UAS.pk
ft⁻ d⁻ stan⁻
ft⁻ d⁻ stan⁻ tub.Gal4 UAS.pk
d⁻
d⁻ tub.Gal4 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

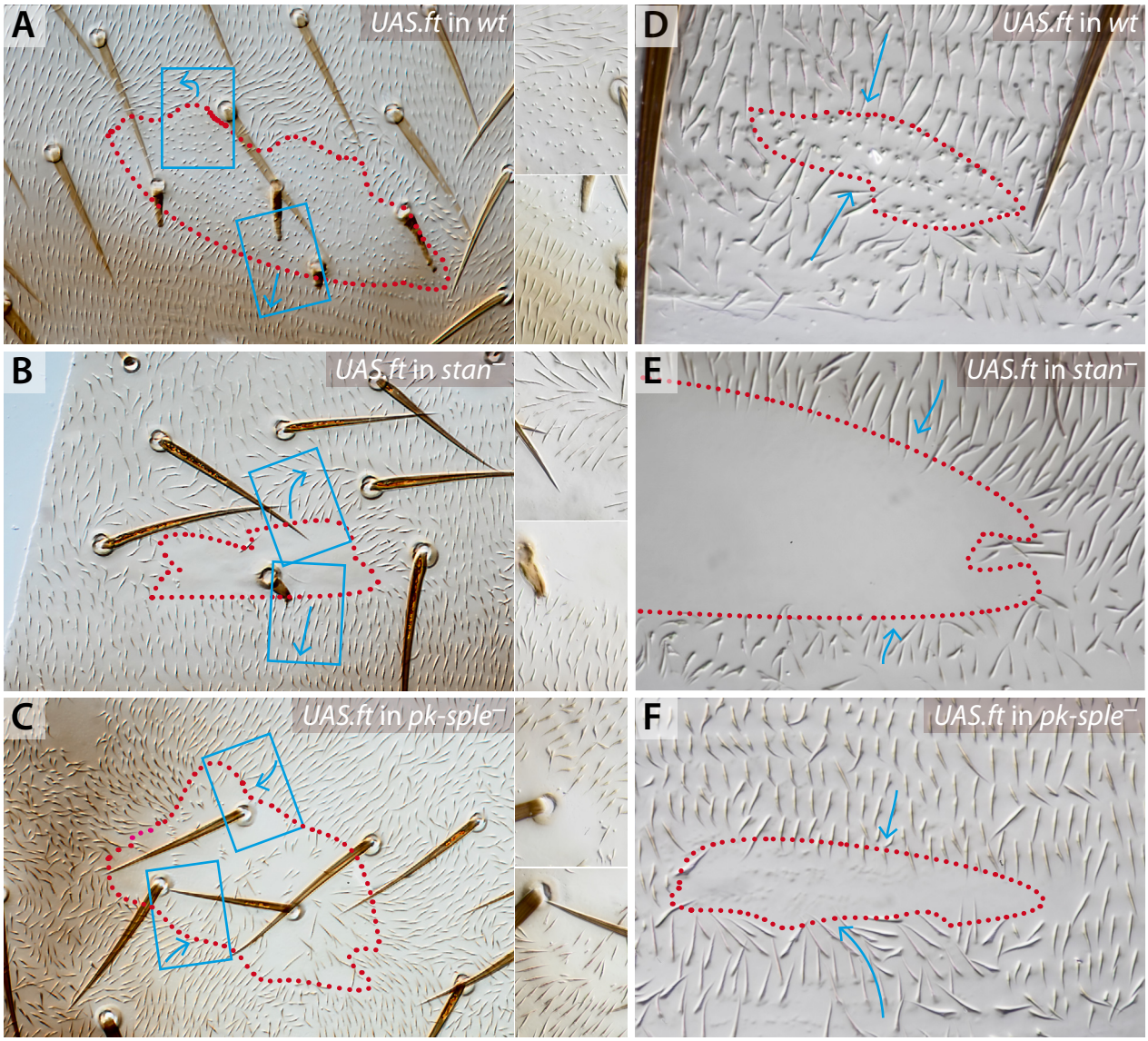
A
UAS.ft in wt
D
UAS.ft in wt
B
UAS.ft in stan⁻
E
UAS.ft in stan⁻
C
UAS.ft in pk-sple⁻
F
UAS.ft in pk-sple⁻

figure 2

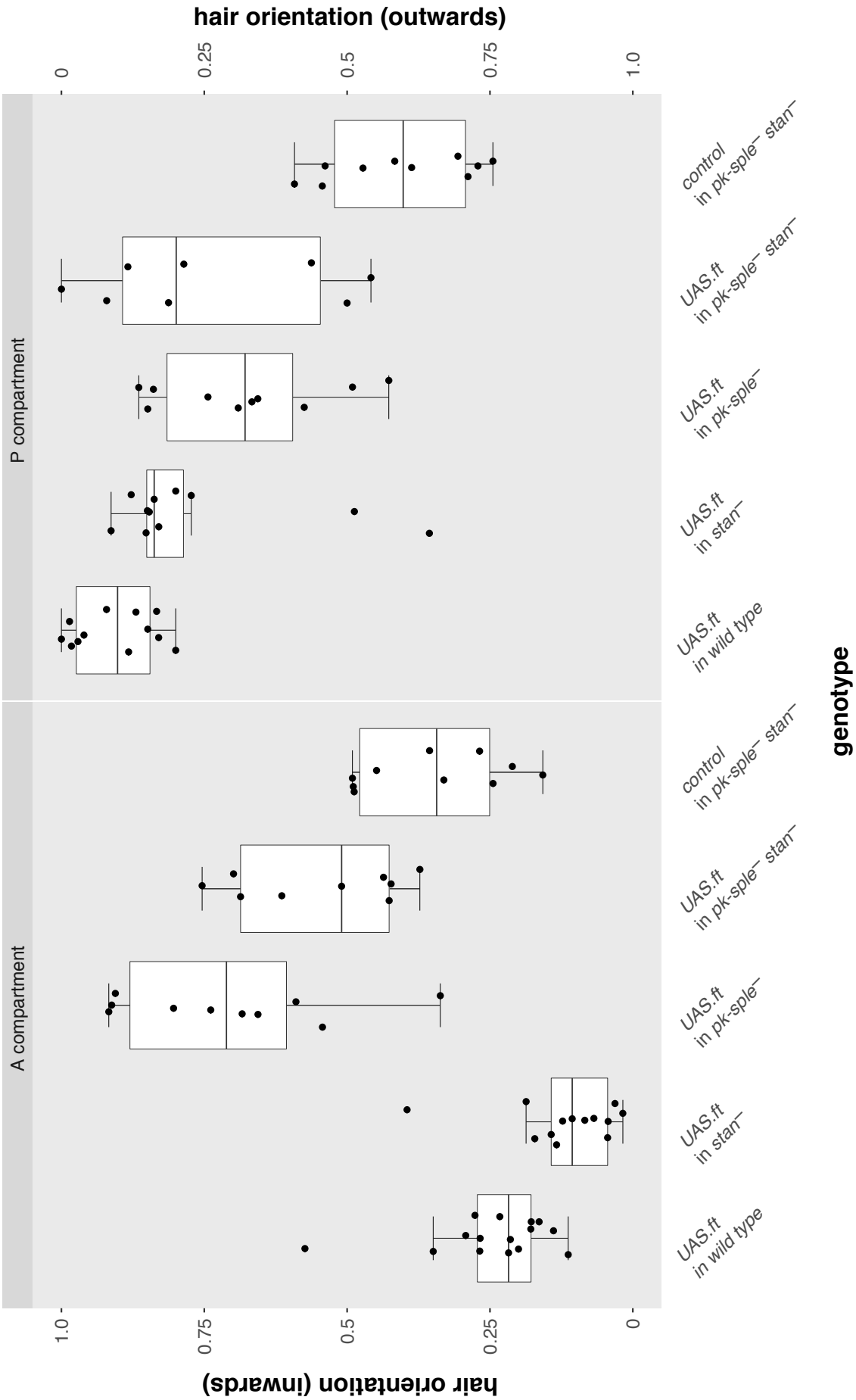
hair orientation (outwards)
0
0.25
0.5
0.75
1.0
P compartment
A compartment
control in pk-sple⁻ stan⁻
UAS.ft in pk-sple⁻ stan⁻
UAS.ft in pk-sple⁻
UAS.ft in stan⁻
UAS.ft in wild type
genotype
hair orientation (inwards)

figure 3

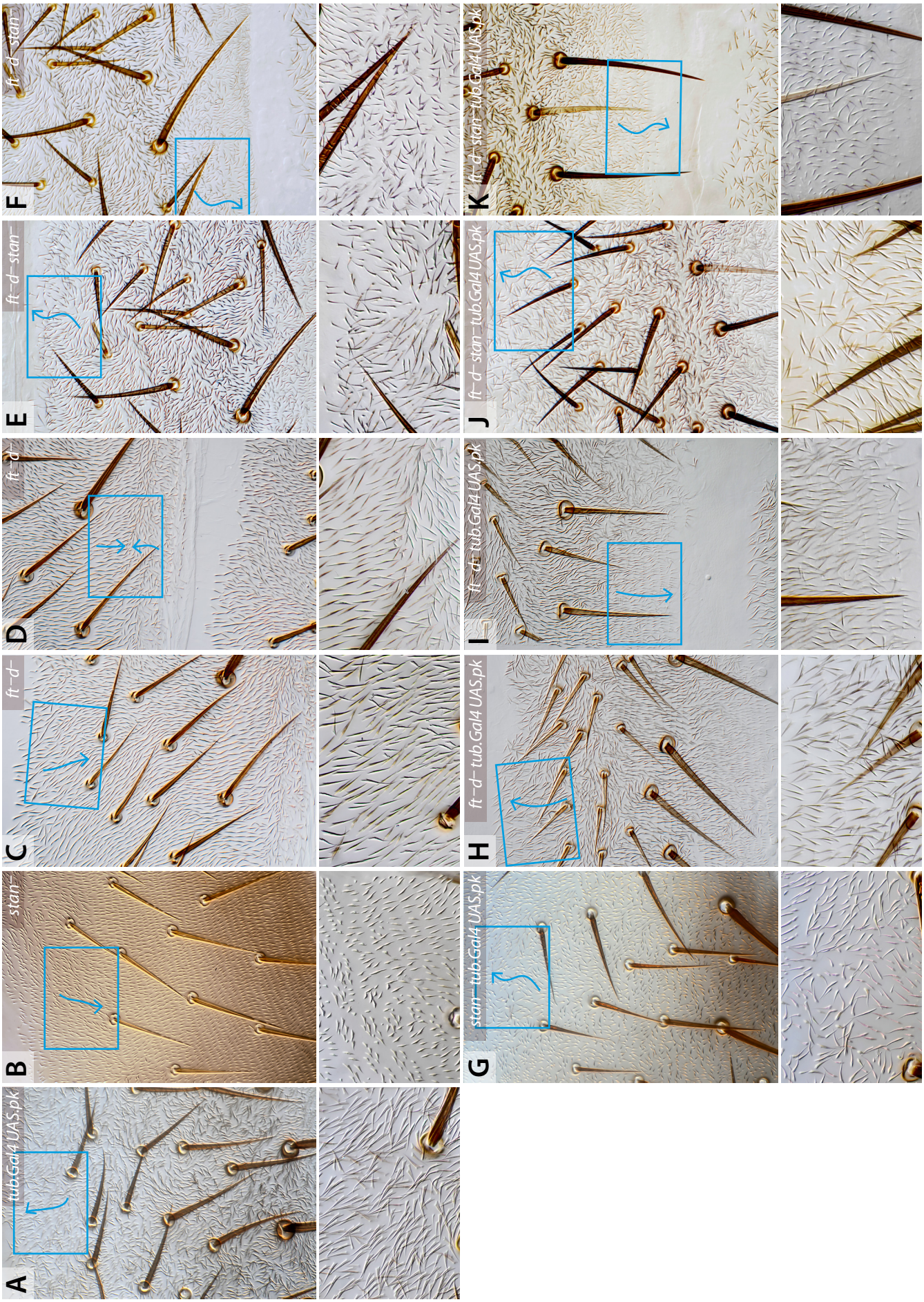
A
tub.Gal4 UAS.pk
B
stan−
C
ft−d−
D
ft−d−
E
ft−d− stan−
F
ft−d− stan−
G
stan− tub.Gal4 UAS.pk
H
ft−d− tub.Gal4 UAS.pk
I
ft−d− tub.Gal4 UAS.pk
J
ft−d− stan− tub.Gal4 UAS.pk
K
ft−d− stan− tub.Gal4 UAS.pk

figure 4

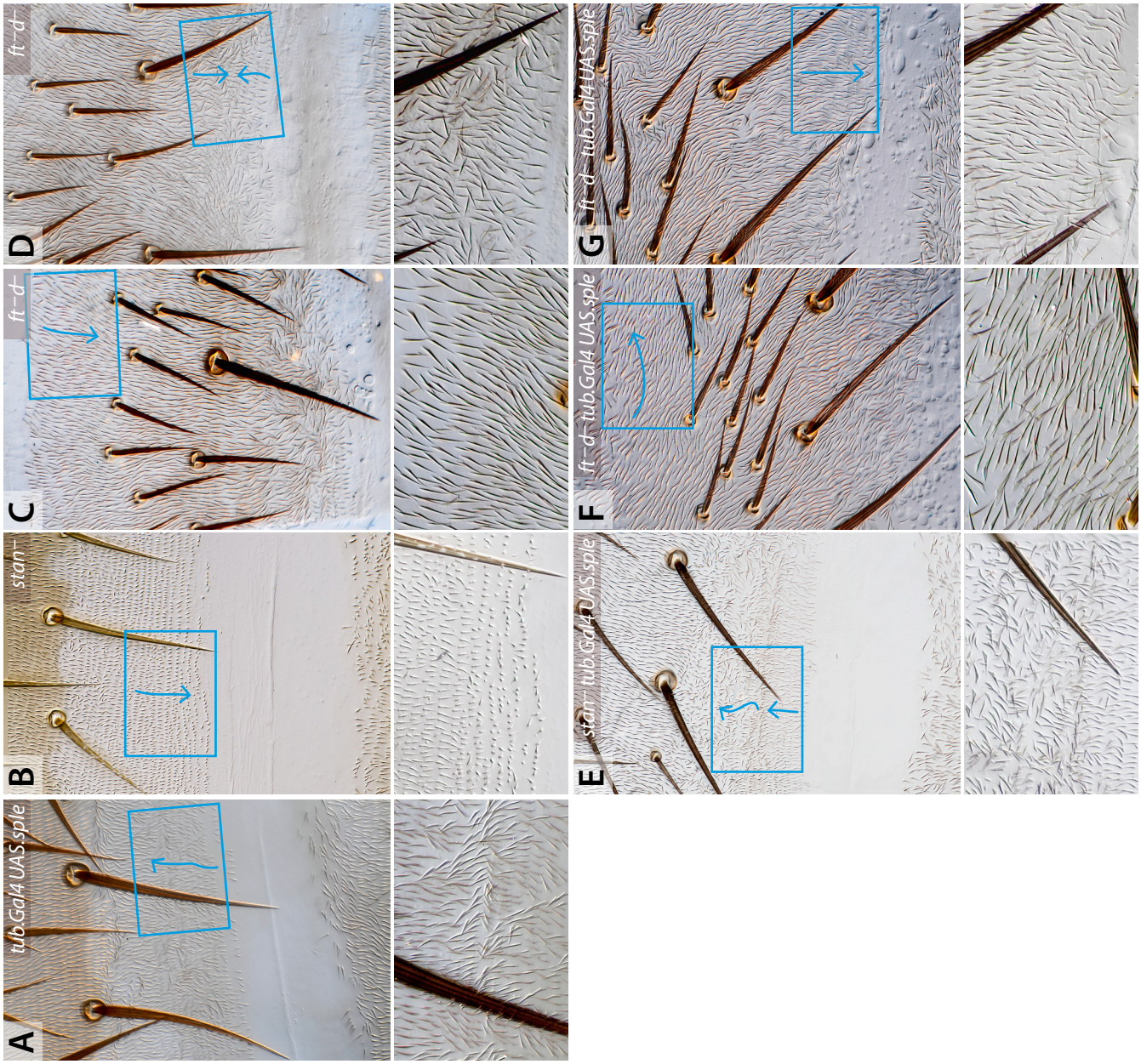
A
tub.Gal4 UAS.sple
B
stan
C
ft −d
D
ft −d
E
stan tub.Gal4 UAS.sple
F
ft −d tub.Gal4 UAS.sple
G
ft −d tub.Gal4 UAS.sple

figure 5

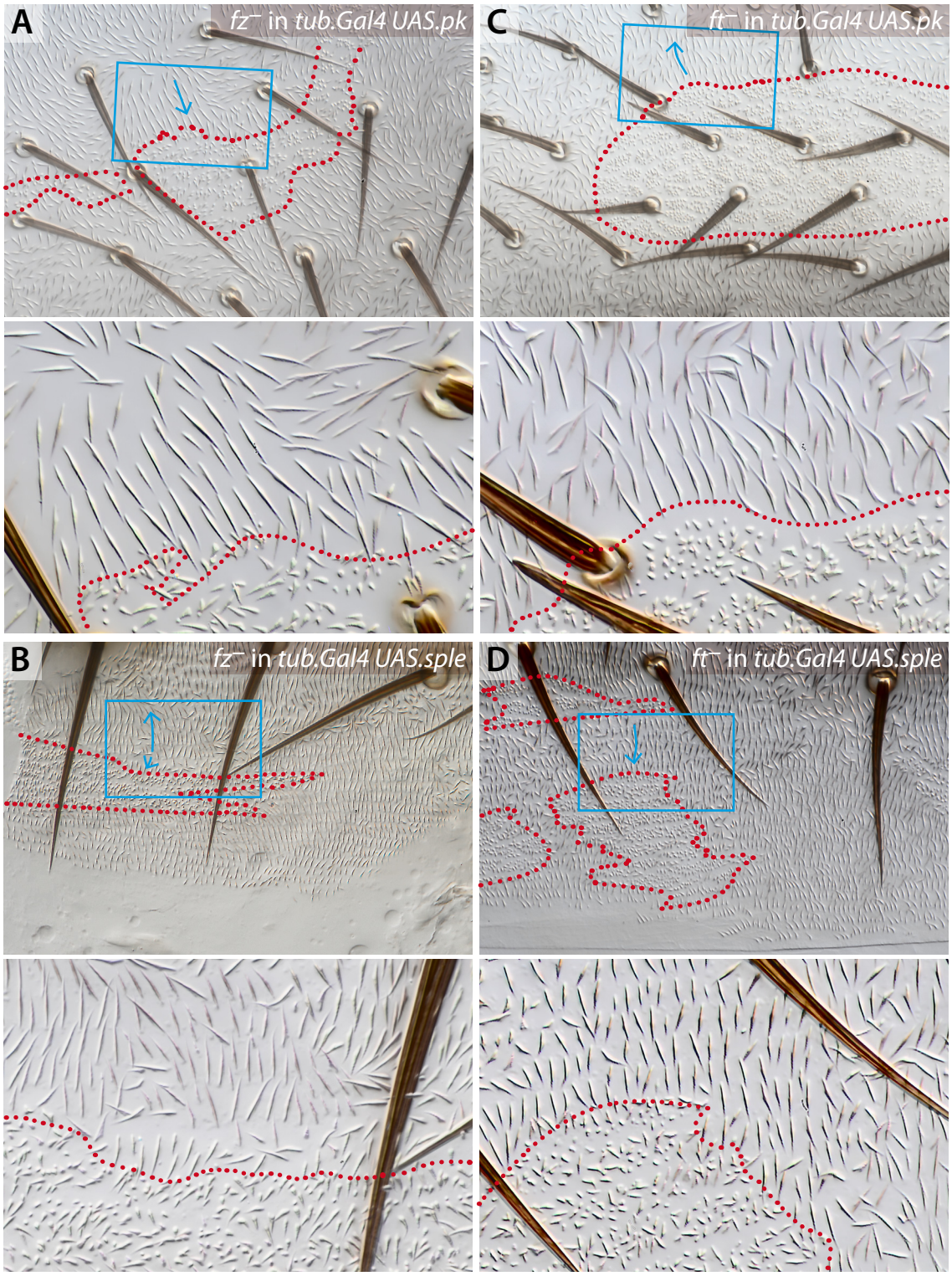
A
fz⁻ in tub.Gal4 UAS.pk
C
ft⁻ in tub.Gal4 UAS.pk
B
fz⁻ in tub.Gal4 UAS.sple
D
ft⁻ in tub.Gal4 UAS.sple

figure 6

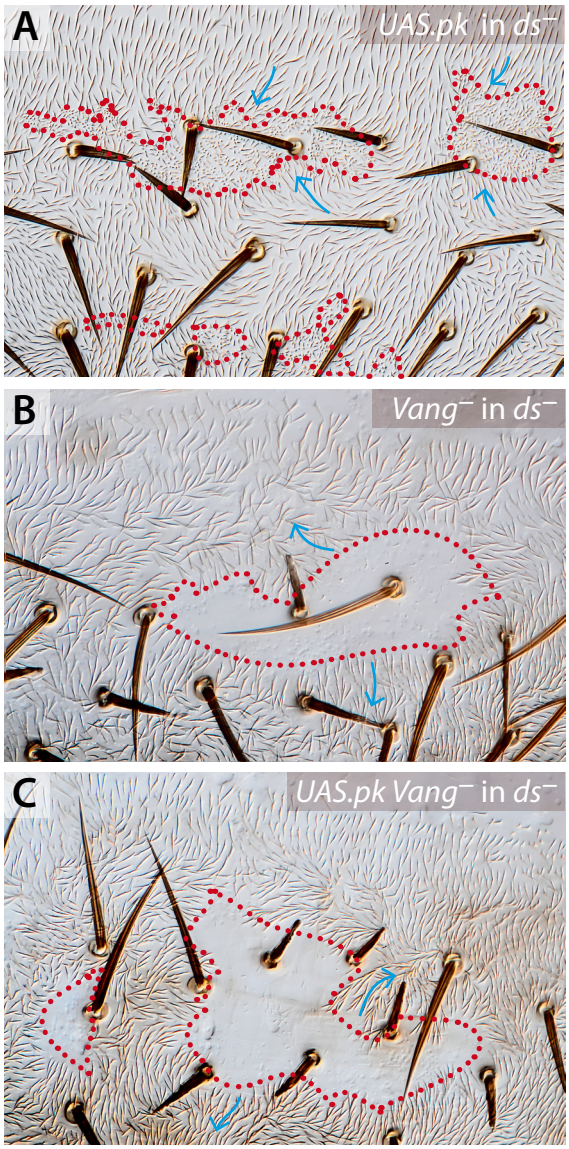

figure 7

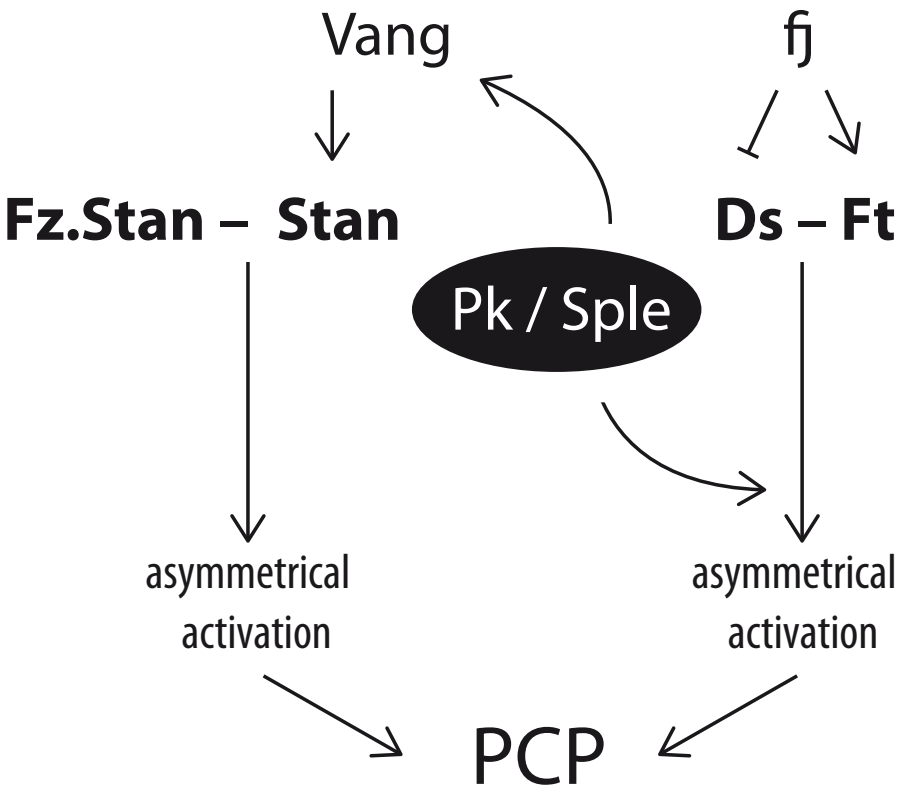
Vang
fj
Fz.Stan – Stan
Ds – Ft
Pk / Sple
asymmetrical activation
asymmetrical activation
PCP

figure 8

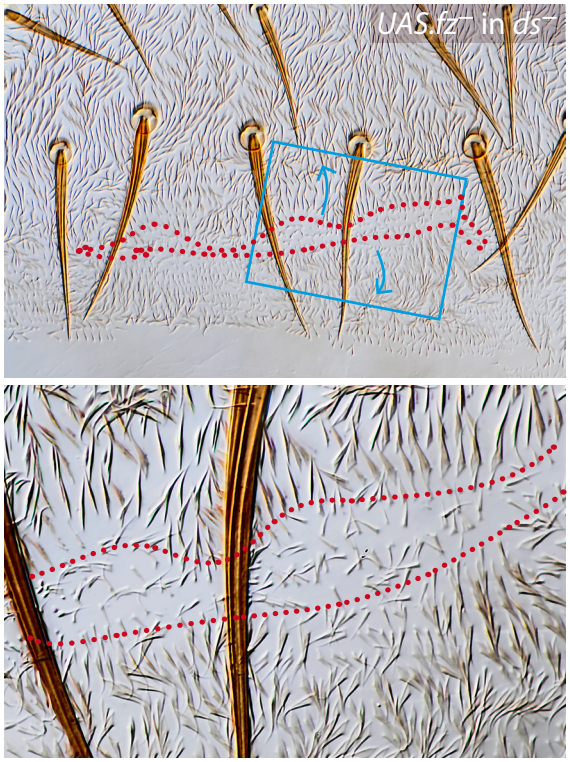

figure S1

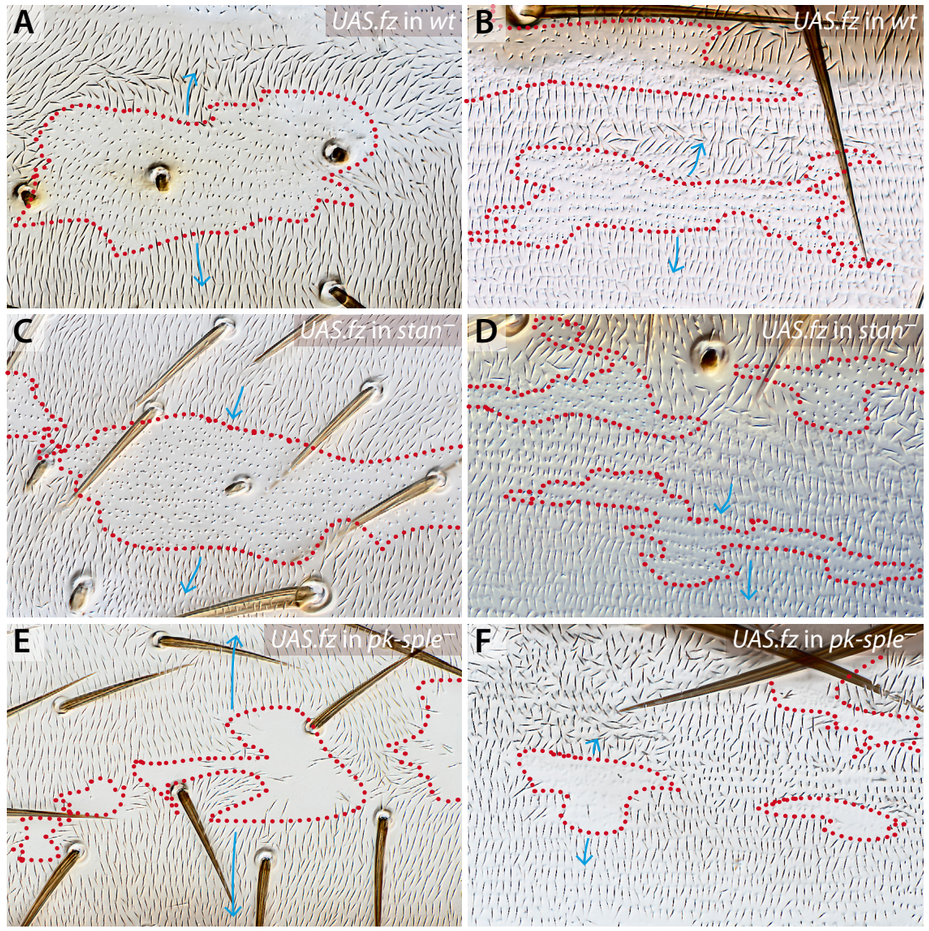

figure S2

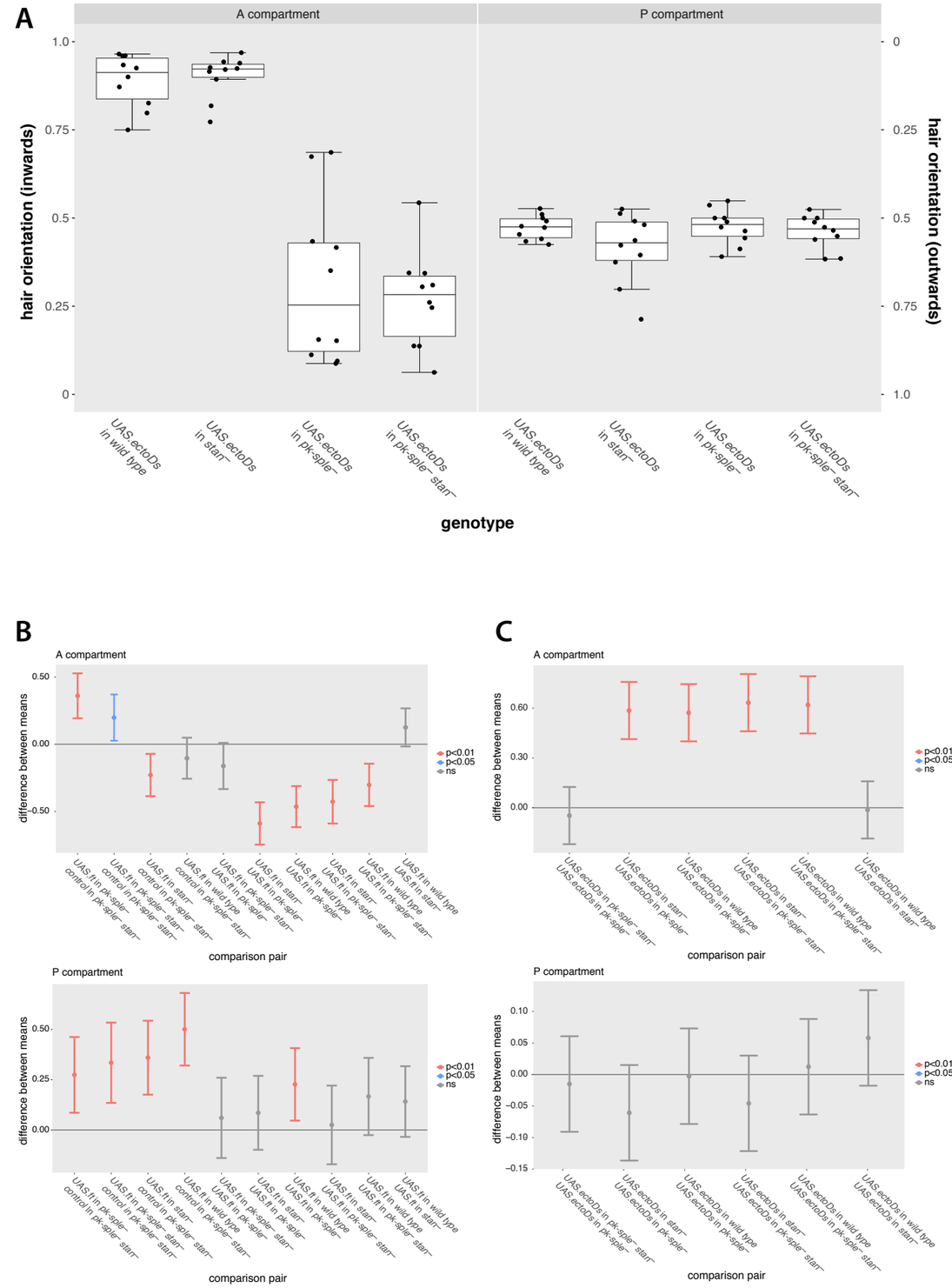

figure S3

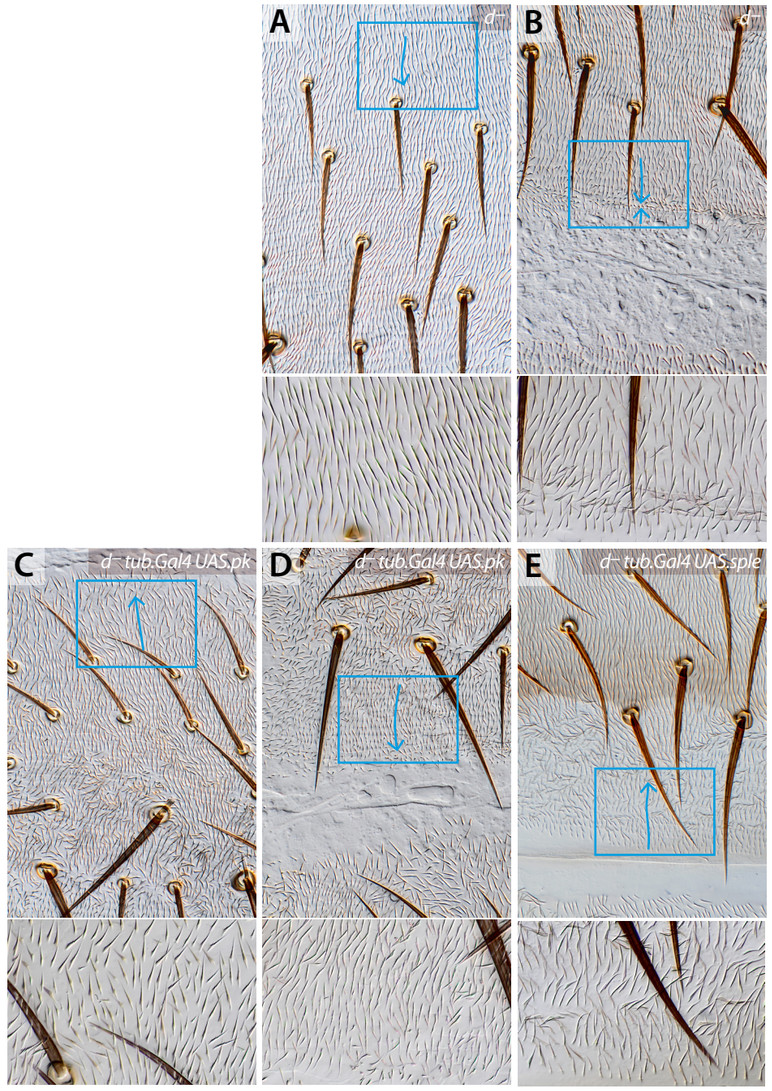

figure S4

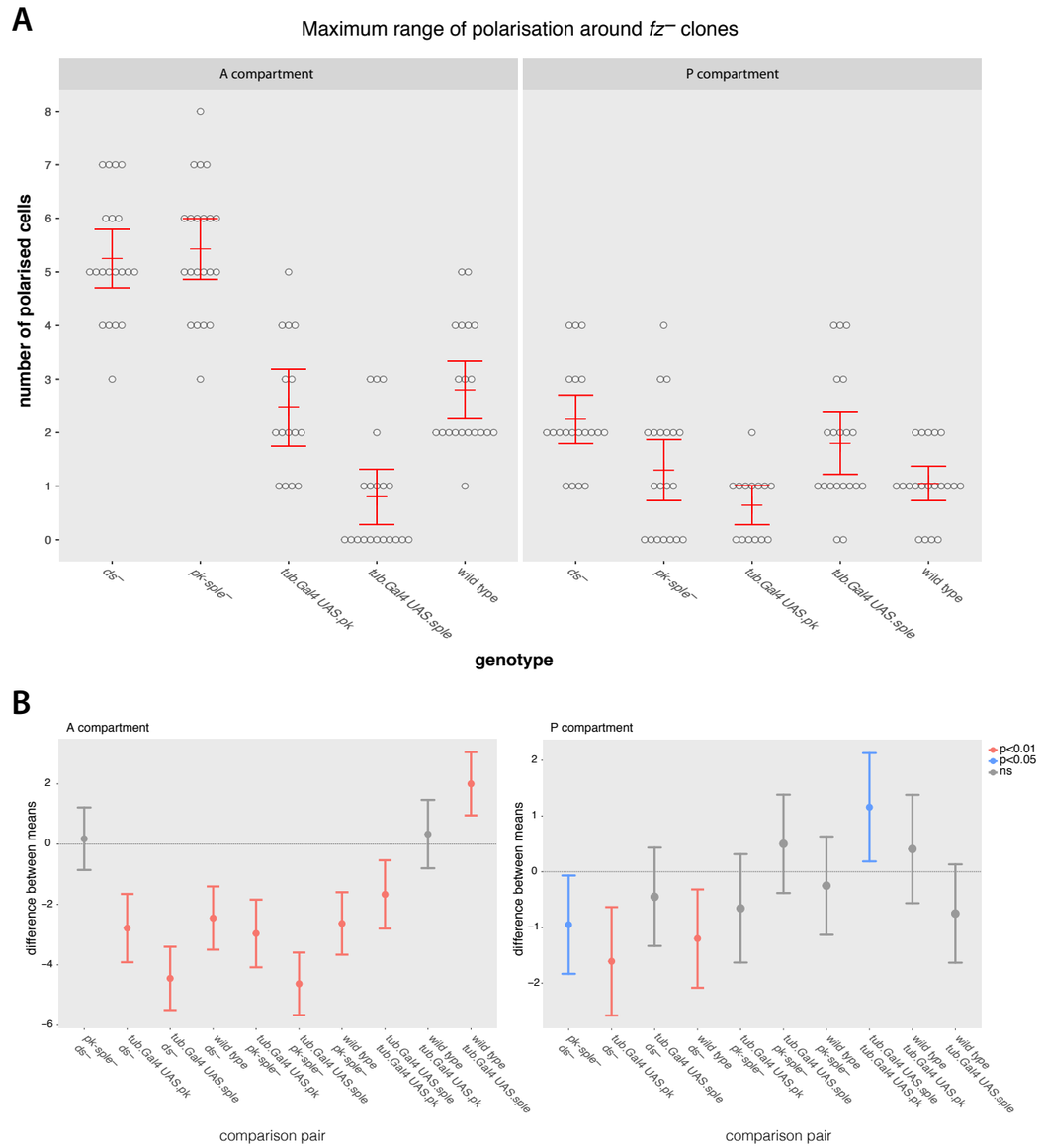

figure S5