

# Core PCP mutations affect short time mechanical properties but not tissue morphogenesis in the *Drosophila* pupal wing

Romina Piscitello-Gómez<sup>1,2#</sup>, Franz S Gruber<sup>1,3#</sup>, Abhijeet Krishna<sup>1,2,4</sup>, Charlie Duclut<sup>5,6,7</sup>, Carl D Modes<sup>1,2,4</sup>, Marko Popović<sup>4,5</sup>, Frank Jülicher<sup>2,4,5</sup>, Natalie A Dye<sup>1,2,8\*</sup>, Suzanne Eaton<sup>1,2,4,9§</sup>

<sup>1</sup>Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany

<sup>2</sup>DFG Excellence Cluster Physics of Life, Technische Universität Dresden, Dresden, Germany

<sup>3</sup>National Phenotypic Screening Centre, University of Dundee, Dundee, United Kingdom

<sup>4</sup>Center for Systems Biology Dresden, Dresden, Germany

<sup>5</sup>Max Planck Institute for the Physics of Complex Systems, Dresden, Germany

<sup>6</sup>Université Paris Cité, Laboratoire Matière et Systèmes Complexes, Paris, France

<sup>7</sup>Laboratoire Physico-Chimie Curie, CNRS UMR 168, Institut Curie, Université PSL, Sorbonne Université, Paris, France

<sup>8</sup>Mildred Scheel Nachwuchszentrum P2, Medical Faculty, Technische Universität Dresden, Dresden, Germany

<sup>9</sup> Biotechnologisches Zentrum, Technische Universität Dresden, Dresden, Germany

\*Corresponding author: [natalie\\_anne.dye@tu-dresden.de](mailto:natalie_anne.dye@tu-dresden.de)

# These authors contributed equally to this work

§ Deceased

December 9, 2022

1

## Abstract

How morphogenetic movements are robustly coordinated in space and time is a fundamental open question in biology. We study this question using the wing of *Drosophila melanogaster*, an epithelial tissue that undergoes large-scale tissue flows during pupal stages. We showed previously ([Etournay et al., 2015](#)) that pupal wing morphogenesis involves both cellular behaviors that allow relaxation of mechanical tissue stress, as well as cellular behaviors that appear to be actively patterned. The core planar cell polarity (PCP) pathway influences morphogenetic cell movements in many other contexts, which suggests that it could globally pattern active cellular behaviors during pupal wing morphogenesis. We show here, however, that this is not the case: there is no significant phenotype on the cellular dynamics underlying pupal morphogenesis in mutants of core PCP. Furthermore, using laser ablation experiments, coupled with a rheological model to describe the dynamics of the response to laser ablation, we conclude that while core PCP mutations affect the fast timescale response to laser ablation, they do not affect overall tissue mechanics. In conclusion, our work shows that cellular dynamics and tissue shape changes during *Drosophila* pupal wing morphogenesis are independent of one potential chemical guiding cue, core PCP.

## 16 1 Introduction

17 The spatial-temporal pattern of mechanical deformation during tissue morphogenesis is often guided  
18 by patterns of chemical signaling. Precisely how chemical signaling couples with the mechanics of  
19 morphogenesis, however, remains an active area of research. One known signal that is organized across  
20 tissues is the core planar cell polarity (PCP) pathway, a dynamic set of interacting membrane proteins  
21 that polarize within the plane of a tissue and globally align to orient structures such as hairs on the fly  
22 wing or animal skin (Cetera et al., 2017; Gubb and García-Bellido, 1982; Guo et al., 2004; Strutt and  
23 Strutt, 2002) and stereocilia of the vertebrate inner ear (Deans, 2021; Eaton, 1997). In many systems, core  
24 PCP also organizes patterns of dynamic cellular behaviors underlying tissue morphogenesis. For example,  
25 in zebrafish, non-canonical Wnt-mediated PCP regulates the actomyosin cytoskeleton to coordinate cell  
26 rearrangements during heart chamber remodelling (Merks et al., 2018). In *Drosophila*, there is also  
27 evidence to suggest that PCP components influence the orientation of cell division and cell shape changes  
28 in the larval wing disc, as well as cell rearrangements in the pupal notum (Baena-López et al., 2005;  
29 Bosveld et al., 2012; Mao et al., 2011; Ségalen et al., 2010). Here, we examine a potential role for core  
30 PCP in the dynamics and mechanics of morphogenesis using the *Drosophila* pupal wing.

31 The *Drosophila* wing is a flat epithelium that can be imaged at high spatial-temporal resolution *in vivo*  
32 during large-scale tissue flows. During the pupal stage the proximal hinge region of the wing contracts  
33 and pulls on the blade region, generating mechanical stress that is counteracted by marginal connections  
34 mediated by the extracellular protein Dumpy (Etournay et al., 2015; Ray et al., 2015; Wilkin et al.,  
35 2000). As a consequence, the tissue elongates along the proximal-distal (PD) axis and narrows along  
36 the anterior-posterior (AP) axis to resemble the adult wing (Etournay et al., 2015). Both cell elongation  
37 changes and cell rearrangements are important for tissue deformation. To some extent, mechanical stress  
38 induces these cell behaviors. However, the reduction of mechanical stress in a *umpy* mutant does not  
39 completely eliminate cell rearrangements, suggesting that there could be other patterning cues that drive  
40 oriented cell rearrangements (Etournay et al., 2015). We therefore wondered whether PCP systems could  
41 orient cell behaviors, such as cell rearrangements, during pupal wing morphogenesis.

42 In the *Drosophila* wing, there are two PCP systems termed Fat and core PCP. The Fat PCP system  
43 consists of two cadherins Fat and Dachsous, a cytoplasmic kinase Four-jointed and an atypical myosin  
44 Dachs (Clark et al., 1995; Ishikawa et al., 2008; Mahoney et al., 1991; Mao et al., 2006). The core PCP  
45 system is composed of two transmembrane cadherins Frizzled (Fz) and Flamingo or Starry night (Fmi,  
46 Stan), the transmembrane protein Strabismus or Van Gogh (Stbm, Vang) and the cytosolic components  
47 Dishevelled (Dsh), Prickle (Pk), and Diego (Dgo) (Adler et al., 1990; Devenport, 2014; Feiguin et al., 2001;  
48 Gubb and García-Bellido, 1982; Klingensmith et al., 1994; Taylor et al., 1998; Vinson and Adler, 1987;  
49 Wolff and Rubin, 1998). Our group has shown that tissue-scale patterns of PCP emerge during larval stages  
50 and then are dynamically reoriented during pupal tissue flows by shear stress (Aigouy et al., 2010; Merkel  
51 et al., 2014; Sagner et al., 2012). At the onset of pupal morphogenesis, both systems are margin-oriented,  
52 however as morphogenesis proceeds, core PCP reorients to point along the proximal-distal axis, whereas  
53 Fat PCP remains margin-oriented until very late, when it reorients towards veins (Fig S1.1A-B) (Merkel

54 et al., 2014). Whether these systems and their reorientation influence tissue mechanics during pupal  
55 morphogenesis is unknown.

56 Here, we examine cellular dynamics in tissues mutant for core PCP and we find that they are largely  
57 unperturbed, indicating that core PCP does not have an essential role in organizing global patterns of cell  
58 rearrangements in the pupal wing. We also performed an extensive analysis of the mechanics using laser  
59 ablation, developing a rheological model to interpret the results. We find that mutants in core PCP differ  
60 from wild type in the initial retraction velocity upon laser ablation. We find, however, that this difference  
61 is produced from the very fast timescale response, which does not appear to affect morphogenesis and  
62 overall tissue stresses, consistent with the lack of phenotype in cellular dynamics.

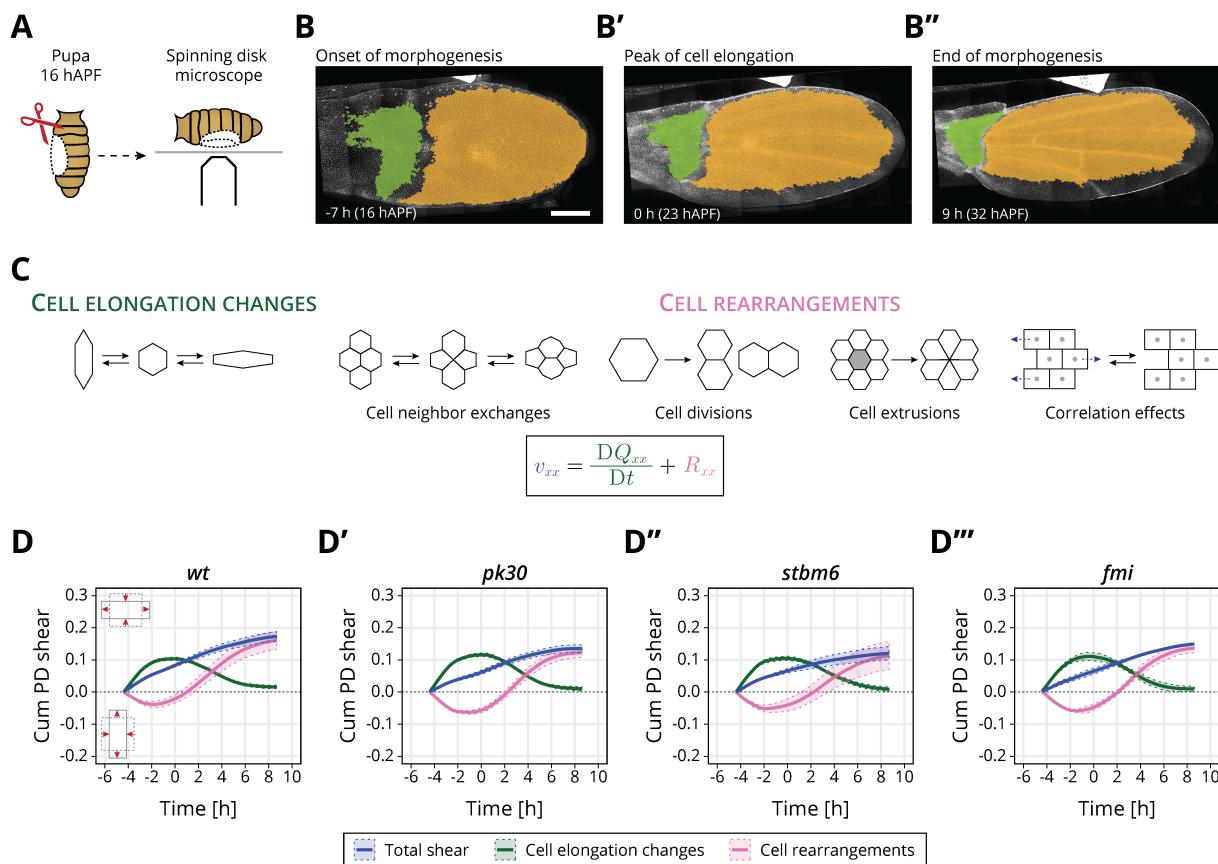
## 63 2 Results

### 64 2.1 Core PCP does not guide cellular dynamics during pupal wing 65 morphogenesis

66 To investigate the role of core PCP in orienting cell behaviors during pupal wing morphogenesis, we  
67 analyzed cell dynamics in wild type (*wt*) and three different *core PCP* mutant tissues: *prickle* (*pk30*),  
68 *strabismus* (*stbm6*) and *flamingo* (*fmi*<sup>frz3</sup>, aka *stan*<sup>frz3</sup>, abbreviated here simply as *fmi*). In *pk30*, the core  
69 and Fat PCP systems remain aligned together toward the margin and the magnitude of Stbm polarity is  
70 reduced (Merkel et al., 2014). In *stbm6* and *fmi*, the core PCP network is absent (Fig S1.1A-B) (Merkel  
71 et al., 2014). We analyzed shape changes of the wing blade during morphogenesis and decomposed  
72 these changes into contributions from cell elongation changes and cell rearrangements, which include cell  
73 neighbor exchanges, cell divisions, cell extrusions, and correlation effects (Fig 1A-C) (Etournay et al.,  
74 2015). Overall, the wing blade elongates along the PD axis by the end of morphogenesis (blue line in  
75 Fig 1D). Cells first elongate along the PD axis and then relax to more isotropic shapes (green line in  
76 Fig 1D). Cell rearrangements, however, go the opposite direction, initially contributing to AP deformation,  
77 before turning around to contribute to PD deformation (magenta line in Fig 1D). We introduce here  
78 a relative timescale, where time 0 h is the peak of cell elongation. This new scale allows us to handle  
79 variation in the timing of the onset of pupal wing morphogenesis, which we have observed recently (see  
80 Appendix 1).

81 Interestingly, we find that the overall deformation of the pupal wing blade is unchanged in *core PCP*  
82 mutants (Fig 1D-D''). Furthermore, the dynamics of cell elongation changes and the cell rearrangements,  
83 when averaged across the entire blade, occur normally. We observe that by the end of the process, only  
84 slightly lower total shear has occurred in the *core PCP* mutants, caused by slightly less cell rearrangements,  
85 but these subtle changes are not statistically significant (Fig 1D-D'', Fig S1.2C). The cellular dynamics  
86 contributing to isotropic tissue deformation also do not show differences between *wt* and *core PCP* mutant  
87 tissues (Fig S1.2D).

88 We also looked for differences in the behavior of regions of the wing blade subdivided along the PD  
89 axis (Fig S1.3E-F), as previous work has shown that distal regions of the wing blade shear more at early  
90 times, while proximal regions start deforming later (Merkel et al., 2017). Again, we find only subtle  
91 differences between *core PCP* mutants and *wt* when we subdivide the wing into regions along the PD axis:  
92 proximal blade regions (Region 1, Fig S1.3F) have slightly more AP-oriented cell rearrangements and end  
93 up with less overall total shear, whereas distal regions (Region 4, Fig S1.3F) shear slightly faster due to  
94 more PD-oriented cell elongation. These differences, however, are not statistically significant (Fig S1.4G).  
95 From this analysis, we conclude that core PCP does not guide the global patterns of cell dynamics during  
96 pupal morphogenesis. The subtle reduction in shear observed in the mutants is nevertheless consistent  
97 with the slight defect in adult wing shape: *pk30* and *stbm6* (but not *fmi*) mutant wings are slightly  
98 rounder and wider than *wt* (Fig S1.5H). These differences between *core PCP* mutants and *wt* may be  
99 detectable in adult wings because we can analyze many more wings, whereas the acquisition of timelapses is



**Figure 1: Core PCP does not orient cellular behaviors and tissue reshaping during pupal wing morphogenesis:** (A) Cartoon of pupal wing dissection at 16 hAPF and imaging using a spinning disk microscope. (B-B'') Images of a *wt* wing at -7, 0 and 9 h (for this movie these times correspond to 16, 23 and 32 hAPF). The green and orange regions correspond to the hinge and blade, respectively. Anterior is up; proximal to the left. Scale bar, 100  $\mu$ m. (C) Schematic of the cellular contributions underlying anisotropic tissue deformation. Total shear is the sum of cell elongation changes (green) and cell rearrangements (magenta). (D-D''') Accumulated tissue shear during morphogenesis in the blade region averaged for (D) *wt* (n=4), (D') *pk30* (n=3), (D'') *stbm6* (n=3) and (D''') *fmi* (n=2) movies. Solid line indicates the mean, and the shaded regions enclose  $\pm$  SEM. Differences in total accumulated shear are not statistically relevant (Fig S1.2C). The time is relative to the peak of cell elongation. SUPPLEMENTARY DATA: S4.1.

100 technically challenging and time-consuming, and we could only reasonably analyze 2-4 wings per genotype.  
 101 Alternatively, core PCP may affect later stages of wing development, after the after the pupal stages  
 102 observed here. To investigate whether the slight differences we observe in the pupal shear patterns and  
 103 adult wing shape could indicate a more subtle effect of core PCP on cell and tissue mechanics, we next  
 104 analyzed mechanical stress and strain in a small region of the wing blade.

## 105 2.2 A rheological model for the response to laser ablation

106 We investigated cell and tissue mechanics in *core PCP* mutants using laser ablation in a small region of  
 107 the wing blade. We used a region located between the second and third sensory organs in the intervein  
 108 region between the L3 and L4 longitudinal veins, which is a region that is easy to identify throughout  
 109 morphogenesis (Fig 2A). We cut 3-4 cells in a line along the AP axis and measure the displacement of the  
 110 tissue (Fig 2A, Supplemental movie 1). Previously, we reported that initial recoil velocity measured along  
 111 the PD axis in *wt* peaks around -8 h (20 hAPF in Iyer et al., 2019), and therefore we first focus on this

112 timepoint. We find that *core PCP* mutants have significantly lower recoil velocity (Fig 2B, Fig S2.1A),  
113 suggesting that there is a mechanical defect in these mutants.

114 As initial recoil velocity is often used as a proxy for mechanical stress (Etournay et al., 2015; Farhadifar  
115 et al., 2007; Iyer et al., 2019; Mayer et al., 2010), this result seems to suggest that the PCP mutant  
116 wings generate less mechanical stress during morphogenesis, even though the cellular dynamics is basically  
117 unperturbed. To explore this phenotype in more detail, we considered that the response to laser ablation  
118 is not exactly a direct measure of mechanical stress, as it is also affected by cellular material properties.  
119 We thus further analyzed the full kinetics of the linear laser ablations, focusing on the *pk30* mutant, and  
120 developed a rheological model to interpret the results. When plotting displacement of the nearest bond  
121 to ablation over time, we observe both a fast ( $<1$  s) and a slow ( $<20$  s) regime (Fig 2C). We therefore  
122 developed a model consisting of two Kelvin-Voigt (KV) elements in series (Fig 2D) to represent the tissue  
123 after ablation. The two KV elements have different elastic constants ( $k_f$  and  $k_s$ ) and viscosities ( $\eta_f$  and  
124  $\eta_s$ ). Before ablation, the system is subjected to a constant stress ( $\sigma$ ) and contains a spring with elastic  
125 constant  $k$ , which represents the cell patch that will be ablated. Upon ablation, the third spring is removed  
126 which leads to change in strain of our rheological model. We represent this strain by a displacement  $\Delta x$   
127 as a function of time given by

$$\Delta x(t) = X_f(1 - e^{-t/\tau_f}) + X_s(1 - e^{-t/\tau_s}), \quad (1)$$

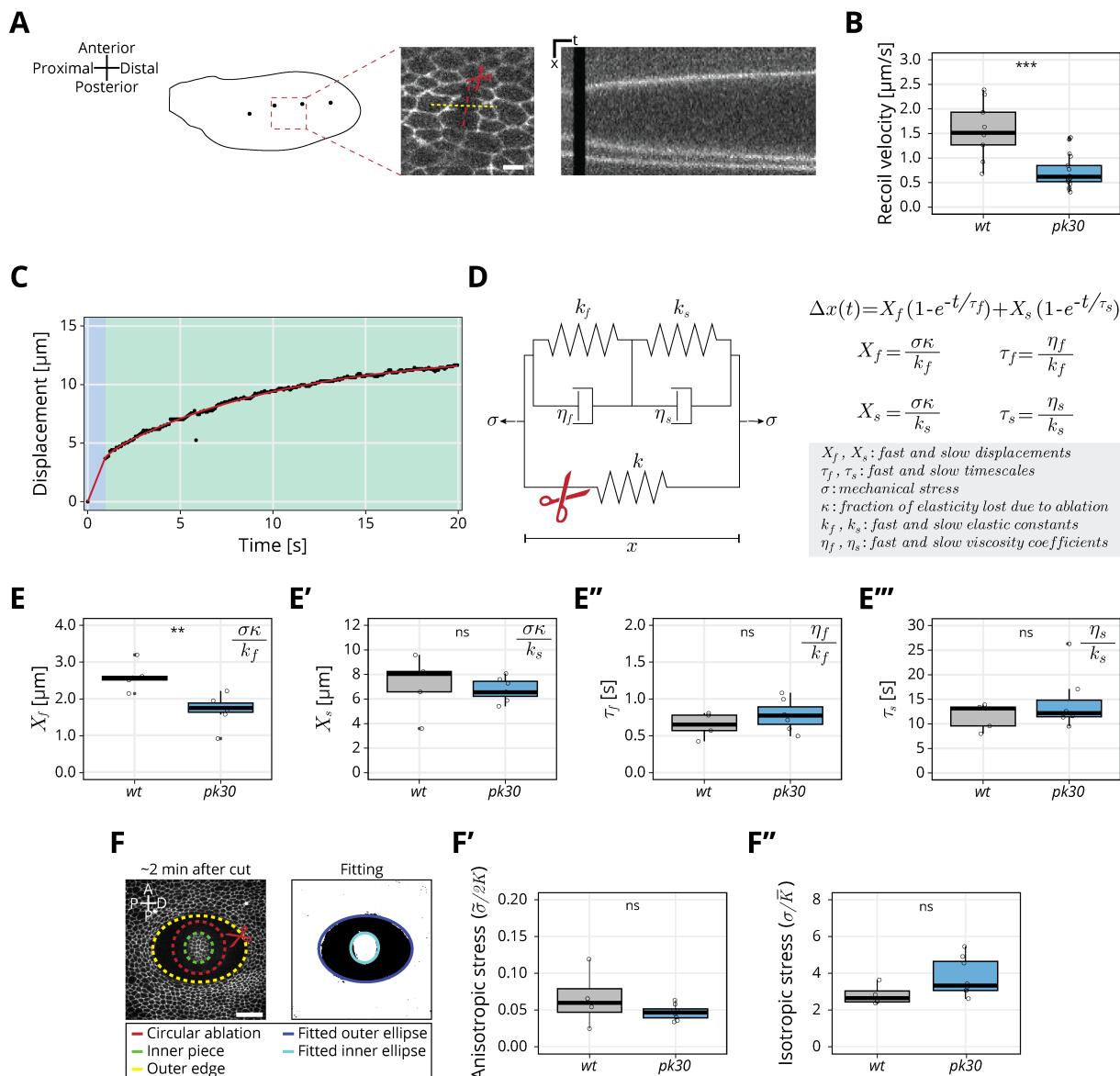
128 where  $X_f = \sigma\kappa/k_f$  is the displacement associated with the fast timescale,  $\tau_f = \eta_f/k_f$ , and  $X_s = \sigma\kappa/k_s$  is  
129 the displacement associated with the slow timescale,  $\tau_s = \eta_s/k_s$ . Here,  $\kappa = k/(k + \bar{k})$  is the fraction of the  
130 overall system elasticity lost due to ablation (see Materials and Methods 5.6.3) and  $\bar{k} = k_s k_f / (k_s + k_f)$  is  
131 the elasticity of the two KV elements connected in series. With this model, we presume the properties of  
132 the ablated cell itself, including its membrane, adhesion proteins, and actomyosin cortex likely dominate  
133 the fast timescale response. The slow timescale response is a collective effect emerging from the ablated  
134 cell together with its surrounding cellular network.

135 We analyzed experimentally measured displacement over time for each ablation and then fit the data  
136 to our model with four parameters ( $X_f$ ,  $X_s$ ,  $\tau_f$  and  $\tau_s$ ) (Eq 1, Fig 2E-E''). Surprisingly, we find that  
137 the only parameter that changes between *pk30* and *wt* is  $X_f$ , the displacement associated with the fast  
138 timescale (Fig 2E-E'').

139 To interpret this result, we consider that these four fitted parameters constrain the five mechanical  
140 model parameters (Fig 2D) but do not provide a unique solution. Since only one measured parameter  
141 changes, we asked what is the simplest set of model parameter changes that could have such an effect. To  
142 this end, we first note that the measured values of  $X_f$  and  $X_s$  ( $1.8 - 2.6 \mu m$  vs  $6 - 8 \mu m$ , respectively)  
143 indicate  $k_f \gg k_s$  and therefore the overall elasticity of our rheological model is largely determined by the  
144 elasticity of the slow relaxation  $\bar{k} \approx k_s$ . If we also consider that the contribution to the elasticity of the  
145 cellular patch from the ablated cells, represented by  $k$  in the model, is small, then we can approximate  
146  $\kappa \approx k/k_s$  and therefore  $X_f \approx \sigma k / (k_s k_f)$ , (see Materials and Methods 5.6.2). To probe whether a change  
147 of  $\sigma/k_s$  can account for the change of  $X_f$  in the *pk30* mutant, we used ESCA (Elliptical Shape after  
148 Circular Ablation) (Dye et al., 2021) to infer anisotropic and isotropic stress in the tissue from the final

149 relaxed tissue shape after a circular ablation (Fig 2F, see Materials and Methods [5.6.2](#)). We find no  
150 significant difference between *wt* and *pk30* mutants (Fig 2F-F''), therefore we conclude that  $\sigma/k_s$  cannot  
151 account for the observed change in  $X_f$ . Furthermore, we find from ESCA that the ratio of shear and  
152 isotropic elastic constants also does not change between *wt* and *pk30* mutant, suggesting that  $\sigma$  does not  
153 change (Fig [S2.1B](#)).

154 Therefore, we can account for the observed changes in the *pk30* mutant with a change of only one  
155 elastic coefficient  $k_f$ . This result suggests that a change in  $k_f$  is inversely proportional to the observed  
156 change in  $X_f$ . In this scenario, the only other parameter that changes is  $\eta_f$  such that  $\tau_f$  does not change,  
157 as observed. The conclusion that only the short time response to the ablation is affected in the *pk30*  
158 mutant is consistent with the lack of a clear phenotype in the large-scale tissue flows.



**Figure 2: Rheological model for the response to laser ablation:** (A) Schematic of a *wt* wing at -8 h. Linear laser ablation experiments were performed in the blade region enclosed by the red square. Dots on the wing cartoon indicate sensory organs. The red line corresponds to the ablation and the kymograph was drawn perpendicularly to the cut (yellow). Scale bar, 5  $\mu\text{m}$ . (B) Initial recoil velocity upon ablation along the PD axis at -8 h for *wt* (gray) and *pk30* (blue) tissues ( $n \geq 9$ ). Significance is estimated using the Mann-Whitney U test. \*\*\*,  $p\text{-val} \leq 0.001$ . (C) Example of the measured displacement after laser ablation (black dots) and corresponding exponential fit of the mechanical model (red curve). The blue and green regions highlight the displacement in the fast and slow timescale, respectively. (D) Description of the mechanical model that was devised to analyze the tissue response upon laser ablation. After the cut, the spring with elastic constant  $k$  is ablated (red scissor), and the tissue response is given by the combination of the two Kelvin-Voigt models arranged in series. These two correspond to the fast response given by  $k_f$  and  $\eta_f$  and the slow response given by  $k_s$  and  $\eta_s$ . The mechanical stress  $\sigma$  is constant. The membrane displacement  $\Delta x(t)$  is calculated as a sum of the displacement ( $X_f$ ) associated with the fast timescale ( $\tau_f$ ) and the displacement ( $X_s$ ) associated with the slow timescale ( $\tau_s$ ). (E-E'') Values obtained for each of the four fitting parameters when fit to the data. (E) Displacement associated with the fast and (E') slow timescale for *wt* (gray) and *pk30* (blue). (E'') Fast and (E''') slow timescale for *wt* (gray) and *pk30* (blue) ( $n \geq 5$ ). Significance is estimated using the Student's t-test. \*\*,  $p\text{-val} \leq 0.01$ ; ns,  $p\text{-val} > 0.05$ . (F) Example of a circular laser ablation used for analysis with ESCA. The left image shows the final shape of the ablation around 2 min after cut, and the right image shows the corresponding segmented image, where the inner and outer pieces were fit with ellipses. After the fitting, the model outputs the anisotropic and isotropic stress (equations shown on the right side). Scale bar, 20  $\mu\text{m}$ . A=Anterior, P\*=posterior, D=distal, P=proximal. (F') Anisotropic stress  $\tilde{\sigma}/2K$  for *wt* (gray) and *pk30* (blue) tissues at -8 h ( $n \geq 4$ ). Significance is estimated using the Mann-Whitney U test. ns,  $p\text{-val} > 0.05$ . (F'') Isotropic stress  $\sigma/\bar{K}$  for *wt* (gray) and *pk30* (blue) tissues at -8 h ( $n \geq 4$ ). Significance is estimated using the Mann-Whitney U test. ns,  $p\text{-val} > 0.05$ . The time is relative to the peak of cell elongation. In all plots, each empty circle indicates one cut, and the box plots summarize the data: thick black line indicates the median; the boxes enclose the 1st and 3rd quartiles; lines extend to the minimum and maximum without outliers, and filled circles mark outliers. SUPPLEMENTARY DATA: [S4.2](#).

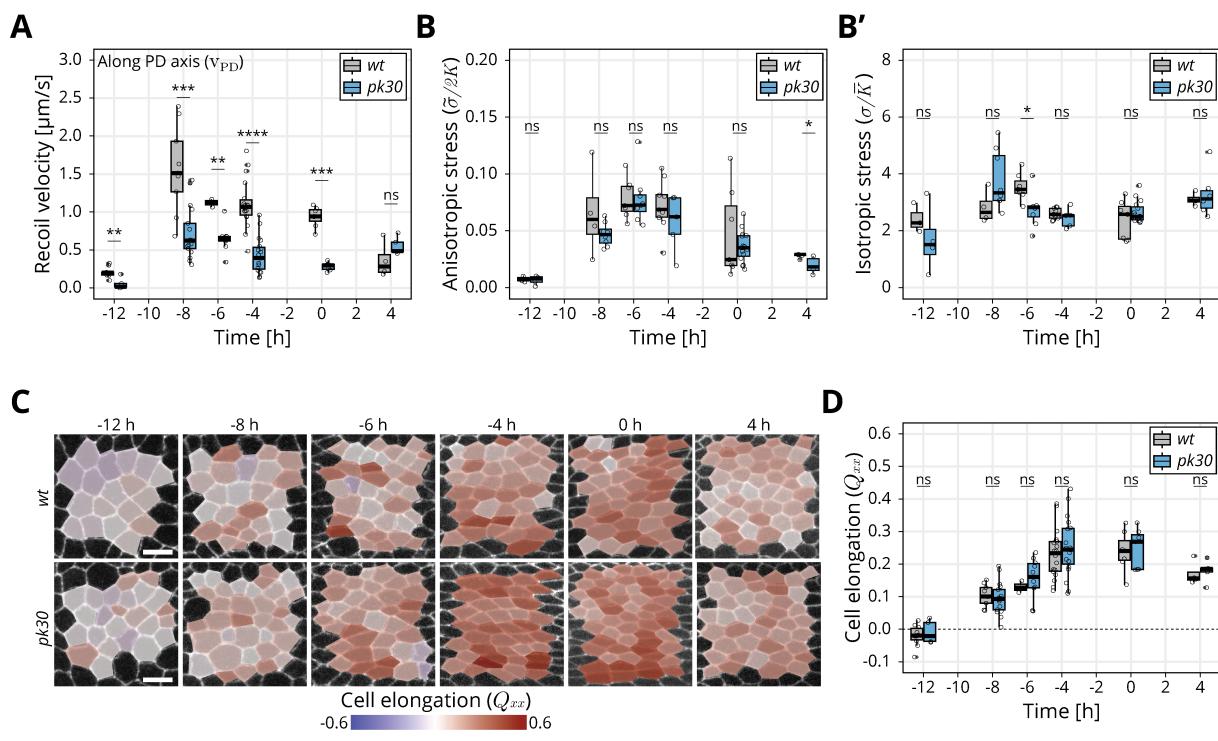
159 **2.3 Dynamics of stress and cell elongation throughout morphogenesis in wild  
160 type and *pk30* mutant**

161 To examine the effect of PCP mutation throughout pupal morphogenesis, we aimed to simplify the time  
162 intensive segmentation of the full ablation dynamics. To this end, we measured the initial recoil velocity  $v$   
163 defined by the displacement after ablation  $\Delta x$  over a short time interval  $\delta t = 0.65$  s. The recoil velocity  
164 can be expressed in terms of our model parameters as  $v = (X_f(1 - e^{-\delta t/\tau_f}) + X_s(1 - e^{-\delta t/\tau_s}))/\delta t$ . Since  
165 the value of  $\delta t$  is comparable to the fast time-scale  $\tau_f$ , about 63% of the  $X_f$  value relaxes over that time,  
166 while at the same time only about 5% of the  $X_s$  value is relaxed. Using the measured values of  $X_f$  and  
167  $X_s$ , we estimate that the fast timescale dynamics contributes about 80% of the  $v$  value. Therefore, the  
168 initial retraction velocity is a good proxy for the fast displacement  $X_f$ .

169 We find that the initial recoil velocity along the PD axis peaks at -8 h before declining again by 4 h  
170 (Fig 3A), consistent with previous work (Iyer et al., 2019). The behaviour of the recoil velocity in the *pk30*  
171 mutant is qualitatively similar throughout morphogenesis, however, with significantly lower magnitude  
172 than *wt* (Fig 3A). We also observed this behavior in *stbm6* and *fmi* mutant tissues (Fig S3.1A). This  
173 result indicates that  $X_f$  is lower in *core PCP* mutants than in *wt* throughout morphogenesis. We also  
174 performed ESCA over time in *pk30* mutants and observe that anisotropic stress ( $\tilde{\sigma}/2K$ ) rises early during  
175 morphogenesis before eventually declining (Fig 3B), whereas isotropic stress ( $\sigma/\bar{K}$ ) remains fairly constant  
176 (Fig 3B'). Importantly, ESCA does not report any difference in measured stresses between *pk30* and  
177 *wt*, nor in the ratio of elastic constants ( $2K/\bar{K}$ , Fig S3.2), indicating that our conclusions based on the  
178 -8 h timepoint are true throughout morphogenesis, and differences in  $\sigma/k_s$  between *pk30* and *wt* do not  
179 account for differences in  $X_f$ .

180 To directly compare linear laser ablations to ESCA, we performed linear ablations also in the  
181 perpendicular orientation. We then compared the difference in retraction velocities in these two orientations  
182 with the anisotropic tissue stress measured by ESCA (Fig S3.1B-C). We find that the magnitude of the  
183 response is lower in *pk30* compared to *wt*, as observed for the PD orientation alone. Interestingly, the  
184 dynamics of the response in *wt* is comparable to the dynamics of anisotropic stress measured by ESCA  
185 (Fig 3B, Fig S3.1C). However, for *pk30*, the dynamics reflected by initial recoil velocity is qualitatively  
186 different from the stress measured by ESCA (Fig 3B, Fig S3.1C), indicating that  $k_f$  could be changing in  
187 time in *pk30* mutants.

188 To further probe the possible role of Prickle in epithelial mechanics, we also measured the dynamics of  
189 the proximal-distal component of cell elongation ( $Q_{xx}$ ) in *wt* and *pk30* (Fig 3C). Interestingly, in both  
190 cases, anisotropic stress peaks around -6 h (Fig 3B), whereas  $Q_{xx}$  peaks significantly later, between -4 h  
191 and 0 h, indicating that active cellular stresses contributing to cell elongation change in time. However,  
192 there are no differences between *wt* and *pk30* (Fig 3D), showing that core PCP also does not affect active  
193 stresses underlying the dynamics of cell elongation during pupal morphogenesis.



**Figure 3: Dynamics of stress and cell elongation throughout morphogenesis in *wt* and *pk30* mutant:** (A) Initial recoil velocity upon ablation along the PD axis throughout morphogenesis for *wt* (gray) and *pk30* (blue) tissues ( $n \geq 3$ ). Significance is estimated using the Mann–Whitney U test. \*\*\*\*\*,  $p\text{-val} \leq 0.0001$ ; \*\*\*,  $p\text{-val} \leq 0.001$ ; \*\*,  $p\text{-val} \leq 0.01$ ; ns,  $p\text{-val} > 0.05$ . (B) ESCA results for anisotropic stress  $\tilde{\sigma}/2K$  for *wt* (gray) and *pk30* (blue) tissues throughout morphogenesis ( $n \geq 3$ ). Significance is estimated using the Mann–Whitney U test. \*,  $p\text{-val} < 0.05$ ; ns,  $p\text{-val} > 0.05$ . (B') ESCA results for isotropic stress  $\sigma/\bar{K}$  for *wt* (gray) and *pk30* (blue) throughout morphogenesis ( $n \geq 3$ ). Significance is estimated using the Mann–Whitney U test. \*,  $p\text{-val} < 0.05$ ; ns,  $p\text{-val} > 0.05$ . (C) Color-coded PD component of cell elongation  $Q_{xx}$  in the blade region between the second and third sensory organs found in the intervein region between L2 and L3. The images correspond to *wt* (top row) and *pk30* (bottom row) wings throughout morphogenesis. Scale bar, 5  $\mu\text{m}$ . (D) Quantification of  $Q_{xx}$  in this region throughout morphogenesis for *wt* (gray) and *pk30* (blue) ( $n \geq 3$ ). Significance is estimated using the Mann–Whitney U test. ns,  $p\text{-val} > 0.05$ . The time is relative to the peak of cell elongation. In all plots, each empty circle indicates one experiment, and the box plots summarize the data: thick black line indicates the median; the boxes enclose the 1st and 3rd quartiles; lines extend to the minimum and maximum without outliers, and filled circles mark outliers. SUPPLEMENTARY DATA: S4.3.

### 194 3 Discussion

195 Here, we used the *Drosophila* pupal wing as a model for studying the interplay between planar polarized  
196 chemical signaling components, specifically the core PCP pathway, and the mechanical forces underlying  
197 tissue morphogenesis. Unlike in other systems, where core PCP seems to organize patterns of cellular  
198 behaviors (Bosveld et al., 2012; Ségalen et al., 2010; Shindo and Wallingford, 2014, reviewed in Wang and  
199 Nathans, 2007), here an extensive analysis of core PCP mutants shows no significant phenotype in pupal  
200 wing morphogenesis. We find no differences in overall tissue shape change, nor in the pattern or dynamics  
201 of underlying cellular contributions. Furthermore, we found no significant differences in tissue mechanical  
202 stress or in cell elongation over time. Generally these results are consistent in mutants that destroy core  
203 PCP polarity (*stbm6* and *fmi*) or prevent its decoupling from Fat (*pk30*).

204 Interestingly, we do observe a phenotype in the initial retraction velocity upon laser ablation between  
205 *pk30* and *wt*. Retraction velocity after a laser ablation is often used a proxy for tissue mechanical  
206 stresses. If the differences between the *wt* and *core PCP* mutant wings observed in the retraction velocity  
207 indeed reflect the difference in mechanical stress, how can we understand lack of phenotype in tissue  
208 shape changes, cell dynamics and cell elongation? A more detailed analysis suggests that the difference  
209 between the two genotypes arises from a difference in the elastic constant  $k_f$  underlying the fast timescale  
210 response ( $\tau_f = 0.65$  s) to the ablation. Our results are consistent with the initial retraction velocity being  
211 proportional to tissue stress, however, the proportionality factor can depend on the genotype and can  
212 change in time (Fig S3.1C). This result highlights a limitation of comparing tissue mechanics in different  
213 genotypes based on retraction velocity measurements.

214 What is the the biophysical nature of the fast response to laser ablation? We hypothesize that processes  
215 that react on time-scales  $< 1$  s could be related to cortical mechanics of cell bonds or possibly changes in  
216 cell hydraulics after an ablation. In our simple model, these processes affect the stiff spring  $k_f$ , which has  
217 a small contribution to the effective cell and tissue elasticity  $\bar{k}$ , which is dominated by the soft spring  $k_s$ ,  
218 see Results 2.2. This could explain why the differences we observed at the fast time scales in *wt* and *pk30*  
219 wings are not reflected in wing morphogenesis. However, it remains unclear how core PCP affects only  
220 the elasticity  $k_f$  of the fast response and it remains an open possibility that there is a mechanism that  
221 compensates for such a change so that there is no change at larger scales.

222 While we have shown that core PCP is not required to organize the dynamic patterns of cellular events  
223 underlying pupal wing morphogenesis, it might still affect later stages of wing development. Furthermore,  
224 there may still be other patterning systems acting redundantly or independently with core PCP. For  
225 example, the Fat PCP system and Toll-like receptors have been shown to influence the orientation of  
226 cellular rearrangements and cell divisions in other contexts (Lavalou et al., 2021; Mao et al., 2006; Paré  
227 et al., 2014, reviewed in Umetsu, 2022). Whether and how other polarity systems influence pupal wing  
228 morphogenesis remains unknown.

229 **Acknowledgements**

230

231 We thank Stephan Grill for giving us access to the microscope used for laser ablation. We thank the Light  
232 Microscopy Facility, the Computer Department, and the Fly Keepers of the MPI-CBG for their support  
233 and expertise. We would like to thank Christian Dahmann and Jana Fuhrmann for comments on the  
234 manuscript prior to publication. This work was funded by Germany's Excellence Strategy – EXC-2068 –  
235 390729961– Cluster of Excellence Physics of Life of TU Dresden, as well as grants awarded to SE from the  
236 Deutsche Forschungsgemeinschaft (SPP1782, EA4/10-1, EA4/10-2) and core funding of the Max-Planck  
237 Society to SE. NAD additionally acknowledges funding from the Deutsche Krebshilfe (MSNZ P2 Dresden).  
238 AK and RPG were funded through the Elbe PhD program. FSG was supported by a DOC Fellowship of  
239 the Austrian Academy of Sciences. CD acknowledges the support of a postdoctoral fellowship from the  
240 LabEx “Who Am I?” (ANR-11-LABX-0071) and the Université Paris Cité IdEx (ANR-18-IDEX-0001)  
241 funded by the French Government through its “Investments for the Future”. We dedicate this work to our  
242 coauthor Prof. Dr. Suzanne Eaton, who tragically passed away before the finalization of the project.

243

244 **Author Contributions**

245

246 **RPP:** Investigation, Formal analysis, Software, Validation, Data curation, Writing - original draft,  
247 Writing - Review and editing, Visualization. **FSG:** Investigation, Formal analysis, Software, Validation,  
248 Data curation, Writing - Review and editing. **AK:** Methodology, Formal analysis, Software, Writing -  
249 Review and editing. **CD:** Methodology, Writing - Review and editing **CDM:** Methodology, Resources,  
250 Supervision, Writing - Review and editing. **MP:** Methodology, Supervision, Writing - original draft,  
251 Writing - Review and editing. **FJ:** Conceptualization, Methodology, Resources, Supervision, Writing -  
252 Review and editing, Funding acquisition, Project administration. **NAD:** Resources, Supervision, Writing  
253 - original draft, Writing - Review and editing, Data curation, Funding acquisition, Project administration.  
254 **SE:** Conceptualization, Methodology, Resources, Supervision, Funding acquisition, Project administration.

255

256 **Competing Interests**

257

258 The authors declare no competing interests.

## 259 References

- 260 Adler, P. N., Vinson, C., Park, W. J., Conover, S., and Klein, L. (1990). Molecular structure of *frizzled*, a  
261 *Drosophila* tissue polarity gene. *Genetics*, 126(2):401–416.
- 262 Aigouy, B., Farhadifar, R., Staple, D. B., Sagner, A., Röper, J.-C., Jülicher, F., and Eaton, S. (2010). Cell  
263 flow reorients the axis of planar polarity in the wing epithelium of *Drosophila*. *Cell*, 142(5):773–786.
- 264 Aigouy, B., Umetsu, D., and Eaton, S. (2016). Segmentation and quantitative analysis of epithelial tissues.  
265 In *Drosophila*, pages 227–239. Springer.
- 266 Baena-López, L. A., Baonza, A., and García-Bellido, A. (2005). The orientation of cell divisions determines  
267 the shape of *Drosophila* organs. *Current Biology*, 15(18):1640–1644.
- 268 Berg, S., Kutra, D., Kroeger, T., Straehle, C. N., Kausler, B. X., Haubold, C., Schiegg, M., Ales, J., Beier,  
269 T., Rudy, M., Eren, K., Cervantes, J. I., Xu, B., Beuttenmueller, F., Wolny, A., Zhang, C., Koethe,  
270 U., Hamprecht, F. A., and Kreshuk, A. (2019). *Ilastik: interactive machine learning for (bio)image  
271 analysis*. *Nature Methods*, 16(12):1226–1232.
- 272 Blasse, C., Saalfeld, S., Etournay, R., Sagner, A., Eaton, S., and Myers, E. W. (2017). *PreMosa: extracting  
273 2D surfaces from 3D microscopy mosaics*. *Bioinformatics*, 33(16):2563–2569.
- 274 Bosveld, F., Bonnet, I., Guirao, B., Tlili, S., Wang, Z., Petitalot, A., Marchand, R., Bardet, P.-L., Marcq,  
275 P., Graner, F., et al. (2012). Mechanical control of morphogenesis by Fat/Dachsous/Four-jointed  
276 planar cell polarity pathway. *Science*, 336(6082):724–727.
- 277 Cetera, M., Leybova, L., Woo, F. W., Deans, M., and Devenport, D. (2017). Planar cell polarity-dependent  
278 and independent functions in the emergence of tissue-scale hair follicle patterns. *Developmental  
279 Biology*, 428(1):188–203.
- 280 Clark, H. F., Brentrup, D., Schneitz, K., Bieber, A., Goodman, C., and Noll, M. (1995). *Dachsous* encodes  
281 a member of the cadherin superfamily that controls imaginal disc morphogenesis in *Drosophila*.  
282 *Genes & Development*, 9(12):1530–1542.
- 283 Classen, A. K., Aigouy, B., Giangrande, A., and Eaton, S. (2008). Imaging *Drosophila* pupal wing  
284 morphogenesis. In *Drosophila*, pages 265–275. Springer.
- 285 Deans, M. R. (2021). Conserved and divergent principles of planar polarity revealed by hair cell development  
286 and function. *Frontiers in Neuroscience*, 15.
- 287 Devenport, D. (2014). The cell biology of planar cell polarity. *Journal of Cell Biology*, 207(2):171–179.
- 288 Dye, N. A., Popović, M., Iyer, K. V., Fuhrmann, J. F., Piscitello-Gómez, R., Eaton, S., and Jülicher, F.  
289 (2021). Self-organized patterning of cell morphology via mechanosensitive feedback. *eLife*, 10:e57964.
- 290 Eaton, S. (1997). Planar polarization of drosophila and vertebrate epithelia. *Current Opinion in Cell  
291 Biology*, 9(6):860–866.
- 292 Etournay, R., Merkel, M., Popović, M., Brandl, H., Dye, N. A., Aigouy, B., Salbreux, G., Eaton, S., and  
293 Jülicher, F. (2016). *TissueMiner: A multiscale analysis toolkit to quantify how cellular processes  
294 create tissue dynamics*. *eLife*, 5:e14334.
- 295 Etournay, R., Popović, M., Merkel, M., Nandi, A., Blasse, C., Aigouy, B., Brandl, H., Myers, G., Salbreux,  
296 G., Jülicher, F., et al. (2015). Interplay of cell dynamics and epithelial tension during morphogenesis

- 297        of the *Drosophila* pupal wing. *eLife*, 4:e07090.
- 298    Farhadifar, R., Röper, J.-C., Aigouy, B., Eaton, S., and Jülicher, F. (2007). The influence of cell mechanics,  
299        cell-cell interactions, and proliferation on epithelial packing. *Current Biology*, 17(24):2095–2104.
- 300    Feiguin, F., Hannus, M., Mlodzik, M., and Eaton, S. (2001). The ankyrin repeat protein Diego mediates  
301        Frizzled-dependent planar polarization. *Developmental Cell*, 1(1):93–101.
- 302    Grill, S. W., Goënczy, P., Stelzer, E. H., and Hyman, A. A. (2001). Polarity controls forces governing  
303        asymmetric spindle positioning in the *Caenorhabditis elegans* embryo. *Nature*, 409(6820):630–633.
- 304    Gubb, D. and García-Bellido, A. (1982). A genetic analysis of the determination of cuticular polarity  
305        during development in *Drosophila melanogaster*. *Development*, 68(1):37–57.
- 306    Gubb, D., Green, C., Huen, D., Coulson, D., Johnson, G., Tree, D., Collier, S., and Roote, J. (1999).  
307        The balance between isoforms of the Prickle LIM domain protein is critical for planar polarity in  
308        *Drosophila* imaginal discs. *Genes & Development*, 13(17):2315–2327.
- 309    Guo, N., Hawkins, C., and Nathans, J. (2004). Frizzled6 controls hair patterning in mice. *Proceedings of  
310        the National Academy of Sciences*, 101(25):9277–9281.
- 311    Huang, J., Zhou, W., Dong, W., Watson, A. M., and Hong, Y. (2009). Directed, efficient, and versatile  
312        modifications of the *Drosophila* genome by genomic engineering. *Proceedings of the National Academy  
313        of Sciences*, 106(20):8284–8289.
- 314    Ishikawa, H. O., Takeuchi, H., Haltiwanger, R. S., and Irvine, K. D. (2008). Four-jointed is a Golgi kinase  
315        that phosphorylates a subset of cadherin domains. *Science*, 321(5887):401–404.
- 316    Iyer, K. V., Piscitello-Gómez, R., Paijmans, J., Jülicher, F., and Eaton, S. (2019). Epithelial viscoelasticity  
317        is regulated by mechanosensitive E-cadherin turnover. *Current Biology*, 29(4):578–591.
- 318    Klingensmith, J., Nusse, R., and Perrimon, N. (1994). The *Drosophila* segment polarity gene *dishevelled*  
319        encodes a novel protein required for response to the *wingless* signal. *Genes & Development*,  
320        8(1):118–130.
- 321    Lavalou, J., Mao, Q., Harmansa, S., Kerridge, S., Lelouch, A. C., Philippe, J.-M., Audebert, S., Camoin,  
322        L., and Lecuit, T. (2021). Formation of polarized contractile interfaces by self-organized Toll-8/Cirr  
323        GPCR asymmetry. *Developmental Cell*, 56(11):1574–1588.
- 324    Mahoney, P. A., Weber, U., Onofrechuk, P., Biessmann, H., Bryant, P. J., and Goodman, C. S. (1991). The  
325        *fat* tumor suppressor gene in *Drosophila* encodes a novel member of the cadherin gene superfamily.  
326        *Cell*, 67(5):853–868.
- 327    Mao, Y., Rauskolb, C., Cho, E., Hu, W.-L., Hayter, H., Minihan, G., Katz, F. N., and Irvine, K. D. (2006).  
328        Dachs: an unconventional myosin that functions downstream of Fat to regulate growth, affinity and  
329        gene expression in *Drosophila*. *Development*, 133(13):2539–2551.
- 330    Mao, Y., Tournier, A. L., Bates, P. A., Gale, J. E., Tapon, N., and Thompson, B. J. (2011). Planar  
331        polarization of the atypical myosin Dachs orients cell divisions in *Drosophila*. *Genes & Development*,  
332        25(2):131–136.
- 333    MATLAB (2017). *MATLAB version 9.2.0.556344 (R2017a)*. The Mathworks, Inc., Natick, Massachusetts.
- 334    Mayer, M., Depken, M., Bois, J. S., Jülicher, F., and Grill, S. W. (2010). Anisotropies in cortical tension  
335        reveal the physical basis of polarizing cortical flows. *Nature*, 467(7315):617–621.

- 336 Merkel, M., Etournay, R., Popović, M., Salbreux, G., Eaton, S., and Jülicher, F. (2017). *Triangles bridge*  
337 *the scales: Quantifying cellular contributions to tissue deformation*. *Physical Review E*, 95(3):032401.
- 338 Merkel, M., Sagner, A., Gruber, F. S., Etournay, R., Blasse, C., Myers, E., Eaton, S., and Jülicher, F.  
339 *(2014). The balance of Prickle/Spiny-legs isoforms controls the amount of coupling between core*  
340 *and Fat PCP systems*. *Current Biology*, 24(18):2111–2123.
- 341 Merks, A. M., Swinarski, M., Meyer, A. M., Müller, N. V., Özcan, I., Donat, S., Burger, A., Gilbert,  
342 Mosimann, C., Abdelilah-Seyfried, S., and Panáková, D. (2018). *Planar cell polarity signalling*  
343 *coordinates heart tube remodelling through tissue-scale polarisation of actomyosin activity*. *Nature*  
344 *Communications*, 9(1):1–15.
- 345 Nickoloff, J. (2016). *Docker in action*. Manning Publications Co.
- 346 Paré, A. C., Vichas, A., Fincher, C. T., Mirman, Z., Farrell, D. L., Mainieri, A., and Zallen, J. A. (2014). *A*  
347 *positional Toll receptor code directs convergent extension in Drosophila*. *Nature*, 515(7528):523–527.
- 348 R Core Team (2020). R: A Language and Environment for Statistical Computing. <https://www.R-project.org/>.
- 350 Ray, R. P., Matamoro-Vidal, A., Ribeiro, P. S., Tapon, N., Houle, D., Salazar-Ciudad, I., and Thompson,  
351 B. J. (2015). *Patterned anchorage to the apical extracellular matrix defines tissue shape in the*  
352 *developing appendages of Drosophila*. *Developmental Cell*, 34(3):310–322.
- 353 RStudio Team (2020). RStudio: Integrated Development Environment for R. <https://rstudio.com>.
- 354 Sagner, A., Merkel, M., Aigouy, B., Gaebel, J., Brankatschk, M., Jülicher, F., and Eaton, S. (2012).  
355 *Establishment of global patterns of planar polarity during growth of the Drosophila wing epithelium*.  
356 *Current Biology*, 22(14):1296–1301.
- 357 Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden,  
358 C., Saalfeld, S., Schmid, B., Tinevez, J.-Y., White, D. J., Hartenstein, V., Eliceiri, K., Tomancak,  
359 P., and Cardona, A. (2012). *Fiji: an open-source platform for biological-image analysis*. *Nature*  
360 *Methods*, 9(7):676–682.
- 361 Ségalen, M., Johnston, C. A., Martin, C. A., Dumortier, J. G., Prehoda, K. E., David, N. B., Doe, C. Q.,  
362 and Bellaïche, Y. (2010). *The Fz-Dsh planar cell polarity pathway induces oriented cell division via*  
363 *Mud/NuMA in Drosophila and zebrafish*. *Developmental Cell*, 19(5):740–752.
- 364 Shindo, A. and Wallingford, J. B. (2014). *PCP and septins compartmentalize cortical actomyosin to direct*  
365 *collective cell movement*. *Science*, 343(6171):649–652.
- 366 Strutt, H. and Strutt, D. (2002). *Nonautonomous planar polarity patterning in Drosophila:*  
367 *Dishevelled-independent functions of Frizzled*. *Developmental Cell*, 3(6):851–863.
- 368 Taylor, J., Abramova, N., Charlton, J., and Adler, P. N. (1998). *Van Gogh: a new Drosophila tissue*  
369 *polarity gene*. *Genetics*, 150(1):199–210.
- 370 Umetsu, D. (2022). *Cell mechanics and cell-cell recognition controls by Toll-like receptors in tissue*  
371 *morphogenesis and homeostasis*. *Fly*, 16(1):233–247.
- 372 Vinson, C. R. and Adler, P. N. (1987). *Directional non-cell autonomy and the transmission of polarity*  
373 *information by the frizzled gene of Drosophila*. *Nature*, 329(6139):549–551.
- 374 Wang, Y. and Nathans, J. (2007). *Tissue/planar cell polarity in vertebrates: new insights and new*

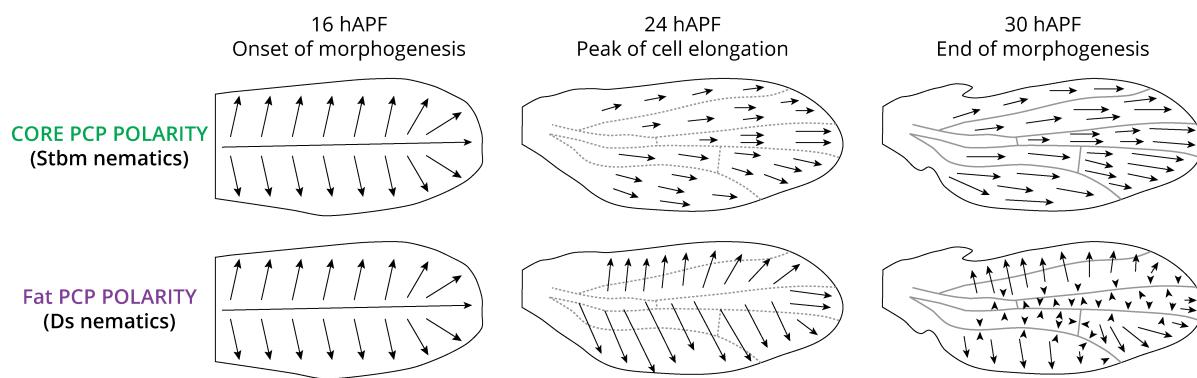
375       questions. *Development*.

- 376 Wilkin, M., Becker, M., Mulvey, D., Phan, I., Chao, A., Cooper, K., Chung, H., Campbell, I., Baron,  
377 M., and MacIntyre, R. (2000). *Drosophila* Dumpy is a gigantic extracellular protein required to  
378 maintain tension at epidermal-cuticle attachment sites. *Current Biology*, 10(10):559–567.
- 379 Wolff, T. and Rubin, G. M. (1998). *strabismus*, a novel gene that regulates tissue polarity and cell fate  
380 decisions in *Drosophila*. *Development*, 125(6):1149–1159.

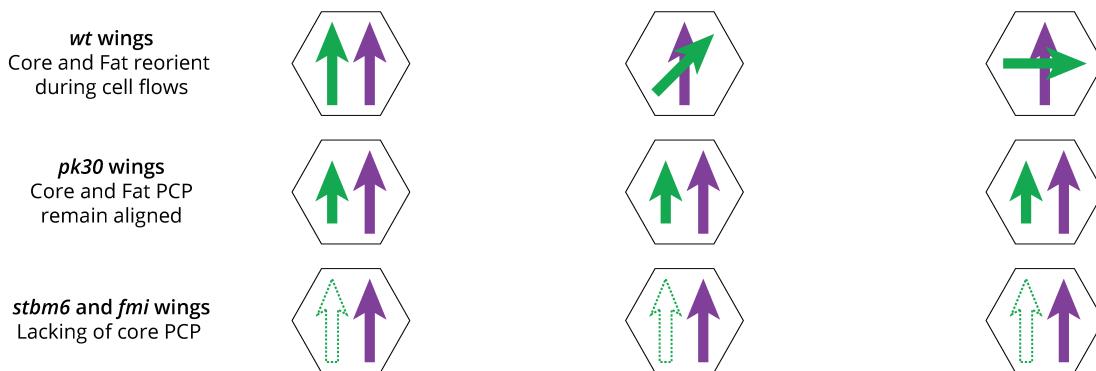
## 381 4 Supplementary Data

### 382 4.1 Fig 1 Supplementary

#### A. REORGANIZATION OF CORE AND FAT PCP DURING PUPAL WING MORPHOGENESIS

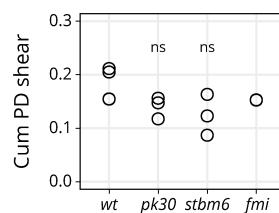


#### B. SCHEMATIC OF THE EVOLUTION OF PCP PATTERNS IN CORE PCP MUTANT WINGS

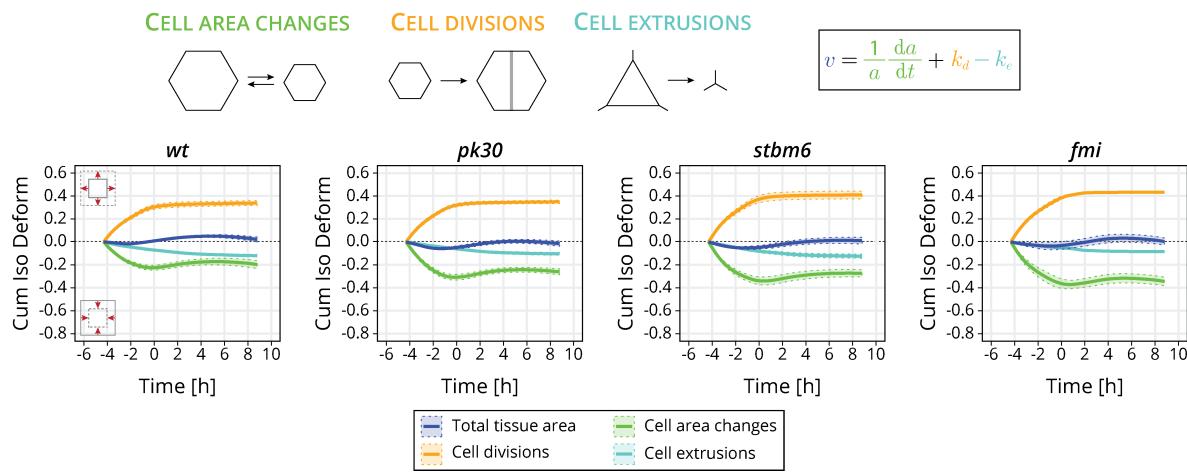


**Figure S1.1: Reorganization of the core and Fat PCP systems during pupal wing morphogenesis:** (A) Evolution of the PCP network during pupal wing morphogenesis. Core PCP polarity is based on Stbm::YFP nematics. Initially, core PCP polarity is radially organized towards the wing margin. As tissue flows occur, it reorients towards the distal tip. Fat PCP polarity is based on the pattern of Ds::EGFP. Fat PCP is initially also radially organized. Tissue flows reorganize Fat PCP and by the end of morphogenesis it is perpendicularly oriented to core PCP. Cartoon adapted from [Merkel et al. \(2014\)](#). (B) Schematic of the core (green arrow) and Fat (purple arrow) PCP patterns in *wt*, *pk30*, and *stbm6* and *fmi* wings. During pupal tissue flows in *wt* wings, core PCP reorients towards the distal tip of the wing. By the end of morphogenesis, core and Fat PCP are perpendicularly aligned. In *pk30* mutant wings, core and Fat PCP remain aligned and core polarity is reduced. In *stbm6* and *fmi* wings, the core PCP network is absent (empty green arrow), while the Fat PCP pattern is unperturbed (purple arrow) ([Merkel et al., 2014](#)).

**C. STATITISTICAL ANALYSIS OF THE FINAL ACCUMULATED TOTAL SHEAR IN *wt*, *pk30*, *stbm6* and *fmi* pupal wings (8.7 h)**

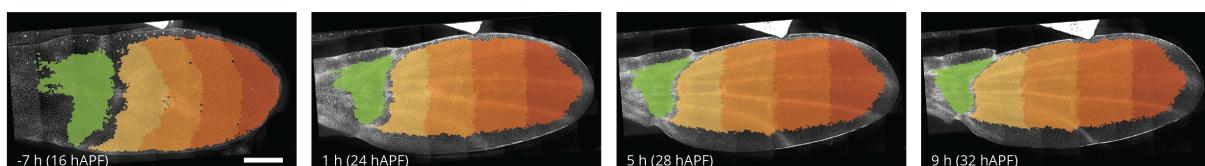


**D. ISOTROPIC TISSUE DEFORMATION IN *wt* AND CORE PCP MUTANT WINGS**

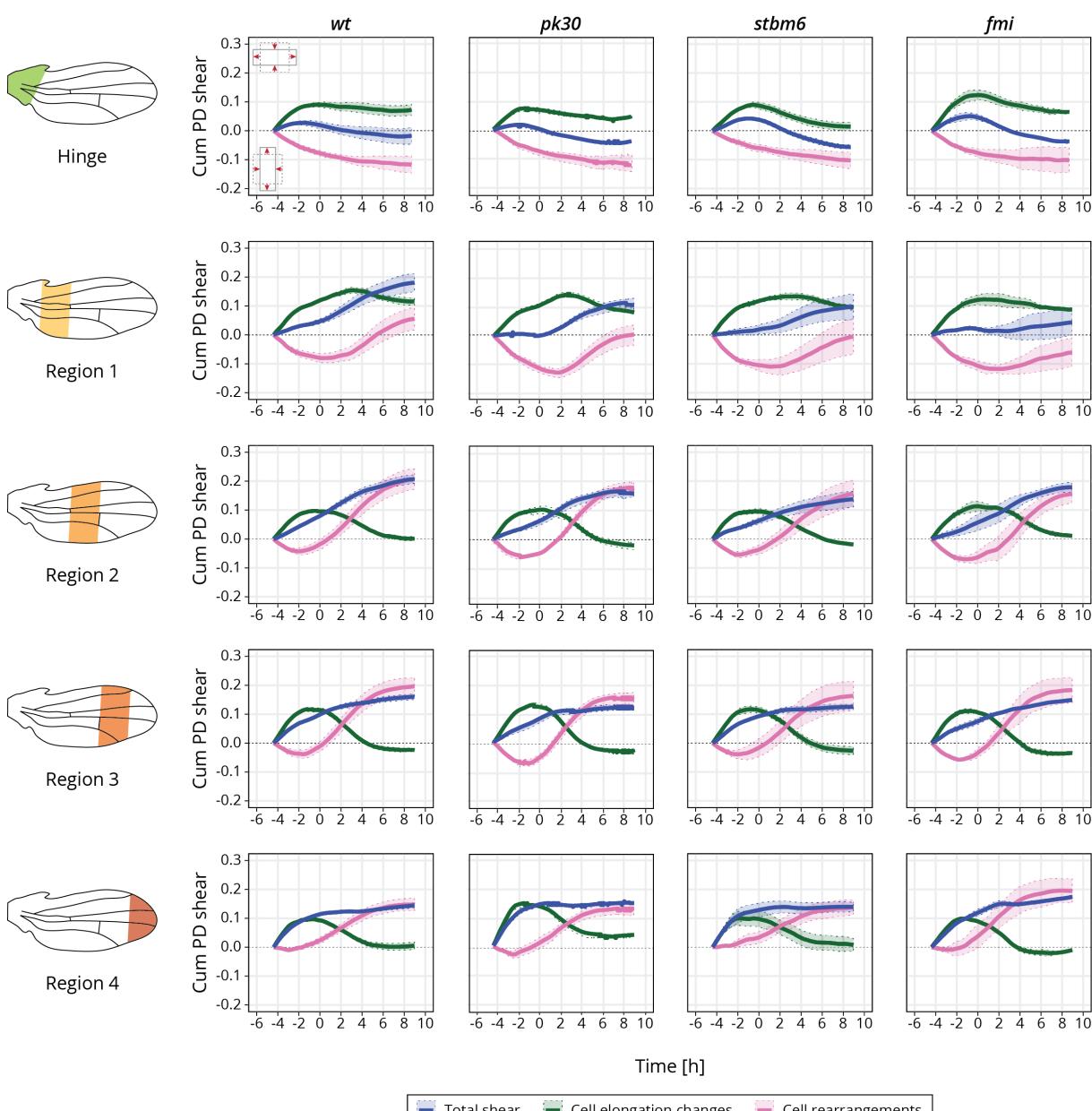


**Figure S1.2: Quantification of final pupal tissue deformation and cellular contributions to isotropic tissue area:** (C) Statistical analysis of the final pupal accumulated total tissue shear in *wt* (n=4), *pk30* (n=3), *stbm6* (n=3) and *fmi* (n=2) movies. Significance is estimated using the Kruskal-Wallis test. ns, p-val>0.05. (D) Quantification of isotropic tissue deformation in *wt* (n=4), *pk30* (n=3), *stbm6* (n=3) and *fmi* (n=2) movies. The cellular contributions are cell area changes (green), cell divisions (yellow) and cell extrusions (cyan). Solid line indicates the mean, and the shaded regions enclose  $\pm$  SEM. The time is relative to the peak of cell elongation.

### E. WING REGIONS USED FOR ANALYSIS OF SHEAR DURING PUPAL WING MORPHOGENESIS



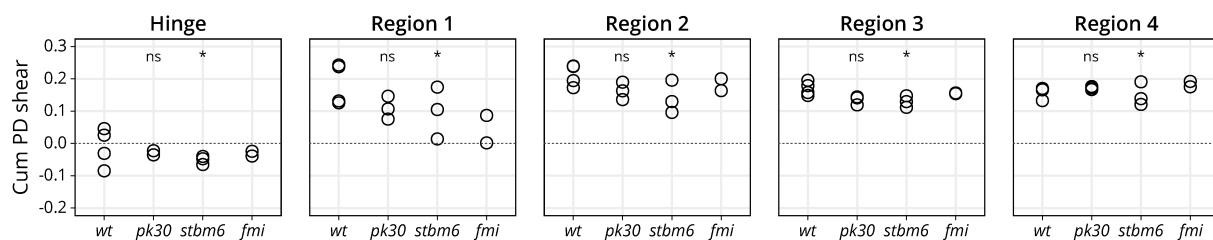
### F. TISSUE SHEAR DECOMPOSITION IN wt AND CORE PCP MUTANT WINGS



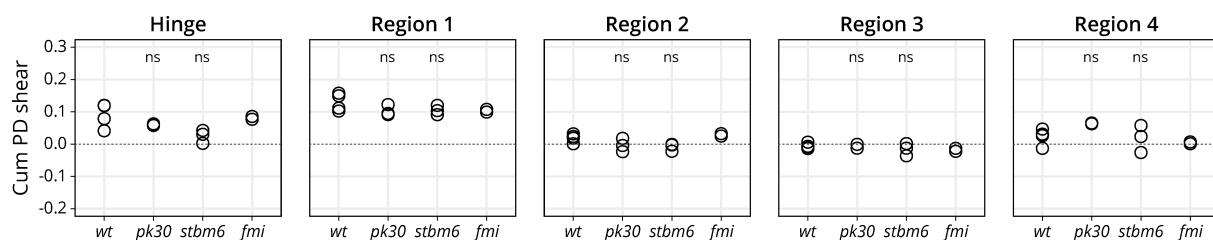
**Figure S1.3: Subregional analysis of tissue shear in the hinge and four blade subregions:** (E) Images of a *wt* wing at -7, 1, 5 and 9 h (for this movie it corresponded to 16, 24, 28 and 32 hAPF). The green region corresponds to the hinge and the four blade subregions are shown in an orange color palette. Scale bar, 100  $\mu\text{m}$ . (F) Total shear (dark blue curve) and decomposition into cell elongation changes (green curve) and cell rearrangements (magenta curve) for the hinge and four blade subregions for *wt* ( $n=4$ ), *pk30* ( $n=3$ ), *stbm6* ( $n=3$ ) and *fmi* ( $n=2$ ). Solid line indicates the mean, and the shaded regions enclose  $\pm$  SEM. The time is relative to the peak of cell elongation.

## G. FINAL TOTAL SHEAR AND DECOMPOSITION FOR THE BLADE REGIONAL ANALYSIS (8.7 h)

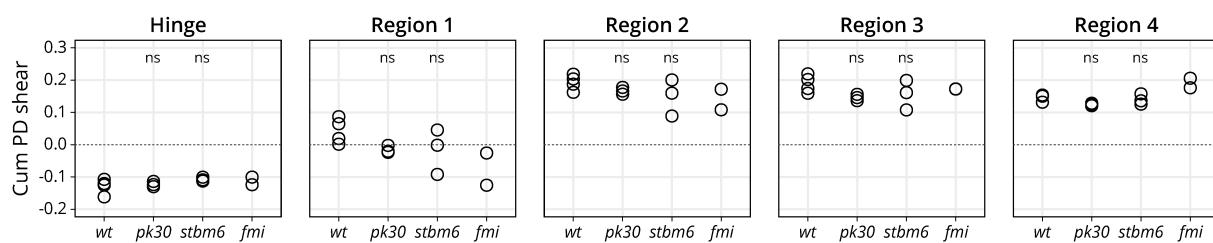
### TOTAL SHEAR



### CELL ELONGATION CHANGES

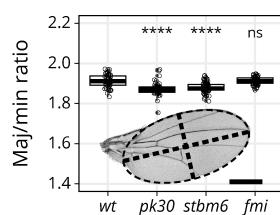


### CELL REARRANGEMENTS



**Figure S1.4: Statistics of final shear in the blade subregions and its cellular contributions.: (G)** Quantification of the final total shear (top row), shear caused by cell elongation changes (middle row) and cell rearrangements (bottom row) in the hinge (left column) and four blade subregions for *wt* (n=4), *pk30* (n=3), *stbm6* (n=3) and *fmi* (n=2). Significance is estimated using the Kruskal–Wallis test. \*, p-val $\leqslant$ 0.05; ns, p-val>0.05.

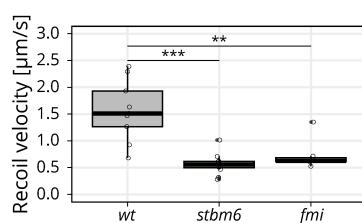
## H. ADULT WING SHAPE QUANTIFICATION IN *wt* AND CORE PCP MUTANT WINGS



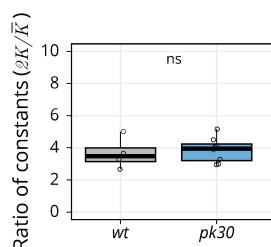
**Figure S1.5: Adult wing shape quantification: (H)** Quantification of the adult wing blade major (maj) to minor (min) ratio for *wt*, *pk30*, *stbm6* and *fmi* wings (n $\geqslant$ 47). Scale bar, 500  $\mu$ m. Each empty circle indicates one wing, and the box plots summarize the data: thick black line indicates the median; the boxes enclose the 1st and 3rd quartiles; lines extend to the minimum and maximum without outliers, and filled circles mark outliers. Significance is estimated using the Kruskal–Wallis test. \*\*\*, p-val $\leqslant$ 0.0001; ns, p-val>0.05.

383 4.2 Fig 2 Supplementary Figures

**A. INITIAL RECOIL VELOCITY UPON ABLATION IN OTHER CORE PCP MUTANT TISSUES AT -8 h**



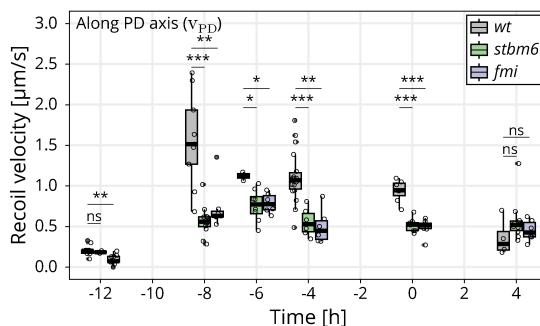
**B. RATIO OF CONSTANT OBTAINED BY ESCA AT -8 h**



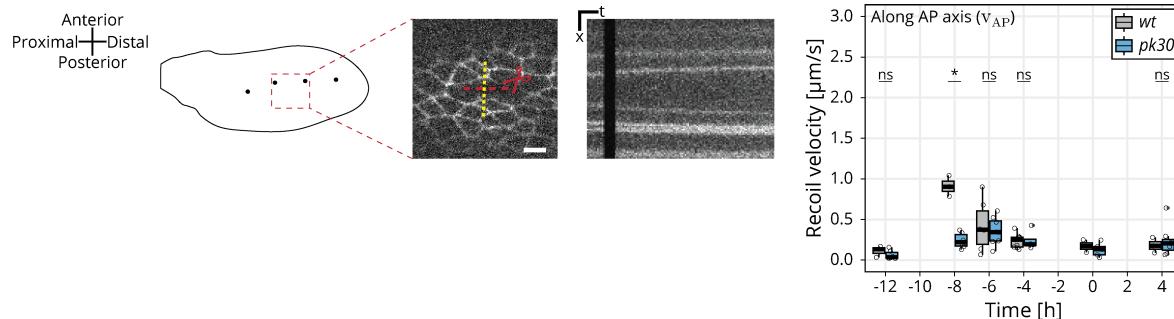
**Figure S2.1: Initial recoil velocity upon linear laser ablation for *stbm6* and *fmi* mutant wings and ratio of elastic constants obtained by ESCA at -8 h:** (A) Initial recoil velocity upon ablation along the PD axis for *wt* (gray), *stbm6* (green) and *fmi* (purple) mutant wings at -8 h ( $n \geq 9$ ). Significance is estimated using the Kruskal-Wallis test. \*\*\*, p-val  $\leq 0.001$ ; \*\*, p-val  $\leq 0.01$ . (B) Ratio of elastic constants ( $2K/\bar{K}$ ) for *wt* and *pk30* (blue) at -8 h ( $n \geq 4$ ). Significance is estimated using the Mann-Whitney U test. ns, p-val  $> 0.05$ . The time is relative to the peak of cell elongation. In both plots, each empty circle indicates one cut, and the box plots summarize the data: thick black line indicates the median; the boxes enclose the 1st and 3rd quartiles; lines extend to the minimum and maximum without outliers, and filled circles mark outliers.

384 **4.3 Fig 3 Supplementary Figures**

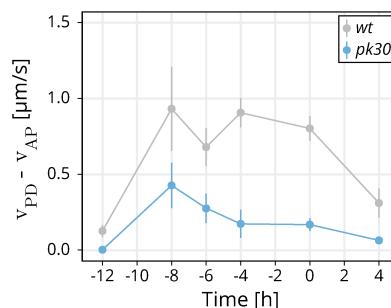
### A. RECOIL VELOCITY ALONG PD AXIS IN OTHER CORE PCP MUTANT TISSUES



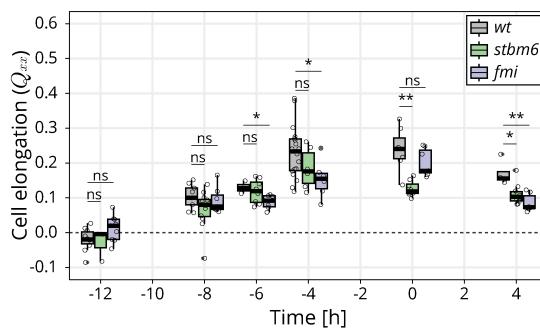
### B. RECOIL VELOCITY ALONG THE AP AXIS FOR wt AND pk30 MUTANT WINGS



### C. PROXY FOR SHEAR STRESS CALCULATED FROM THE DIFFERENCE IN RECOIL VELOCITIES ALONG PD AND AP AXES

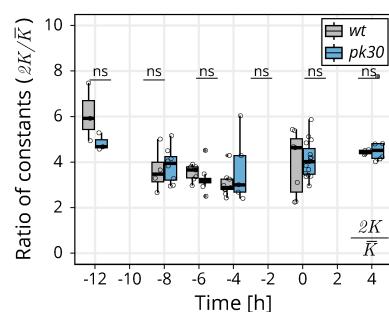


### D. CELL ELONGATION $Q_{xx}$ IN OTHER CORE PCP MUTANT WINGS



**Figure S3.1: Study of pupal wing mechanics over time:** (A) Initial recoil velocity upon ablation along the PD axis for *wt* (gray), *stbm6* (green) and *fmi* (purple) mutant wings throughout morphogenesis ( $n \geq 4$ ). Significance is estimated using the Kruskal-Wallis test. \*\*\*\*,  $p\text{-val} \leq 0.0001$ ; \*\*\*,  $p\text{-val} \leq 0.001$ ; \*\*,  $p\text{-val} \leq 0.01$ ; \*,  $p\text{-val} \leq 0.05$ ; ns,  $p\text{-val} > 0.05$ . (B) Left: Schematic of a *wt* wing at -8 h. Linear laser ablation experiments were performed in the blade region enclosed by the red square. Dots on the cartoon indicate sensory organs. Red line corresponds to the ablation; the kymograph was drawn perpendicularly to the cut (yellow). Scale bar, 5  $\mu\text{m}$ . Right: Initial recoil velocity upon ablation along the AP axis for *wt* (gray) and *pk30* (blue) mutant wings throughout morphogenesis ( $n \geq 3$ ,  $n=2$  in *wt* wings at 0 h). Significance is estimated using the Mann-Whitney U test. \*,  $p\text{-val} \leq 0.05$ ; ns,  $p\text{-val} > 0.05$ . (C) Proxy for shear stress calculated as the difference between the initial recoil velocity along the PD ( $v_{PD}$ ) and AP ( $v_{AP}$ ) axes for *wt* (gray) and *pk30* (blue) mutant wings. Filled colored dots correspond to the mean value, and the error bars report the SEM. (D) Quantification of  $Q_{xx}$  in the blade subregion throughout morphogenesis for *wt* (gray), *stbm6* (green) and *fmi* (purple) mutant wings ( $n \geq 4$ ). Significance is estimated using the Kruskal-Wallis test. \*\*,  $p\text{-val} \leq 0.01$ ; \*,  $p\text{-val} \leq 0.05$ ; ns,  $p\text{-val} > 0.05$ . The time is relative to the peak of cell elongation. In (A), (B) and (D), each empty circle indicates one experiment, and the box plots summarize the data: thick black line indicates the median; the boxes enclose the 1st and 3rd quartiles; lines extend to the minimum and maximum without outliers, and filled circles mark outliers.

**D. RATIO OF CONSTANT OBTAINED BY ESCA**



**Figure S3.2: Ratio of elastic constants throughout morphogenesis:** (D) Ratio of elastic constants ( $2K/\bar{K}$ ) for *wt* and *pk30* (blue) throughout morphogenesis ( $n \geq 3$ ). Significance is estimated using the Kruskal-Wallis test. ns, p-val > 0.05. The time is relative to the peak of cell elongation.

385 **4.4 Supplemental Movie 1**

386 Shown here is an example of a linear laser ablation, cutting 3-4 cells, in wild type (left) or *pk30* pupal  
387 wings. The movie goes dark during the ablation itself. Thereafter, the tissue displaces. Anterior is up;  
388 proximal is left.

389 **5 Materials and Methods**

390 **5.1 Key resources table**

RESOURCE	SOURCE	IDENTIFIERS
<b>Experimental models: Organisms/strains</b>		
<i>D. melanogaster</i> wt: <i>w<sup>-</sup></i> ; <i>EcadGFP</i> ;;	Huang et al. (2009)	
<i>D. melanogaster</i> <i>pk30</i> : <i>w<sup>-</sup></i> ; <i>EcadGFP</i> , <i>pk30</i> ;;	Gubb et al. (1999)	BDSC 44229
<i>D. melanogaster</i> <i>stbm6</i> : <i>w<sup>-</sup></i> ; <i>EcadGFP</i> , <i>stbm6</i> ;;	Wolff and Rubin (1998)	BDSC 6918
<i>D. melanogaster</i> <i>fmi</i> : <i>w<sup>-</sup></i> ; <i>EcadGFP</i> , <i>fmi</i> ;;	Wolff and Rubin (1998)	BDSC 6967
<b>Chemicals</b>		
Euparal	Carl Roth	7356.1
Holocarbon oil 700	Sigma-Aldrich	H8898
Isopropanol (2-propanol)	Sigma-Aldrich	1.01040
<b>Software and algorithms</b>		
Fiji (v. 2.0.0-rc-68/1.52e)	Schindelin et al. (2012)	
Ilastik (v. 1.2.2)	Berg et al. (2019)	
MATLAB (v. 9.2.0.1226206 (R2017a))	MATLAB (2017)	
PreMosa	Blasse et al. (2017)	
R (v. 3.4.1)	R Core Team (2020)	
RStudio (v. 3.6.1)	RStudio Team (2020)	
TissueMiner (v. TM_1.0.2)	Etournay et al. (2016)	
<b>Other equipment</b>		
Coverslip	Paul Marienfeld GmbH	0107052
Microscope slides	Paul Marienfeld GmbH	1000200
Dumont #55 Forceps	Fine Science Tools	11295-51
Vannas Spring Scissors	Fine Science Tools	15000-08

391 **5.2 Fly husbandry**

392 Flies were maintained at 25°C and fed with standard fly food containing cornmeal, yeast extract, soy flour,  
393 malt, agar, methyl 4-hydroxybenzoate, sugar beet syrup and propionic acid. Flies were kept at 25°C in a  
394 12 h light/dark cycle. Vials were flipped every 2-3 days to maintain a continuous production of pupae and  
395 adult flies. All experiments were performed with male flies, since they are slightly smaller and therefore  
396 the wings require less tiling on the microscope to be imaged than females.

397 **5.3 Long-term timelapse imaging of pupal wing morphogenesis**

398 **5.3.1 Acquisition**

399 White male pupae were collected, slightly washed with a wet brush and transferred to a vial with standard  
400 food. At 16 hAPF, the pupal case was carefully dissected so that the wing would be accessible. The pupae  
401 was then mounted onto a 0.017 mm coverslip on a self-built metal dish with a drop of Holocarbon oil 700  
402 ([Classen et al., 2008](#)). Pupal wing morphogenesis was imaged every 5 min for approximately 24 h, as in  
403 [Etournay et al. \(2015\)](#).

404 Two different microscopes were used for acquisition of long-term timelapses. All *wt*, *pk30* and *stbm6*  
405 movies were acquired using a Zeiss spinning disk microscope driven by ZEN 2.6 (blue edition). This  
406 microscope consists of a motorized xyz stage, an inverted stand, a Yokogawa CSU-X1 scan head, and a  
407 temperature-controlled chamber set to 25°C. The sample was illuminated with a 488 nm laser, and the  
408 emission was collected using a 470/40 bandpass filter, through a Zeiss 63x 1.3 W/Glyc LCI Plan-Neofluar  
409 objective and a Zeiss AxioCam Monochrome CCD camera with a 2x2 binning. The whole wing was  
410 imaged in 24 tiles with an 8% overlap. Each tile consisted of 50-60 stacks with a Z-spacing of 1 μm. The  
411 laser power was set to 0.1 mW.

412 The two *fmi* movies were acquired with an Olympus IX 83 inverted stand driven by the Andor iQ 3.6  
413 software. The microscope is equipped with a motorized xyz stage, a Yokogawa CSU-W1 scan head and an  
414 Olympus 60x 1.3 Sil Plan SApo objective. The setup was located inside a temperature-controlled chamber  
415 set to 25°C. The sample was illuminated with a 488 nm laser, and the emission was collected using a  
416 525/50 bandpass filter. The whole wing was imaged by tiling with 8 tiles with a 10% overlap. Each tile  
417 consisted of 50-60 stacks with a distance of 1 μm between them. The laser power was set to 0.75 mW.

418 **5.3.2 Processing, segmentation, tracking and database generation**

419 Raw stacks were projected, corrected for illumination artifacts, and stitched using PreMosa ([Blasse et al.,](#)  
420 [2017](#)). The stitched images of individual timepoints were cropped to fit the wing size, registered using the  
421 Fiji plugin “Descriptor-based series registration (2D/3D + t)” and converted to 8 bit with Fiji ([Schindelin](#)  
422 [et al., 2012](#)). The segmentation was performed with the Fiji plugin TissueAnalyzer ([Schindelin et al., 2012;](#)  
423 [Aigouy et al., 2010; Aigouy et al., 2016](#)). Segmentation errors were identified and manually corrected by  
424 looking at the cell divisions and deaths masks.

425 Subsequent processing and quantifications were performed using TissueMiner [Etournay et al. \(2016\)](#).  
426 Before generating the relational database, we rotated the movies so that the angle formed by a manually

427 drawn line connecting the sensory organs would be 0. We manually defined the regions of interest, such  
428 as the blade, hinge and the anterior and posterior regions, using the last frame of the movie. Next, we  
429 generated the relational database containing information about the cellular dynamics during morphogenesis  
430 using *TissueMiner* ([Etournay et al., 2016](#)).

431 We queried and worked with the data using the Dockerized version of RStudio ([Nickoloff, 2016](#)), which  
432 loads all packages and functions required to work with *TissueMiner*. Movies were aligned by the peak of  
433 cell elongation by fitting a quadratic function around the cell elongation values 40 frames before and after  
434 the absolute maximum of cell elongation in the blade region for each movie. The maximum of this curve  
435 was identified and set as the timepoint 0 h.

#### 436 5.4 Adult wing preparation and analysis of wing shape

437 Adult male flies were fixed in isopropanol for at least 12 h. One wing per fly was dissected in isopropanol,  
438 transferred to a microscope slide and covered with 50% euparal in isopropanol. Wings were mounted with  
439 50-70  $\mu\text{L}$  75% euparal/isopropanol.

440 *wt*, *pk30* and *stbm6* wings were imaged using a Zeiss widefield AxioScan Z1 microscope equipped with  
441 a Zeiss 10x 0.45 Air objective. *fmi* wings were imaged using a Zeiss widefield Axiovert 200M microscope  
442 equipped with a Zeiss 5x 0.15 Plan-Neofluar air Zeiss objective.

443 Wing blade parameters were quantified using a custom-written Fiji macro ([Schindelin et al., 2012](#)). The  
444 shape or major-to-minor ratio was calculated using a custom RStudio script ([R Core Team, 2020](#); [RStudio](#)  
445 [Team, 2020](#)).

#### 446 5.5 Quantification of the PD component of cell elongation $Q_{xx}$

447 Prior to all laser ablation experiments, we acquired a stack of 50  $\mu\text{m}$  thick that was projected using  
448 PreMosa ([Blasse et al., 2017](#)). We cropped a region that enclosed the region that was ablated, segmented  
449 cells using *TissueAnalyzer* ([Aigouy et al., 2010,1](#)) and generated a relational database with *TissueMiner*  
450 ([Etournay et al., 2016](#)).

451 The definition of cell elongation was first presented in ([Aigouy et al., 2010](#)) and it describes the angle  
452 and magnitude of the tensor. The cell elongation tensor is given by

$$\begin{pmatrix} \epsilon_{xx} & \epsilon_{xy} \\ \epsilon_{xy} & -\epsilon_{xx} \end{pmatrix}, \quad (2)$$

453 where

$$\epsilon_{xx} = \frac{1}{A_c} \int \cos(2\phi) dA \quad (3)$$

454 and

$$\epsilon_{xy} = \frac{1}{A_c} \int \sin(2\phi) dA. \quad (4)$$

455 Cell elongation is normalized by the cell area ( $A_c$ ) of each cell. The magnitude of cell elongation is:

$$\epsilon = (\epsilon_{xx}^2 + \epsilon_{xy}^2)^{1/2} \quad (5)$$

456 Here we plot  $\epsilon_{xx}$  as  $Q_{xx}$ , which we describe as the PD component of cell elongation.

## 457 5.6 Laser ablation experiments

458 Pupae were dissected and mounted as described for the long-term timelapses. Ablations were always  
459 performed in the same region of the wing blade, found in the intervein region between the longitudinal  
460 veins L3 and L4 and between the second and third sensory organs. This region was chosen because these  
461 landmarks are easily visible in all timepoints. Laser ablations were performed using a Zeiss spinning disc  
462 microscope equipped with a CSU-X1 Yokogawa scan head, an EMCCD Andor camera, a Zeiss 63x 1.2  
463 water immersion Korr UV-VIS-IR objective and a custom-built laser ablation system using a 355 nm,  
464 1000 Hz pulsed ultraviolet (UV) laser (Grill et al., 2001; Mayer et al., 2010). The imaging and cutting  
465 parameters for line and circular laser ablations are shown in Table 1.

**Table 1:** Parameters used to perform laser ablations.

	LINEAR ABLATIONS	CIRCULAR ABLATIONS
Exposure time [s]	0.05	0.05
488 nm laser intensity [%]	50	50
Time interval [s]	0.09	2.55
Pulses per shot	25	25
Shots per $\mu\text{m}$	2	2
Shooting time [s]	0.67	147.28
Thickness of stack ablated [ $\mu\text{m}$ ]	1	20

### 466 5.6.1 Linear laser ablations to calculate the initial recoil velocity

467 We performed both types of linear ablations in only one plane of the tissue, in order to minimize the time  
468 required for ablation and therefore be able to acquire the initial recoil velocity upon ablation (no imaging  
469 is possible during ablation). The length of the linear laser ablations was 10  $\mu\text{m}$ , ablating 3-4 cells. We drew  
470 kymographs perpendicularly to the cut to follow the two edges of one ablated cell using Fiji (Schindelin  
471 et al., 2012). The initial recoil velocity was calculated as the average displacement of two membranes  
472 of the same cell that occurred during the black frames of the ablation itself. This calculation was made  
473 using a self-written MATLAB script (MATLAB, 2017). The image acquired prior to the laser ablation  
474 was used to compute  $Q_{xx}$  in that region, as described in Subsection 5.5, and the time corresponding to  
475 the maximum of cell elongation was defined as 0 h.

476 **5.6.2 Elliptical Shape after Circular Ablation (ESCA)**

477 Circular laser ablations used for ESCA were  $20 \mu\text{m}$  in radius (approximately 10 cells). This radius was  
478 selected was that it would fit into the same blade region throughout morphogenesis. Due to the bigger size  
479 of these cuts and the curvature of the tissue, we cut the tissue along a stack of  $20 \mu\text{m}$  thick. Approximately  
480 2 min after the ablation, we acquired a stack of  $50 \mu\text{m}$ . This image was projected using PreMosa ([Blasse et al., 2017](#)) and preprocessed by applying a Gaussian blur ( $\sigma=1$ ) and background subtraction (rolling  
481 ball radius = 30) in Fiji ([Schindelin et al., 2012](#)). The next steps were performed as in [Dye et al. \(2021\)](#):  
482 the image of the final shape of the cut was segmented using Ilastik ([Berg et al., 2019](#)) by defining three  
483 regions: membrane, cell and dark regions. The segmented image was thresholded to obtain a binary image  
484 of the final shape of the cut. We fitted two ellipses to this image: one to the inner piece and another  
485 one to the outer outline of the cut. Based on the shape of these ellipses, the method outputs the  
486 anisotropic  $\frac{\tilde{\sigma}}{2K}$  and isotropic stress  $\frac{\sigma}{K}$  as a function of their respective elastic constants, and the ratio of  
487 elastic constants  $\frac{2K}{K}$ . A small number of experiments were fitted poorly (defined as an error per point  
488 greater than 0.3) and were therefore excluded from analysis. Prior to the circular ablation, a stack of  
489  $50 \mu\text{m}$  was acquired and used to calculate cell elongation before ablation (Subsection 5.5). The time  
490 corresponding to the maximum of cell elongation was set to be 0 h.

492 **5.6.3 Kymograph analysis and fit to model**

493 The ablations used to calculate the mechanical stress along the PD axis for *wt* and *pk30* were further  
494 analyzed with the rheological model. To do so, we processed the kymographs by applying a Gaussian  
495 blur ( $\sigma=1$ ) ([Schindelin et al., 2012](#)), and then we segmented these kymographs with Ilastik ([Berg et al., 2019](#)). Using a self-written Fiji macro ([Schindelin et al., 2012](#)), we extracted the intensity profile for  
496 each timepoint. Next, we wrote an R script ([R Core Team, 2020](#); [RStudio Team, 2020](#)) to identify the  
497 membrane displacement over time and obtained a unique curve per kymograph, which could be fitted  
498 with our model. We modelled a local patch of tissue as a combination of a spring with spring constant  
499  $k$ , representing the ablated cells and two KV elements with spring constants  $k_f$  and  $k_s$  and viscosity  
500 coefficients  $\eta_f$  and  $\eta_s$ , representing the unablated cells, as shown in (Fig 2C-D). Because the local tissue  
501 strain in the experimental measurement is expressed by the displacement of the bond nearest to the  
502 ablation, in the rheological model we represent tissue strain by displacements of the two KV elements.  
503 In principle, the strain can be recovered by normalising the displacements by the width of ablated cells.  
504 Displacements of the two KV elements are defined as a change in the distance between the end points  
505 of the KV elements  $x_i(t)$ , relative to their initial values  $x_i(0)$ , where  $i \in \{f, s\}$  for fast ( $f$ ) and slow ( $s$ )  
506 element.

508 Mechanical stress in the tissue is represented by the  $\sigma$  acting on our model, and we assume that  $\sigma$  is  
509 not changed by the ablation. Before the ablation, the model is in mechanical equilibrium and we can  
510 write

$$\sigma = (k + \bar{k})x(0) \quad , \quad (6)$$

where  $x(0)$  is the initial distance between the two end points of the model, and  $\bar{k} = k_f k_s / (k_f + k_s)$  is the

elastic constant of the two KV elements connected in series. Upon ablation, the spring  $k$  is removed and stresses in the model are imbalanced. The distance between the end points of the model  $x(t)$  then evolves towards the new equilibrium position. The distance  $x(t)$  can be decomposed as  $x(t) = x_f(t) + x_s(t)$ , where  $x_f(t)$  and  $x_s(t)$  are the time-dependent distances between end points of the two KV elements, representing their strains. The dynamics of  $x(t)$  is then obtained by writing the force balance equation for the two KV elements

$$\sigma = k_f x_f(t) + \eta_f \frac{dx_f(t)}{dt} , \quad (7)$$

$$\sigma = k_s x_s(t) + \eta_s \frac{dx_s(t)}{dt} , \quad (8)$$

We solve for  $x_f(t)$  and  $x_s(t)$  to obtain

$$x_f(t) = \frac{\sigma}{k_f} (1 - e^{-t/\tau_f}) + x_f(0) e^{-t/\tau_f} , \quad (9)$$

$$x_s(t) = \frac{\sigma}{k_s} (1 - e^{-t/\tau_s}) + x_s(0) e^{-t/\tau_s} , \quad (10)$$

where

$$x_{f,s}(0) = \frac{\sigma(1 - \kappa)}{k_{f,s}} , \quad (11)$$

where  $\kappa = k/(k + \bar{k})$  is the fraction of the overall model elasticity  $k + \bar{k}$  destroyed by the ablation. The displacement relative to the initial configuration  $\Delta x(t) = x(t) - x(0)$  is therefore

$$\Delta x(t) = X_f \left( 1 - e^{-t/\tau_f} \right) + X_s \left( 1 - e^{-t/\tau_s} \right) , \quad (12)$$

where we introduced the long time displacements associated with the two KV elements

$$X_{f,s} = \frac{\sigma\kappa}{k_{f,s}} . \quad (13)$$

511 For simplicity, in the main text we refer to the long time displacements  $X_f$  and  $X_s$  of the two KV elements  
 512 simply as displacements.

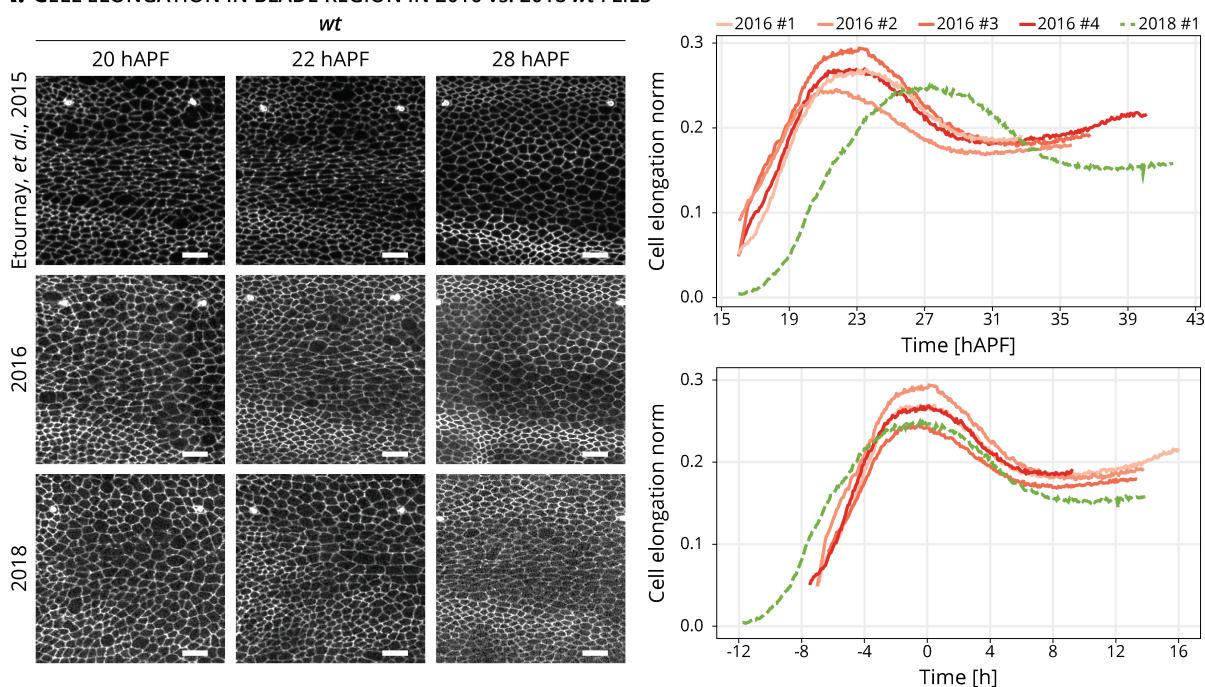
## 513 5.7 Statistical analysis

514 Statistical analysis was done using R ([R Core Team, 2020](#); [RStudio Team, 2020](#)). We first tested normality  
 515 of the data using the Shapiro–Wilk test. When data were normal, we used Student’s t-test to test statistical  
 516 significance between two groups. When data were not normally distributed, significance was tested using  
 517 the Mann-Whitney U test for two groups or Kruskal-Wallis test for multiple groups. Statistical test results  
 518 are shown on the figure captions.

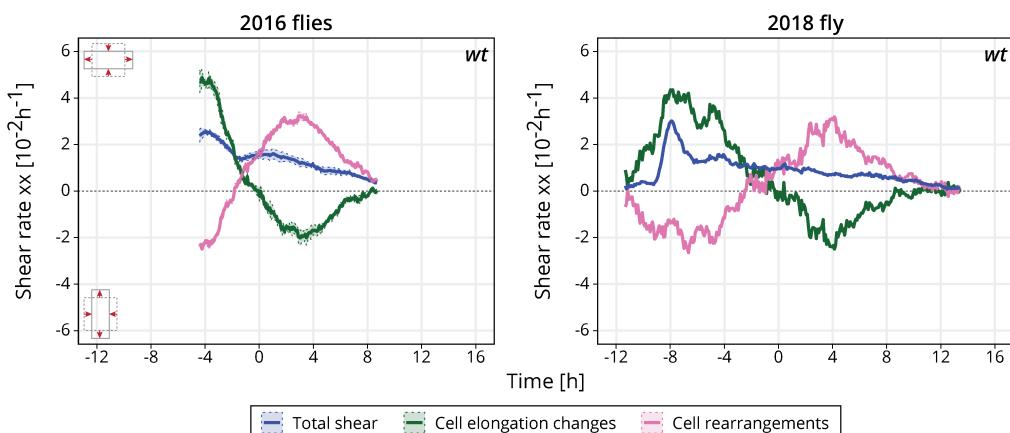
## 519 1 Appendix 1

520 During the course of this work, we identified a delay on the onset of pupal wing morphogenesis compared  
 521 to previous work (Etournay et al., 2015). In the past, cells reached their maximum of cell elongation at  
 522  $22.9 \pm 0.4$  hAPF, while now they reach it at 28 hAPF (Fig A1.1A). To combine data acquired at different  
 523 times, we present cell dynamics data aligned in time by the peaks of cell elongation, and we refer to this  
 524 timepoint as 0 h (Fig A1.1B). We investigated the cell dynamics underlying tissue morphogenesis in the  
 525 delayed flies and observed that the shear rates were comparable with the older flies (Fig A1.1B). Thus, it  
 526 is reasonable to shift the curves by aligning them to a new reference time.

### A. CELL ELONGATION IN BLADE REGION IN 2016 vs. 2018 wt FLIES



### B. TISSUE SHEAR RATES OF NEW AND OLD wt FLIES



**Figure A1.1: Delay and time alignment of old and newer wt flies:** (A) Left: snapshots of the blade region of long-term timelapses of pupal wing morphogenesis acquired in different years. Scale bar, 10  $\mu$ m. Right: cell elongation norm during pupal wing morphogenesis for old flies (orange palette, 2016 flies) and new flies (green curve, 2018 fly). Top plot: cell elongation magnitude for each movie not aligned in time. The peak of cell elongation is delayed from around 23 hAPF to 28 hAPF. Bottom plot: cell elongation magnitude aligned in time. The time 0 h corresponds to the peak of cell elongation. (B) Cell dynamics contributions underlying anisotropic tissue deformation for older ( $n=4$ ) and newer ( $n=1$ ) wt flies. The time is relative to the peak of cell elongation.