

15 **ABSTRACT**

16 Cell polarity is essential for the architecture and function of numerous epithelial tissues. Here we show
17 how planar cell polarity (PCP), so far studied principally in flat epithelia, is deployed during the
18 morphogenesis of a tubular organ. Using the mammalian pancreas as a model, we report that
19 components of the core PCP pathway such as the transmembrane protein Van Gogh-like (VANGL), are
20 progressively apically-restricted. VANGL expression becomes asymmetrically localized at the apical
21 surface of ductal cells, revealing a planar polarization of the pancreatic duct. We further show that
22 restricting VANGL to these discrete sites of expression is crucial for epithelial integrity. Expansion of
23 expression on basolateral membranes of the progenitors leads to their death and extrusion from the
24 epithelium, as previously observed for perturbations of apico-basal polarity. Using organoids and *in vivo*
25 analyses, we show that cell elimination is induced by a decrease of Rock activity *via* Dishevelled.

26 **INTRODUCTION**

27 Establishment of polarity is essential for the function of many cell types. In epithelia two axes of polarity
28 have been characterized: 1) apico-basal polarity orients cells from the free surface or the lumen to the
29 basal lamina and 2) planar polarity coordinates polarization of cell structures or behaviors in the plane of
30 the tissue, orthogonal to the apico-basal axis. While visualizing the apico-basal axis is relatively easy, few
31 cell types present overtly polarized structures suitable for the study of planar polarity. Much of what is
32 known regarding planar cell polarity (PCP) has been gleaned from studies of flat monolayers in which
33 planar polarity coordinates the orientation of cell appendices such as trichomes in fly wings or
34 stereocilia bundles in the inner ear¹. Here, we investigate its organization and function in more complex
35 epithelia, such as tubes.

36 A key feature of planar polarity is the asymmetric localization of core molecules of the pathway along
37 both the apical-basal polarity axis and the plane of the epithelium. An evolutionarily-conserved core

38 group of six families of proteins is required to coordinate planar polarization between neighboring cells:
39 the transmembrane proteins Frizzled (vertebrate: FZD; fly: FZ), CELSR (CELSR; FMI) and Van Gogh-like
40 (VANGL; STBM/VANG) and the cytosolic-submembrane proteins Dishevelled (DVL; DSH),
41 Inversin/Diversin (INV/ANKRD6; DGO), and Prickle (PK). The PCP signaling system employs intra- and inter-
42 cellular feedback interactions between its core components to establish their characteristic asymmetric
43 cellular distributions. In the most comprehensively-characterized system, the *Drosophila* wing blade, the
44 FZ-DSH-DGO complex localizes to intercellular junctions facing the VANG-PK complex in the adjacent
45 cell. FMI, an atypical cadherin, localizes to both sides of the apical junction and bridges the FZ and VANG
46 complexes between neighboring cells. These intercellular interactions are counterbalanced
47 intracellularly by reciprocal inhibitory interactions between the two complexes^{1,2}.

48 While traditionally depicted in flat epithelia that require directional motion or an oriented structure, it is less
49 clear how these proteins are organized or function in complex epithelia forming tubular structures³. The
50 developing pancreas consists of a network of tubes lined by polarized progenitors, the apical membranes of
51 which line the duct lumen^{4,5}. This network is dynamic and remodeled throughout development while the
52 pancreatic epithelium grows and progenitors commit to various specialized fates⁶. At embryonic day
53 E10.5, multipotent progenitors are organized around discrete micro-lumens^{7,8}. By E12.5, these micro-
54 lumens have fused to form a mesh of interconnected ducts. Distal “Tip” progenitors committed to an
55 exocrine fate are restricted to the periphery while the core of this plexus contains bipotent “Trunk”
56 progenitors that subsequently either remain in the epithelium to form mature duct cells or delaminate
57 to give rise to endocrine cells. By birth, the ducts are remodeled into an arborized structure,
58 transporting duct- and acinar-secreted bicarbonate and digestive enzymes respectively to the
59 duodenum, while the endocrine cells coalesce into islets of Langerhans embedded near the ductal
60 system.

61 As endocrine differentiation is decreased as a result of mutation of CELSR2/3, it suggests PCP
62 components are essential regulators linking pancreas morphogenesis with cell fate⁹. However, how
63 would these components be localized in a radially symmetric tubular epithelium and what would their
64 function be in tubular organs remains to be determined. VANGL, a component specific to PCP, plays a
65 central role in the establishment of the core protein complex¹⁰. Mutations in the VANGLs underlie a
66 broad spectrum of developmental disorders in humans¹¹ such as neural tube defects^{12,13} and renal^{14,15},
67 heart^{16,17} and lung diseases¹⁸. Moreover, recent studies have shown that VANGL2 is consistently
68 upregulated and amplified in breast, ovarian and uterine carcinomas¹⁰ and a tumorigenic function has
69 been established in rhabdomyosarcoma¹⁹.

70 In this study, we investigated how planar polarity is deployed in the development of a complex tubular
71 organ, the pancreas, and how that spatial information is integrated to enable proper morphogenesis.
72 We show that expression of the core PCP molecule VANGL2 is planar polarized and we highlight an
73 enrichment of that protein at tricellular junctions. By perturbing the localization of VANGL2, we
74 demonstrate that a stringent regulation of the level and localization of this protein at the membrane of
75 progenitors is required to maintain epithelial integrity. In particular, we show that apical restriction of
76 VANGL is essential for progenitor survival as an extension of its expression domain on the basolateral
77 membrane leads to apoptosis *via* downregulation of the ROCK pathway. Moreover, systematic
78 examination of the localization of other core PCP components reveals a cell-autonomous negative
79 regulation of VANGL on Dishevelled, reinforcing the model of inhibitory intracellular interaction
80 currently proposed in lower vertebrates.

81 **RESULTS**

82 **Planar polarization of PCP components in the tubular epithelium of the pancreas**

83 We have previously shown that the expression of VANGL1/2 is initiated in the pancreatic epithelium at
84 around E11 when apico-basal polarity is first established⁹. Its cellular localization becomes progressively
85 restricted to apical foci at cell junctions (Fig. 1a,b, S1d). In order to determine whether VANGL1/2 is
86 asymmetrically expressed in the plane of the epithelium, we performed whole mount staining which
87 allowed us to visualize pancreatic ducts in three dimensions (3D) (Fig. 1e-g). Co-staining for Mucin1 and
88 β -Catenin, respectively marking apical and lateral membranes, revealed that ductal cells were
89 anisotropic, exhibiting elongated apical surfaces along the longitudinal axis of the ducts (Fig. 1f,g).

90 VANGL1/2 was distributed asymmetrically in the apical plane, the protein being enriched on the
91 transverse membranes (perpendicular to the longitudinal axis of the duct) while it was almost absent on
92 the longitudinal membranes (Fig. 1f,h and movie 1). While an asymmetric expression pattern
93 characterizes the localization of PCP core components in flat monolayered epithelia, these findings
94 reveal that this is conserved in a branched, tubular network. We also observed an enrichment of
95 VANGL1/2 protein at tricellular junctions, “hot-spots” of epithelial tension²⁰ (Fig. 1g).

96 We then investigated the expression of Frizzled proteins which is also planar polarized in flat epithelia.
97 Although both FZD3 and FZD6 receptors can mediate PCP signaling²¹, we were only able to detect FZD3.
98 Similarly to VANGL1/2 expression, at E10.5, small foci of FZD3 protein were detected around forming
99 micro-lumens (Fig. S1a). At E12.5, FZD3 expression was enriched apically in the branching epithelium
100 (Fig. S1b). Though largely restricted to the membrane, some cytoplasmic expression was visible (Fig.
101 S1b). By E14.5, FZD3 was restricted to the apical-most regions of the lateral membranes, appearing as
102 sub-apical striations of expression (Fig. S1c), which, by E18.5, condensed to foci at the apical cell
103 junctions (Fig. 1d). Both FZD3 and VANGL1/2 were expressed in distal tip and proximal trunk cells of the

104 epithelium, with VANGL1/2 expression becoming enriched in the trunk at later stages (Fig. 1a,c). Both
105 were subsequently detected in islets of Langerhans at E18.5 (data not shown). Taken together, the
106 membranous proteins of the PCP display progressive apical restriction in tubes as well as planar
107 expression for at least VANGL.

108 **Ectopic expression of VANGL2 on baso-lateral membranes leads to cell death and pancreatic**
109 **hypoplasia**

110 Though the importance of asymmetric expression of PCP proteins in the plane is well established in both
111 *Drosophila*²² and vertebrates²³, little is known regarding their apical restriction. To test the significance
112 of VANGL2 expression and localization, we generated transgenic mouse lines in which Cre-mediated
113 recombination inactivates β -actin promoter-driven GFP expression and induces expression of a Cherry-
114 VANGL2 fusion protein (Fig. 2d). This construct was previously reported to express a functional
115 protein²⁴. When expressed in pancreatic progenitors using *Pdx1*-Cre, the fusion protein was initially
116 detected at the cell cortex in of most pancreatic epithelial cells at E10.5 and by E12.5 became
117 membrane-enriched (Fig. S2a-b). Mesenchymal cells surrounding the epithelium expressed GFP, as
118 expected from the non-recombined transgene (Fig. S2c). In contrast to endogenous VANGL proteins
119 which are restricted to the apical junction in *wild-type* pancreata (Fig. 2a), expression of the Cherry-
120 VANGL2 fusion protein showed no such delimitation and was detected throughout the membrane (Fig.
121 2b-c).

122 To determine whether this ectopic expression of VANGL protein throughout the cell membrane
123 impacted upon pancreas development, we analyzed pancreatic epithelial size in double-transgenic *Pdx1*-
124 *Cre; Cherry-Vangl2* (*Ch-Vgl2*) mice *versus* control (single-transgenic) littermates. At E10.5, when Cherry-
125 VANGL2 protein was first detectable in double-transgenic animals, their pancreata were equivalent in
126 size to controls (Fig. S2d) but by E12.5, we observed a ~60% decrease in *Ch-Vgl2* pancreatic epithelial

127 size (Fig. 2e, S2e). Pancreatic hypoplasia in *Ch-Vgl2* embryos was maintained at E14.5 (Fig. 2f,g) and was
128 exacerbated at E16.5 (Fig. S2f). At this stage, we observed an ~85% decrease in PDX1⁺ progenitors and
129 endocrine (Glucagon⁺ or Insulin⁺) cells (Fig. S2f). Pancreatic hypoplasia was consistently observed in
130 double-transgenic embryos generated from three independent lines of *Cherry-Vangl2* mice with varying
131 transgene expression levels (Fig. S2e). Of note, for each line, the variability in pancreatic size between
132 distinct *Ch-Vgl2* embryos likely reflects heterogeneity observed in the recombination rate between
133 individuals.

134 To test whether hypoplasia of Cherry-VANGL2-expressing pancreata resulted from reduced
135 proliferation, we quantified the proportions of phospho-HistoneH3-expressing cells in the epithelium.
136 No significant difference was detected between the ratios of proliferating epithelial progenitors in E12.5
137 *Ch-Vgl2* versus control siblings (Fig. S2g-h). In contrast, while TUNEL assays performed on E12.5 litters
138 revealed only a few (0.07% in average) apoptotic cells in control pancreatic epithelia, we observed a 2.2-
139 fold increase in TUNEL⁺ pancreatic epithelial cells in *Ch-Vgl2* littermates (Fig. 2h). Moreover, the extent
140 of hypoplasia positively correlated with the proportion of apoptotic cells (Pearson correlation test:
141 $r=0.8$; $p<0.05$) (Fig. S2k). In the *Ch-Vgl2* embryos, we also observed elevated TUNEL signal among the
142 mesenchymal cells located just in the periphery of the epithelium (Fig. 2h, arrow and Fig. S2j). We
143 presume that these cells, located at a distance of one or two cell diameters from the epithelium, are
144 epithelial cells that have recombined and extruded from the distal-most epithelial layer. Staining for
145 Caspase3 confirmed these observations, revealing an increased number of Caspase3⁺ cells in the
146 epithelium expressing the Cherry-VANGL2 fusion protein (Fig. 2i,j), with some of those cells departing
147 the outer-most epithelium (Fig. 2l). Furthermore, we detected dying (Caspase3⁺) cells retaining Cherry
148 expression, suggesting that Cherry-VANGL2 expression cell-autonomously manifests in cell death (Fig.
149 2k). Taken together, these data show that ectopic expression of Cherry-VANGL2 protein in the

150 pancreatic epithelium leads to apoptosis of the progenitors and, subsequently, to hypoplasia of the
151 pancreas.

152 **Epithelial exit and destruction of epithelial integrity upon VANGL mislocalization to all cell membranes**

153 To test the hypothesis that VANGL extension to all cell membranes may promotes epithelial exit, we
154 conducted live imaging on pancreatospheres. These structures comprise a spherical monolayered
155 pancreatic progenitor epithelium and are thus simpler than the branched pancreatic epithelium, and
156 more amenable for observation of epithelial exit in live imaging^{25,26}. We generated pancreatospheres
157 from *Ch-Vgl2* and control littermate pancreatic epithelium at E13.5, after PCP was established. We
158 consistently observed increased cell delamination in spheres originating from *Ch-Vgl2* pancreata (movies
159 2, 3). These events lead to a disruption of the epithelium, resulting in inward collapse of
160 pancreatospheres (Fig. 2m, n and movies 4, 5, 6). We have recently shown that pancreatic progenitors
161 lining ducts secrete fluid into the lumen from E10.5⁶. We interpret that disruption of epithelium integrity
162 leads to collapse because of either a release of internal fluid pressure or disruption of supracellular apical
163 cortical tension.

164 **VANGL misexpression leads to decreased actomyosin contractility**

165 Actomyosin contraction, mediated by activation of Rho kinase, is well-recognised as an important
166 downstream effector of the PCP^{11,27,28} and VANGL, in particular, has been involved in remodeling of actin
167 microfilaments²⁹⁻³³. Moreover, actomyosin contractility is associated with apoptosis in different
168 contexts³⁴. For these reasons, we examined phosphorylation of myosin regulatory light chain (pMLC), a
169 substrate of Rho kinase and effector/read-out of actomyosin-based contraction³⁵ in pancreata from *Ch-*
170 *Vgl2*. Immunostaining showed pMLC to be apically polarized in E12.5 control pancreata while the
171 protein was undetectable in *Ch-Vgl2* pancreatic epithelia (Fig. S3a-b). At E14.5, pMLC was expressed at
172 the apical cell junction in controls (Fig. 3a,c), mirroring the distribution of core PCP components, but

173 pMLC was undetectable in Cherry-VANGL2-expressing epithelial domains (Fig. 3b). Western blotting on
174 E12.5 (Fig. S3c) and E14.5 (Fig. 3d-e) dorsal pancreata confirmed these observations, revealing a 60%
175 decrease in average of pMLC expression in E14.5 pancreata from *Ch-Vgl2* embryos compared with
176 control siblings (Fig. 3e). Taken together, these results show a decrease of the ROCK pathway activity
177 following ectopic VANGL expression.

178 Jun N terminal Kinase (JNK) has been reported as a downstream effector of the PCP pathway although
179 its function downstream of the core components and especially of VANGL protein remains unclear³⁶.
180 Western blots for phospho-c-jun did not reveal any significant dysregulation of the JNK pathway activity
181 in E14.5 *Ch-Vgl2* compared with control pancreata (Fig. S3d-e). This suggests that the mechanism of
182 VANGL misexpression-induced cell death is independent of the JNK pathway.

183 **RhoA pathway activation rescues cell death and pancreatic hypoplasia induced by VANGL** 184 **misexpression**

185 So far, our analysis showed an increase of cell death and a decrease of ROCK pathway activity following
186 the misexpression of VANGL protein. In order to determine whether the decrease of ROCK pathway
187 activity was responsible for apoptosis in *Ch-Vgl2* pancreata, we tested whether pharmacological
188 activation of the ROCK pathway in *Ch-Vgl2* pancreatic epithelium is sufficient to rescue apoptosis and
189 hypoplasia. To manipulate the ROCK pathway conveniently, we used an *ex vivo* culture system similar to
190 our previously-published pancreatic organoid model²⁵, but in which the whole E10.5 pancreatic
191 epithelium was seeded in Matrigel enabling growth in 3D in the absence of mesenchyme. The growth of
192 the developing pancreas was evaluated every day by measuring the circumference of the epithelial bud.
193 The pancreatic buds expressing Cherry-VANGL2 protein grew slower and remained smaller than those
194 from control littermates at the end of the culture period (five days), mimicking the phenotype observed
195 *in vivo* (Fig. 4a,b). Inactivation of ROCK activity (*via* H1152, 5 μ M) in *wild-type* epithelium manifested in

196 an almost indistinguishable phenotype than the one observed for the *Ch-Vgl2* buds (Fig. 4c,d). Of note,
197 we also observed a delay in branching of the pancreatic epithelium when the ROCK pathway was
198 inhibited (Fig. 4c; asterisk). On the contrary, *wild-type* buds treated with a RhoA activator (CN03, 1
199 $\mu\text{g}/\text{ul}$) grew more rapidly and branched precociously compared with control vehicle-treated buds (Fig.
200 4e,f). Finally, activation of RhoA in *Ch-Vgl2* pancreatic epithelium rescued the hypoplasia (Fig. 4g,h,
201 normalized data; for details, see *Materials & Methods*). In order to assess the level of activity of the
202 ROCK pathway resulting from the various perturbations above, we performed Western blotting for
203 pMLC on individual treated buds (Fig. 4i). While the amount of pMLC protein was decreased by $\sim 70\%$ in
204 untreated *Ch-Vgl2* buds ($70 \pm 4\%$) and *wild-type* buds treated with ROCK inhibitor ($68 \pm 7\%$), in *Ch-Vgl2*
205 buds treated with RhoA activator, pMLC levels were comparable to controls, indicating that ROCK
206 pathway activity was restored to endogenous levels (Fig. 4i). Lastly, we observed that *Ch-Vgl2* buds as
207 well as those treated with ROCK inhibitor H1152 exhibited extensive apoptosis (Fig. 4j-l). In combination,
208 these *ex vivo* experiments demonstrate that the cell death and pancreatic hypoplasia resulting from
209 VANGL2 misexpression is a consequence of decreased ROCK pathway activity.

210 **Ectopic VANGL expression interferes with Dishevelled but not with Prickle or Frizzled localization**

211 VANGL physically interacts with the majority of the other PCP components²³ and, depending on the
212 context, manipulation of VANGL expression can affect the level and/or the localization of these proteins.
213 Hence, we systematically examined the expression pattern of VANGL2 partners possibly involved in the
214 downregulation of actomyosin contractility.

215 Frizzled and VANGL interact across the membranes of adjacent cells, bridged by the atypical cadherin
216 CELSR, enabling the propagation of polarity from cell to cell. In the *Ch-Vgl2* pancreas, Frizzled3
217 expression remained at the apical junction and the expression level of the protein was unaffected (Fig.
218 5a-d). Thus, VANGL basolateral expansion fails to induce a similar expansion of Frizzled3. This suggests

219 that intercellular communication is not drastically dysregulated following ectopic VANGL expression, as
220 Frizzled receptor in the neighboring cells acts as a limiting factor.

221 Intracellularly, Prickle directly binds to VANGL and can bind to Dishevelled to antagonize its Frizzled-
222 mediated membrane recruitment³⁷⁻⁴⁰. Like VANGL1/2 and Frizzled3, Prickle2 is expressed at the apical
223 junctions in the pancreatic epithelium (Fig. S4a-b). In *Ch-Vgl2*, we did not detect any changes in the
224 pattern of expression of Prickle2 at E13.5 or E14.5 (Fig. 5e-h and data not shown).

225 Dishevelled can be physically bound by VANG in *Drosophila*, and in vertebrates, these interactions are
226 believed to antagonize the formation of the FZD-DVL complex by affecting DSH/DVL levels and/or
227 stability^{28,37,41,42}. In the E12.5 pancreatic epithelium, DVL2 protein is enriched apically in the duct and
228 becomes cytoplasmic in the newly-formed endocrine clusters (Fig. S4c). This expression pattern is
229 maintained at E15.5 (Fig. S4d-g). High resolution expression analysis at this stage showed that contrary
230 to the other core components, DVL2 protein is not tightly restricted to the apical junction (Fig. S4g) but
231 instead extends sub-apically, forming a ring just below the apical membrane (Fig. S4e). In *Ch-Vgl2*
232 pancreata at E13.5 and E15.5, we observed a decrease in DVL2 expression in the cells expressing Cherry-
233 VANGL2 protein (Fig. S4h ; Fig. 5i-k). These results support the notion that ectopic expression of VANGL2
234 perturbs the level and/or stability of DVL2 in a cell-autonomous manner, which in turn leads to a
235 decreased activity of the downstream effectors involved in actomyosin contractility.

236 Although VANGL is mainly known to regulate PCP, previous reports have shown that VANGL2 may be
237 involved in maintaining apico-basal polarity^{10,31,43-45}. Therefore, we investigated whether the extension
238 of the VANGL2 expression domain to the basolateral membrane affects the localization of apico-basal
239 markers. The cellular localization of the apical membrane glycoprotein Mucin1 (Fig. 5a-h), the apical
240 junction marker PAR3, the tight junction protein ZO-1 and the basolateral marker Scribble are all

241 unchanged in Cherry-VANGL2-expressing cells (Fig. S5a-e), indicating that the integrity of the apico-basal
242 axis is maintained in *Ch-Vgl2* pancreata.

243 **Apical overexpression of VANGL protein due to inversin mutation does not perturb actomyosin**
244 **contractility or induce cell death**

245 Since the function of VANGL relies on its cellular localization, we sought to distinguish whether cell
246 death and epithelial exit in *Ch-Vgl2* pancreata are due to the ectopic expression of VANGL on the
247 basolateral membrane or overexpression of the protein in general. Diego and its distant vertebrate
248 homologue Inversin, are known to bind directly with Van Gogh/VANGL protein^{46,47} and genetically
249 interfere with VANG function^{47,48}. Analysis of *the Inversin* mutant (*Invs^{Inv/Inv}*)^{47,49,50} pancreata revealed
250 increased levels of VANGL protein at the apical side of pancreatic ducts (Fig. 6a-d). However, conversely
251 to our observations in *Ch-Vgl2*, we showed that the expression domain of VANGL did not extend to the
252 baso-lateral membrane but rather to the apical membrane in the *Invs^{Inv/Inv}* pancreas (Fig. 6b,d).

253 This model of apical overexpression of VANGL without lateral expansion does not display any defect in
254 the size of the pancreas at E12.5 and E14.5, nor increased apoptosis (Fig. 6e,f). Moreover, the level and
255 the localization of pMLC were unchanged in *Invs^{Inv/Inv}* mutant pancreata relative to controls (Fig. 6g-i),
256 suggesting that the activity of the ROCK pathway is unaffected. Finally, DVL2 protein expression levels
257 and localization were also unaffected in the *Invs^{Inv/Inv}* pancreatic epithelium (Fig. 6j,k).

258 Overall, these findings show that while both *Invs^{Inv/Inv}* and *Ch-Vgl2* exhibit overexpression of VANGL at
259 the membrane, only ectopic expression of VANGL specifically on the basolateral membrane leads to cell
260 death and epithelial exit. This strongly suggests that an apical restriction of VANGL protein activity is
261 essential to maintain epithelial integrity.

262

263 **Misexpression of VANGL2 protein in the neural tube also induces apoptosis**

264 In order to determine whether we could extend our findings to other epithelia besides the pancreas, we
265 induced the expression of Cherry-VANGL2 protein in the neural tube using the *Rosa26^{CreER}* driver. VANGL
266 protein is polarized apically in this tissue and plays an essential role in neural tube closure and
267 elongation⁵¹. Induction of the transgene was performed at E11 by injection of 4OH-tamoxifen into the
268 pregnant female and embryos were harvested at either 6, 14, 24 or 48 hours post-injection. Staining for
269 Caspase3 revealed a significant 3.3-fold increase in the proportion of apoptotic cells within the neural
270 epithelium following the induction of the transgene, peaking at 14 hours post-injection (Fig. 7a-c).
271 Therefore, we can conclude that a VANGL misexpression induces apoptosis not only in pancreas but also
272 in other epithelia such as the neural tube.

273 **DISCUSSION**

274 In this study we elucidate how planar polarity is deployed in a complex tubular organ and we
275 demonstrate that proper localization of the PCP core component VANGL is required for survival of
276 pancreatic progenitors and, hence, for normal pancreatic morphogenesis. Using transgenic lines driving
277 the expression of Cherry-VANGL2 protein all around the membrane of pancreatic progenitors, we show
278 that ectopic expression of this protein in the basolateral domain leads to apoptosis *via* downregulation
279 of the ROCK pathway. Live imaging of pancreatospheres revealed this phenomenon to be associated
280 with a disruption of epithelial integrity. Moreover, systematic expression analyses of core PCP
281 components allowed us to decipher a mechanism of intracellular inhibitory interactions of possible
282 relevance to other organs. We propose a model whereby extension of the VANGL membrane expression
283 domain increases the cell-autonomous repression exerted on Dishevelled, hence preventing its
284 localization at the apical membrane (Fig. 7d). As VANGL can bind Dishevelled in the fly³⁷ as well as in
285 vertebrates⁴¹, VANGL may directly destabilize DVL and inhibit its recruitment to the junctions by FZD.

286 This hypothesis is strongly supported by the work of the Tomlin lab in *Drosophila* showing the ability of
287 VANG to cell-autonomously block recruitment of DSH at the membrane⁵², resembling the previously-
288 reported repression of DSH by PK^{39,40,52}. In our system, an expansion of Prickle does not result from
289 misexpression of VANGL protein and is therefore unlikely to contribute to the repression exerted on
290 DVL. Recruitment of Dishevelled at the membrane is essential for its function in PCP^{53,54}. More
291 specifically, its membrane localization in *Xenopus* gastrulating cells is required for activation of Rho and
292 Rac⁵⁵, the small GTPases active downstream of the Frizzled-Dishevelled module. Our results corroborate
293 these data in mammals as we show a decrease of ROCK activity in Dishevelled^{low} (Cherry⁺) cells. Precisely
294 how VANGL perturbs DVL level/stability remains unclear but it may operate *via* ubiquitin-mediated
295 degradation, a mechanism recently proposed to modulate PCP protein levels^{56,57}. In particular VANGL1/2
296 promotes NRDP1-mediated Dishevelled ubiquitination in cell lines⁵⁸. Interestingly, in the *Inversin* mutant
297 which exhibits an upregulation of VANGL protein exclusively at the apical side, such a downregulation of
298 Dishevelled protein is not observed. Neither increased cell death, epithelium integrity defects nor ROCK
299 activity dysregulation were detected. Therefore, it appears that the ectopic localization of VANGL on the
300 basolateral membrane is the key determinant in the mechanism we propose and that restriction of
301 VANGL protein activity to the apical membrane is essential for cell survival.

302 Interactions between apico-basal polarity and planar polarity have been reported^{11,59} but, so far, only
303 apico-basal polarity defects have been associated with apoptosis. In the fly it is well established that
304 disruption of apico-basal components such as crumbs, stardust or bazooka lead to apoptosis⁶⁰⁻⁶². Our
305 work hereby reveals that disruption of a PCP core component expression domain can also manifest in
306 cell death. The downstream pathways involved differ as in fly, activation of the JNK pathway triggers
307 apoptosis⁶³ while we show that a decrease of ROCK pathway activity induces apoptosis following ectopic
308 expression of VANGL. ROCK signaling can serve as either a pro-apoptotic or pro-survival cue in a cell type
309 and context-dependent manner⁶⁴. In particular, in airway epithelial cells, ROCK inhibition induces

310 apoptosis *via* activation of the caspase cascade⁶⁵. Our studies suggest that this is similarly operating in
311 the pancreatic epithelium. We also show that Cherry-VANGL2-positive cells exit the epithelium,
312 suggesting that ectopic VANGL expression promotes delamination in a cell-autonomous manner. The
313 causal relationships between actomyosin dynamics, delamination and cell death have been debated in
314 recent investigations³⁴. Hence, one hypothesis is that mosaic transgene expression results in varying
315 levels of actomyosin activity between cells, leading cells with low actomyosin activity to be excluded by
316 neighboring cells exerting higher tension before or after cell death. Such mosaic patterns are
317 reminiscent of cell competition processes observed in apico-basal polarity-deficient clones. In both fly
318 and MDCK cells, cells lacking basolateral determinants such as Scribble, Mahjong and Lethal giant larvae
319 undergo apoptosis if *wild-type* cells surround them⁶⁶. Interestingly, Scribble-deficient clones are also
320 eliminated by extrusion and, ultimately, apoptosis^{67,68}. These competition mechanisms which eliminate
321 abnormal cells, are essential to prevent tumor development and such a mechanism may be at play in
322 the case of abnormal VANGL-polarized cells. Vang Gogh-like proteins are upregulated in many types of
323 aggressive cancers and this upregulation is associated with poor patient prognosis in breast cancer^{69,70,71}.

324 In addition to revealing the crucial nature of PCP components in maintaining epithelial integrity in two
325 tissues, using whole mount staining enabled us to visualize planar polarity in three dimensions in a
326 complex ductular network. VANGL and other PCP proteins have been shown to control the development
327 of tubular organs such as the branching of the lung⁷² or the diameter of the duct in the kidney⁷³ ³ where
328 planar-oriented proteins and cell behaviors have been inferred from 2D sections^{74,75}. Our study showing
329 PCP organization in 3D reveals that a chevron organization of VANGL orients the cells along the
330 longitudinal axis of the duct (Fig. 1h). This organization, the striking elongation of cells along the duct
331 length and VANGL enrichment at tricellular junctions, known “hot-spots” of epithelial tension, are all
332 suggestive of a link between PCP component localization and anisotropic stresses in tubes^{20,76}. To our
333 knowledge, such enrichment of a PCP core component at tricellular junctions has never been reported.

334 As these specialized junctions are involved in cell translocation between epithelial layers or cell
335 intercalation²⁰, an increase of VANGL at the tricellular junctions may affect the delamination of
336 endocrine progenitors, thereby linking morphogenesis/architecture to the endocrine differentiation
337 program. Further work investigating the integrity of the ductal network as well as cellular differentiation
338 in the absence of VANGL protein will be essential to answer these questions.

339 **Acknowledgements**

340 We would like to thank Manuel Figueiredo-Larsen for his contribution to the experiments with whole-
341 pancreas organoids. We thank Danelle Devenport for the *Cherry-Vangl2* construct, Nancy Thompson
342 and the EPFL transgenesis facility for generating the transgenic lines. Thanks to J. Bulkescher and G.
343 Karemore for their advice on imaging and statistics respectively and to Josh Brickman and Phil Seymour
344 for their comments on the manuscript. Many thanks to Toshihisa Ohtsuka and Jeremy Nathans for the
345 Prickle2 and Frizzled3 antibodies respectively. The Novo Nordisk Foundation Center for Stem Cell Biology
346 is supported by a Novo Nordisk Foundation grant number NNF17CC0027852

347 **Author contributions**

348 L. F. contributed to project design as well as most experimental data and analyses; S. Y. contributed to
349 all experiments with the ex vivo systems ; I. S. B. contributed to the quantification of Caspase3⁺ cells in
350 the inversin mutant and in the neural tube and participated to the analysis of the pancreatosphere
351 movies ; C.C. performed characterization of hypoplasia in *Cherry-Vangl2* line at E16.5; M. K. quantified
352 pancreatic size of Inversin mutant at E14.5; C.C. , M. K. and L.F established VANGL up-regulation in *Inv*
353 mice. A. G-B. supervised the project and helped design the project. All authors contributed to drafting or
354 revising the manuscript.

355

356 **Competing interests statement**

357 The authors declare no competing financial interests.

358 **METHODS**

359 **Mice**

360 Mice (*Mus musculus*) of mixed background were housed at the University of Copenhagen. All
361 experiments were performed according to ethical guidelines approved by the Danish Animal
362 Experiments Inspectorate (Dyreforsøgstilsynet). The following genetically modified mouse lines were
363 used: *Gt(ROSA)26Sortm1(cre/ERT2)Tyj/J (Rosa26CreER)⁷⁷; Invs^{inv 50}; Tg(Ipf1-cre)1Tuv (Pdx1-CRE)⁷⁸. For
364 the *Cherry-Vangl2* lines (*Tg (pCAGGS-LoxP-Cherry-Vgl2)AGB*) : the amino-terminally tagged *Cherry-*
365 *Vangl2* fusion protein construct²⁴ was inserted into a *CMV-CAG-loxP-eGFP-Stop-loxP-IRES-bGal*
366 expression vector⁷⁹. Three lines (7979; 9140 and 9139) expressing different level of the transgene were
367 generated by random insertion, the two lines expressing the highest level of the Cherry-VANGL2 protein
368 (7979 and 9140) were used to collect the data. *Pdx1-Cre; Cherry-Vangl2* double-transgenic embryos
369 were obtained by crossing heterozygous *Pdx1-Cre* with heterozygous *Cherry-Vangl2* transgenic mice.
370 After checking that the single transgenic did not present any phenotype, we used WT and single
371 transgenic (*Pdx1-Cre Tg/+* or *Cherry-Vangl2 Tg/+*) indiscriminately as controls. Conditional induction of
372 Cherry-VANGL2 protein was performed by intraperitoneal injection of 4-OH tamoxifen (4-OHTm, Sigma,
373 H6278) at a concentration of 66,6 µg/g in *Rosa26CreER* females mated with *Cherry-Vangl2* males. The
374 4-OHTm solution was prepared following instructions described in Chevalier et al.⁸⁰*

375 **Whole-mount immunohistochemistry**

376 E18.5 dorsal and ventral pancreata embedded in the first loop of duodenum were fixed in 4%
377 paraformaldehyde (PFA) for 2h at room temperature (RT) while E14.5 dorsal pancreata were fixed 30
378 min or 1h at RT. After washing in PBS and stepwise dehydration in methanol (MeOH), fixed tissue was

379 stored in 100% MeOH at -20°C . To quench autofluorescence, samples were incubated with freshly
380 prepared MeOH:DMSO:H₂O₂ (2:1:3, 15% H₂O₂) for 12–24 hours at RT. Samples were washed twice in
381 100% MeOH for 30 minutes at RT. To improve permeability of the tissue, 3 cycles of incubation at -80°C
382 were used (1h at -80°C followed by 1h at RT). Samples were rehydrated stepwise in TBST (50 mM Tris-
383 HCl pH 7.4, 150 mM NaCl, 0.1% TritonX-100) 33%, 66%, and 100% for 15 min at each step at RT. For
384 VANGL staining, an antigen retrieval step was performed: tissue was immersed in 10mM trisodium
385 citrate buffer pH6.0 and brought, under low pressure setting, to 110°C for 15 min using the
386 TintoRetriever system from BioSB. After blocking 24h at 4°C in TNB blocking solution (Perking Elmer TSA
387 kit, NEL704A001KT) for VANGL staining or in CAS-Block (Thermo Fisher, #8120) for the other stainings,
388 the specimen were incubated with primary antibodies for 48 hours at 4°C . Samples were washed
389 extensively in TBST (at least 5 incubations of 15 min) and incubated with secondary antibodies (for
390 VANGL staining, anti-rabbit biotinylated was used) for 48 hours at 4°C . After extensive washes in TBST, a
391 48h incubation with Streptavidin-HRP (Perking Elmer TSA kit) was performed to amplify VANGL signal.
392 Other stainings performed in whole-mount did not require any amplification, the samples were
393 therefore dehydrated stepwise in MeOH at this step. Samples stained with VANGL antibody were
394 washed again in TBST before incubation with Cy3 for 1h at RT (Perking Elmer TSA kit). Then, after
395 washing in TBST, the specimen were dehydrated in MeOH and stored at -20°C . All samples were cleared
396 in a solution of 1:2 Benzyle Alcohol and Benzyle Benzoate (BABB) for 12–24 hours prior to imaging.
397 Cleared specimens were subsequently mounted in glass concavity slides in BABB. Imaging was
398 performed using a Leica SP8 confocal microscope with a 20x/0.75 IMM CORR objective and Hybrid
399 detectors at 1024×1024 resolution in an 8-bit format. Three-dimensional reconstructions and movies
400 were performed with the Imaris software (Bitplane).

401 **Immunohistochemistry on sections**

402 The whole pancreas and the surrounding tissue (duodenum, stomach and spleen) were isolated in one
403 piece and fixed in 4% PFA for 30min (E10.5 and E12.5), 1h (E14.5) or 2h (E18.5) at RT. E13.5 and E15.5
404 samples prepared for DVL2 staining were fixed 30 min in PFA diluted to 4% in water rather than the
405 usual PBS. After several washes in PBS, samples were equilibrated in 15% sucrose/PBS 0.12 M solution
406 overnight at 4°C prior to embedding in gelatin (7.5% gelatin diluted in 15% sucrose/PBS 0.12 M
407 solution). Gelatin blocks were subsequently frozen at -65°C in 2-methylbutane (isopentane, Acros
408 Organics) and kept at -80°C until sectioning. Frozen sections (8 to 10 µm) were then dried at room
409 temperature and washed in PBS Triton 0.1%. The slides were incubated 10min in PBS Triton 0.3% to
410 permeabilize the tissue. If necessary, an antigen retrieval step was performed at this point (see below).
411 If secondary antibodies directly conjugated to HorseRadish Peroxidase (HRP) were used, a 7 min
412 incubation in 3% H₂O₂ was performed at this point. The tissues were rinsed and then blocked 1h at RT in
413 the appropriate blocking reagent (Table 1, supp. Methods). Primary antibodies were incubated
414 overnight at 4°C (Table 2, supp. Methods). Following 3 washes in PBS Triton 0.1%, the samples were
415 incubated with secondary antibodies for 1h at RT then rinsed again. For the antibodies requiring
416 amplification (Table1, supp. Methods): if the secondary antibody was directly conjugated to HRP, a
417 tyramide labeling was performed using the TSA kit (Molecular probes T20949) reagents while, if the
418 secondary antibody was directly conjugated to Biotin, an incubation with streptavidin-HRP of 1h was
419 performed and followed by Cy3 labeling (Perking Elmer TSA kit, NEL704A001KT). The sections were
420 washed extensively before mounting in 80% glycerol/PBS. All the images (beside the one use for
421 quantification, see below) were taken on the Leica SP8 confocal microscope with a 63x/1.30 Glycerol
422 CORR objective and Hybrid detectors at 1024×1024 resolution in an 8-bit format. Brightness and
423 contrast were adjusted using Fiji software and 3D reconstructions were performed with the Imaris
424 software (Bitplane).

425 Antigen retrieval was performed using the TintoRetriever system from BioSB. The tissue was immersed
426 in 10mM trisodium citrate buffer pH6.0 and brought to high temperature for 15 min using either the low
427 pressure setting (106°C -110°C) or the high pressure setting (114°C -121°C) depending of the antibody
428 (Table 1, supp. Methods). The samples were brought back gradually to RT before proceeding to the next
429 step. For Frizzled3 staining we used a cycle of 20min at 95°C.

430 TUNEL assays were performed with an ApopTag Peroxidase *in situ* apoptosis detection kit (Millipore,
431 S7100) and followed by immunofluorescence staining as described above.

432 **Image quantification**

433 For quantification on the pancreatic epithelium, the entire pancreas was serially sectioned (8µm thick
434 section) and the immunostained sections were imaged using a Leica DM5500 upright wide-field
435 microscope with a 20X air objective. The positive pixels of the section stained for PDX1, SOX9 or β-
436 +DAPI were measured on every 8th (E16.5), 6th (E14.5), 5th (E12.5) or every section (E10.5) using a
437 customized Macro in Fiji software (Schindelin et al., 2012). The area obtained for the sampled sections
438 were summed for each pancreas, which gives an estimation of the size of the epithelium. pHH3, CAS3
439 and TUNEL⁺ cells (matching a DAPI nucleus) were counted manually using Imaris software. For each
440 pancreas sample, ratio between the number of counted cells (pHH3, CAS3 or TUNEL⁺) and the
441 estimation of the size of the epithelium (based on SOX9, PDX1 or β-Catenin+DAPI areas of expression)
442 were calculated and then normalized to the control littermates.

443 For the nervous system analysis, the whole embryo was serially sectioned (10µm thick section). After
444 being stained for β-Catenin, DAPI and Caspase3 the sections were imaged using a Leica DM5500 upright
445 wide-field microscope with a 20X air objective. Quantifications were made every 30th section, which
446 corresponds to 10 measurements per embryo. The neural tube was manually delimited based on β-
447 Catenin staining using a free-hand-tool of Fiji. The area of neural tube was then calculated by measuring

448 the positive pixels of β -Catenin+DAPI staining included in the drawn perimeter using the customized
449 Macro of Fiji (Schindelin et al., 2012). Within the area of the neural tube we then calculated the area of
450 Caspase3 expression with the same Macro. For each sample the ratio between the total area of
451 Caspase3 expression/total neural tube area was calculated and normalized to control littermates.

452 **Western blot**

453 E12.5 and E15.5 dorsal pancreata were snap-frozen and processed as described in⁸¹. Antibodies are
454 listed in Table2 (supp. Methods). The blots were visualized using a Chemidoc MP (Bio-Rad) and Images
455 were quantified based on pixel density using the ImageJ software package. The protein of interest was
456 normalized to alpha tubulin level (internal control) then compared to the average value obtained for the
457 control littermate.

458 **Pancreatic bud and sphere culture**

459 For pancreatic bud culture, E10.5 dorsal bud epithelia were recovered, the mesenchyme was
460 mechanically removed and the intact epithelium was seeded in 3D Matrigel culture. The buds were
461 cultured in organoid medium (without Y27632)²⁵ supplemented with either RhoA activator (CNO3,
462 Cytoskeleton Inc), Rock inhibitor (H-1152, #555550 Millipore) or an equal amount of DMSO for vehicle
463 control. They were cultured for 5 to 7 days and the buds were imaged every day on a AF6000 Leica
464 microscope with a 10x dry objective. In order to determine the working concentration of the drugs, a
465 range of concentrations were initially tested (H1152: 0.25, 0.5, 1, 2.5, 5 and 10 μ M; CNO3: 0.25, 0.5 and
466 1 μ g/ml), the buds were collected after treatment and western blot for pMLC was performed to quantify
467 the activity of RhoA/Rock pathway. 5 μ M H1152 and 1 μ g/ml CNO3 concentration were chosen as this
468 respectively led to an average decrease of 68% and an average increase of 133% of pMLC protein
469 amount while allowing an the epithelium to grow and branch. For the quantifications, the
470 circumference of the growing epithelium was measured using Imaris software and exponential growth

471 curves were generated for each bud using the GraphPad prism software. The doubling time of each
472 individual growth curve was determined and used for statistical analysis. Normalization of the data (Fig.
473 4g) was done for each experimental condition by calculating the ratio (Δ) of the circumference (c)
474 measured at each time point (t(x)) to the average circumference of the control littermate (C) at the
475 same time point ($\Delta_{t(x)} = c_{t(x)} / C_{t(x)}$). Δ values were plotted against the time points of measurement and a
476 linear regression was applied using GraphPad prism software. The slope of each individual line was
477 determined and used for statistical analysis (Fig 4h). Of note, for the sake of clarity only the average
478 growth curves or the average normalized linear regressions are shown on the graphs for each
479 experimental condition.

480 For the sphere cultures, E13.5 dorsal pancreata were isolated, the cells were dispersed, and seeded in
481 Matrigel as described previously^{25,82}. Spheres were cultured for 4 days prior to live imaging and imaging
482 was performed using either a SP8 Leica microscope with 20x glycerol objective for 5 hours (Litter1, 16
483 positions imaged for control and 22 positions imaged for *Ch-Vgl2* sample) or using a Zeiss LM780 with
484 20x dry objective for 3 hours (Litter2, 14 positions imaged for control, 13 for one *Ch-Vgl2* sample and 12
485 for the second *Ch-Vgl2* sample of the litter). Representative movies were made using Fiji software.

486 **Statistical analysis**

487 Statistical analyses were performed with GraphPad 6 software packages and Microsoft Excel.
488 Normalization to control littermates was obtain by making a ratio between the value obtained in the
489 transgenics (or mutants) and the average value of controls present in the same litter, multiplied by 100
490 to convert to percentages. All statistical tests in the paper are unpaired non-parametric student t-tests.
491 Normality of the data cannot be tested, we thus assume that the data have a Gaussian distribution.
492 Results were indicated by the mean \pm SEM. Fold change calculations were made according to the
493 formula: (final value- initial value)/initial value

494 **REFERENCES**

- 495 1 Goodrich, L. V. & Strutt, D. Principles of planar polarity in animal development. *Development*
496 **138**, 1877-1892, doi:10.1242/dev.054080 (2011).
- 497 2 Peng, Y. & Axelrod, J. D. Asymmetric protein localization in planar cell polarity: mechanisms,
498 puzzles, and challenges. *Curr Top Dev Biol* **101**, 33-53, doi:10.1016/B978-0-12-394592-1.00002-8
499 (2012).
- 500 3 Kunitomo, K. *et al.* Disruption of Core Planar Cell Polarity Signaling Regulates Renal Tubule
501 Morphogenesis but Is Not Cystogenic. *Curr Biol* **27**, 3120-3131 e3124,
502 doi:10.1016/j.cub.2017.09.011 (2017).
- 503 4 Villasenor, A., Chong, D. C., Henkemeyer, M. & Cleaver, O. Epithelial dynamics of pancreatic
504 branching morphogenesis. *Development* **137**, 4295-4305 (2010).
- 505 5 Kesavan, G. *et al.* Cdc42-mediated tubulogenesis controls cell specification. *Cell* **139**, 791-801,
506 doi:10.1016/j.cell.2009.08.049 (2009).
- 507 6 Dahl-Jensen, S. B. *et al.* Deconstructing the principles of ductal network formation in the
508 pancreas. *PLoS Biol* **16**, e2002842, doi:10.1371/journal.pbio.2002842 (2018).
- 509 7 Larsen, H. L. & Grapin-Botton, A. The molecular and morphogenetic basis of pancreas
510 organogenesis. *Semin Cell Dev Biol* **66**, 51-68, doi:10.1016/j.semcdb.2017.01.005 (2017).
- 511 8 Pan, F. C. & Wright, C. Pancreas organogenesis: from bud to plexus to gland. *Dev Dyn* **240**, 530-
512 565, doi:10.1002/dvdy.22584 (2011).
- 513 9 Cortijo, C., Gouzi, M., Tissir, F. & Grapin-Botton, A. Planar cell polarity controls pancreatic beta
514 cell differentiation and glucose homeostasis. *Cell Rep* **2**, 1593-1606,
515 doi:10.1016/j.celrep.2012.10.016 (2012).

- 516 10 Hatakeyama, J., Wald, J. H., Printsev, I., Ho, H. Y. & Carraway, K. L., 3rd. Vangl1 and Vangl2:
517 planar cell polarity components with a developing role in cancer. *Endocr Relat Cancer* **21**, R345-
518 356, doi:10.1530/ERC-14-0141 (2014).
- 519 11 Butler, M. T. & Wallingford, J. B. Planar cell polarity in development and disease. *Nat Rev Mol*
520 *Cell Biol* **18**, 375-388, doi:10.1038/nrm.2017.11 (2017).
- 521 12 Iliescu, A., Gravel, M., Horth, C. & Gros, P. Independent mutations at Arg181 and Arg274 of
522 Vangl proteins that are associated with neural tube defects in humans decrease protein stability
523 and impair membrane targeting. *Biochemistry* **53**, 5356-5364, doi:10.1021/bi500400g (2014).
- 524 13 Merello, E. *et al.* Expanding the mutational spectrum associated to neural tube defects:
525 literature revision and description of novel VANGL1 mutations. *Birth Defects Res A Clin Mol*
526 *Teratol* **103**, 51-61, doi:10.1002/bdra.23305 (2015).
- 527 14 Papakrivopoulou, E. *et al.* Vangl2, a planar cell polarity molecule, is implicated in irreversible and
528 reversible kidney glomerular injury. *J Pathol*, doi:10.1002/path.5158 (2018).
- 529 15 Schnell, U. & Carroll, T. J. Planar cell polarity of the kidney. *Exp Cell Res* **343**, 258-266,
530 doi:10.1016/j.yexcr.2014.11.003 (2016).
- 531 16 Yuan, Y. *et al.* Promoter methylation and expression of the VANGL2 gene in the myocardium of
532 pediatric patients with tetralogy of fallot. *Birth Defects Res A Clin Mol Teratol* **100**, 973-984,
533 doi:10.1002/bdra.23291 (2014).
- 534 17 Li, D. & Wang, J. Planar Cell Polarity Signaling in Mammalian Cardiac Morphogenesis. *Pediatr*
535 *Cardiol* **39**, 1052-1062, doi:10.1007/s00246-018-1860-5 (2018).
- 536 18 Yates, L. L. & Dean, C. H. Planar polarity: A new player in both lung development and disease.
537 *Organogenesis* **7**, 209-216, doi:10.4161/org.7.3.18462 (2011).

- 538 19 Hayes, M. N. *et al.* Vangl2/RhoA Signaling Pathway Regulates Stem Cell Self-Renewal Programs
539 and Growth in Rhabdomyosarcoma. *Cell Stem Cell* **22**, 414-427 e416,
540 doi:10.1016/j.stem.2018.02.002 (2018).
- 541 20 Higashi, T. & Miller, A. L. Tricellular junctions: how to build junctions at the TRICKiest points of
542 epithelial cells. *Mol Biol Cell* **28**, 2023-2034, doi:10.1091/mbc.E16-10-0697 (2017).
- 543 21 Wang, Y., Chang, H., Rattner, A. & Nathans, J. Frizzled Receptors in Development and Disease.
544 *Curr Top Dev Biol* **117**, 113-139, doi:10.1016/bs.ctdb.2015.11.028 (2016).
- 545 22 Strutt, H., Gamage, J. & Strutt, D. Robust Asymmetric Localization of Planar Polarity Proteins Is
546 Associated with Organization into Signalosome-like Domains of Variable Stoichiometry. *Cell Rep*
547 **17**, 2660-2671, doi:10.1016/j.celrep.2016.11.021 (2016).
- 548 23 Bailly, E., Walton, A. & Borg, J. P. The Planar Cell Polarity Vangl2 protein: from genetics to
549 cellular and molecular functions. *Semin Cell Dev Biol*, doi:10.1016/j.semcdb.2017.10.030 (2017).
- 550 24 Devenport, D. & Fuchs, E. Planar polarization in embryonic epidermis orchestrates global
551 asymmetric morphogenesis of hair follicles. *Nat Cell Biol* **10**, 1257-1268 (2008).
- 552 25 Greggio, C. *et al.* Artificial three-dimensional niches deconstruct pancreas development in vitro.
553 *Development* **140**, 4452-4462, doi:10.1242/dev.096628 (2013).
- 554 26 Sugiyama, T. *et al.* Reconstituting pancreas development from purified progenitor cells reveals
555 genes essential for islet differentiation. *Proc Natl Acad Sci U S A* **110**, 12691-12696,
556 doi:10.1073/pnas.1304507110 (2013).
- 557 27 Devenport, D. Tissue morphodynamics: Translating planar polarity cues into polarized cell
558 behaviors. *Semin Cell Dev Biol* **55**, 99-110, doi:10.1016/j.semcdb.2016.03.012 (2016).
- 559 28 Yang, Y. & Mlodzik, M. Wnt-Frizzled/planar cell polarity signaling: cellular orientation by facing
560 the wind (Wnt). *Annu Rev Cell Dev Biol* **31**, 623-646, doi:10.1146/annurev-cellbio-100814-
561 125315 (2015).

- 562 29 Chen, H., Mruk, D. D., Lee, W. M. & Cheng, C. Y. Planar Cell Polarity (PCP) Protein Vangl2
563 Regulates Ectoplasmic Specialization Dynamics via Its Effects on Actin Microfilaments in the
564 Testes of Male Rats. *Endocrinology* **157**, 2140-2159, doi:10.1210/en.2015-1987 (2016).
- 565 30 Galea, G. L. *et al.* Vangl2 disruption alters the biomechanics of late spinal neurulation leading to
566 spina bifida in mouse embryos. *Dis Model Mech* **11**, doi:10.1242/dmm.032219 (2018).
- 567 31 Lindqvist, M. *et al.* Vang-like protein 2 and Rac1 interact to regulate adherens junctions. *J Cell Sci*
568 **123**, 472-483, doi:10.1242/jcs.048074 (2010).
- 569 32 McGreevy, E. M., Vijayraghavan, D., Davidson, L. A. & Hildebrand, J. D. Shroom3 functions
570 downstream of planar cell polarity to regulate myosin II distribution and cellular organization
571 during neural tube closure. *Biol Open* **4**, 186-196, doi:10.1242/bio.20149589 (2015).
- 572 33 Lopez-Escobar, B. *et al.* The non-canonical Wnt-PCP pathway shapes the mouse caudal neural
573 plate. *Development* **145**, doi:10.1242/dev.157487 (2018).
- 574 34 Ohsawa, S., Vaughen, J. & Igaki, T. Cell Extrusion: A Stress-Responsive Force for Good or Evil in
575 Epithelial Homeostasis. *Dev Cell* **44**, 532, doi:10.1016/j.devcel.2018.02.007 (2018).
- 576 35 Vicente-Manzanares, M., Ma, X., Adelstein, R. S. & Horwitz, A. R. Non-muscle myosin II takes
577 centre stage in cell adhesion and migration. *Nat Rev Mol Cell Biol* **10**, 778-790,
578 doi:10.1038/nrm2786 (2009).
- 579 36 Lapebie, P., Borchiellini, C. & Houliston, E. Dissecting the PCP pathway: one or more pathways?:
580 Does a separate Wnt-Fz-Rho pathway drive morphogenesis? *Bioessays* **33**, 759-768.
- 581 37 Bastock, R., Strutt, H. & Strutt, D. Strabismus is asymmetrically localised and binds to Prickle and
582 Dishevelled during *Drosophila* planar polarity patterning. *Development* **130**, 3007-3014 (2003).
- 583 38 Carreira-Barbosa, F. *et al.* Prickle 1 regulates cell movements during gastrulation and neuronal
584 migration in zebrafish. *Development* **130**, 4037-4046 (2003).

- 585 39 Jenny, A., Darken, R. S., Wilson, P. A. & Mlodzik, M. Prickle and Strabismus form a functional
586 complex to generate a correct axis during planar cell polarity signaling. *EMBO J* **22**, 4409-4420,
587 doi:10.1093/emboj/cdg424 (2003).
- 588 40 Tree, D. R. *et al.* Prickle mediates feedback amplification to generate asymmetric planar cell
589 polarity signaling. *Cell* **109**, 371-381 (2002).
- 590 41 Park, M. & Moon, R. T. The planar cell-polarity gene *stbm* regulates cell behaviour and cell fate
591 in vertebrate embryos. *Nat Cell Biol* **4**, 20-25, doi:10.1038/ncb716 (2002).
- 592 42 Seo, H. S., Habas, R., Chang, C. & Wang, J. Bimodal regulation of Dishevelled function by Vangl2
593 during morphogenesis. *Hum Mol Genet* **26**, 2053-2061, doi:10.1093/hmg/ddx095 (2017).
- 594 43 Tao, H. *et al.* Mouse *prickle1*, the homolog of a PCP gene, is essential for epiblast apical-basal
595 polarity. *Proc Natl Acad Sci U S A* **106**, 14426-14431, doi:10.1073/pnas.0901332106 (2009).
- 596 44 Vandenberg, A. L. & Sassoon, D. A. Non-canonical Wnt signaling regulates cell polarity in female
597 reproductive tract development via *van gogh-like 2*. *Development* **136**, 1559-1570,
598 doi:10.1242/dev.034066 (2009).
- 599 45 Milgrom-Hoffman, M. & Humbert, P. O. Regulation of cellular and PCP signalling by the Scribble
600 polarity module. *Semin Cell Dev Biol* **81**, 33-45, doi:10.1016/j.semcdb.2017.11.021 (2018).
- 601 46 Das, G., Jenny, A., Klein, T. J., Eaton, S. & Mlodzik, M. Diego interacts with Prickle and
602 Strabismus/Van Gogh to localize planar cell polarity complexes. *Development* **131**, 4467-4476,
603 doi:10.1242/dev.01317 (2004).
- 604 47 Simons, M. *et al.* Inversin, the gene product mutated in nephronophthisis type II, functions as a
605 molecular switch between Wnt signaling pathways. *Nat Genet* **37**, 537-543, doi:10.1038/ng1552
606 (2005).

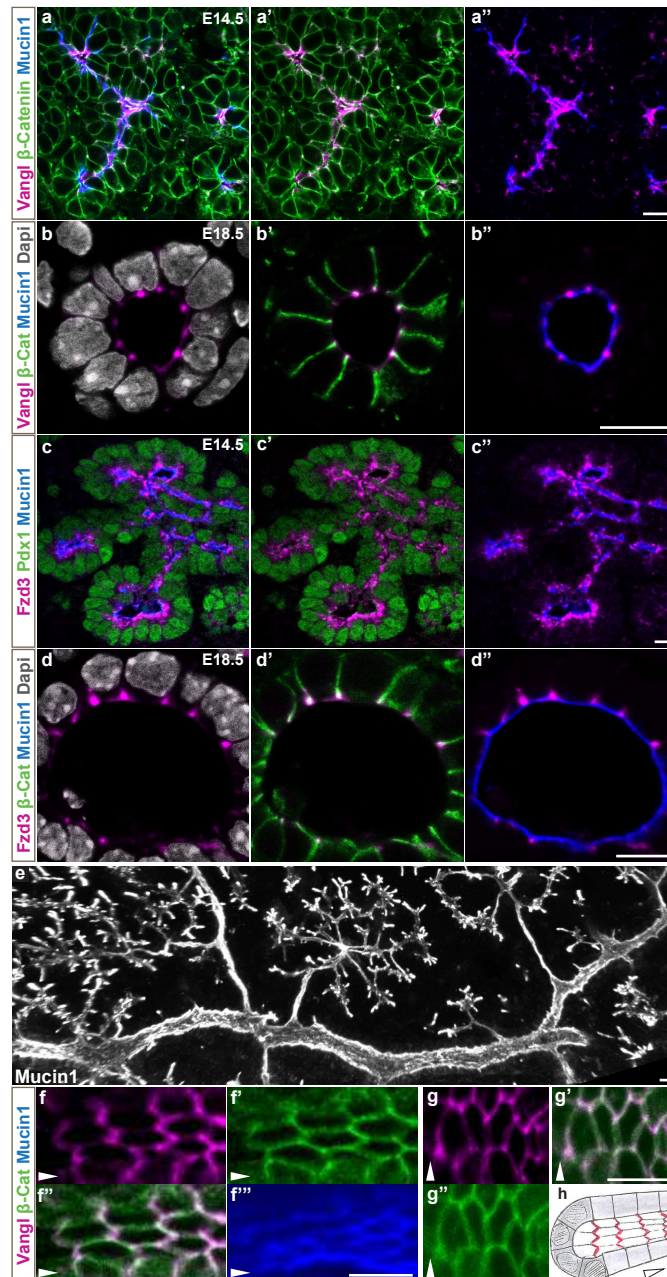
- 607 48 Jenny, A., Reynolds-Kenneally, J., Das, G., Burnett, M. & Mlodzik, M. Diego and Prickle regulate
608 Frizzled planar cell polarity signalling by competing for Dishevelled binding. *Nat Cell Biol* **7**, 691-
609 697, doi:10.1038/ncb1271 (2005).
- 610 49 Morgan, D. *et al.* Inversin, a novel gene in the vertebrate left-right axis pathway, is partially
611 deleted in the *inv* mouse. *Nat Genet* **20**, 149-156, doi:10.1038/2450 (1998).
- 612 50 Yokoyama, T. *et al.* Reversal of left-right asymmetry: a situs inversus mutation. *Science* **260**, 679-
613 682 (1993).
- 614 51 Torban, E., Iliescu, A. & Gros, P. An expanding role of Vangl proteins in embryonic development.
615 *Curr Top Dev Biol* **101**, 237-261, doi:10.1016/B978-0-12-394592-1.00005-3 (2012).
- 616 52 Amonlirdviman, K. *et al.* Mathematical modeling of planar cell polarity to understand
617 domineering nonautonomy. *Science* **307**, 423-426, doi:10.1126/science.1105471 (2005).
- 618 53 Axelrod, J. D. Unipolar membrane association of Dishevelled mediates Frizzled planar cell
619 polarity signaling. *Genes Dev* **15**, 1182-1187, doi:10.1101/gad.890501 (2001).
- 620 54 Axelrod, J. D., Miller, J. R., Shulman, J. M., Moon, R. T. & Perrimon, N. Differential recruitment of
621 Dishevelled provides signaling specificity in the planar cell polarity and Wingless signaling
622 pathways. *Genes Dev* **12**, 2610-2622 (1998).
- 623 55 Park, T. J., Gray, R. S., Sato, A., Habas, R. & Wallingford, J. B. Subcellular localization and signaling
624 properties of dishevelled in developing vertebrate embryos. *Curr Biol* **15**, 1039-1044,
625 doi:10.1016/j.cub.2005.04.062 (2005).
- 626 56 Narimatsu, M. *et al.* Regulation of planar cell polarity by Smurf ubiquitin ligases. *Cell* **137**, 295-
627 307, doi:10.1016/j.cell.2009.02.025 (2009).
- 628 57 Strutt, H., Searle, E., Thomas-Macarthur, V., Brookfield, R. & Strutt, D. A Cul-3-BTB ubiquitylation
629 pathway regulates junctional levels and asymmetry of core planar polarity proteins.
630 *Development* **140**, 1693-1702, doi:10.1242/dev.089656 (2013).

- 631 58 Wald, J. H. *et al.* Suppression of planar cell polarity signaling and migration in glioblastoma by
632 Nrdp1-mediated Dvl polyubiquitination. *Oncogene* **36**, 5158-5167, doi:10.1038/onc.2017.126
633 (2017).
- 634 59 Apodaca, G. Role of Polarity Proteins in the Generation and Organization of Apical Surface
635 Protrusions. *Cold Spring Harb Perspect Biol* **10**, doi:10.1101/cshperspect.a027813 (2018).
- 636 60 Knust, E., Tepass, U. & Wodarz, A. crumbs and stardust, two genes of Drosophila required for
637 the development of epithelial cell polarity. *Dev Suppl*, 261-268 (1993).
- 638 61 Muller, H. A. & Wieschaus, E. armadillo, bazooka, and stardust are critical for early stages in
639 formation of the zonula adherens and maintenance of the polarized blastoderm epithelium in
640 Drosophila. *J Cell Biol* **134**, 149-163 (1996).
- 641 62 Tepass, U., Theres, C. & Knust, E. crumbs encodes an EGF-like protein expressed on apical
642 membranes of Drosophila epithelial cells and required for organization of epithelia. *Cell* **61**, 787-
643 799 (1990).
- 644 63 Kolahgar, G., Bardet, P. L., Langton, P. F., Alexandre, C. & Vincent, J. P. Apical deficiency triggers
645 JNK-dependent apoptosis in the embryonic epidermis of Drosophila. *Development* **138**, 3021-
646 3031, doi:10.1242/dev.059980 (2011).
- 647 64 Street, C. A. & Bryan, B. A. Rho kinase proteins--pleiotropic modulators of cell survival and
648 apoptosis. *Anticancer Res* **31**, 3645-3657 (2011).
- 649 65 Moore, M., Marroquin, B. A., Gugliotta, W., Tse, R. & White, S. R. Rho kinase inhibition initiates
650 apoptosis in human airway epithelial cells. *Am J Respir Cell Mol Biol* **30**, 379-387,
651 doi:10.1165/rcmb.2003-0019OC (2004).
- 652 66 Di Gregorio, A., Bowling, S. & Rodriguez, T. A. Cell Competition and Its Role in the Regulation of
653 Cell Fitness from Development to Cancer. *Dev Cell* **38**, 621-634,
654 doi:10.1016/j.devcel.2016.08.012 (2016).

- 655 67 Vaughn, J. & Igaki, T. Slit-Robo Repulsive Signaling Extrudes Tumorigenic Cells from Epithelia.
656 *Dev Cell* **39**, 683-695, doi:10.1016/j.devcel.2016.11.015 (2016).
- 657 68 Norman, M. *et al.* Loss of Scribble causes cell competition in mammalian cells. *J Cell Sci* **125**, 59-
658 66, doi:10.1242/jcs.085803 (2012).
- 659 69 Saito, Y., Desai, R. R. & Muthuswamy, S. K. Reinterpreting polarity and cancer: The changing
660 landscape from tumor suppression to tumor promotion. *Biochim Biophys Acta Rev Cancer* **1869**,
661 103-116, doi:10.1016/j.bbcan.2017.12.001 (2018).
- 662 70 VanderVorst, K., Hatakeyama, J., Berg, A., Lee, H. & Carraway, K. L., 3rd. Cellular and molecular
663 mechanisms underlying planar cell polarity pathway contributions to cancer malignancy. *Semin*
664 *Cell Dev Biol* **81**, 78-87, doi:10.1016/j.semcdb.2017.09.026 (2018).
- 665 71 Daulat, A. M. & Borg, J. P. Wnt/Planar Cell Polarity Signaling: New Opportunities for Cancer
666 Treatment. *Trends Cancer* **3**, 113-125, doi:10.1016/j.trecan.2017.01.001 (2017).
- 667 72 Yates, L. L. *et al.* The PCP genes *Celsr1* and *Vangl2* are required for normal lung branching
668 morphogenesis. *Hum Mol Genet* **19**, 2251-2267, doi:10.1093/hmg/ddq104 (2010).
- 669 73 Yates, L. L. *et al.* The planar cell polarity gene *Vangl2* is required for mammalian kidney-
670 branching morphogenesis and glomerular maturation. *Hum Mol Genet* **19**, 4663-4676,
671 doi:10.1093/hmg/ddq397 (2010).
- 672 74 Fischer, E. *et al.* Defective planar cell polarity in polycystic kidney disease. *Nat Genet* **38**, 21-23,
673 doi:10.1038/ng1701 (2006).
- 674 75 Karner, C. M. *et al.* Wnt9b signaling regulates planar cell polarity and kidney tubule
675 morphogenesis. *Nat Genet* **41**, 793-799, doi:10.1038/ng.400 (2009).
- 676 76 Eaton, S. & Julicher, F. Cell flow and tissue polarity patterns. *Curr Opin Genet Dev* **21**, 747-752,
677 doi:10.1016/j.gde.2011.08.010 (2011).

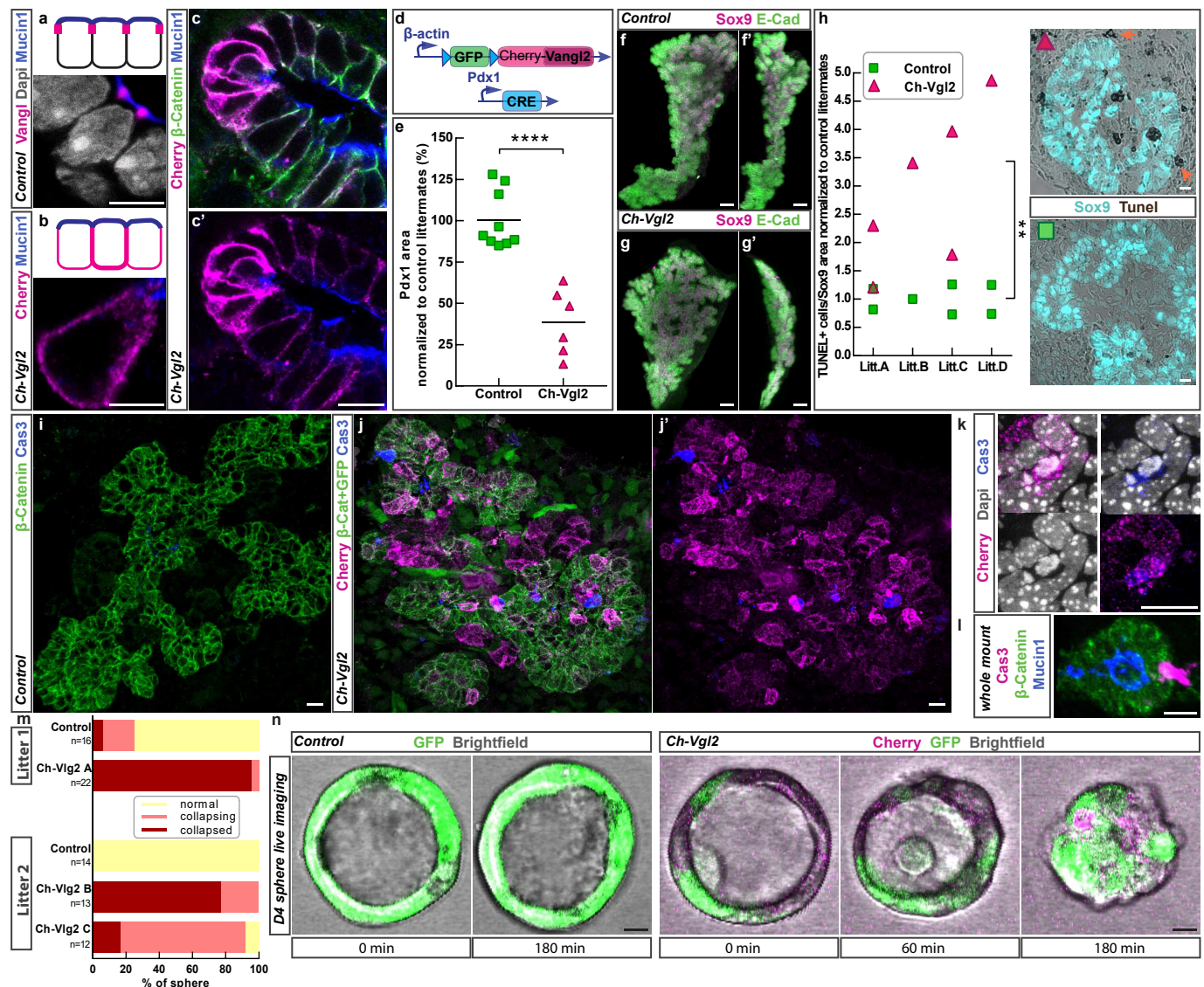
- 678 77 Ventura, A. *et al.* Restoration of p53 function leads to tumour regression in vivo. *Nature* **445**,
679 661-665, doi:10.1038/nature05541 (2007).
- 680 78 Hingorani, S. R. *et al.* Preinvasive and invasive ductal pancreatic cancer and its early detection in
681 the mouse. *Cancer Cell* **4**, 437-450 (2003).
- 682 79 Collombat, P. *et al.* Embryonic endocrine pancreas and mature beta cells acquire alpha and PP
683 cell phenotypes upon Arx misexpression. *J Clin Invest* **117**, 961-970, doi:10.1172/JCI29115
684 (2007).
- 685 80 Chevalier, C., Nicolas, J. F. & Petit, A. C. Preparation and delivery of 4-hydroxy-tamoxifen for
686 clonal and polyclonal labeling of cells of the surface ectoderm, skin, and hair follicle. *Methods*
687 *Mol Biol* **1195**, 239-245, doi:10.1007/7651_2013_63 (2014).
- 688 81 Lemaire, L. A. *et al.* Bicaudal C1 promotes pancreatic NEUROG3+ endocrine progenitor
689 differentiation and ductal morphogenesis. *Development* **142**, 858-870, doi:10.1242/dev.114611
690 (2015).
- 691 82 Greggio, C., De Franceschi, F., Figueiredo-Larsen, M. & Grapin-Botton, A. In vitro pancreas
692 organogenesis from dispersed mouse embryonic progenitors. *J Vis Exp*, doi:10.3791/51725
693 (2014).

Figure 1



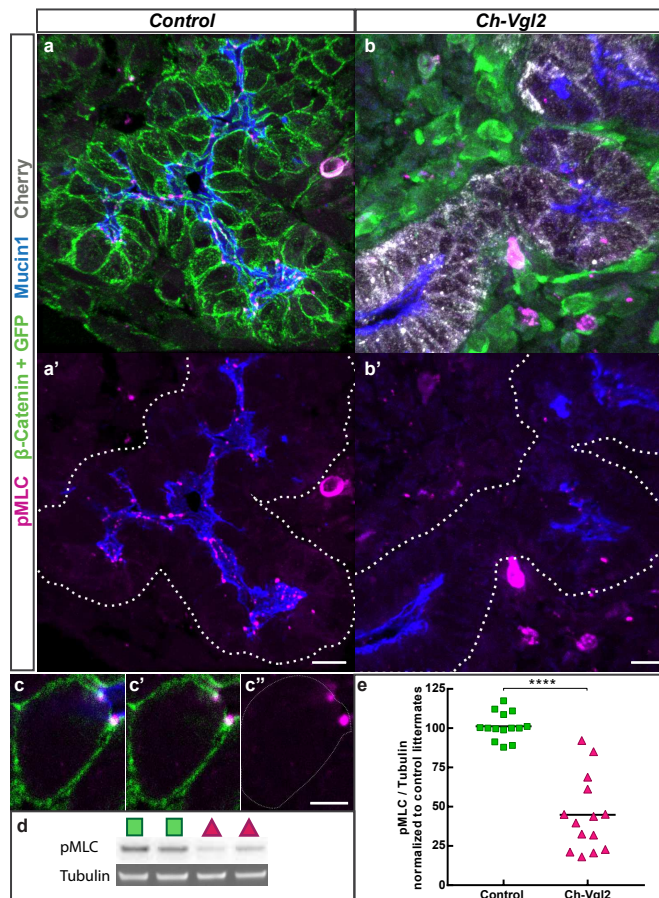
Planar polarization of PCP components in the tubular epithelium of the pancreas. Confocal images of embryonic pancreata at E14.5 (**a, c**) or E18.5 (**b, d-g**). β -Catenin labels all the membranes and Mucin1 the apical side. **a-d**, Representative images of VANGL (a-b) and FZD3 (c-d) expression, in a general view of the epithelium (a, c) or a close up of a transverse section of a duct (b, d), showing expression of those proteins at the apical cell junctions. **e**, 3D projection of a whole mount staining of Mucin1 depicting the architecture of the pancreatic duct tree. **f-g**, Confocal section of whole mount staining for VANGL showing representative apical views of the epithelium in a large duct, the arrowhead is pointing along the longitudinal axis of the duct. **f** illustrates the chevron-like localization of VANGL protein on the transverse membranes revealing the planar polarized organization of this protein. **g** illustrates the enrichment of VANGL protein at the tricellular junctions. **h**, scheme depicting VANGL (red) planar polarization in pancreatic ducts. Additional representation of VANGL expression pattern is shown in **movie 1**. Scale bar 10 μ m.

Figure2



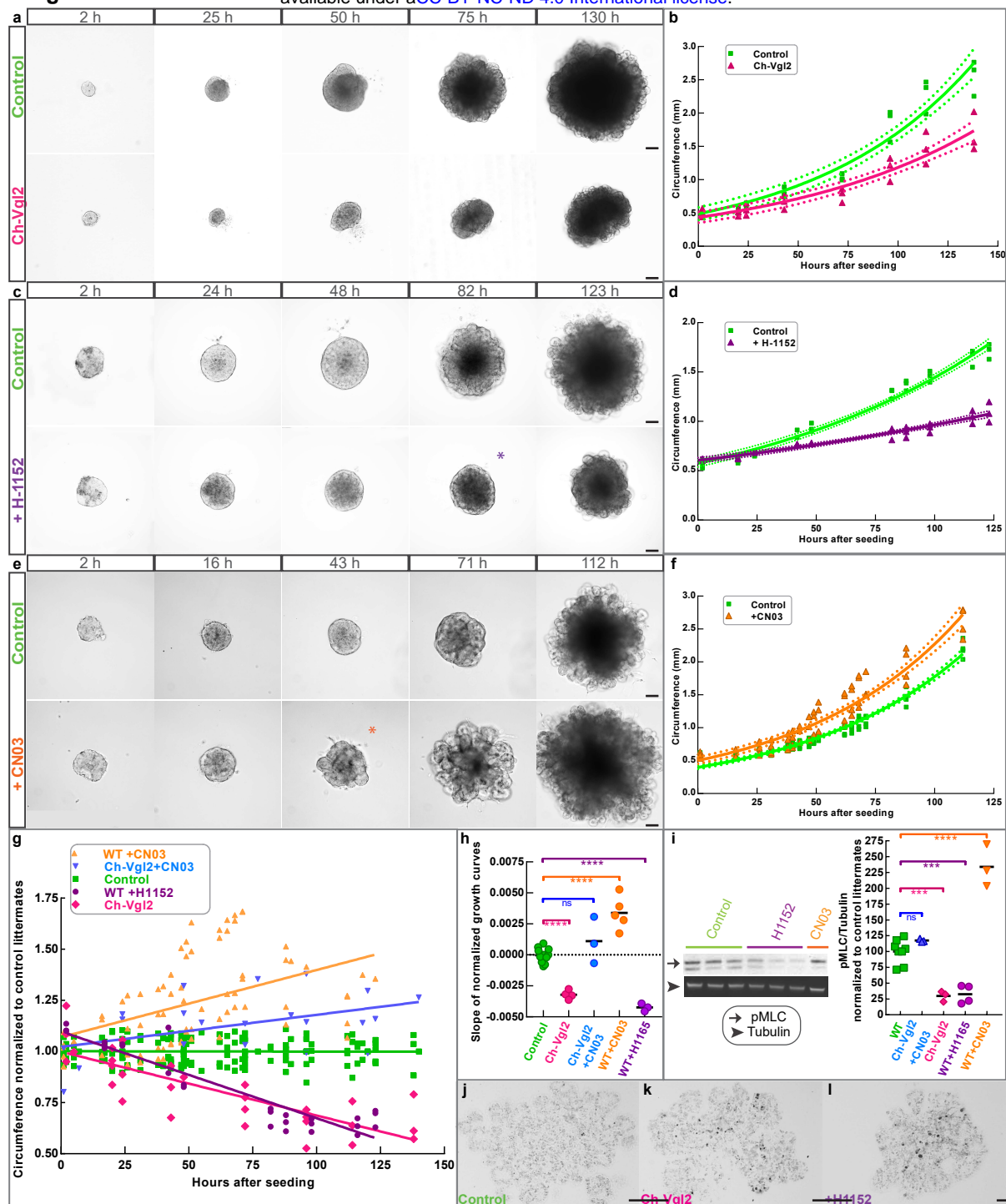
Ectopic expression of VANGL2 on baso-lateral membranes leads to disruption of epithelium integrity, cell death and pancreatic hypoplasia. **a-c**, confocal images showing localization of VANGL in a WT (**a**) or double-transgenic *Ch-Vgl2* (*Pdx1-Cre; Cherry-Vangl2*) (**b**) on an E16.5 pancreatic section. **d**, illustration of the construct used to express the fusion protein Cherry-VANGL2 in the pancreatic progenitors. **e**, PDX1 area quantified on E12.5 embryonic sections of control (green) and *Ch-Vgl2* (pink) pancreas showing a significant decrease of the epithelium size in the double-transgenic *Ch-Vgl2*. Each dot represents one pancreas, data normalized against control littermate. (****) p-value < 0.0001 by t-test. **f-g**, whole mount immunostaining on E14.5 dorsal pancreas labelling progenitors (SOX9) and epithelial membrane (E-CAD). **h**, TUNEL assay labelling apoptotic cells in pancreatic epithelium (SOX9+) sections showing a significant increase of cell death in *Ch-Vgl2*. The ratios (number of TUNEL+ cells/epithelium area) are normalized against the control littermates and data are presented by litters. Each dot represents one pancreas. (**) p-value=0.004 by t-test. The orange arrow points at dead cells that left the epithelial layer. **i-k**, Caspase3 immuno staining on E12.5 pancreas sections showing dying cells in Cherry+ areas. The close-up in **k** shows one dying cell (CAS3+ and fragmented nuclei) expressing Cherry-VANGL2 protein. **l**, representative area of whole mount immunostaining illustrating delamination of a CAS3+ cell. **m-n**, pancreatospheres generated from control or *Ch-Vgl2* pancreata were imaged 4 days after seeding for either 3 hours (litter1) or 5 hours (litter2). **m**, Phenotypes were scored at the end of the movie: complete loss of lumen (collapsed), reduction of lumen size (collapsing), lumen diameter unchanged or increased (normal). A Chi-square test shows a significant difference between spheres generated from control or *Ch-Vgl2* with respect to the phenotype 'reduced lumen size' (collapsing + collapsed). Chi-square value = 52.6, p-value < 0.00001. **n**, Snapshots of representative spheres at the beginning and end of the movie. Additional examples of collapsing spheres are shown in **movie 2-7**. Scale bar 5 μm (**a,b**); 10 μm (**c,h-l,n**); 50 μm (**f-g**)

Figure 3



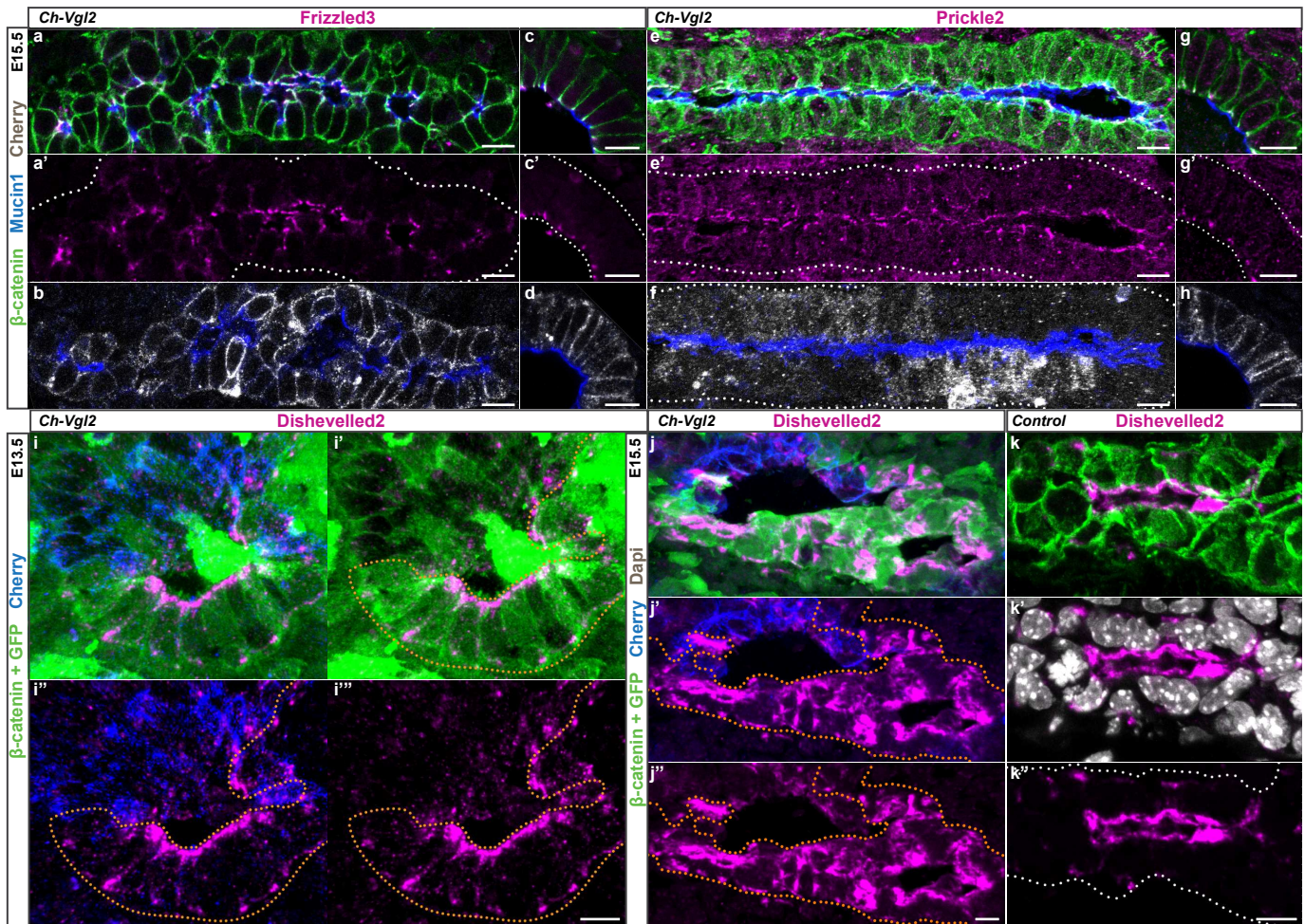
VANGL misexpression leads to a decrease of actomyosin contractility. a-b, 3D projection of confocal images showing expression of pMLC on E14.5 pancreas sections. Note the disappearance of pMLC apical foci in the *Ch-Vgl2* (*Pdx1-Cre; Cherry-Vangl2*) section (b). c, close up of a z section from the staining above highlighting the localization of pMLC protein at the apical cell junction in a WT sample. d, representative image of the pMLC western blots used for the quantification in e. e, pMLC/Tubulin ratios are normalized against the control littermates, each dot corresponds to one pancreas. Unpaired t-test reveals a significant decrease of pMLC protein level in the transgenic population compared to the control. (****) p-value < 0.0001. Scale bar 5 μm (c); 10 μm (a-b).

Figure 4



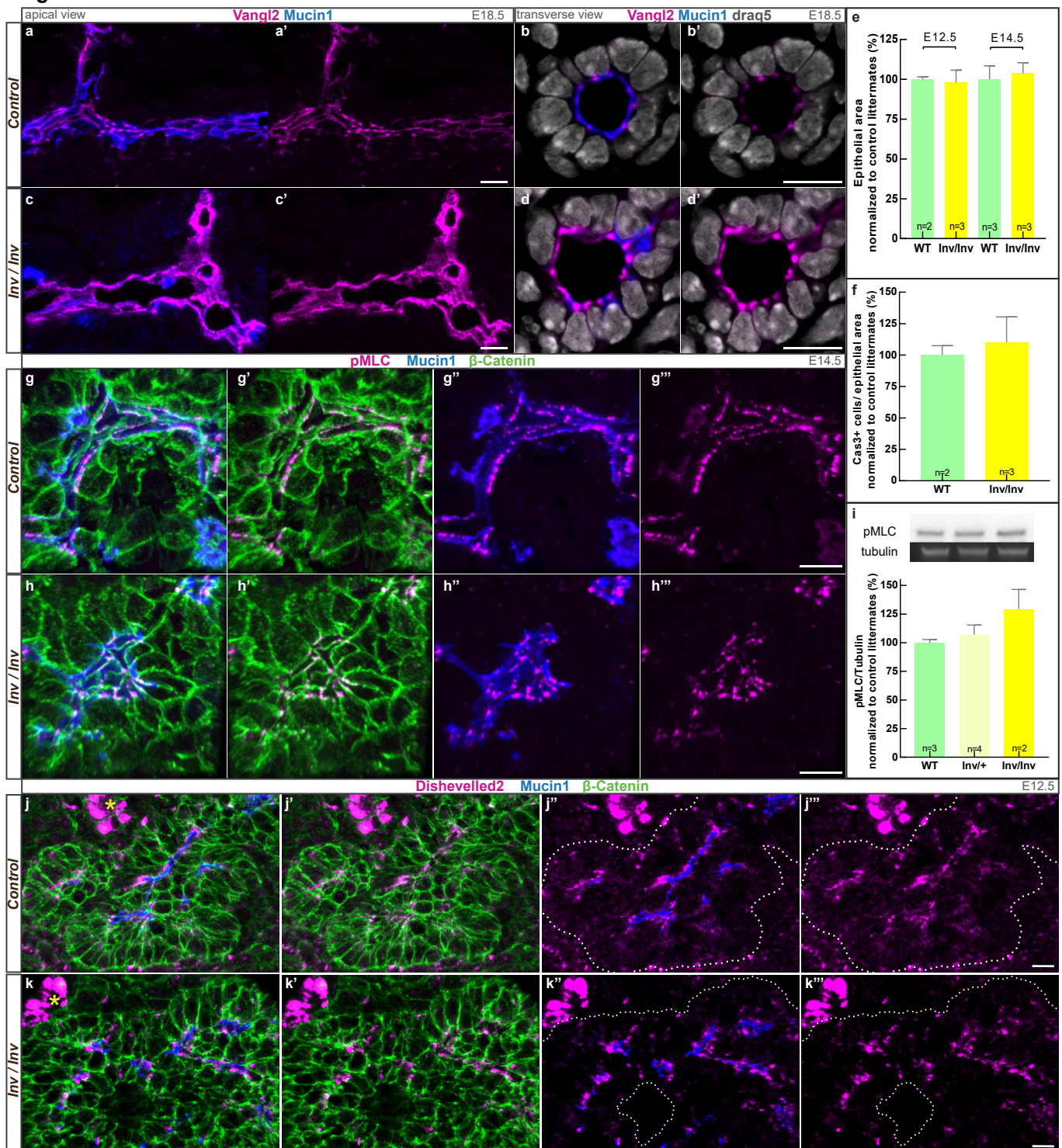
Activation of the RhoA pathway rescues cell death and hypoplasia induced by VANGL2 misexpression. a-f, Culture in 3D Matrigel of E10.5 pancreatic epithelium collected from WT or *Ch-Vgl2* (*Pdx1-Cre; Cherry-Vangl2*) embryos and submitted to treatment with ROCK inhibitor (H-1152) or RhoA activator (CNO3). **a, c, e,** pictures of the growing epithelium in each experimental condition at different time points of the culture (h=hours post seeding). **b, d, f,** graphs representing the quantification of the size of the epithelium, performed at each time point by measuring the circumference of the bud and fitting an exponential growth curve for each individual bud. For clarity, only an average growth curve is presented in each experimental condition. T-test computed on the doubling time of each curve demonstrates that double-transgenic (*Ch-Vgl2*, pink) and WT buds treated with ROCK inhibitor (purple) grow slower than their control littermates, p-value=0.01 and 0.006 respectively (n=3 in each condition). The buds treated with RhoA activator (orange) behave as controls (p-value= 0.8, n=3). T-test performed on the size of the bud at the end of the culture show that the *Ch-Vgl2* buds and the buds treated with ROCK inhibitor are smaller (p-value=0.02 and 0.001, respectively) while the bud treated with the activator became bigger (p-value=0.02). **g,** normalization of all the growth curves to the control littermates (see material and methods) showing that activation of the ROCK pathway in *Ch-Vgl2* can rescue the hypoplasia phenotype. **h,** statistical analysis computed on the slope of the normalized data presented in g. Each dot represents the growth of one bud; colors indicated the different experimental condition. (****) p-value <0.0001; ns=non-significant. **i,** representative image of a western blot against pMLC and quantification of the band intensities in the different experimental conditions showing that the rescued buds display similar level of Rock pathway activity relative to the control. One dot represents the pMLC/Tubulin ratio for an individual bud. Data were normalized to the control littermates. (****) p-value <0.0001; (***) p-value ≤0.0005; ns=non-significant. **j-l,** TUNEL assay on bud sections shows an increase of apoptosis in the *Ch-Vgl2* buds (k) as well as in the bud treated with ROCK inhibitor (l) compared to the control sibling (j). Scale bar 100 μm.

Figure 5



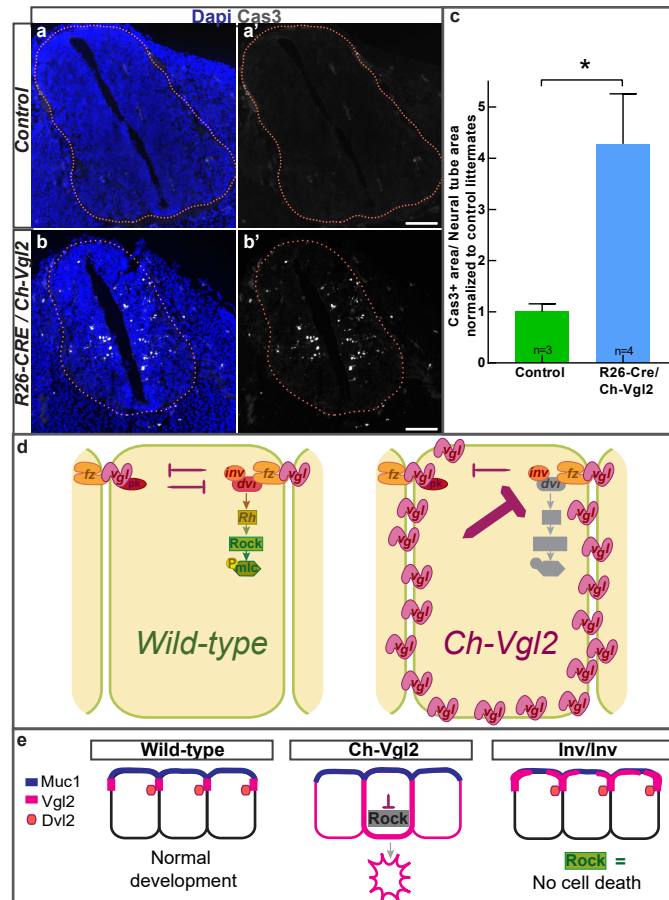
Ectopic VANGL expression interferes with Dishevelled but not with Prickle nor Frizzled localization. Confocal images of E15.5 (a-h, j-k) or E13.5 (i) pancreatic sections, showing the expression pattern of PCP components in Cherry-VANGL2+ area. (a-d, g-h) Z-sections and (e-f, i-k) 3D projections are displayed. Longitudinal (a-b; e-f) and transversal (c-d; g-h; i-k) sections of pancreatic ducts. **a-d**, Frizzled3 (FZD3) is expressed at the apical cell junctions in the area expressing the fusion protein Cherry-VANGL2 showing that VANGL basolateral expansion fails to induce a similar expansion of FZD3. Section b and d are adjacent to a and c respectively. **e-h**, Prickle2 (PK2) is expressed at the apical cell junction in the Cherry+ area. No extension of PK2 expression domain on the basolateral membrane is observed. Sections f and h are adjacent to e and g respectively. **i-k**, Dishevelled2 expression (magenta) is decreased in the Cherry+ cells (blue) compared to the adjacent cells that are not expressing the Cherry-VANGL2 protein (GFP+ cell; green and highlighted by orange dots) or the WT cells (k). Scale bar 10 μ m.

Figure 6



Apical overexpression of VANGL protein due to Inversin mutation does not lead to the perturbation of actomyosin contractility nor cell death. **a-d**, longitudinal (**a**, **c**) and transversal (**b**, **d**) sections of E18.5 pancreatic duct immunolabelled for VANGL (magenta) show an increase of the protein at the apical membrane in the inversin mutant (**c**, **d**) compared to the WT littermate (**a**, **b**). **e**, quantification of the epithelium area in the inversin mutant and the control littermates at E12.5 and E14.5 shows no difference in the size of the epithelium. p -value>0.5 by t-test. **f**, quantification of the number of Caspase3 positive (CAS3+) cells in E12.5 inversin mutant shows no significant difference in apoptotic cells compared to the control. The CAS3+ cells/ epithelium area ratio was normalized against the control littermates. p -value>0.5 by t-test. **g-h**, staining for pMLC in E14.5 WT and Inversin pancreatic sections shows no differences in the localization of the protein. **i**, representative image of a western blot against pMLC. Quantification of the pMLC/Tubulin ratio shows no difference between inversin homozygote, heterozygote mutant and their control littermates. p -value>0.1, by t-test. **j-k**, Z-projection of confocal images showing Dishevelled2 (DVL2) expression on E12.5 pancreatic sections. No differences of localization or level of DVL2 were observed between the control (**j**) and the inversin mutant (**k**). *, highlighted endocrine islet. Scale bar 10 μ m.

Figure 7



Misexpression of VANGL2 protein in the neural tube also induces apoptosis. **a,b**, representative images of Caspase3 immunostaining on whole embryo sections showing an increase of apoptotic cell in the neural tube of *Rosa26^{CreER}; Cherry-Vangl2* embryos collected 14h after induction of transgene expression relative to control (*Rosa26^{CreER}*). **c**, Quantification of the ratio CAS3⁺ area/neural tube area shows a 3.3 fold increase of apoptosis following expression of Cherry-VANGL2 protein. (*) p-value <0.05 by t-test. Scale bar 100 μ m. **d**, Working model: in wild-type cells (left) VANGL expression is restricted to the apical junctions and the Rock pathway is activated downstream of the FZD/DVL complex. In the *Ch-Vgl2* double-transgenic (right) VANGL expression is extended to the latero/basal membranes and Rock pathway activity is decreased. Since FZD protein level is not increased at the apical cell junctions of the transgenic cells, the intercellular activation of the distal FZD/DVL/INV complex by VANGL is not increased. Rather, cell autonomous misexpression of VANGL leads to a decrease of DVL protein and consequently of the downstream ROCK activity. **e**, Results summary.