Supplementary Information

Supplementary methods

Production and affinity-purification of the chicken Prickle1 antibody.

A segment at the C-terminus (amino acid 512-801; protein ID: XP_416036.2) of the chicken *Prickle1* gene was PCR cloned into the expression vector pET-32a, using the following primer pair: sense 5'-GGTACGGAGGTTCACTTGAA-3', antisense 5'-GATAACGCAGTAGTTGGACC-3'. Protein expression was induced by 1mM IPTG in BL21 bacteria. The Hig-tag fusion protein was affinity purified by a Nickel resin column (GeneScript). The purified lysate was further concentrated by running a SDS-PAGE gel and cut off the band at the correct size. The gel band was then used to immunize the mice with Freud's adjuvant (Sigma). The antiserum was collected and affinity purified by using the Prickle1-conjugated Nickel resin column.

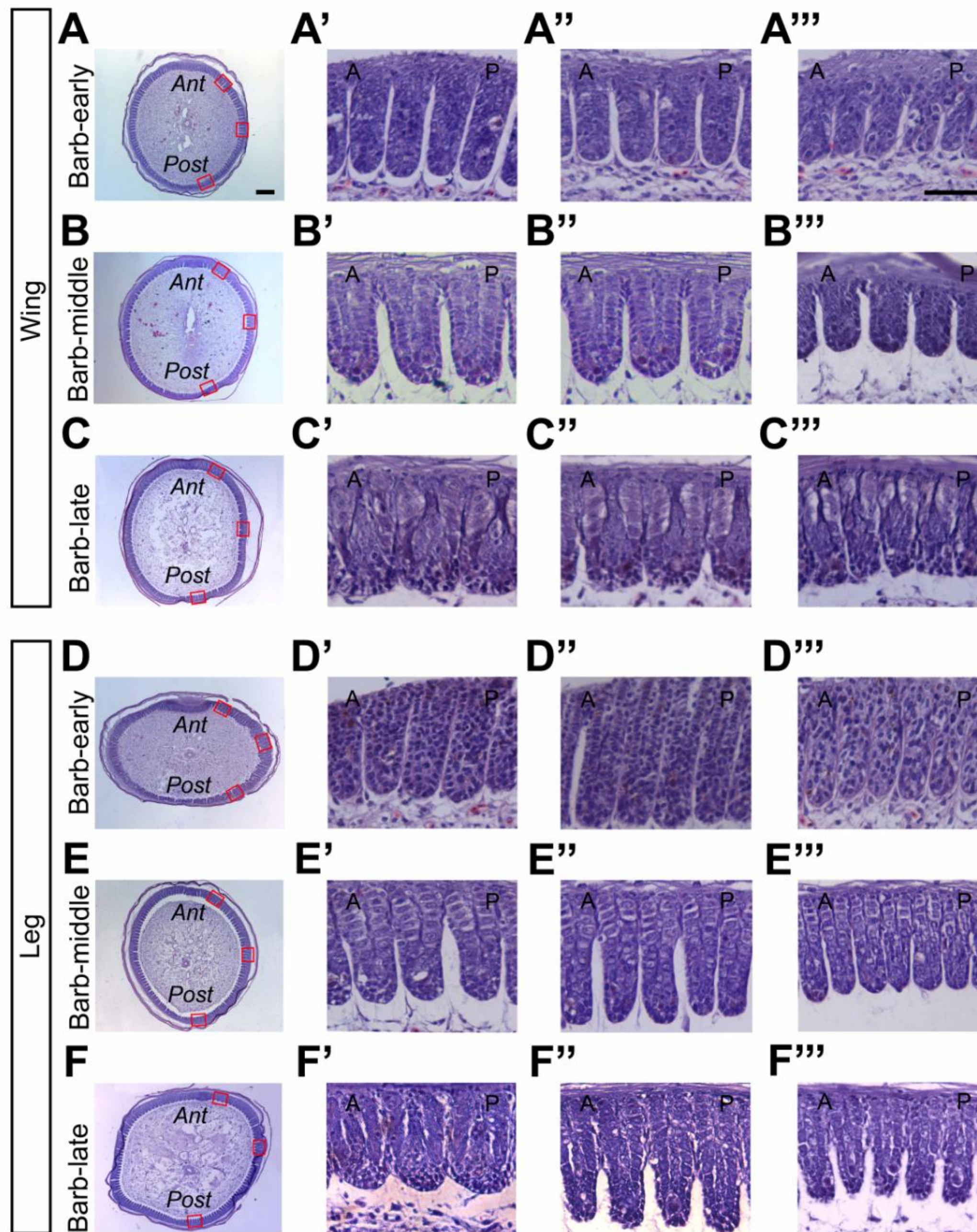

Fig. S1. Additional analysis of cell shape change in feather development. (A-C) Wing contour feathers as bilateral feathers. (D-F) Leg feathers as an approximation of radial feathers. The anterior, middle and posterior regions (red boxes) are shown as enlarged images. In bilateral feathers, the anterior barbule cells are elongated earlier, but eventually the posterior barbule cells are also elongated. This is in contrast to the radial feathers where the posterior barbule cells do not elongate. Scale bar: 50μm.

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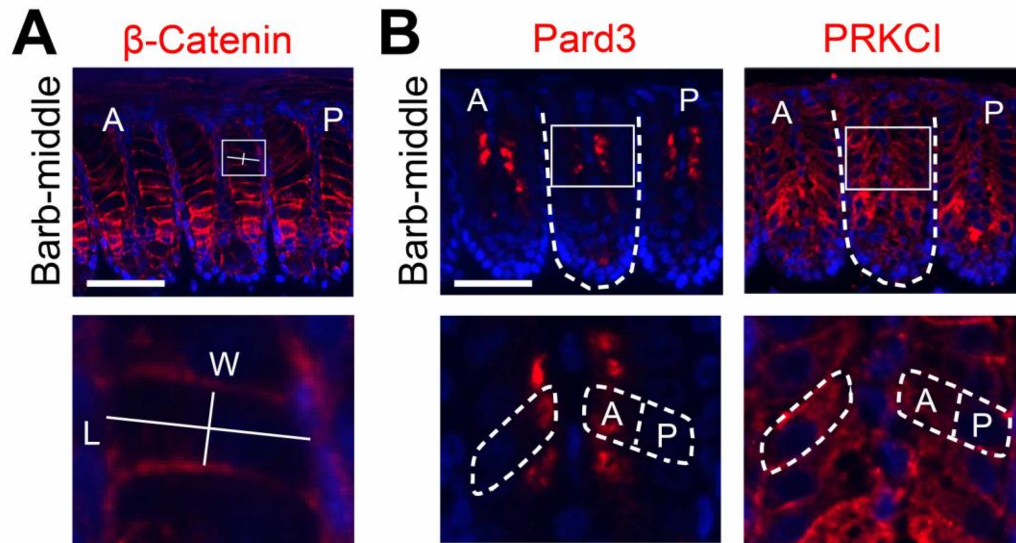

Fig. S2. Diagrams showing the measurement of barbule cell parameters. (A) The length (L) and width (W) of barbule plate cells. (B) The barbule cells were bisected into the anterior and posterior halves, and signal strengths were measured in each half using the ImageJ program. Scale bars: 50μm.

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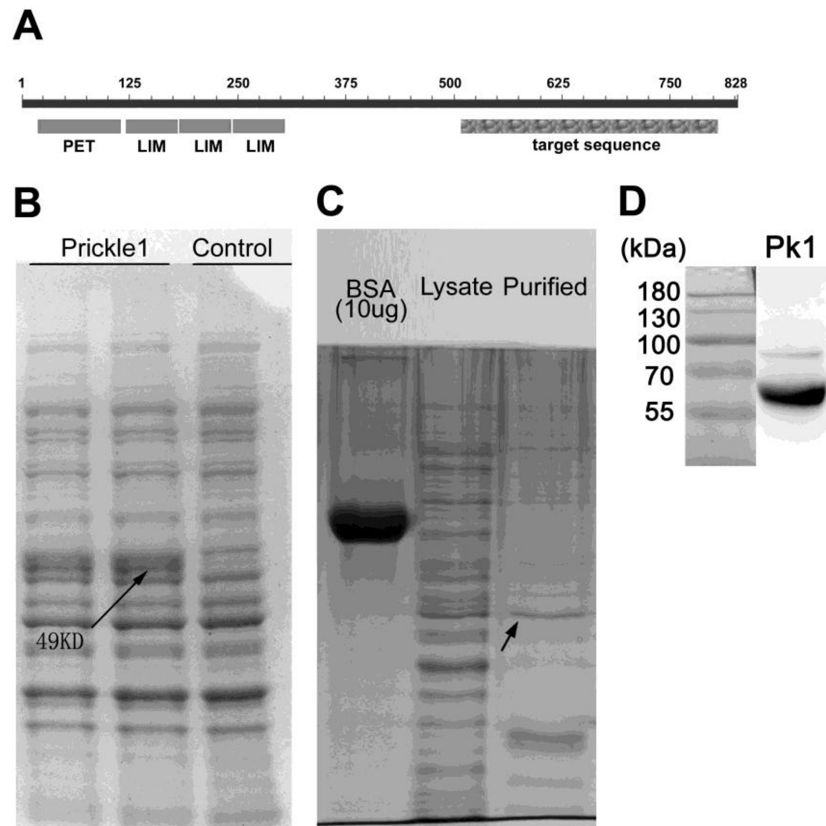

Fig. S3. Production and characterization of the chicken Pk1 antibody. (A) A segment near the C-terminus (amino acid 512-801) of chicken *Prickle1* gene was cloned into the pET-32a vector and expressed in BL-2 bacteria. (B) SDS-PAGE gel showing the induced expression of fusion protein by 1mM IPTG, which has the predicted size. (C) The fusion protein was purified using a Ni column. This protein was further concentrated by SDS-PAGE gel and the band was cut off at the predicted size to immunize the mice. (D) After purification, the antibody was verified in whole-feather lysate by Western blot analysis. A strong band at about 60kd was detected, as well as a weaker band at about 90kd, possibly corresponding to the different isoforms of this protein (similar to the Proteintech antibody 22589-1-AP and Abcam antibody #139077).

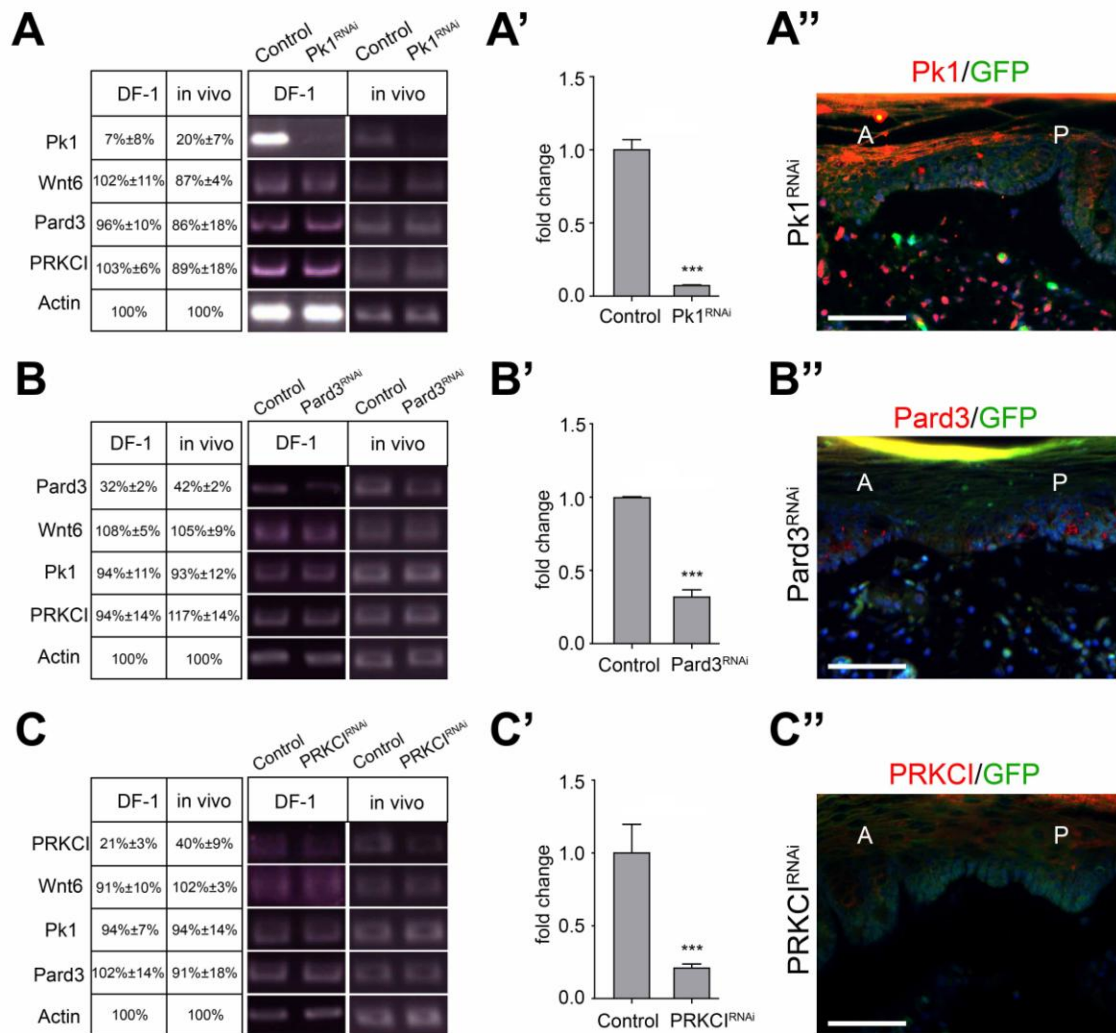

Fig. S4. The RNAi knockdown efficiency as examined both in vitro and in vivo. (A) Pk1, (B) Par3, and (C) aPKC. The constructs were transfected into DF-1 cells for 48 hours, or infect the feather follicle for 4 days before sample collection. A scramble viral vector was used as control. Gene expression levels were monitored by semi-quantitative RT-PCR and further quantified by qRT-PCR (A', B', C'; results are from DF-1 cells). In addition, local injection of the virus into the developing follicles were performed, and samples were collected 2 days later to examine the protein expression (red) and virus expression (GFP; A'', B'', C''). ***, $p < 0.001$. Scale bars: 50μm.

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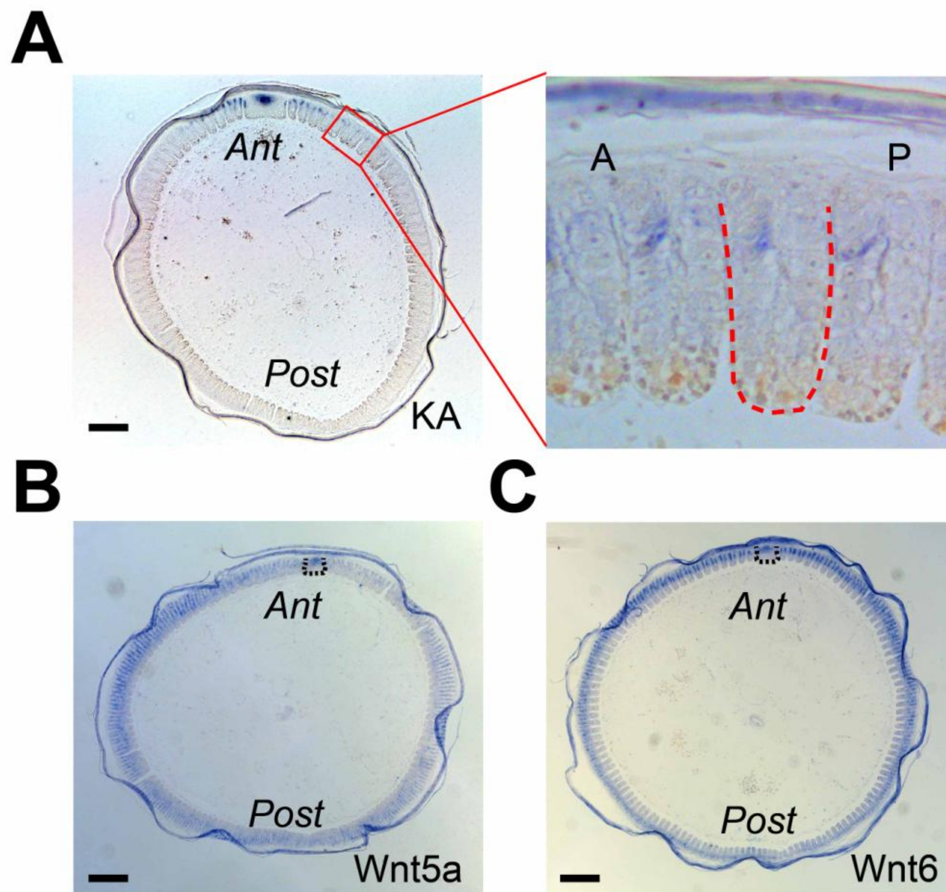

Fig. S5. Characterization of molecular expression in the feather follicle. (A) *Keratin-A* (*KA*) expression in bilaterally symmetric feathers. *KA* is expressed in the anterior barbule plate cells earlier than in the posterior barbule plate cells. (B-C) *Wnt5a* and *Wnt6* are weakly expressed in radially symmetric feathers. Scale bars: 100µm.

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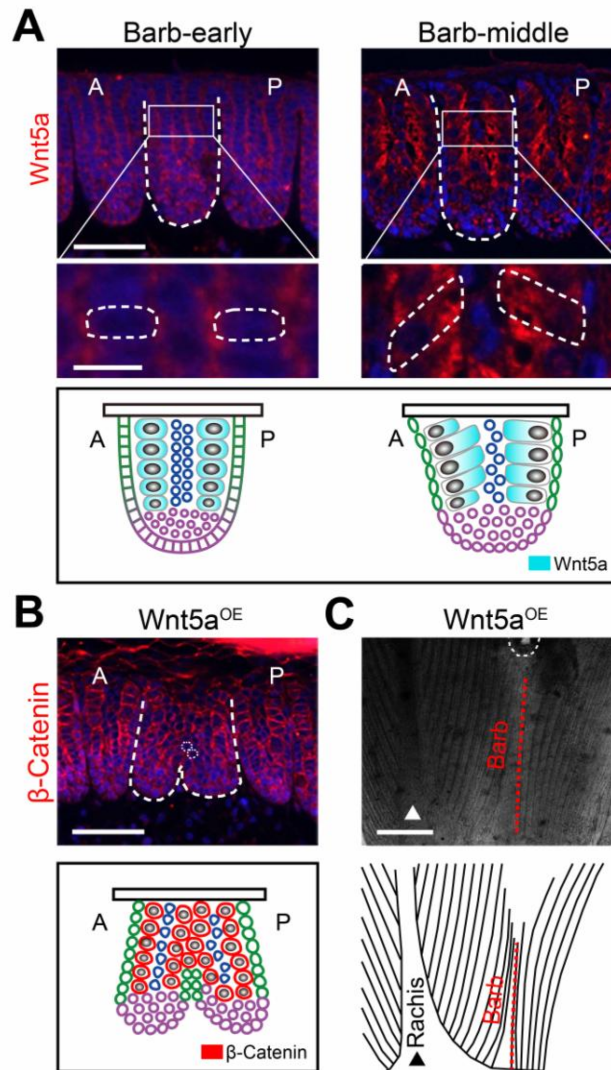

Fig. S6. Wnt5a expression and function in feather development. (A) Wnt5a was initially homogenously expressed, which then enriched toward the axial plate. (B) Overexpression of Wnt5a disrupted the programmed feather cell shape change. (C) Overexpression of Wnt5a disrupted directional tilting of barbs. Scale bars: 50μm in A, B; 200μm in C.

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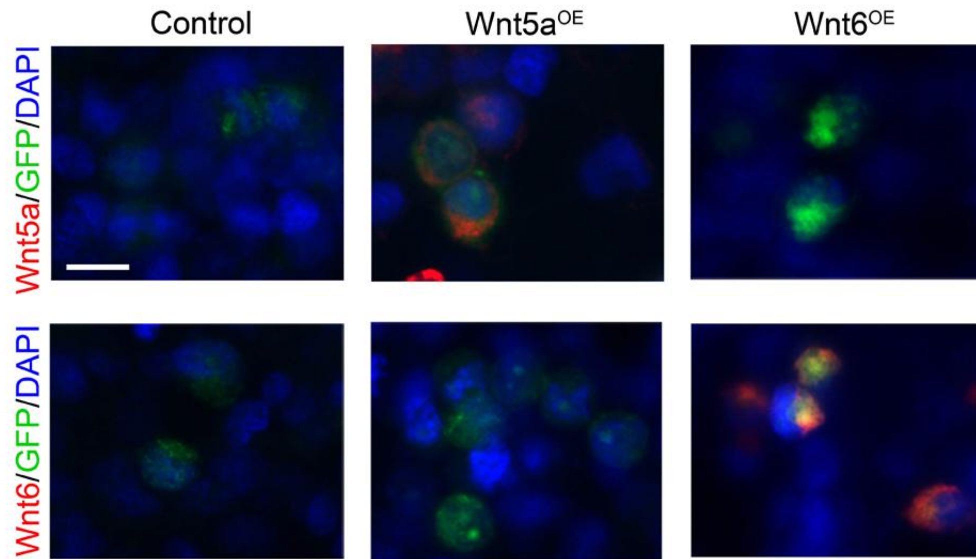

Fig. S7. Specificity of the antibodies for Wnt5a and Wnt6. The antibodies for Wnt5a and Wnt6 do not cross-react with each other. A control vector contains GFP is co-transfected into 293T cells, together with the plasmids containing the Wnt ligand Wnt5a or Wnt6, respectively. Scale bar: 20μm.

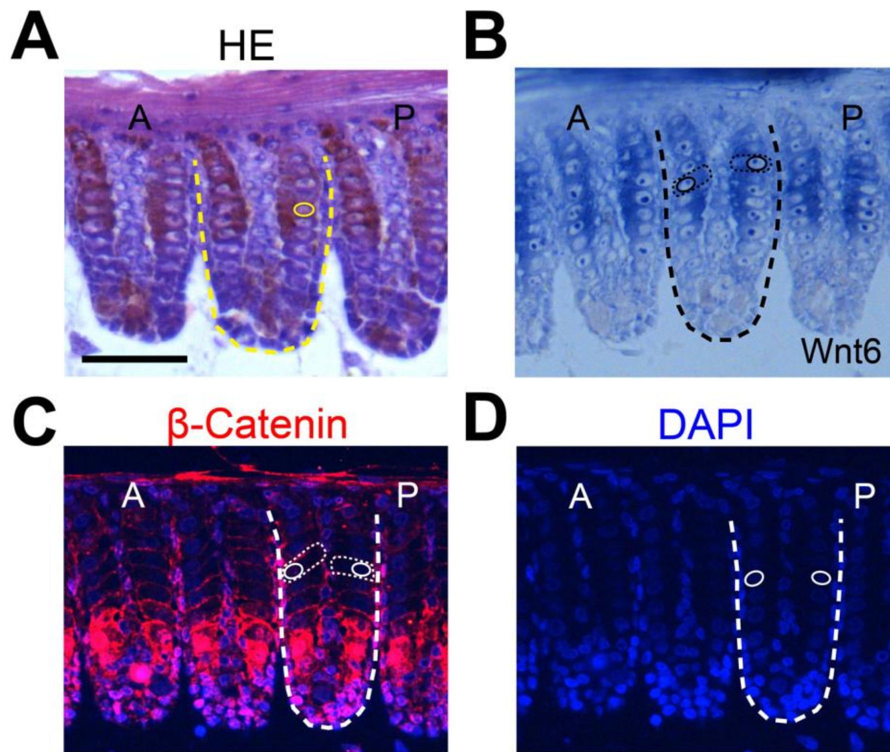

Fig. S8. Morphological features of apical-basal polarity in barbule plate cells. (A) HE staining of a pigmented feather (brown) showing the enrichment of cytoplasmic melanin content facing the axial plate, with the cell nucleus localized to the opposite side. (B) In situ hybridization of *Wnt6* showing the cytoplasmic mRNA in barbule plate cells were enriched facing the axial plate. (C-D) β-catenin and DAPI staining showing the relative localization of cell nuclei in barbule plate cells. Scale bar: 50μm.

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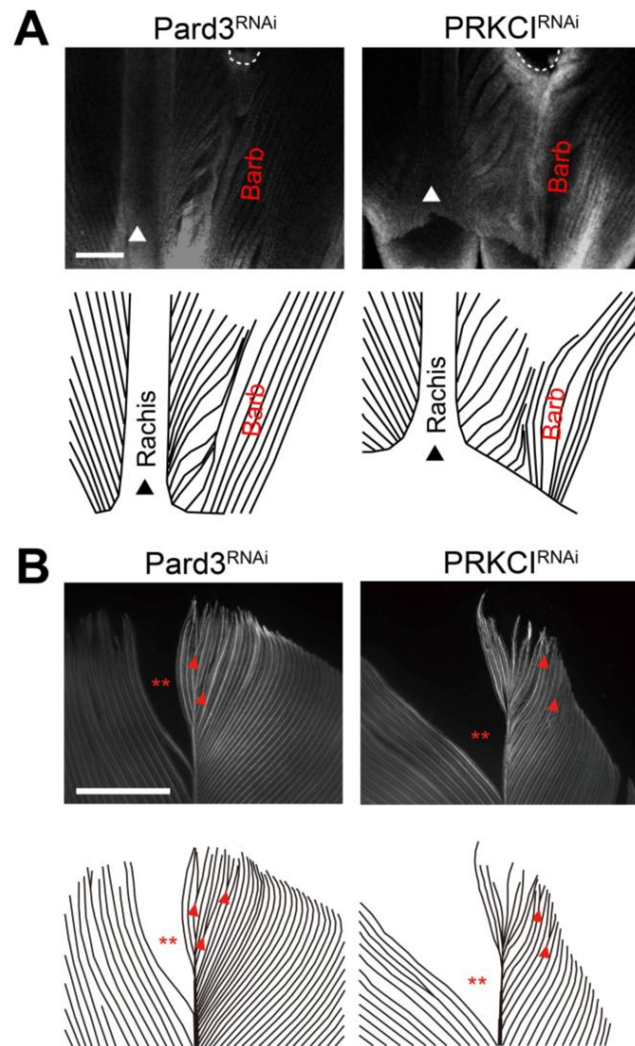

Fig. S9. Phenotypes of Par3 and aPKC perturbation. (A) Local perturbation of *Par3* or *aPKC* expression via injection of shRNA lentivirus disrupted barb tilting. (B) Gross morphology of feathers after knockdown of *Par3* or *aPKC* in vivo. Arrowheads, ectopic barb fusion; stars, regions showing lost of barbs. Scale bars: 200µm in A, 1cm in B.

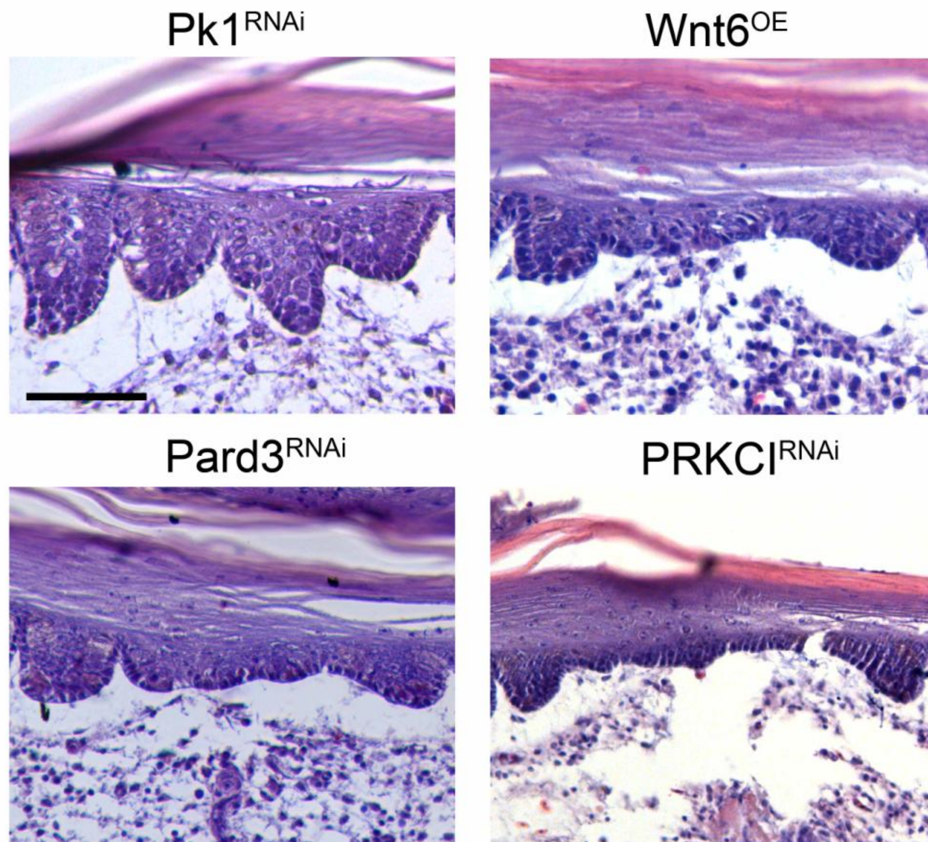

Fig. S10. Phenotypes of the feather barb after gene perturbation. Wnt6 overexpression (OE), Par3-RNAi or PRKCI-RNAi, and to a less extend Pk1-RNAi all reduced the cell number, and disrupted cell shape change within the barb. The branching of feather epithelium is also reduced/disrupted. Scale bar: 50µm.