Supplemental Figure 1. (A) Genomic structure and mutant alleles of Prickle 1 gene (Liu et al, 2014). Prickle 1a (hypomorphs) and Prickle 1b (null) alleles were bred together to produce Prickle 1a/b compound mutant mice. (B) Immunoblot of tissue extracts from E13.5 embryonic hindpaws was probed with a customized polyclonal Prickle 1 antibody. Prickle 1 was barely detectable in Prickle 1b/b and Prickle 1a/b mutants.
Supplemental Figure 2

Supplemental Figure 2. (A) Illustration of measurement of eyelid closure at E15.5. Same measurement was performed at E16.5. The maximal vertical distance (white lines) between the upper and lower eyelid edges (n>5, dashed line circles) were measured to indicate progress of eyelid closure. (a), Plotted measured distances. (B) Peridermal cells revealed by DAPI staining at E15.5 (dashed circles) were counted and plotted in (b). Five sections of each
embryo were subjected to counting. Total 3 embryos for each genotype were used. (C) Illustration of measurement of the eyelid thickness of a E15.5 wild type embryo. Same measurement was performed at each age presented. Because of the uneven thickness along the eyelid meridian axis on cross sections, thickness index was generated as follows: 1) line “a” is drawn from the apex of the eyelid wedging epithelium; 2) line “b” is drawn to skin surface vertical to “a”; 3) line “c” is drawn along skin surface connecting “a” and “b”; 4) the closed area contained within “a”, “b”, and “c” were measured by NIH ImageJ; and 5) measured areas were divided by length of “a” to generate thickness indices. Note “a” is drawn in same length through ages of eyelids. (c), Plotted eyelid thickness indices. Multiple sections (n>5) from each eyelid were measured. Three or more embryos were used for each genotype. Student’s t-test was performed to detect p-values for all measurements.
Supplemental Figure 3. (A-C), SEM of ocular surface of E15.5 wild type, Prickle 1<sup>a/b</sup>, and Prickle 1<sup>b/b</sup> embryos. Note the similar degree of eyelid opening of Prickle 1<sup>a/b</sup> and Prickle 1<sup>b/b</sup> embryos. (A’-C’) Magnified images in boxed areas respective to (A-C). Arrows point to the peridermal cells. (D-F) Bright field microscopy of ocular surface of whole mount embryos at E16.5.
Note the entirely closed wild type eyelid and partially closed *Prickle 1*<sup>a/b</sup> and *Prickle 1*<sup>b/b</sup> mutant eyelid. (G-I) Eyelid from each genotype was completely closed at E17.5.

Supplemental Figure 4. (A), Defined areas for counting apoptotic cells at E15.5 and E17.5. Tunel labeling is in green. (a), Plotted cell counts per square millimeter. Lines demarcate the areas chosen for apoptotic cell counting. Five
sections of each embryo were subjected to counting. More than 3 embryos for each genotype were used. No significant differences were detected by Student's t-test. (B), Illustration of measurement of the amount of eyelid opening in postnatal mutant mice. P8 mutant eyelids were not counted as open because they were connected with connective tissues (green arrow). Distances between upper and lower eyelids (green lines) through the meridian were measured on multiple sections (>3) of each of the multiple animals (>3). Student's t-test was performed to detect the p-values. (b), Plotted measured distances of the postnatal eyelids.
Supplemental Figure 5

Supplemental Figure 5. For all panels, keratinocytes markers K1 and K4 were used for immunostaining of vertical sections through meridian of the eyelids. Letters “s”, “pc”, and “c” indicate skin, palpebral conjunctiva and cornea, respectively. Red, K1; Blue, DAPI; Green, K4. Red, yellow and white
arrows indicate skin, conjunctiva and cornea epithelium, respectively. (A-C) K1 and K4 were expressed in complementary domains of skin and conjunctiva epithelium in both wild type and the mutants at P5. (C) merged from (A) and (B). Part of stained area in (C) (small dashed box) was zoomed in (solid box right corner) to structurally distinguish palpebral conjunctiva from the underneath cornea epithelium for their close proximity. The seemingly expansion of K1 in the mutant skin (arrows in (A) and (C) right panels) is likely due to the deformed mutant eyelid with a lying “v”-shape rather than a “u”-shape, which normally presents in the wild type. The anterior chamber staining in (B) and (C) was non-specific. (D-F) K1 and K4 expression at P8 is similar to at P5. (F) is a merged image from (D) and (E). (G-I) Mutant K1 and K4 expressing in their respective skin and conjunctiva domains retained normal at P10. However, a precocious eyelid reopening was observed (right panels). Cornea was disorganized revealed by DAPI staining (right panels of (G) and (I)), and K4 expression was extending toward cornea epithelium. (I) is merged from (G) and (H). (J-L) At P15, both wild type and mutant eyelids opened, and mutant K1 and K4 expression in respective skin and conjunctiva epithelium retained normal. Ectopic expression of K4 in the mutant cornea epithelium extended further through the central cornea (K). (L) is merged from (J) and (K). (M) Quantification of domain size of K1 expression. Dashed lines illustrated the K1 expression domain under skin tissue of both wild type and mutant eyelids at P5. K1 expression domain is measured from its conjunction
point of skin/eyelid expression to its ending point of the inner lid expression. Length indices of dashed lines were generated by NIH ImageJ. (m), Plotted domain size of K1 expression. Multiple sections (>3) from meridian region of each eyelid were measured. More than 3 embryos are for each genotype. Student’s t-test was performed to detect the p-values.

Supplemental Figure 6

Supplemental Figure 6. (A), Generation of the eyelid thickness indices for postnatal mice. The method is described in Supplemental Fig. 2C and Materials and Methods section. Basically, area of “abc” (Area(abc)) was measured and divided by length of “a”, which is arbitrarily fixed for wild type
and mutant eyelids of the same age. (B) For analysis of eyelid cell infiltration, Prickle 1/EYFP positive mesenchymal cells were counted from E17.5 to P15. Because cell infiltration is positively correlated with the area of eyelid pocket but not with the surficial circumference of the eyelid pocket, the number of counted cells on each section was normalized to the length of surficial circumference (white lines). Plotted data is shown in Fig. 5G. (C) Analysis of cell size. For measuring cell size, staining of beta-catenin was used to demarcate the cell boundary. Cell size indices were generated by total measured area (red dashed lines) divided by total cell number. P5 and P8 wild type (left) and mutant (right) eyelids were subjected to analysis. (D), Plotted cell indices. Multiple sections (>3) from each of embryos (>3) were counted for all ages. Student’s t-test was performed to detect p-values for all measurements.
Supplemental Figure 7. (A) Ki67 stained P8 eyelids showing choosen areas for quantification. Quantified method is described in Supplemental Fig. 2C for P8 and for all other ages of eyelids. The results of quantification are plotted in Fig. 6E. (B) An example for apoptotic cell counting at P8. Dashed lines demarcated cells that were chosen for counting. Hair follicle apoptotic cells (arrowhead) were excluded from counting. Tunel-labeled apoptotic cells are in green. DAPI in blue. Separate channels for Tunel labeling are shown in (C). Quantification results are plotted in Fig. 6J. Multiple sections (>5) from each mouse were counted. More than 3 mice were used (n>3). Statistics was performed with Student’s t-test.
**Supplemental Figure 8.** (A) Immunostained Phosphorylated C-Jun expression at E15.5 for illustration of quantification methodology. Lines and dashed lines marked chosen areas for quantification. White lines were drawn in same length. Defined areas were enclosed within dashed and solid white lines. Fractions of counted phosphor-c-Jun cells in the counted areas are plotted in Fig. 8K. (B, C), β-catenin expression (green) in the eyelid front areas at E15.5. To quantify fluorescence intensity, the eyelid front area was divided into two sub domains: the wedging epithelium “a” (red lines) and the protruding front cells “b” (dashed lines). Fluorescence intensity from these two domains were separately quantified using NIH ImageJ. (C) Separate channels
for β-catenin expression. Data is plotted as intensity ratio of “a” to “b” in Fig. 8P. Multiple sections (>3) from each of embryos (>3) were subjected to counting. P-values were generated by performing Student’s t-test.

Supplemental Figure 9. Prickle 1 expression after eyelid closure and before eyelid reopening. (A) Immunostaining of eYFP reporter at E17.5 indicated broad expression of Prickle 1 in eyelid tissues. Bracket indicates eyelid junction, arrow point to the cornea epithelium. (B) Expression of Prickle 1 at P1. Note the downregulation in eyelid junction tissue (bracket). Asterisks
indicate cornea stroma cells. (C) Expression of *Prickle 1* at P5. Left and right arrows point to a hair follicle and Meibomian gland, respectively.