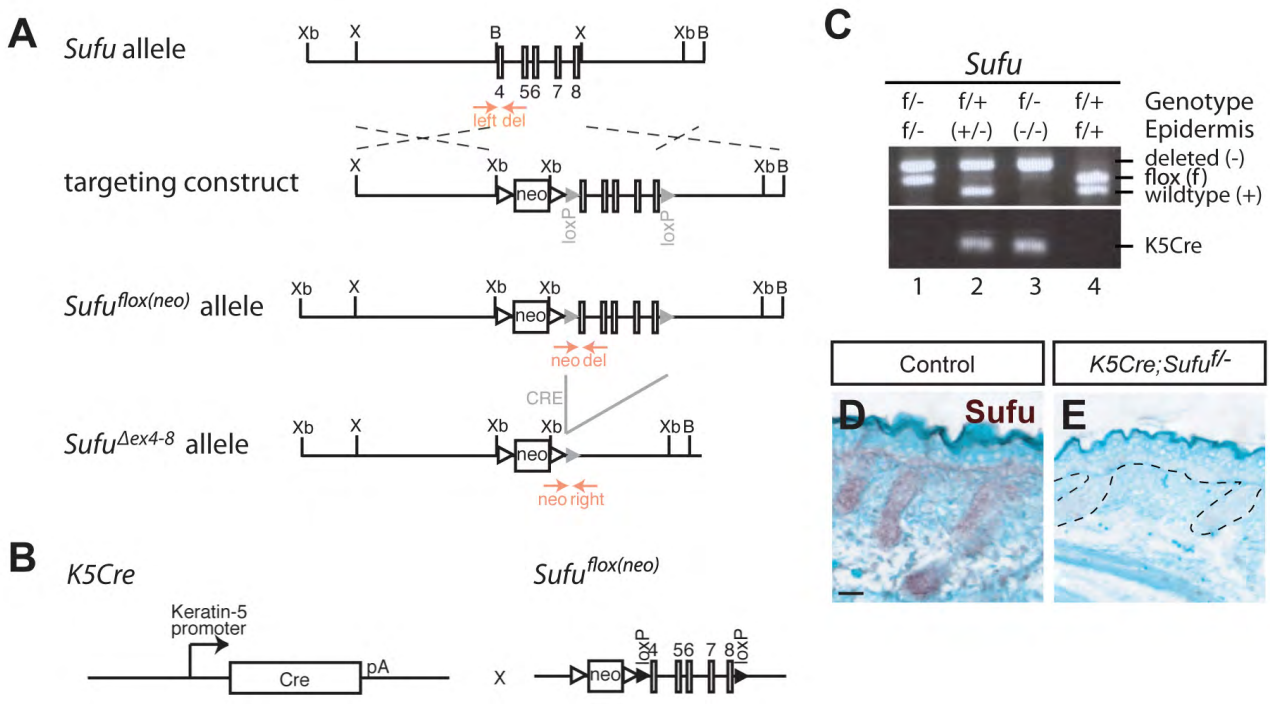

Fig. S1. Skin-specific deletion of *Sufu*. (**A**) Schematic of the targeting strategy. The conditional *Sufu* allele contains a pair of loxP sites flanking exons 4 to 8, and a neomycin-cassette flanked by Frt-sites. (**B**) Schematic diagrams of *K5Cre* and *Sufu*^{flox} alleles. (**C**) PCR genotyping of E18.5 epidermis to confirm the deletion of *Sufu*. (**D**,**E**) Immunohistochemistry reveals Sufu protein expression in hair follicle and epidermis of control skin, but absent upon Cre-mediated deletion at E18.5.

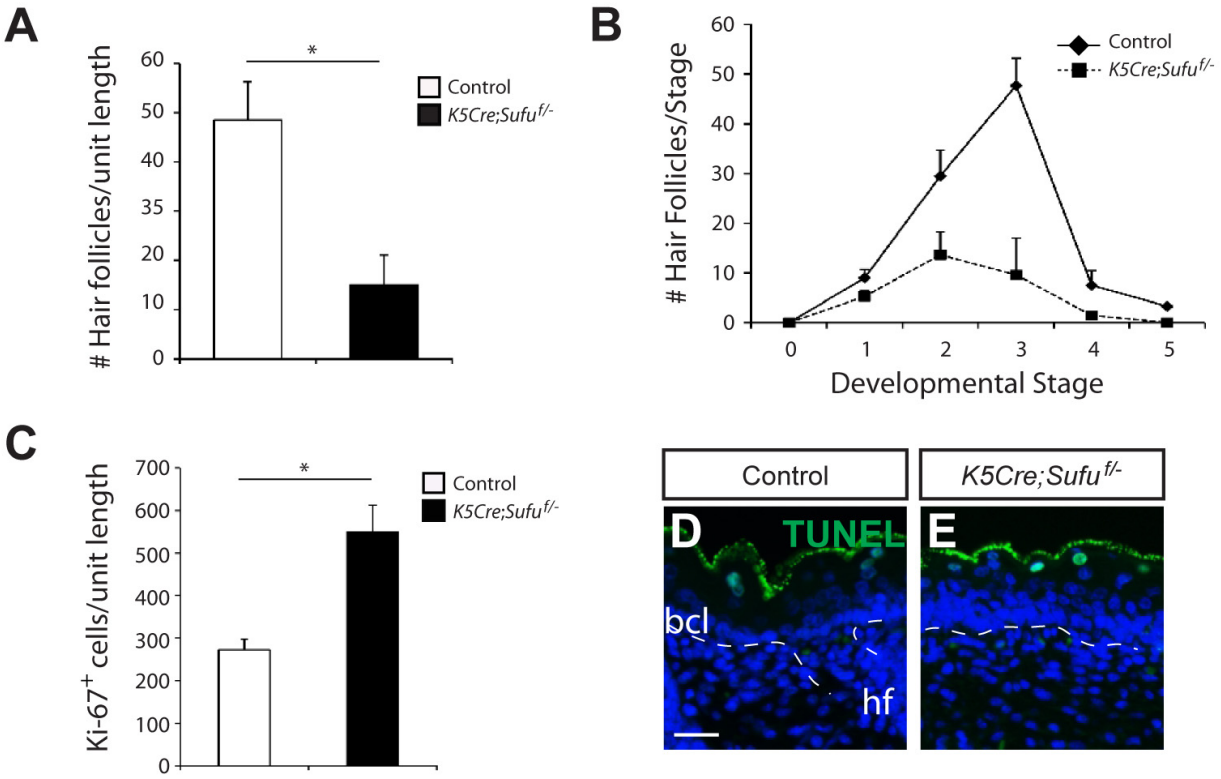

Fig. S2. Hair follicle morphogenesis defects in *Sufu* mutant skin at E18.5. (**A**,**B**) Histomorphometric studies show a developmental delay and reduction in number of hair follicles in *Sufu* mutants compared with control. Data are given as the means and error bars represent s.e.m. (*P<0.05, with t-test). (**C**) A significant increase in proliferation is observed in *Sufu* mutant skin compared with control, as indicated by the number of Ki-67 positive cells. Data are given as means and error bars represent s.e.m. (n=3, *P<0.05, with t-test). (**D**,**E**) TUNEL staining does not reveal a difference in apoptosis in the basal cell layer of *Sufu* mutant skin compared with control. bcl, basal cell layer; hf, hair follicle. Scale bar: 50 μm.

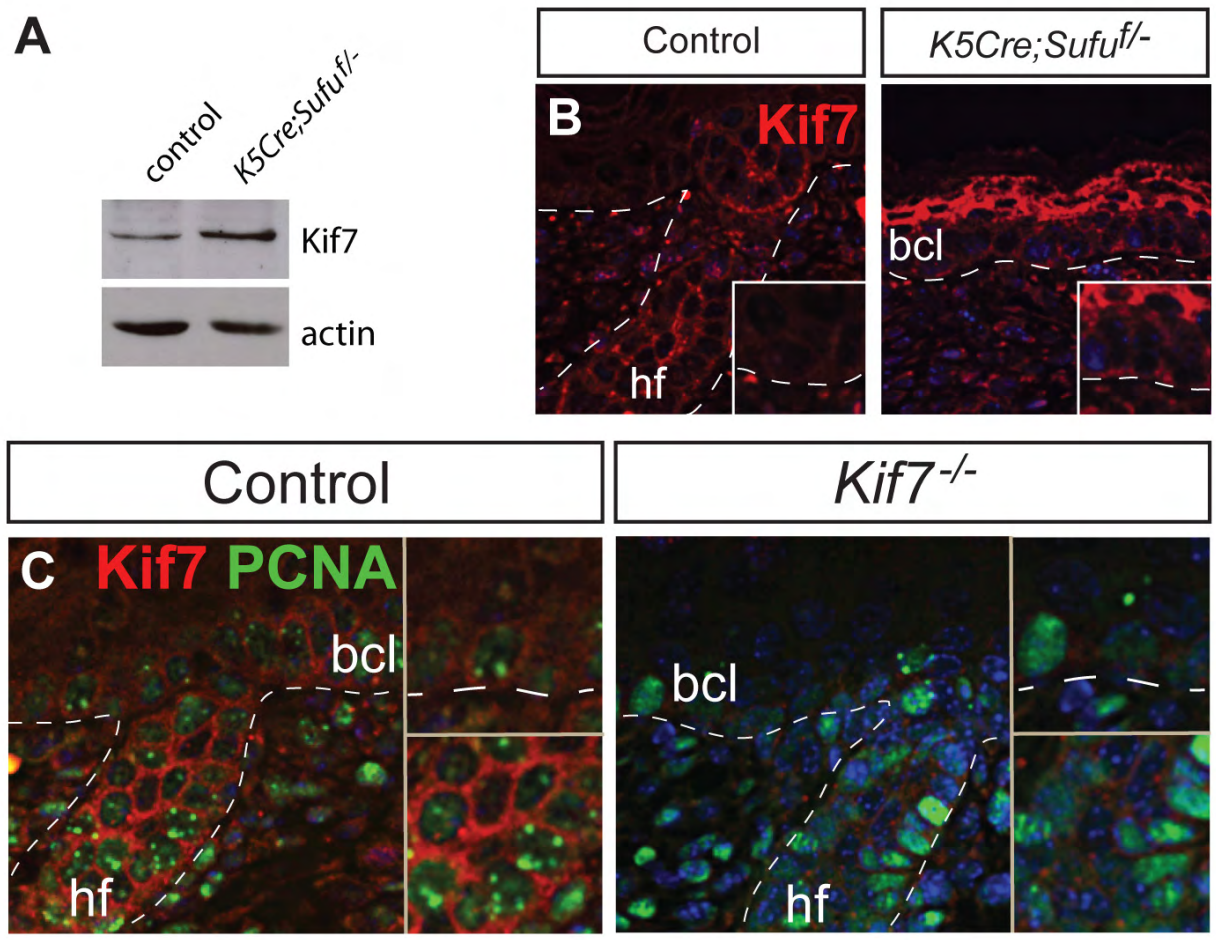

Fig. S3. Kif7 expression in the skin of wild-type and *Sufu* mutants at E18.5. (**A**) Western blot analysis reveals that Kif7 protein level is upregulated in *Sufu* KO keratinocytes compared with control. A representative western blot is shown from three independent sets of *Sufu* KO and control keratinocyte lysates. (**B**) Immunostaining shows an upregulation of Kif7 expression in the epidermis of *Sufu* mutant skin (see also inset for a higher magnification). (**C**) Immunostaining of Kif7 reveals that Kif7 is expressed in the proliferative basal cell layer (bcl), and the hair follicles (hf) and dermal cells of embryonic skin. Kif7 expression is higher in the keratinocytes of the hair follicles than in the basal cell layer (compare lower with upper inset).

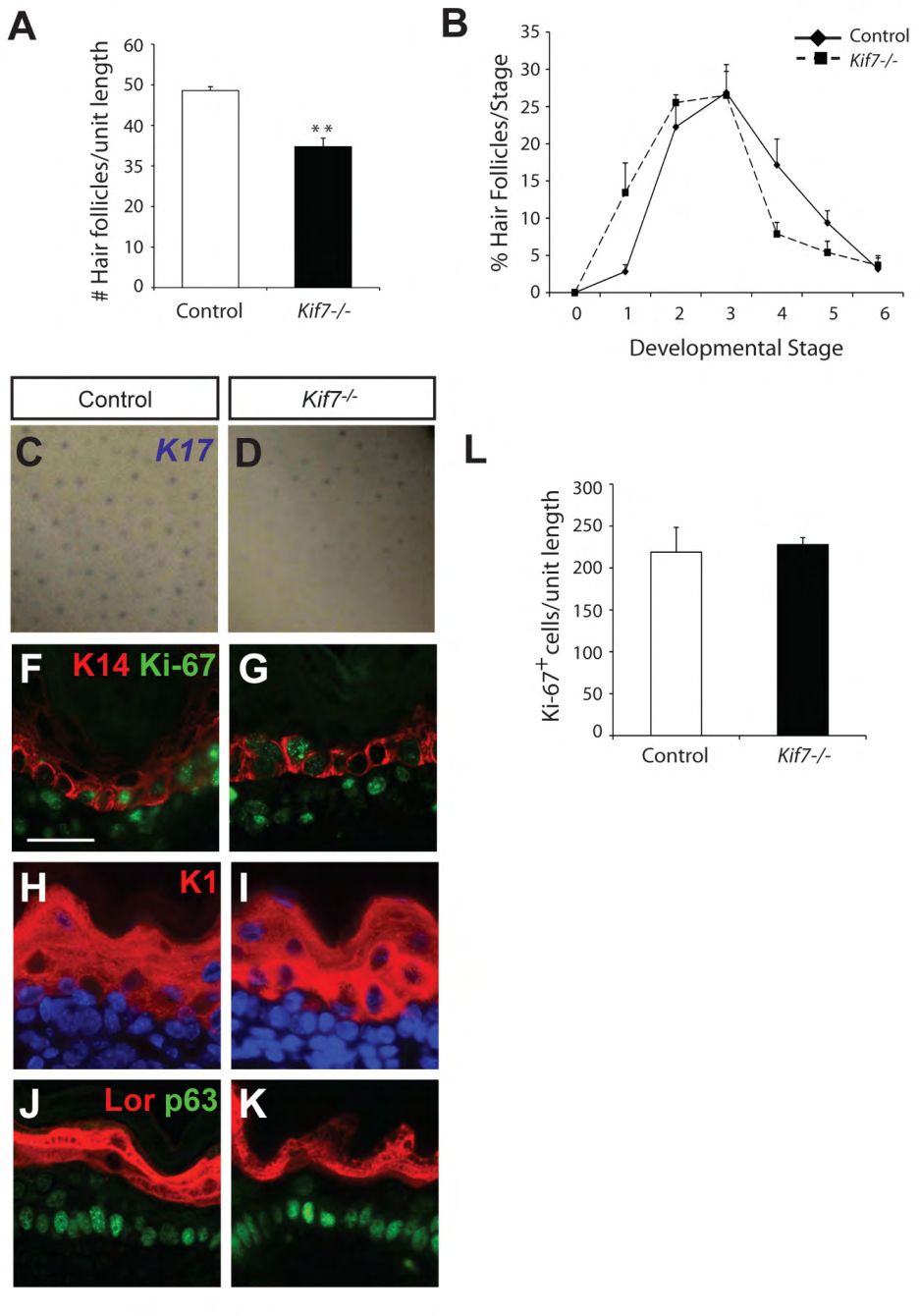

Fig. S4. *Kif7*$^{-/-}$ skin displays hair follicle defects while epidermal stratification appears normal. (**A**,**B**) *Kif7*$^{-/-}$ skin displays a reduction in the number of hair follicles and delayed hair follicle development at E18.5. Data are given as means and error bars represent s.e.m. (**P<0.01, with t-test). (**C**,**D**) Whole-mount in situ hybridization analysis of *K17* expression at E15.5 reveals a reduction in the number of hair follicles *Kif7*$^{-/-}$ embryos. (**F-K**) Immunostaining of K14, K1 and Loricrin show that the stratification of the epidermis is not affected in *Kif7*$^{-/-}$ skin at E18.5. Ki-67 and p63 expression appear similar between *Kif7*$^{-/-}$ and control skin. (**L**) Proliferation is not significantly affected in *Kif7*$^{-/-}$ skin compared with control at E18.5, as indicated by the number of Ki-67-positive cells. Data are given as means and error bars represent s.e.m. (n=3). Scale bar: 50 μm.

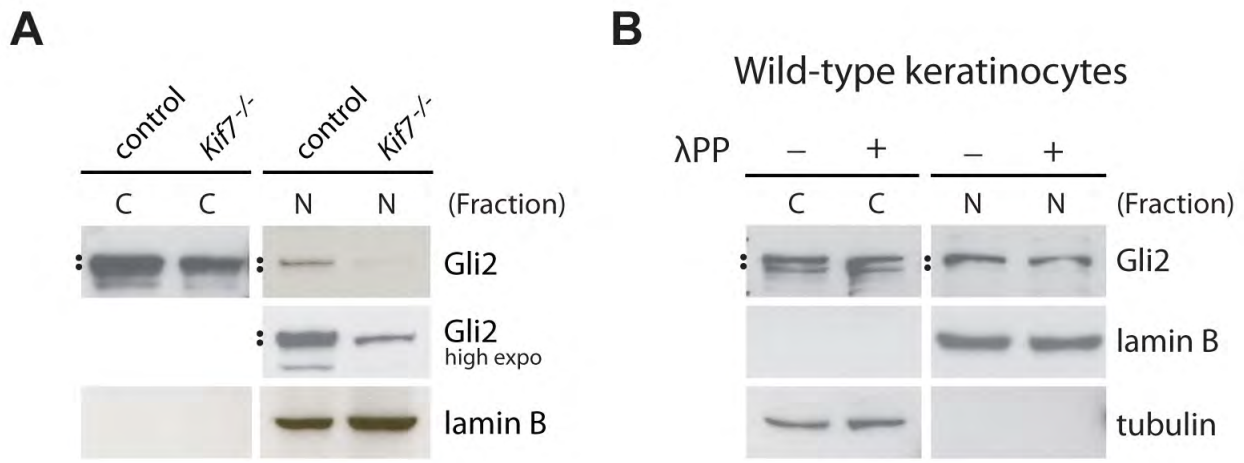

Fig. S5. Characterization of Gli2 protein in *Kif7*$^{-/-}$ and control keratinocytes. (**A**) Subcellular fractionation of *Kif7*$^{-/-}$ keratinocytes shows that the higher molecular weight of Gli2 is not detectable in the nucleus. This is a similar image to Fig. 4K. Dots to the left of the blot indicate the two different molecular weight of Gli2. (**B**) Treatment with lamda phosphatase (λPP) did not change the mobility of Gli2 on the SDS-PAGE gel.

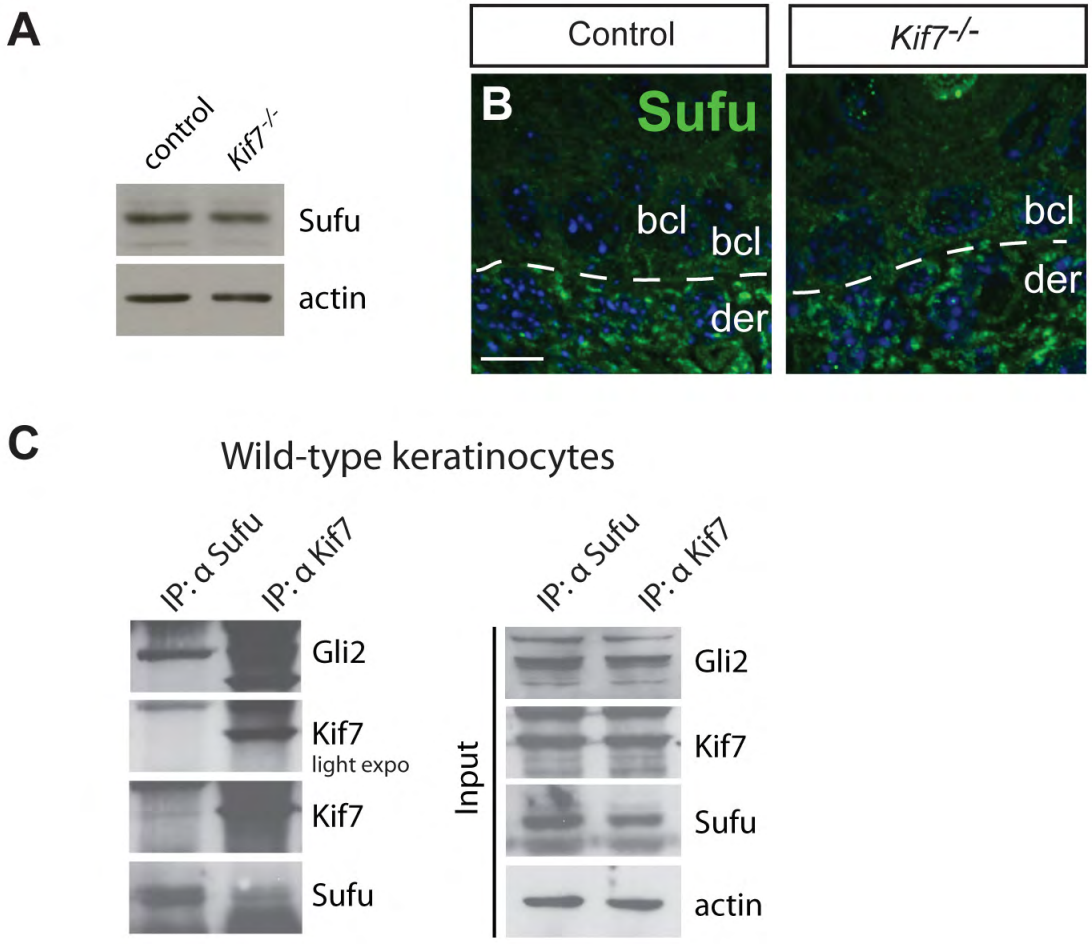

Fig. S6. Sufu expression in *Kif7* mutants at E18.5. (**A**) Western blot analysis reveals that Sufu protein level is similar in *Kif7* KO keratinocytes compared with control. A representative western blot is shown from four independent sets of *Kif7* KO and control keratinocyte lysates. (**B**) Immunostaining shows that Sufu is expressed throughout the skin, including the basal cell layer and the dermis. A similar pattern of Sufu expression is found when comparing control with *Kif7* mutant skin. (**C**) Endogenous Sufu-Kif7 complexes were detected by co-immunoprecipitation in E18.5 wild-type keratinocyte lysates. bcl, basal cell layer; der, dermis; hf, hair follicle. Scale bar: 50μm.

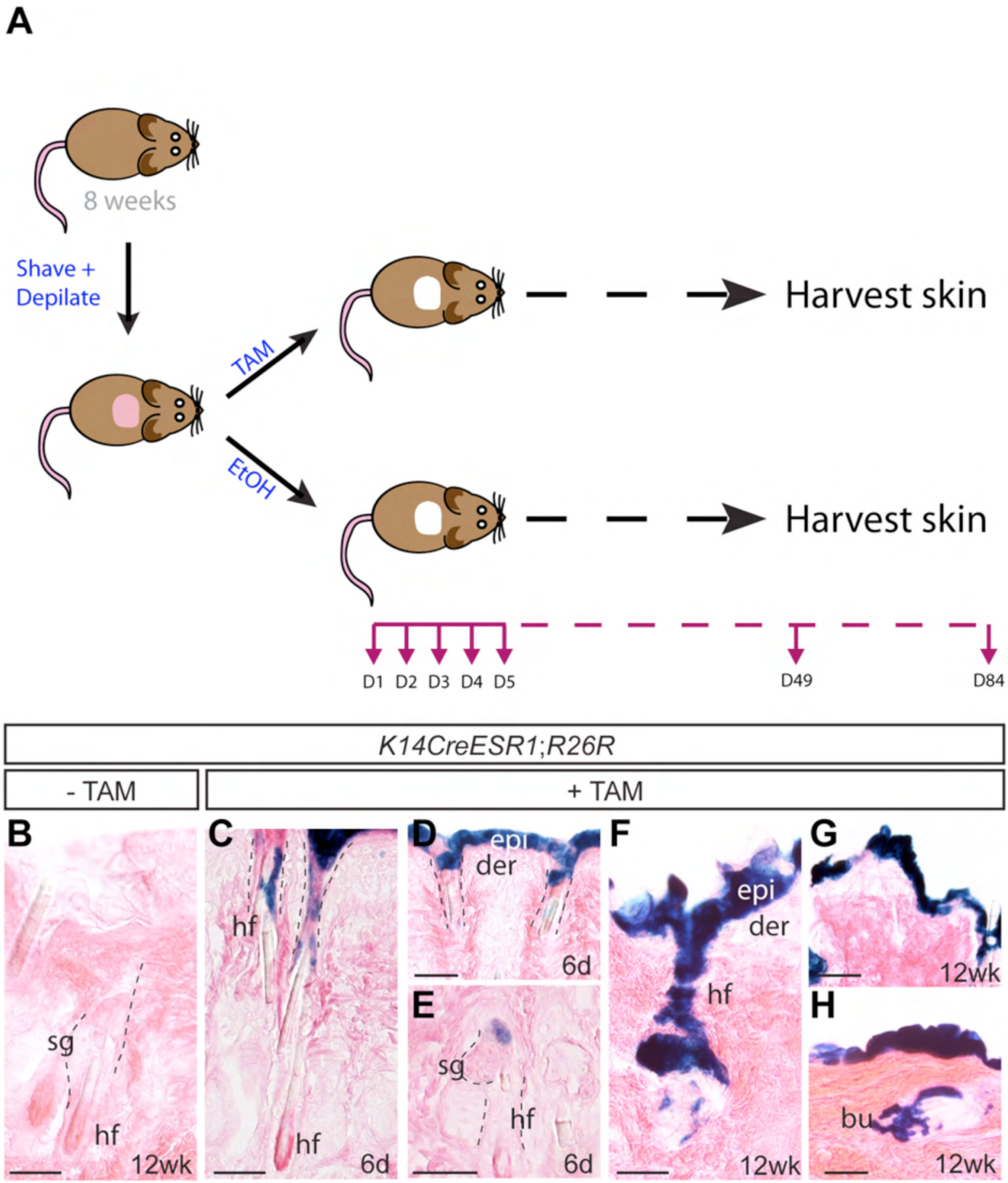

Fig. S7. Tamoxifen administration and β-galactosidase staining of K14CreESR1 deletion in the adult skin. (**A**) Transgenic mice (*K14CreESR1*;*Kif7*$^{f/-}$, *K14CreESR1*;*Sufu*$^{f/-}$ or *K14CreESR1*;*Kif7*$^{f/-}$;*Sufu*$^{f/-}$) were aged to 8 weeks. A dorsal region was shaved and depilated followed by topical application of tamoxifen (TAM) or ethanol control for 5 consecutive days. The skin was harvested for analysis by 7-12 weeks. (**B-H**) β-Galactosidase (β-gal) staining of *K14CreESR1*;*R26R* skin harvested at the indicated time after 5 consecutive daily treatments with either ethanol (B; –TAM) or TAM (C-H; +TAM). In the TAM-treated animals, K14Cre-positive cells contributed to all lineages of the skin: the interfollicular epidermis, hair follicles and sebaceous glands. No β-gal staining is detected in the skin of ethanol-treated mice. bu, bulge; der, dermis; epi, epidermis; hf, hair follicle; sg, sebaceous gland. Scale bar: 100 μm.

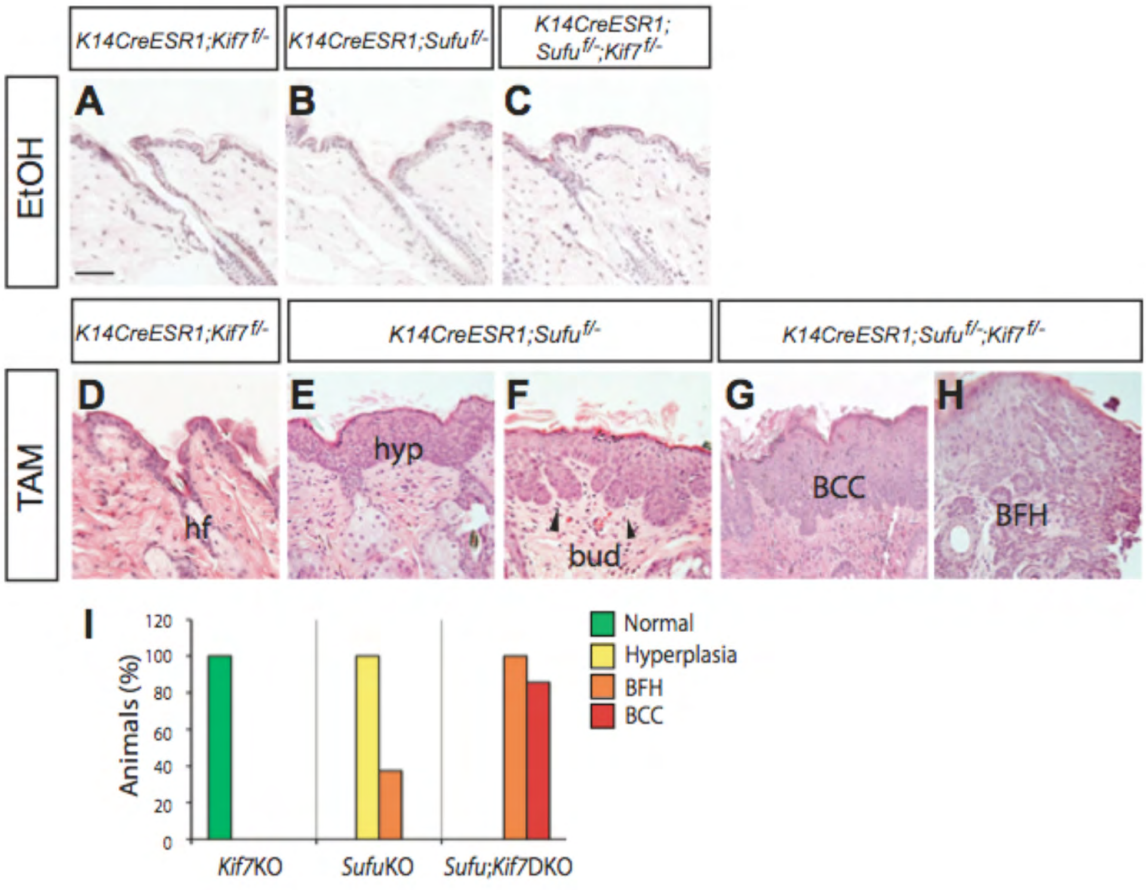

Fig. S8. Histology of ethanol or TAM-treated *Kif7*, *Sufu* and *Sufu*;*Kif7* mutant mice. (**A-C**) Hematoxylin and Eosin staining of *Kif7* (A), *Sufu* (B) and *Sufu*;*Kif7* (C) mutant skin 12 weeks after ethanol treatment. (**D-H**) Histological staining of *Kif7* (D), *Sufu* (E,F) and *Sufu*;*Kif7* (G,H) mutant skin 12 weeks after tamoxifen-induced gene deletion. The skin of TAM-treated *Kif7* mutants appears normal (D), whereas the skin of TAM-treated *Sufu* mutants shows hyperplasia (E) and some epithelial budding (F, arrowhead). TAM-treated *Sufu*;*Kif7* skin develop BCC (G) and/or BFH (H). (**I**) Graphic representation of the percentage of TAM-treated mice from each genotype with the indicated skin phenotype based on histological assessment (normal skin, epidermal hyperplasia, basaloid follicular hamartoma, and BCC). BCC, basal cell carcinoma; BFH, basaloid follicular hamartoma; bud, epithelial budding; hf, hair follicle; hyp, hyperplasia. Scale bar: 50 μm.

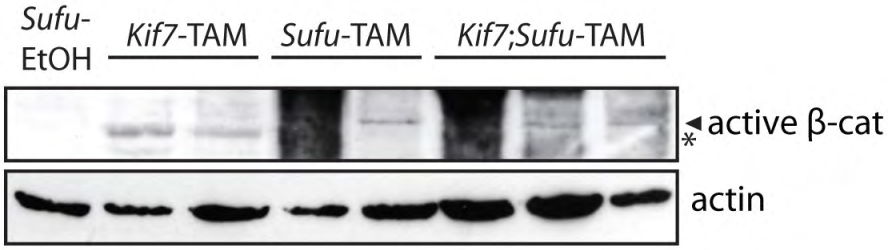

Fig. S9. Activated β-catenin expression in TAM-treated adult transgenic mice. Western blot analysis using protein lysates from the skin of ethanol or TAM-treated adult transgenic mice shows the presence activated (nonphosphorylated) β-catenin in lysates from TAM-treated *Sufu* and *Sufu*;*Kif7*, but not *Kif7* mutant mice and ethanol-treated *Sufu* mutant mice at 7 weeks post-treatment.