

## **Supplemental Figure Legends**

**Supplemental Figure 1. Histologic sections of mouse back skin showing that the epidermis and HFs could be peeled thoroughly from dermis of the 2-month-old mutant and control mice.** (A-D) H&E-stained histologic sections of back skin of 2-month-old wild type (A and B) and *Smad4* mutant mice (C and D). (A and C) were sections of skin before peeling, (B and D) were the remaining dermis parts of peeled mouse skin. Bar: (A-D) 130  $\mu\text{m}$ .

**Supplemental Figure 2. Percentages of *Smad4* mutant mice with skin tumors at different time points.** Most skin tumors developed in *Smad4* mutants younger than 9 months.

**Supplemental Figure 3. The effects of *Smad4* deletion on the IFE.** Immunofluorescence staining with K14 (A-F), K1 (G-L) and involucrin (M-R) on dorsal skin of 3 (A, D, G, J, M and P), 6 (B, E, H, K, N and Q) and 12-month-old (C, F, I, L, O and R) wild type (A-C, G-I, M-O) and *Smad4* mutant mice (D-F, J-L, P-R). Bar, 20  $\mu\text{m}$ .

**Supplemental Figure 4. *Smad4* deletion resulted in gradually diminished expression of CD34.** Immunofluorescence staining of CD34 at day 22 (A and B), day 42 (C and D) and day 72 (E and F) showed CD34 expression was progressively lost from the mutant bugles. Bar, 25  $\mu\text{m}$ .

**Supplemental Figure 5. The percentages of side population (SP) cells in control and *Smad4* mutant epidermis.** SP analysis of keratinocytes from 6-8-week-old mouse epidermis showed that there was no significantly change in the percentage of SP cells in the mutants ( $0.33\pm 0.2\%$ ) compared with that in wild types ( $0.27\pm 0.17\%$  n=3).

**Supplemental Figure 6. Wound re-epithelialization in control and *Smad4* mutant mice.** The 3-mm diameter full-thickness punch wounds were made on the back of wild type (A, C, E and G) and *Smad4* mutant (B, D, F and H) mice of 2 months (A, B, E and F) and 10 months (C, D, G and H) respectively. Sections of wounded skin were stained with BrdU (A-D) and K14 antibodies (E-H) at day 3 post injury. (I) Number of BrdU positive cells per 20 $\times$  field at wound edge. (J) Quantification of re-epithelialization rate of the wound at day 3 post injury. n=6 for each time point and genotype. \*, P<0.05, \*\*, P < 0.01. Bar: (A-D) 40  $\mu$ m; (E-H) 250  $\mu$ m.

## **Supplemental Material and Methods**

### **Side population (SP) analysis**

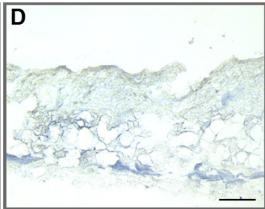
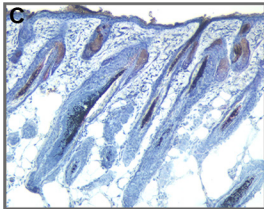
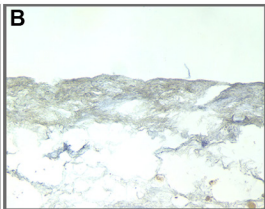
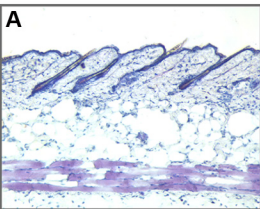
SP analysis was done as described (Yano *et al.*, 2005). Briefly, epidermal cells were suspended at  $10^6$  cells/ml in 2% fetal bovine serum, pre-incubated at 37°C for 15 minutes and incubated for 90 minutes at 37°C with 10 µg/ml Hoechst 33342 (Sigma). Hoechst exclusion was inhibited by adding 100 µM verapamil. After terminating the staining, the cells were washed and then analyzed and sorted by flow cytometer. They were first excited with 50 mW of UV (351–364 nm), and then the emission was detected through a 450/20 nm (Hoechst blue) band-pass filter and a 675 nm (Hoechst red) long-pass filter.

### **Wound healing studies**

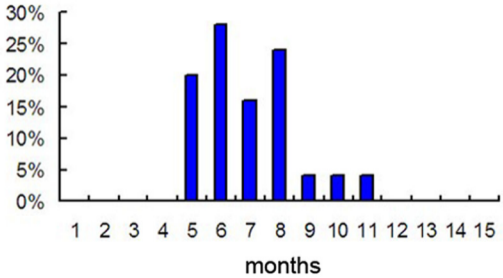
For full-thickness skin wound healing experiments, mice were anaesthetized with tribromoethanol, and four full-thickness 3 mm diameter wounds were made using a sterile biopsy punch on the shaved backs of wild-type and *Smad4* mutant mice at 2 and 10 months of age. Wounds were left undressed, and all mice were housed separately after wounding. At 3 days after injury, mice were injected intraperitoneally with 100 µg/g body weight BrdU (Sigma) and sacrificed 2 hours later. The wounds with a 0.5 cm unwounded skin border were harvested and processed for histochemistry.

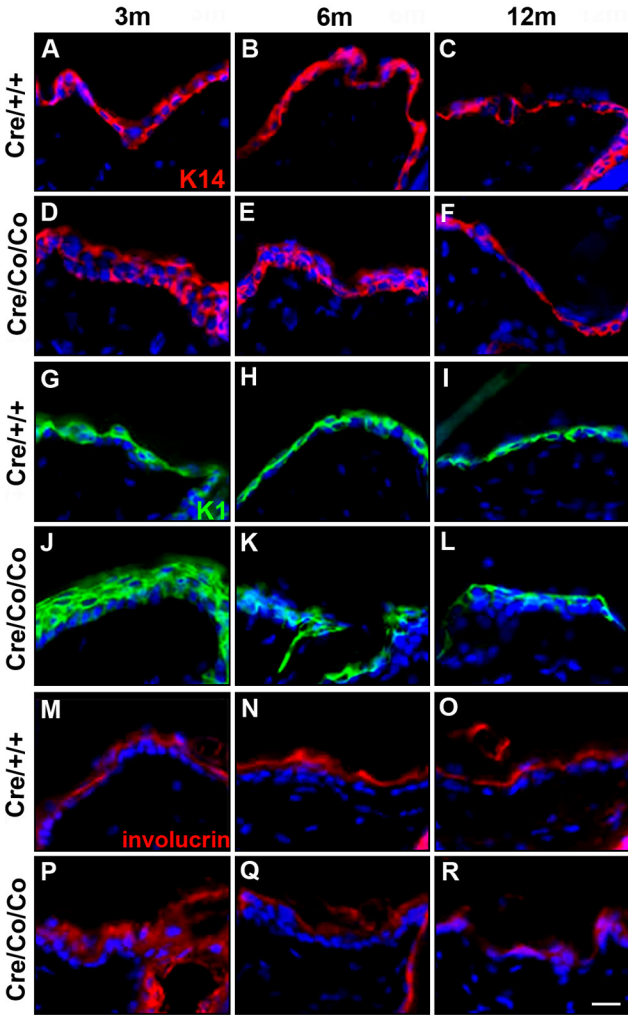
## **Supplemental References**

Yano, S., Ito, Y., Fujimoto, M., Hamazaki, T.S., Tamaki, K., and Okochi, H. (2005). Characterization and localization of side population cells in mouse skin. *Stem Cells* 23, 834-841.



% of skin tumors developed  
in *Smad4* mutants

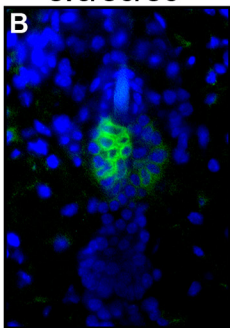
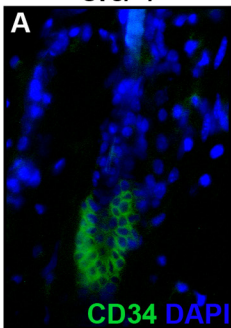




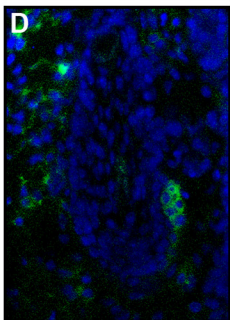
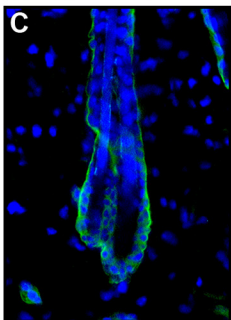
Cre+/+

Cre/Co/Co

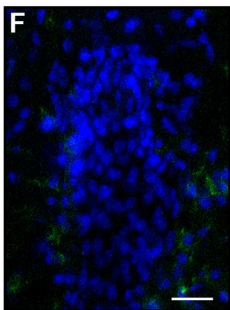
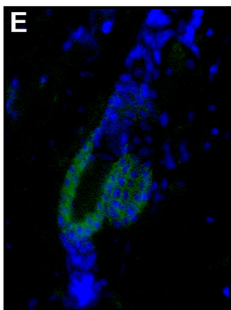
d22



d42



d72

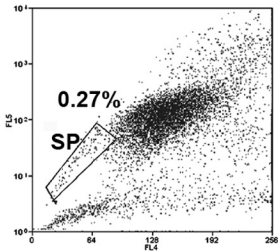
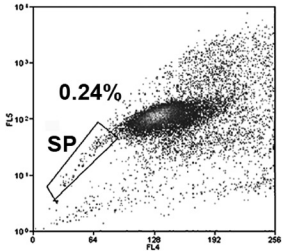




**Cre+/+**

**Cre/Co/Co**

**Hoechst Blue**



**Hoechst Red**

Cre+/+

Cre/Co/Co

