SUPPLEMENTAL METHODS

Histology and immunofluorescence microscopy. Dorsal skin from similar areas in each animal was harvested. The tissues were either fixed in 4% PFA and embedded in paraffin, or embedded in OCT compound and frozen). For histological analyses, 5- or 7-μm paraffin sections were subjected to hematoxylin/eosin or Masson's trichrome staining. For immunofluorescence microscopy, 5- to 7-μm paraffin sections were treated with citrate buffer (10mM citric acid, 0.05% Tween 20, pH 6.0) and microwaved for 7 min for antigen retrieval. Alternatively, frozen sections (7 μm) were fixed in 4% PFA. The tissues were then blocked (10% goat serum, 1% BSA, 0.1% Triton-X-100) for 1 hr at 22°C and incubated overnight at 4°C with primary antibodies. Antibodies diluted in blocking solution used on paraffin sections were: chicken anti-GFP (1:100, AbCam), involucrin (1:100, Covance), keratin 14 (1:100, Thermo Fisher Scientific), Ki67 (1:200, AbCam), LEF1 (1:100, Cell Signaling Technology) and SOX9 (1:25, Santa Cruz Biotechnology). Antibodies used with frozen sections were: Mouse K15 (1:50, LHK15, Abcam), ILK (mouse monoclonal 1:500, BD Biosciences Pharmingen, as well as rabbit polyclonal, 1:500, Cell Signaling Technology). Mouse monoclonal antibodies were used with an M.O.M. Kit (Vector Laboratories) as directed by the manufacturer. For the LHK15 antibody, a biotinylated anti-mouse IgG was used as secondary antibody, and visualized with Alexa 488-labelled streptavidin (Molecular Probes). Other secondary antibodies conjugated to Alexa Fluors (Invitrogen) were diluted in blocking solution and incubated for 1 hr at 22°C. DNA was visualized with Hoescht 33342. For apoptosis measurements, paraffin sections were stained with a TUNEL-FITC kit (Roche Applied Science), as directed by the manufacturer. Photomicrographs were obtained with a Leica DMIRBE microscope equipped with an Orca II digital camera (Hamamatsu) and Volocity 4.3.2 software (Improvision).

Isolation and culture of hair follicle bulge stem cells. The dorsal skin of female *K15.CrePR1YFP-Ilk$^{f/+}$* and *K15.CrePR1YFP-Ilk$^{f/f}$* mice in the second telogen (50-55 day-old; 3-9 mice/experiment) was shaved and treated daily with 1% RU486 for 5 d. Twenty-four hours after the last drug treatment, the skin was harvested and epidermal keratinocyte suspensions were obtained following published procedures (Nowak and Fuchs, 2009), with minor modifications. Specifically, mice were euthanized by CO_2 inhalation and skin was rinsed with ethanol and harvested. The subcutaneous fat and blood vessels were removed, and the skin was digested dermis side down in 0.25% trypsin for 16 h at 4°C. After trypsin digestion, the epidermis was mechanically separated from the dermis and rocked to obtain a single-cell suspension. The cell suspension was strained (70 μm strainers, Falcon, BD), and cold, calcium-free E medium (Calcium-free DMEM: F12 3:1 v/v containing 15% v/v Chelex-treated fetal bovine serum, prepared as described in (Nowak and Fuchs, 2009)) was added. This diluted cell suspension was filtered through 40-μm strainers, centrifuged and the resulting cell pellet was resuspended in cold E-medium, followed by one more round of centrifugation and resuspension. After the last centrifugation step, the cells were suspended in staining buffer as described in (Nowak and Fuchs, 2009) and incubated with antibodies for fluorescence activated cell sorting (FACS). Cells were labelled with Alexa Fluor 649-conjugated anti-CD34 (Biolegend) and phycoerythrin-conjugated anti-α6 integrin (BD Pharmingen) antibodies for FACS. Cells were sorted on a Becton Dickinson FACSAria cell sorter, using FACSDiVa software. The sorter is equipped with a 13 mW Coherent Sapphire solid state 488 nm blue laser, and 11 mW JSD Uniphase HeNe 633 nm red laser. The blue laser octagon was used to detect YFP (detector E, 530/30 bandpass filter), phycoerythrin (detector D, 575/40 bandpass filter), and 7-amino-actinomycin to discard apoptotic/necrotic cells (detector B, 695/20 bandpass filter). The red laser trigon was used to detect APC (detector B or C, 660/20 bandpass filter). The cells were sorted using a 100 μm

nozzle at low pressure (20 psi). The cell populations sorted and analyzed were: α6 integrin^{HIGH}, CD34^{HIGH}, YFP^{+}, as well as α6 integrin^{HIGH}, CD34^{HIGH}, YFP^{-}. The purity of the cell populations, assessed by post-sorting FACS analysis, was consistently ≥ 90%. Sorted cells were seeded in 24-well culture dishes coated with laminin-332 matrix and collagen (Ho *et al*., 2009) for attachment experiments. To assess spreading, cells were fixed and processed for microscopy, using Alexa 594-conjugated phalloidin, to visualize the actin cytoskeleton. The surface area occupied by the cells was traced from micrographs and measured using Openlab software (Improvision).

For apoptosis assessment, 1 x 10^5 sorted cells were cultured for 96 h in 24-well plates coated with laminin-332 matrix and collagen, and incubated in conditioned medium from NIH-3T3 feeder cells. A colorimetric enzyme immunoassay was used to quantify cytoplasmic mono- and oligonucleosomes using Cell Death ELISA kits (Roche Applied Science), as per manufacturer's directions. Cytoplasmic lysates were prepared as per manufacturer's instructions, and, after color development, the absorbance at 405 nm was measured (reference wavelength 490 nm).

We also verified that the efficiency of Cre-mediated activation of YFP from the *Rosa26* locus was similar to that of *Ilk* gene excision in sorted cell populations (Supplemental Figure 4). To this end, mRNA was isolated using RNeasy Plus Micro kits (Qiagen). RNA quality was determined on an Agilent 2100 Bioanalyzer, and 25 ng of high quality RNA for each sample were subjected to reverse transcription and amplification using TaqMan Assays on Demand (Applied Biosystems Inc.). The primer/probe sets used to determine relative transcript abundance corresponded to YFP and to the C-terminus of ILK, which is excised by Cre in these mice (White *et al*., 2006). For normalization, we used primers corresponding to RPL20 and RPL30 (de Jonge *et al.,* 2007). Amplified sequences were detected with a Prism

7900HT sequence detector (Applied Biosystems Inc.). The results were analyzed using SDS v2.1 software (Applied Biosystems Inc.). Relative YFP and ILK transcript levels were calculated using the ΔΔCt method.

REFERENCES FOR SUPPLEMENTAL METHODS.

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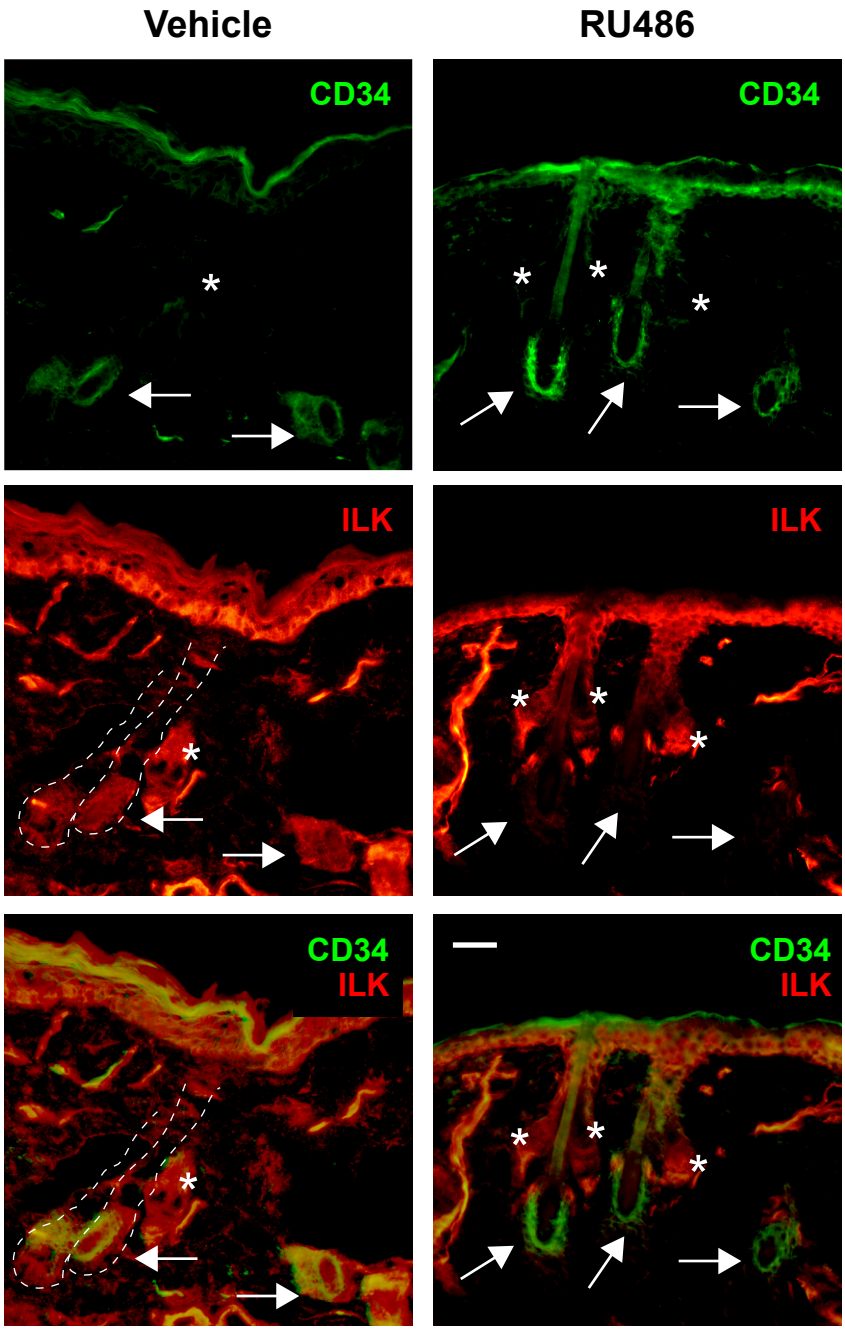
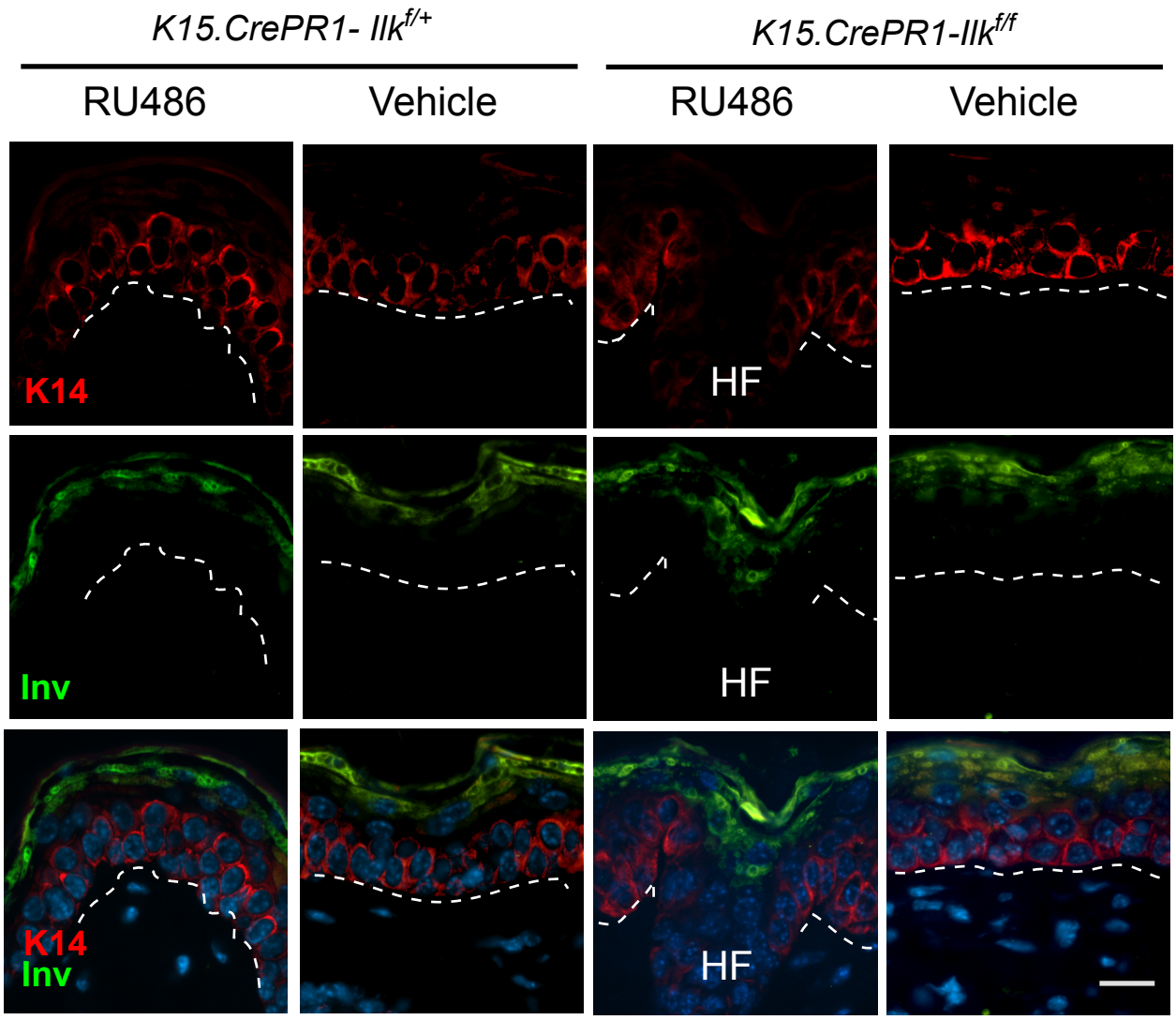
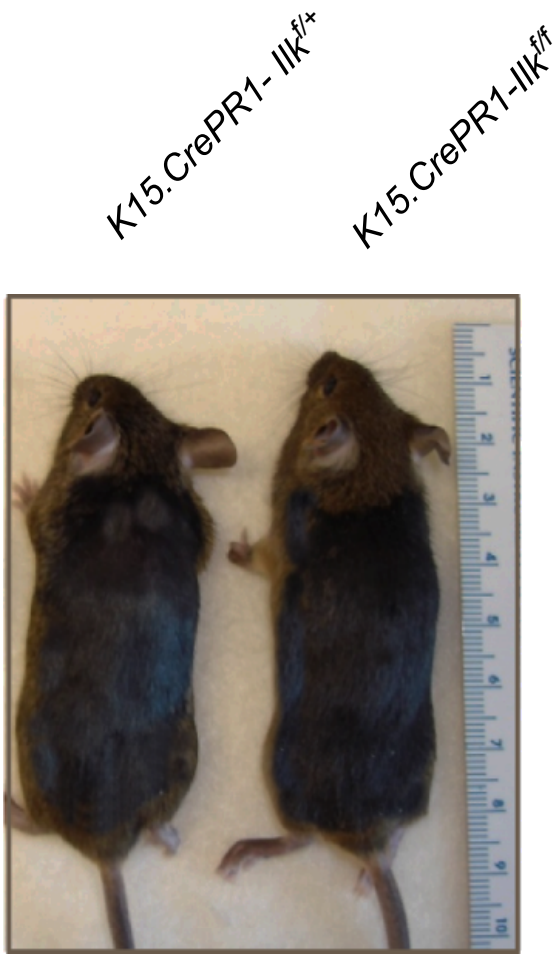


K15.CrePR1-Ilk$^{f/f}$

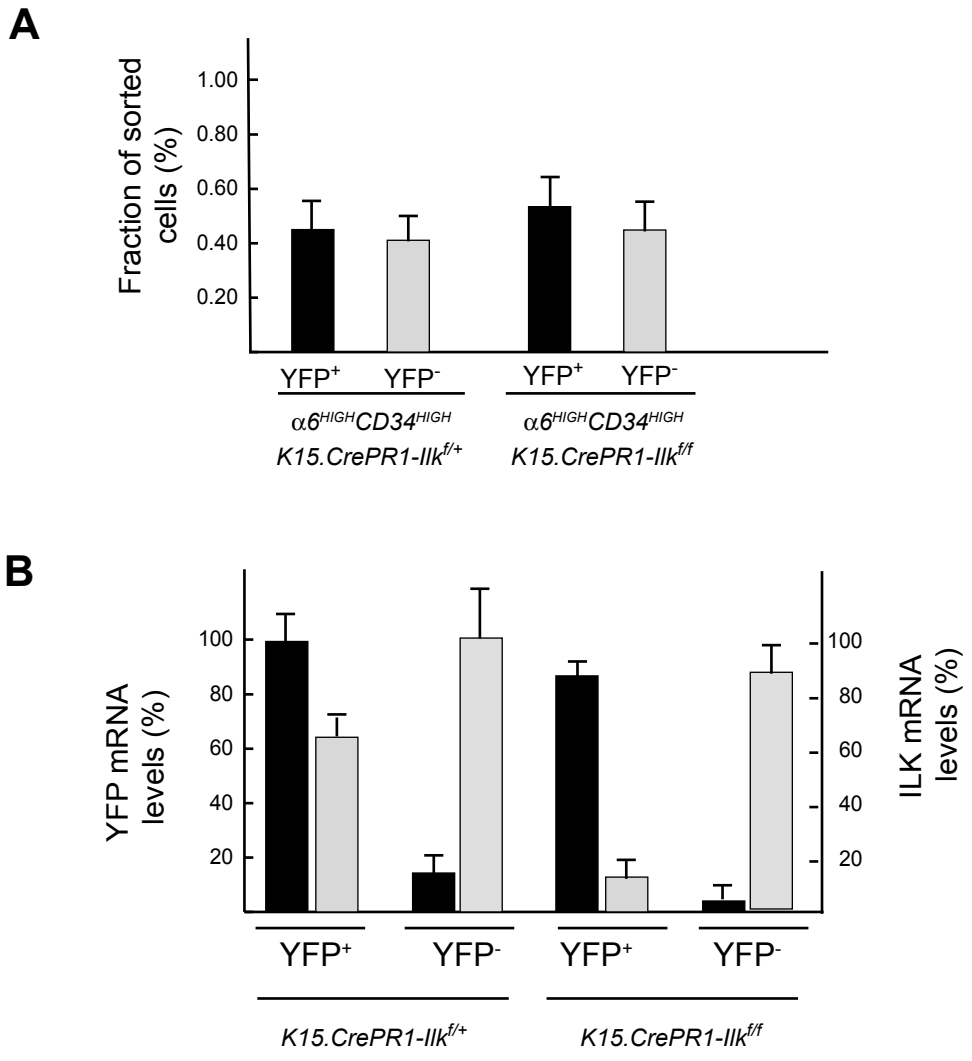
Supplemental Figure 1. The dorsal skin of 50 day-old *K15.CrePR1-Ilk$^{f/f}$* mice was treated with vehicle or with RU486, as indicated, for 5 days. Six days after the last treatment, the skin was harvested and processed for immunofluorescence microscopy with antibodies against the bulge stem cell marker CD34 or ILK. Arrows indicate the CD34-positive bulge stem cell population, and asterisks indicate sebaceous glands. Dashed lines contour hair follicles. Bar, 100 μm.



Supplemental Figure 2. Dorsal skin of 50 day-old *K15.CrePR1-Ilkf/+* or *K15.CrePR1-Ilkf/f* mice was treated daily with topical RU486 (+) or vehicle (-) for 5 days. Tissue sections were obtained and probed with antibodies against keratin 14 (K14) and involucrin. Nuclear DNA was visualized with Hoescht 33258. The dotted line represents the basement membrane underneath the basal keratinocyte layer. Occasionally, we observed K14 immunoreactivity in cells localized just above the basal layer. HF, hair follicle. Bar, 50 μm.



Supplemental Figure 3. Pelage of 75 day-old mice of the indicated genotype treated daily with RU486 for 5 days at 20 days of age.



Supplemental Figure 4. (A) Quantification of YFP-positive bulge stem cells after FACS purification. The dorsal skin of 50 day-old *K15.CrePR1-Ilk$^{f/+}$* or *K15.CrePR1-Ilk$^{f/f}$* mice was treated with RU486 for 5 days. One day after the last treatment, the epidermis was harvested and keratinocytes were isolated and incubated with antibodies against the bulge stem cell marker CD34 and integrin α6, and sorted. The CD34High/α6High population was further sorted according to YFP expression. The graph shows the % of YFP-positive or -negative bulge stem cells relative to the total cell population isolated from the entire epidermis.

(B) FACS-sorted hair follicle stem cells as in (A) were used to prepare mRNA. The mRNA was subjected to reverse transcription followed by qPCR. Relative levels of YFP mRNA (left Y axis, black bars),and ILK mRNA (right Y axis, gray bars) were determined. The results for YFP are expressed relative to abundance in *K15.CrePR1-Ilk$^{f/+}$* YFP-positive stem cells (set to 100%). The results for ILK are expressed relative to abundance in *K15.CrePR1-Ilk$^{f/+}$* YFP-negative stem cells (set to 100%).