ONLINE METHODS

Generation of constructs. The Tcf3 targeting vector used the pGKneobpAloxX2 PGKDTA vector kindly provided by P. Soriano and was previously described[3].

Tcf4 B-isoform cDNA was cloned out from E17.5 total skin cDNA using forward primer Tcf4F1-BamH1 and reverse primer Tcf4bR-BamH1R to amplify the full-length *Tcf4* B isoform. Sequences of cloning primers are listed in **Supplementary Table 1**. The PCR product was digested with BamH1 and ligated to a *Krt14* promoter cassette that was digested with BamH1 and phosphatase treated with shrimp alkaline phosphatase (SAP; Roche). After its sequences were verified, the *Krt14-Tcf4* template was used to amplify the *Tcf4* gene with a BglII restriction site introduced in its 5′ end so as to be in-frame when ligated into the pCMVmyc vector (Clontech). The BglII- and BamH1-digested insert was then ligated to a pCMVmyc vector that was digested with BlgII and treated with SAP. We then used primers to amplify the Myc epitope–tagged, full-length *Tcf4* (*Myc-Tcf4*) gene, digested the DNA with BamH1, and ligated it a to *Krt14* promoter cassette that was cut with BamH1 and treated with SAP.

To generate *Krt14-Tcf4*$^{\Delta N}$, *Krt14-Tcf4* was used as a template for PCR with forward primer Tcf4F166-BamH1 and reverse primer Tcf4bR-BamH1. The PCR product was digested with BamH1 and ligated to a *Krt14* promoter cassette that was cut with BamH1 and treated with SAP.

To generate *Krt14-Tcf4*$^{\Delta C}$, the template *Krt14-Tcf4* was amplified using forward primer Tcf4F1-BamH1 and reverse primer Tcf4R948-stopXba1. The PCR product was cut with BamH1 and Xba1 and ligated to a *Krt14* promoter cassette that was cut with BamH1 and Xba1. To generate *Krt14-Tcf4*$^{\Delta G}$, the *Krt14-Tcf4* template was amplified using forward primer Tcf4F1-BamH1 and reverse primer Tcf4R385 to generate fragment A, and primers Tcf4R-BamH1 and Tcf4F359-aattII to generate fragment B. Fragment B was cut with AattII to delete nucleotides 920–1,400 and then ligated to fragment A, which was digested with AattII and amplified using primers Tcf4F1BamH1 and Tcf4b-RBamH1. The PCR product was cut with BamH1 and ligated to a *Krt14* cassette that was digested with BamH1 and treated with SAP.

To generate pCMVmyc vectors expressing *Tcf4* constructs (full-length, ΔN, ΔG and ΔC), *Krt14-Tcf4* constructs (full-length, ΔN, ΔG and ΔC) were amplified with forward primer BglII-Tcf4F1 and reverse primer BglII-stop-Tcf4 R12889. The PCR products were cut with BglII and ligated to a pCMVmyc vector that was digested with BglII and treated with SAP. To generate *Krt14-Myc-Tcf4* transgenes (full-length, ΔN, ΔG and ΔC), the respective pCMVmyc vectors were used as templates with forward primer mycBamH1F824 and reverse primer mycBamH1R939 to amplify the Myc-tagged versions. All Myc-tagged inserts were digested with BamH1 and ligated to a *Krt14* promoter vector cassette that was digested with BamH1 and treated with SAP. The SacI and HindIII fragments of *Krt14-Myc-Tcf4*, *Krt14-Myc-Tcf4*$^{\Delta N}$ and *Krt14-Myc-Tcf4*$^{\Delta G}$ were then used for injection to engineer transgenic mice as previously described[17].

Generation and analysis of *Tcf3/4*-null and *Krt14-Tcf4* transgenic mice. To conditionally inactivate the *Tcf3* gene, a targeting strategy was designed to allow Cre-mediated deletion of exon 2. NotI-linearized *Tcf3* targeting construct DNA (30 μg; **Supplementary Fig. 1**) was electroporated into GS1 embryonic stem cells. After 6 d of G418 selection, ~300 individual colonies were screened for homologous recombination by PCR and Southern blotting (**Supplementary Fig. 1**). A second electroporation was done to introduce a CMV-Cre expression plasmid into a G418-resistant clone that had undergone homologous recombination with the targeting construct DNA. After 5 d of culture in the absence of G418 selection, ~300 individual colonies were screened for G418 sensitivity and screened by PCR for loss of the floxed *neo* cassette between exons 2 and 3. An embryonic stem cell clone containing a floxed *Tcf3* allele, as confirmed by Southern blotting and PCR (**Supplementary Fig. 1**), was injected in blastocysts of 57Bl/6 mice to generate chimeric mice carrying the *Tcf3* targeted allele.

We bred the chimeric mice containing the *Tcf3*$^{fl/+}$ allele to germline transmission and then mated them to *Krt14-Cre* mice. Both *Tcf3*$^{fl/fl}$; *Krt14-Cre* mouse lines, which appeared phenotypically wild type, were then bred to *Tcf4*$^{+/-}$mice until mice doubly null in their skin epithelium for *Tcf3* (conditional) and *Tcf4* (straight) were generated. Because *Tcf4*$^{-/-}$ mice die at birth, we used skin grafting as previously described[31] for long-term skin monitoring.

Split-thickness and chamber graft assays were done as described previously[6,32]. For cell mixing chamber graft experiments, 5×10^5 cells from dermal fractions enriched for hair follicles[18] were combined with 5×10^5 keratinocytes from dispase- and trypsin-treated pure epidermis. Cell mixtures were then added to chambers that had been inserted onto the back skin of nude mice. Chambers were removed after healing (7–10 d), and grafted skins were collected and analyzed after 30 d. Full-thickness wounds were produced with a 3-mm Miltex biopsy punch administered to grafted skins at day 30, and skins were analyzed 7 d later.

Cell isolation and *in vitro* culture. Primary mouse keratinocytes were isolated, cultured and assayed as described[32]. Cell proliferation was assessed using a Beckman Z2 Coulter counter. Cell adhesion assays were done with equal numbers of freshly isolated primary mouse keratinocytes placed on glass cover slips coated with 10 μg ml^{-1} fibronectin (Upstate Biotechnology), 10 μg ml^{-1} laminin (BD Biosciences) or 100 μg ml^{-1} poly-D-lysine (BD Biosciences). After 1 h, nonadherent cells were removed with PBS, and adherent cells were fixed, stained and counted using ImageJ software.

Colony formation efficiency was analyzed as follows: 2×10^4 live, freshly isolated primary mouse keratinocytes were plated onto mitomycin-treated fibroblast feeders, and fresh medium was changed every other day. Feeders were removed 10 or 12 d later, and colonies were stained with rhodamine B. TOPFlash Wnt reporter assay was done as previously described[2], with FOPFlash (mutant Tcf-Lef binding sites) as a negative control.

Immunoblots, immunofluorescence and histology. To analyze Tcf3 expression, 40 μg protein from flow cytometry–sorted E17.5 epidermal cell lysates were resolved by SDS-PAGE, immunoblotted, probed with primary antibodies to Tcf3 (guinea pig, 1:3,000; Fuchs laboratory) and β-actin (mouse, 1:1,000; Sigma), and detected by chemiluminescence (ECL reagents, Amersham Biosciences).

Immunofluorescence was done on 10-μm OCT-frozen back skin sections as described[4] using a block-diluent solution of 5% normal donkey serum, 2% gelatin and 0.2% Triton X-100 in PBS. We used primary antibodies to Tcf3 (guinea pig, 1:300; Fuchs laboratory), Tcf4 (goat, 1:100; Santa Cruz Biotechnology), Krt5 (rabbit, 1:300; Fuchs laboratory), Krt1 (rabbit, 1:200; Fuchs laboratory), loricrin (rabbit, 1:200; Fuchs laboratory), β4 integrin (rat, 1:200; BD Biosciences), Krt6 (rabbit, 1:500; Fuchs laboratory), Ki67 (rabbit, 1:500; Novocastra Laboratories), Cd3 (1:100; Chemicon), F4/80 (rat, 1:100; Serotec), Gr1 (mouse, 1:100; R&D Systems), β-catenin (mouse, 1:500; Sigma), active caspase 3 (rabbit, 1:1,000; R&D). DNA fragmentation analyses were done using a TUNEL kit (Chemicon). Y-FISH chromosome analyses were done using a Cy3 Star*FISH detection kit (Cambio).

Histological analyses were done on tissues that were fixed for ≥1 h in 0.05 M sodium cacodylate buffer containing 2% glutaraldehyde, 4% formaldehyde and 2 mM $CaCl_2$, and then processed for Epon embedding. One-micron sections were cut, stained with toluidine blue and examined with a Zeiss Axioplan microscope.

Real-time PCR gene expression analysis. Total RNAs were purified with an Absolutely RNA kit (Stratagene) and reverse-transcribed using oligo(dT) primers (Superscript III First-Strand Synthesis System, Invitrogen). The Roche LightCycler system and software and DNA Master SYBR Green II reagents were used for real-time PCR. Differences between samples and controls were normalized to the level of *Gapd* and calculated based on the $2^{-\Delta\Delta CP}$ method. Primer sequences are listed in **Supplementary Table 1**.

Sample preparation for microarrays. After enzymatic treatment with dispase, back-skin epidermis was removed from E17.5 embryos and incubated in 0.05% trypsin for 10 min at 37 °C. After straining to remove debris, dissociated cells were exposed to phycoerythrin-conjugated antibody to α6 integrin (BD Biosciences), and basal cells were flow cytometry–sorted by surface α6 integrin levels. RNAs were isolated with an RNeasy Micro kit (Qiagen) and fluorometrically quantified (RiboGreen, Molecular Probes). Quality was assessed by RNA 6000 Pico assay (Agilent), and 800 ng were primed with oligo(dT)-T7 primer and reverse-transcribed (Superscript III cDNA synthesis kit; Invitrogen). One round of amplification and labeling was done to obtain biotinylated cRNA (MessageAmp aRNA kit, Ambion), and 10 μg of labeled cRNA was hybridized at 45 °C for 16 h to Affymetrix GeneChip Mouse Genome 430 2.0 arrays. Processed chips were read by an argon-ion laser confocal scanner (Genomics Core Facility, Memorial Sloan-Kettering Cancer Center).

31. Kaufman, C.K. *et al.* GATA-3: an unexpected regulator of cell lineage determination in skin. *Genes Dev.* **17**, 2108–2122 (2003).
32. Blanpain, C., Lowry, W.E., Geoghegan, A., Polak, L. & Fuchs, E. Self-renewal, multipotency, and the existence of two cell populations within an epithelial stem cell niche. *Cell* **118**, 635–648 (2004).