ONLINE METHODS

Mouse Models. C57Bl/6 *K17*$^{-/-}$ mice[10] C57Bl/6 *K14*$^{-/-}$ mice[11] were crossed to C57Bl/6 Gli2^{tg} transgenic mice[6] to create *Gli2*^{tg}*/K17*$^{-/-}$ mice and relevant controls. *Gli2*^{tg} mice were backcrossed into the *K17*$^{-/-}$ strain for over 10 generations to generate congenic mice for experimentation. Genotyping was performed by PCR as described for the *K17*[10] and *K14* [11] loci (Supplementary Table 1). PCR screening for the flag-tagged Gli2^{tg} transgene was done using primers listed in Supplementary Table 1. Mice were provided rodent chow and water *ad libitum*. The Johns Hopkins Institutional Animal Care and Use Committee approved all protocols involving mice.

TPA treatments of mice. Age-matched littermates were topically treated with 30µl of vehicle (acetone) or 12-O-tetradecanoylphorbol-13-acetate (TPA; 25ng/ml; Sigma), on days 0 and 2 and ear tissue was harvested on day 4. Ear tissue (unfixed) was either embedded in OCT (see below) for morphological analyses or placed in RNAlater (Invitrogen) for use in qPCR experiments.

Antibodies. The primary antibodies used in this study include rabbit polyclonal antisera against K17[39]; K16[40]; K6[39]; K14, K5, and K1 (from Covance; Gaithersburg, Maryland); Thy-1, MOMA-1, NOS2, Il-1ß, and PECAM-1 (from Santa Cruz Biotechnology, Inc. Santa Cruz, CA); p-Histone H3, NF-kappa b p65, phospho NF-kappa B p65, Erk1/2, phospho Erk1/2, and E-cadherin, Akt, p-Akt(ser473), S6k, and p-S6k(Thr389) (from Cell Signaling, Danvers, MA), and Gli2 (from Abcam, Cambridge, MA). Mouse monoclonal antibodies used were directed against bromodeoxyuridine (BrdU), α-tubulin, and actin (Sigma Chem. Co., St-Louis MO). FITC- or Rhodamine-labeled secondary antibodies (Kirkegaard and Perry Laboratories, Gaithersburg, Maryland) were used for indirect immunofluorescence.

Tissue harvesting, Histological analyses, BrdU labeling, Myeloperoxidase activity assays. Histological assays were done on mouse ear tissue of age- and gender-matched littermates. Surgically-removed ear tissue (unfixed) was embedded in Sakura Tissue-Tec OCT compound (Torrance, CA). 8 µm thick serial sections were fixed for 10 min with neutral buffered formalin, and either stained with hematoxylin-eosin (H&E) or processed for indirect immunofluorescence using relevant antibodies (cf. above). TUNEL staining was carried out using the DeadEnd™

Fluorometric TUNEL System (Promega, Madison, WI). DNA was stained using Hoechst (Sigma Chem Co., St-Louis, MO). To detect mitotic cells, mice were intraperitoneally injected with BrdU (40mg/kg body weight; Sigma) and sacrificed 1 h later. BrdU-labeled cells were localized by indirect immunofluorescence in 8 μm tissue sections, as described[34]. A quantitative assessment of specific features of ear lesions (hamartoma, eschar, cysts, and sebocytes; see refs. 6,7) in *Gli2*^{tg} and *Gli2*^{tg}*/K17*$^{-/-}$ mice was done in a blind fashion on H&E- stained sections by a skin tumor expert (author A.A.D.). Integrity of the epidermal barrier was assessed via in situ beta-galactosidase staining, as described[16]. Myeloperoxidase (MPO) activity was measured as described[15], with minor modifications.

Measurement of ear thickness and epidermal downgrowth. H&E-stained sections from P80 *Gli2*^{tg} and *Gli2*^{tg}*/K17*$^{-/-}$ male mice were used for measurements made using the Zeiss Axiovision software (Thornwood, NY) For average ear thickness, three randomly selected sites along the ear were measured from nine mice to assess ear tissue expansion. For epidermal downgrowth, sections of total ear tissue (base to apex) were scanned to identify areas of greatest basal layer penetration into the underlying dermis. Maximum epithelial thickness was measured on nine different mice in each group to establish the average reported.

Protein extraction, western immunoblotting and co-immunoprecipitation. Protein lysates were prepared in RIPA buffer, after pulverization of liquid nitrogen-frozen tissue samples , and total protein quantified via the Bradford assay (Sigma-Aldrich, St. Louis, MO) 10μg of total protein was loaded for each sample, and subjected to separation on a 8.5% denaturing/reducing SDS-PAGE gel. After transfer onto nitrocellulose membrane via electroblotting, antigens of interest were detected using relevant primary antibodies (cf. above) followed by a peroxidase-conjugated secondary antibody (Sigma-Aldrich, St. Louis, MO) for chemiluminescence-based detection. For co-immunoprecipitation, tissue extracts were prepared from GLi2^{tg} ear lesions according to the rabbit trueblot system protocol (eBioscience, San Diego, CA), and the K17 immune serum used as described[35].

qRT-PCR. Quantitative RT-PCR was performed on ear tissue samples from P80 *Gli2*^{tg} and *Gli2*^{tg}*/K17*$^{-/-}$ male, or TPA-treated *K17*$^{-/-}$ and wildtype control mice as described above. Ear

tissue from a minimum of four mice was used to yield a given pooled RNA sample, and all experiments were performed in triplicate using distinct samples. Total RNA was isolated using Trizol (Invitrogen, Carlsbad, CA) as per the manufacturer's instructions, and treated with DnaseI using the RNeasy Mini Kit and on-column Rnase free Dnase kit (Qiagen, Valenica, CA). RNA concentration and purity was assessed by spectrophotometry. 1.0μg RNA was reverse-transcribed with the RT^2 First Strand Kit (SABiosciences location) as recommended. qPCR was performed on the first strand cDNA through the RT^2 Profiler^{TM} PCR Array for mouse inflammatory cytokines and chemokines, and the NFκB signaling pathway, as described in the manufacturer manual. Data analysis was performed with the template provided by SABiosciences, which yield fold change and p-values.

Primary cultures of skin keratinocytes. Skin keratinocytes were isolated from newborn pups and cultured as described[34]. At 24 hours post plating, cells were transfected with a pBK-CMV/K17 construct or empty vector using LipofectAMINE 2000 (Invitrogen, Carlsbad, CA) as described by the manufacturer. This was followed by TPA treatment, (0.2μM) for 12 hours starting at 24 hours post-transfection, after which RNA was extracted, and qPCR analysis performed as described above. In some studies cells were cultured for 36 hours, treated with TPA for 12 hours, exposed to 50μM BrdU for 2 hours, fixed and processed for BrdU staining.

Status of Hedgehog signaling in mouse skin and keratinocytes in primary culture. Semi-quantitative RT-PCR was performed on total RNA isolated from p80 *Gli2*^{tg} and *Gli2*^{tg}*/K17*$^{-/-}$ male mice using Trizol (Invitrogen, Carlsbad, CA) following the manufacturer's protocol. Total RNA pools from 3-5 mice were used for each genotype. First-strand cDNA was produced using the Omniscript RT Kit (Qiagen, Valencia, CA). Results shown are representative of results from 3 independent experiments. Primer sequences and PCR cycling conditions are given in Supplementary Table 1. Primary keratinocytes were isolated from P2 pups of both genotype [41] and were subsequently transfected with either the promoterless pGL3-Basic or the 8xK17bs-luc vector [9] along with the pRL-TK vector for internal normalization. Transfections were carried out using LipofectAMINE 2000 (Invitrogen, Carlsbad, CA) following the suggested manufacturer's protocol. Luciferase activity of cell lysates were quantified using the Dual Luciferase Kit

(Promega, Madison, WI) and a Spectramax M5 plate reader (Molecular Devices, Sunnyvale, CA). Results reported are compiled rom three independent experiments.