MATERIALS AND METHODS

In situ hybridization

In situ hybridization of mouse skin was performed on 10 µm frozen sections from PFA-fixed tissues cut on SuperFrost Plus slides. Sections were dried for 40 minutes at 60°, rinsed twice in PBS, placed in 0.1 M triethanolamine (pH 8.0)/0.25% acetic anhydride for ten minutes, rinsed twice in PBS, and transferred to prehybridization solution (4X SSC/50% formamide) at 37° for one hour. Digoxigenin-labeled probes were diluted, generally 1:1000, in hybridization buffer (0.2 M NaCl, 0.01 M Tris pH 7.5, 0.01 M Na-phosphate pH 6.8, 5 mM EDTA, 50% formamide, 10% dextran sulfate, 1mg/ml tRNA, 1X Denhardt's solution, in DEPC-treated water) pre-warmed to 65°. Probes were denatured at 70° for ten minutes, vortexed, and 100 µl volumes of probe added per slide. A cover slip was placed on the slide, which was transferred to a humid chamber for overnight hybridization at 60°. Three washes were performed at 65° (15-30 minutes each) using buffer containing 1X SSC, 50% formamide, and 0.1% Tween 20, followed by two washes in buffer containing 150 mM NaCl, 100 mM maleic acid pH 7.5, and 0.1% Tween 20 (MABT). For visualization of dixogenin-labeled probe, sections were blocked using MABT containing 2% blocking reagent (Roche) and 20% heat-inactivated sheep serum, and incubated overnight with anti-digoxigenin at a 1:1000-1:2000 dilution at 4° in a humid chamber. After incubation with primary antibody, sections were washed four times (20 minutes each wash) in MABT with rocking, and equilibrated in staining buffer (100mM NaCl, 50mM MgCl2, 100mM Tris pH 9-9.5, 0.1% Tween 20) without

NBT or BCIP for 2 x 10 minutes. Staining was performed at room temperature or 37° (*SV40* probe only) in staining buffer containing 10% polyvinyl alcohol, NBT, and BCIP, for 12-36 hours. Sections were washed several times in PBS, mounted using a 1:1 mixture of glycerol in PBS, and stored at 4°.

In situ analysis of human tissue, obtained according to University of Michigan Institutional Guidelines, was performed using 5 μm sections cut from NBF-fixed, paraffin embedded tissue according to a similar protocol, but with the following modifications. Sections were heated for 2 hours at 56°, deparaffinized in xylene (2 x 5 minutes); transferred to 100% EtOH (2 x 3 minutes), rehydrated in a graded alcohol series (95%, 90%, 70%, 50%, 20% EtOH; 3 minutes each), and rinsed in 1xSSC (2 x 2 minutes). To permealize, tissue sections were treated with Proteinase K (20 μg/ml) in buffer (50 mM Tris pH 7.5, 5 mM EDTA) for ten minutes at 37°, rinsed twice in 1xSSC, postfixed in 4% PFA for ten minutes, and rinsed twice in 1xSSC. Sections were treated with 0.1 M triethanolamine followed by two washes in 1xSSC and transferred to prehybridization solution. All subsequent steps were as described above for *in situ* using mouse sections.

Semi-Quantitative RT-PCR, and Real-Time PCR Analysis

Semiquantitative RT-PCR was performed using 1 μg samples of total RNA for first-strand DNA synthesis using the Superscript II RT kit (Invitrogen). Transgene-specific primers were designed to detect human *SMO*: forward primer, 5'-aagcggatcaagaagagcaa-3'; reverse primer, 5'-gaggcagtcgaggaatggta-3.' The 435 bp product was amplified using

the following parameters: 3 min "hot start;" 95° X 50 sec denaturation, 56° X 30 sec annealing, and 72° X 60 seconds extension, for 30 cycles; 72° for seven minutes. A 421 bp product for actin was detected using the following primers: forward 5'-taccacaggcattgtgatgga-3,' reverse 5'-caacgtcacacttcatgatgg-3' {7253}, with the following parameters: 3 min "hot start;" 95° X 50 sec, 58° X 30 sec, and 72° X 60 seconds, for 25 cycles; 72° for seven minutes.

Quantitative PCR was performed using real-time "TaqmanTM" technology and analyzed on a Model 7700 Sequence Detector (Applied Biosystems). Two PCR primers and a hybridization probe labeled with a reporter dye, FAM, on the 5' nucleotide and a quenching dye, TAMRA, on the 3' nucleotide were used (sequences will be provided on request). Fifty µl reactions contained: 50 ng of total RNA, 12.5 units murine leukemia virus reverse transcriptase, 1.25 units AmpliTaq Gold DNA polymerase, 0.2 units of RNase Inhibitor, 1X PCR reaction buffer containing 5mM Magnesium Chloride, 165 ng (or 500nM) of each primer, 300 mM dNTPs, and 100 ng (or 300nM) TaqmanTM probe. CT values corresponding to the cycle number at which the fluorescent emission monitored in real-time reaches a threshold of 10 standard deviations above the mean baseline emission from cycles 3 up to 15 were measured. Cycling parameters were: 30 min at 48° C, 10 min at 95° C followed by forty cycles of 15 sec at 95° C and 1 min at 60° C.