Supplemental Experimental Procedures

Mouse Strains and constructs

All animals used for the experiments in this manuscript were generated previously: *K14-rtTA, ShhCreER, Sox9CreER, Smo$^{fl/fl}$, Shh^{neo}, Gli1^{Lacz}, Rosa26$^{Flox\text{-}Stop\text{-}Flox\text{-}YFP}$, Ctnnb1$^{fl/fl}$, Axin2LacZ, Fucci*, *Apc$^{fl/fl}$*, and *Rosa26mTmG$^{fl/+}$*. (Bai et al., 2002; Brault et al., 2001; Chiang et al., 1999; Corrales et al., 2006; Dassule et al., 2000; Harfe et al., 2004; Kuraguchi et al., 2006; Litingtung et al., 1998; Long et al., 2001; Lustig et al., 2002; Mao et al., 1999; Muzumdar et al., 2007; Nguyen et al., 2006; Sakaue-Sawano et al., 2008; Soeda et al., 2010; Srinivas et al., 2001) were described previously. *Lhx2-EGFP* mice were from The Gene Expression Nervous System Atlas (GENSAT) Project, NINDS Contracts N01NS02331 & HHSN271200723701C to The Rockefeller University (New York, NY, USA). K14-H2BGFP transgenic mice were generated with standard pronuclear injections (Fuchs Lab). Lentiviral doxycycline-inducible Shh overexpression construct (LV-TRE-Shh-PGK-H2BGFP) has been previously described (Hsu et al., 2014). Construct for the lentiviral CreER^{T2} has been previously described (Williams et al., 2014). Construct for the lentiviral Cre has been previously described (Beronja et al., 2010). Dkk1 from pCS2+ Dkk1-flag (gift from Xi He (Addgene plasmid # 16690)) was cloned by PCR to replace Shh in the LV-TRE-Shh-PGK-H2BGFP.

Embryo Preparation, Immunofluorescence and *In Situ* Hybridization

4h prior to the desired stage of development, EdU (500 μg/g, Life Technologies) was injected intraperitonally in pregnant females and embryos were then processed. Typically, >3 embryos from independent experiments were analyzed for each condition.

For immunofluorescence, embryos were fixed in 4% PFA in PBS for 1h at room temperature and washed extensively in PBS. For whole-mount or tissue sections, samples were permeabilized for 3 hours in 0.3% Triton X-100 in PBS, or embedded in OCT (Tissue Tek), cut at a thickness of 10μm and permeabilized for 10 min 0.3% in Triton X-100. Tissue samples were then blocked in Gelatin Block (2.5% fish gelatin, 5% normal donkey serum, 1% BSA, 0.3% Triton, 1× PBS). When immunolabeling with mouse antibodies, sections were incubated with the M.O.M. blocking kit according to manufacturer's instructions (Vector Laboratories). The following primary antibodies were used: P-Cadherin (goat, 1:400, R&D AF761), phospho-Tyr397 FAK (rabbit, 1:200, Cell Signaling D20B1), active β1-integrin (rat, 1:150, BD, 9EG7) Survivin (rabbit, 1:300, Cell Signaling 2808), LEF1 (rabbit, 1:300, Fuchs Lab), SOX9 (rabbit, 1:300, Fuchs Lab), LHX2 (rabbit, 1:2000, Fuchs Lab), anti-GFP/YFP (chicken, 1:2000, Abcam), Itga6-PE (1:1000, BD), β-catenin (mouse, 1:1000, BD 610154), acetylated tubulin (mouse, 1:500, Sigma T7451), Beta-Gal (rabbit, 1:10000, MP Bio). Primary antibodies were incubated at 4C overnight. After washing with 0.3% Triton X-100 in PBS, samples were incubated for 2h at room temperature with secondary antibodies conjugated with Alexa 488, RRX, or 647 (respectively, 1:1000, 1:500, and 1:100, Life Technologies). Samples were washed, counterstained with 4'6'-diamidino-2-phenilindole (DAPI) and mounted in Prolong Gold, and EdU incorporation was detected by Click-It EdU AlexaFluor 647 Imaging Kit (Life Technologies).

LacZ-derived β-galactosidase activity was assayed on frozen sections (10μm) fixed with 0.5% glutaraldehyde in PBS for 2 min, washed with PBS, and then incubated with 1 mg/ml Xgal substrates in PBS with 1.3 mM $MgCl_2$, 3 mM K3Fe(CN)6, and 3 mM K4Fe(CN)6 for 1 hr at 37C. *In situ* hybridization for *Shh* was performed as described previously (DasGupta and Fuchs, 1999).

Immunohistochemistry

Pre-fixed (4% PFA in PBS) paraffin embedded embryos were cut at 10μm. Immunohistochemistry was performed by incubating sections at 4 °C overnight with primary antibodies against mouse anti-β-catenin (mouse, 1:1000, Sigma, 15B8). For brightfield immunohistochemistry, biotinylated species-specific secondary antibodies followed by detection using (ImmPRESS reagent kit peroxidase Universal - Vector Labs) and DAB kit (ImmPACT DAB Peroxidase (HRP) SubstrateVector Labs) were used according to the manufacturer's instructions.

Confocal and Epifluorecence Imaging

Epifluorescence images were acquired with an Axio Oberver.Z1 microscope equipped with a Hamamatsu ORCA-ER camera (Hamamatsu Photonics), and with an ApoTome.2 (Carl Zeiss) slider that reduces the light scatter in the fluorescent samples, using 20×, 40×, and 63× objectives, controlled by Zen software (Carl Zeiss). Confocal images were acquired with a Zeiss LSM780 laser-scanning microscope (Carl Zeiss MicroImaging) through a 40× or 63× oil objective. For whole mount imaging, *z* stacks of 20–40 planes (0.25mm) were acquired.

Fluorescence Activated Cell Sorting

Back skins from E17.5 Lhx2-EGFP/Fucci (Figure 4) or E17.5 K14-H2BGFP/Fucci (Figure 6) embryos were dissected and treated overnight with dispase (Gibco, 0.4mg/ml) at 4°C, which selectively removed the epidermis, hair placodes, and hair germs from the skin. This epidermal fraction was placed in Trypsin (GIBCO) at 37°C for 20 min on an orbital shaker. Back skins from E14.5 APC/YFP/Fucci embryos (Figure 4 and 6) were dissected and immediately placed in Trypsin. After centrifugation (300g × 10min), cells were rinsed with PBS and single cell suspensions were obtained. Antibodies and

epifluorescence markers are as indicated in the text. DAPI was used to exclude dead cells. Cell isolations were performed on FACSAria sorters equipped with DIVA software (BD Biosciences), and analyzed using FlowJo.

Lentiviral Lineage Tracing

LV-CreER^{T2} was used for short-term lineage tracing as previously described (Williams et al., 2014). In our hands, this construct was found to have no detectable leakiness in the absence of tamoxifen both *in vitro* and *in vivo*. The *R26flox-stop-flox-YFP* mouse (see above) was used as a reporter for Cre activation and to trace progeny. The fluorescent signal was detected using a polyclonal antibody against GFP, which recognizes cytoplasmic YFP, whose progeny could then be discriminated on the basis of their proximity. Low-titer virus containing the CreER^{T2} cassette was transduced into E9.5 embryos and clonal recombination was induced by administering a single dose of tamoxifen (4mg per dam) by oral gavage, as intraperitoneal injection of tamoxifen at doses sufficient to induce recombination frequently led to aborted litters in our hands. 48h following tamoxifen administration was empirically determined to be sufficient to allow most clones labelled to consist of 1–3 cells. Tamoxifen was administered at E15.5 to monitor peak placode formation. Although CreER^{T2} was delivered at the time when the epidermis was a single layer of basal cells, it will be expressed in all of their descendants owing to its ubiquitous PGK promoter. Only cells within early epithelial buds were counted as it is unlikely that they were present at the time of labelling given the rapid rate of HF morphogenesis at this age. Clones were imaged with Pcad and SOX9 as markers.

Spindle Orientation and Division Measurements

The method for measurement of division angles has been described previously (Williams et al., 2014). Briefly, late-stage mitotic cells were identified by the presence of survivin immunoreactivity at the midbody/cleavage furrow. Cells were scored only if both daughter nuclei surrounding the survivin staining could be unambiguously identified. Angles were measured by drawing a line through the centers of the two nuclei, and parallel to the basement membrane. To reduce any bias in data collection, all data from each group were not analyzed until all images were collected. *n* values are indicated in the main text; each experiment was repeated with at least two replicates and data from at least 3 embryos. No statistical method was used to predetermine sample size, but data were collected from all available embryos of the indicated genotypes. All graphs and statistical analyses (Fisher's exact tests) were produced using Prism.

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