Supplementary Methods

Immunofluorescence studies

Tissue preparation

Frozen sections. Tissue was placed dermal side down on cellulose filter paper (Millipore) for increased stability, mounted in Tissue-Tek cryo-OCT (Fisher Scientific) and frozen using a methyl butane/ dry ice bath. Blocks were cryosectioned at -22ºC taking care to never allow sections to dry. Sections were stored at -80ºC until use.

Whole mounts. Tissue was placed dermal side down on filter paper (see above) and immediately fixed with 4% PFA for 30 minutes at 4ºC. It was then carefully peeled from the paper, washed 3x in PBS (without Ca and Mg) and prepared for immunofluorescence.

IF-EM. Tissue was treated as for wholemount immunofluorescence (see above) and then given to the Penn Microscopy core for further fixation and cutting (see Materials and Methods). A small sample was kept and analyzed for antibody penetration using the membrane staining procedure described below.

Antibody staining for membrane proteins:

For frozen sections, tissue was pre-fixed in 4% PFA for 5 minutes at 4ºC. For either frozen sections or wholemounts, tissue was then incubated in PBS containing 5% FCS (blocking solution) for 10-30 minutes at 4ºC, then incubated with antibody for 1 hour to overnight at 4ºC using dilutions provided in Supplementary Table 1. Tissue was carefully washed 3 times in PBS and incubated with the appropriate secondary antibody (1:500 dilution) for 30-45 minutes followed by washing, fixation and mounting in Vectashield with DAPI ().

Antibody staining for internal proteins (including nuclear):

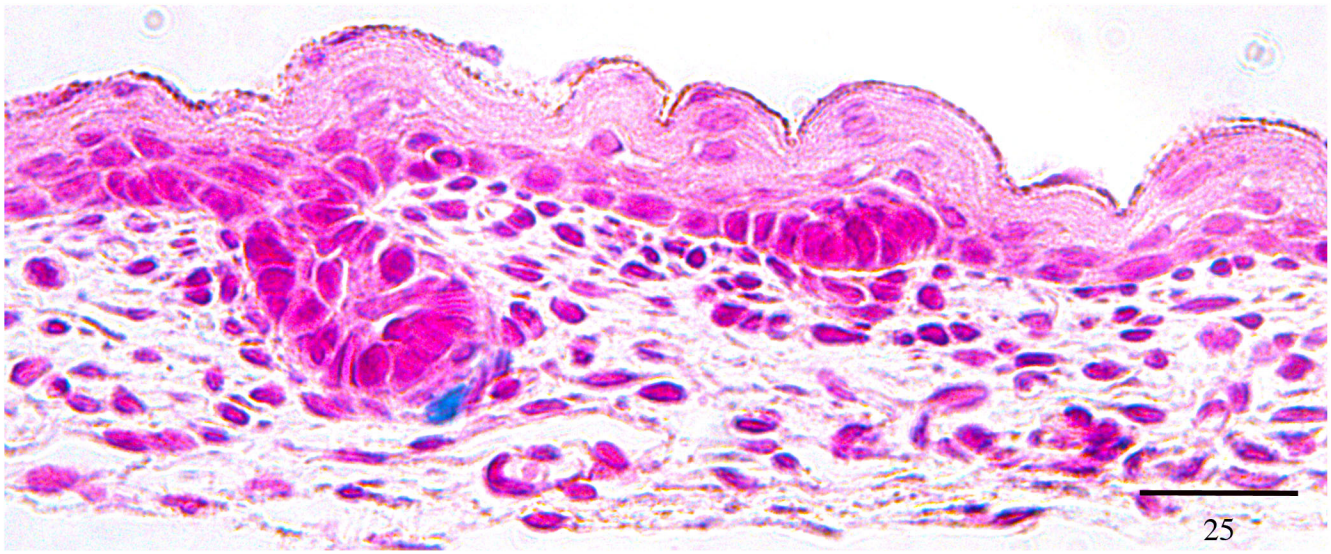
Post-fixed tissue was incubated with block solution containing 0.01% saponin, 0.25% fish gelatin (Sigma), 0.02% sodium azide in PBS to permeabilize cell membranes. Antibody and secondary antibody staining was also undertaken using this block solution. For nuclear detection of activated beta catenin, tissue was first treated with 0.1% Triton X for 3 minutes, washed and then incubated with antibody in saponin block as described above. Detection of other nuclear proteins did not require this additional step. Following antibody staining, tissue was treated as described for membrane antigens.

Isotype controls were run in each experiment to determine background staining, which was typically negligible.

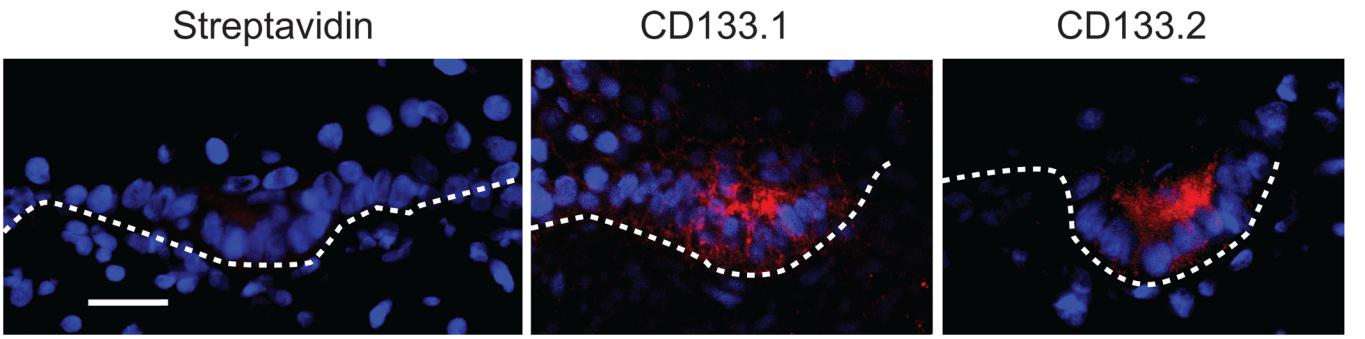
Supplementary Table I. List of antibodies, clone names, companies and dilutions used for studies.

Immunofluorescence	Clone name	Company	Dilution
CD133/1	AC133	Miltenyi Biotec	1:50
CD133/2	293C3	Miltenyi Biotec	1:50
EpCAM	Ber-EP4	Dako	1:100
α6-integrin (CD49f)	GoH3	BD Pharmingen	1:100
E-cadherin	36	BD Pharmingen	1:100
P-cadherin	Ncc-cad-299	Zymed	1:100
active beta-catenin	8E7	Millipore	1:50
beta-catenin	14	BD Pharmingen	1:400
Lef1	C18A7	Cell Signaling	1:400
TGF-beta	Ab66043	Abcam	1:100
Wif1	Ab89935	Abcam	1:100

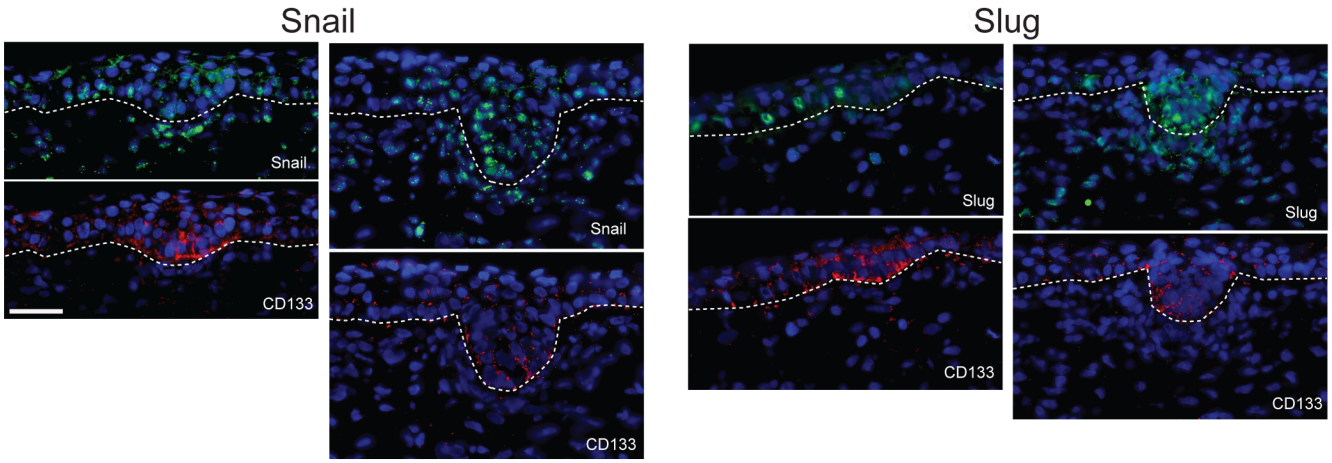
Ki-67	B58	BD Pharmingen	1:100
Snail	Ab53519	Abcam	1:100
Slug	Ab51772	Abcam	1:100
ZO1	33911	Invitrogen	1:40
HDAC6	H-300	Santa Cruz	1:50
PLA Studies			
E-cadherin	24E10	Cell Signaling	1:4000
CD133/2	293C3	Miltenyi Biotec	1:200
CD133 C-term	Ab16518	Abcm	1:400
EpCAM	D1B3	Cell Signaling	1:2500
Beta-catenin	15B8	Sigma	1:15000
HDAC6	Ab117900	Abcam	1:1000



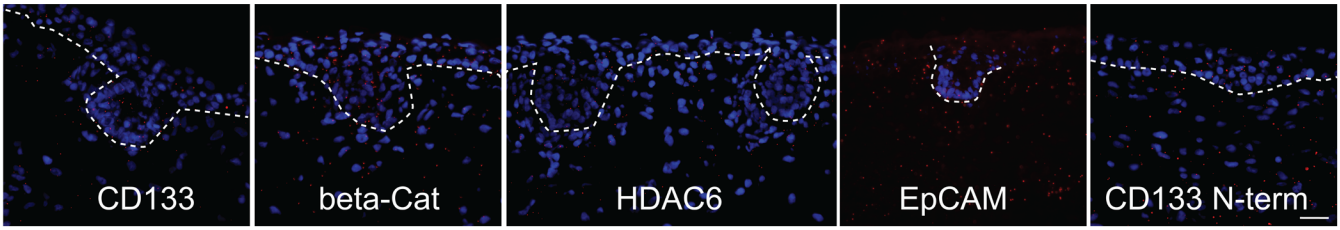
Supplementary Figure 1 – Fetal mouse placodes and pegs do not express CD133 transcripts. Paraffin-embedded E16.5 *Prom1-lacz* reporter mouse back skin was stained for beta-galactosidase expression and counterstained with nuclear fast red. CD133 transcription is seen in dermal condensate cells (blue) but not interfollicular or follicular epithelial cells. Scale bar 25 μm.



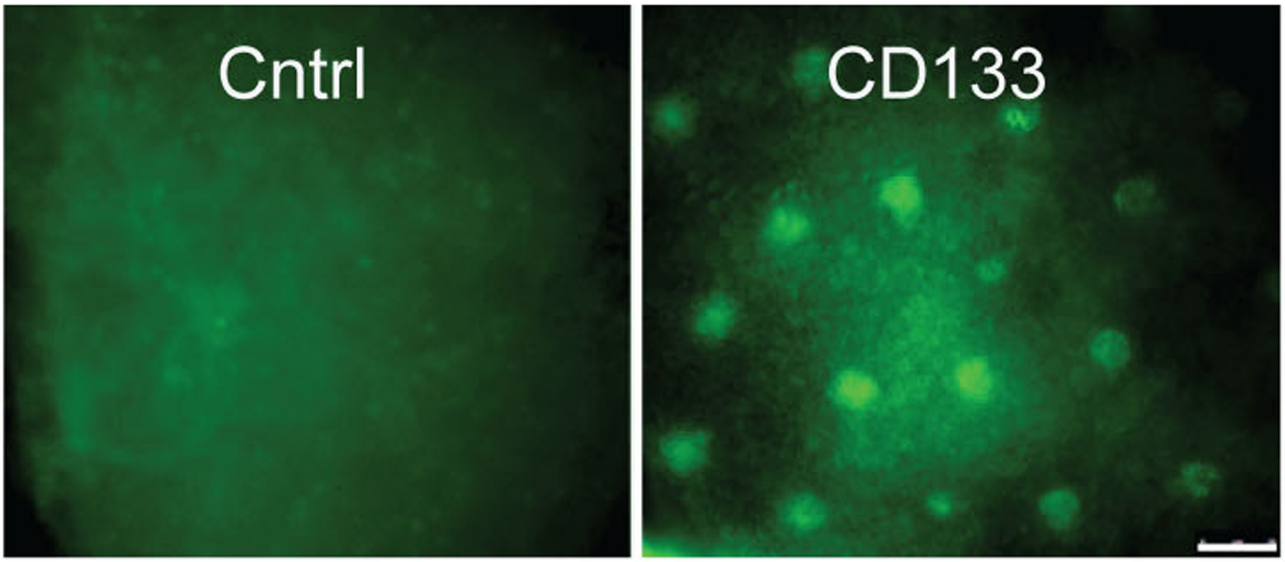
Supplementary Figure 2 – Analyses of human fetal scalp frozen sections with anti-CD133 antibodies. Immunofluorescence analyses using biotinylated anti-CD133 antibodies directed against distinct CD133 epitopes. First panel shows streptavidin negative control. Scale bar is 25 μm.



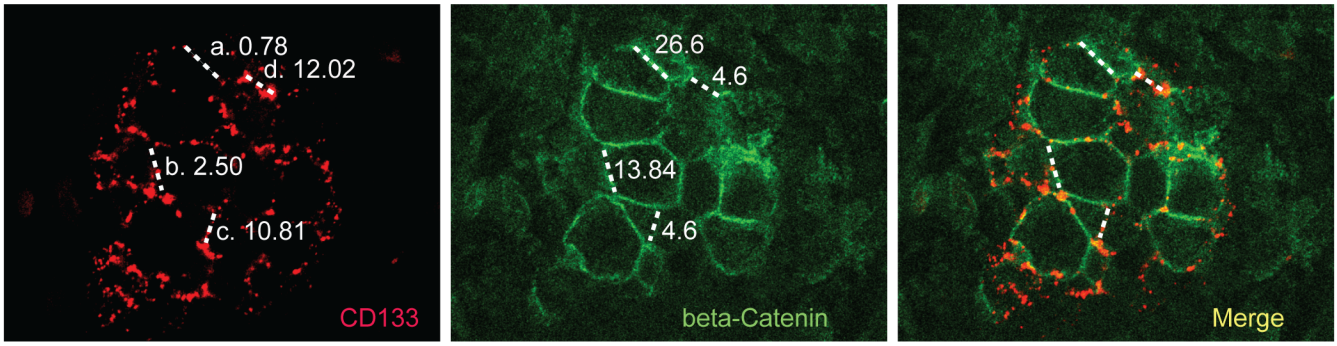
Supplementary Figure 3 – Co-localization of Snail or Slug (top panels) and CD133 (bottom panels) in early and late placodes. Frozen sections of 13 week fetal scalp stained for expression of Snail or Slug (green) and CD133 (red). Scale bar is 25 μm.



Supplementary Figure 4 – Frozen sections stained with single antibodies and processed for PLA. Frozen tissue sections incubated with only one of the antibody combinations and treated for PLA analyses. DAPI-stained nuclei are blue. Red dots represent areas of PLA activity. Scale bar 25 μm.



Supplementary Figure 5 – Localization of tagged immunogold nanoparticles to anti-CD133 or control antibody. Pre-fixed fetal scalp whole mounts were incubated with biotin-anti-CD133 or a biotinylated control isotype antibody, followed by incubation with ultra small 1.4nm Alexa fluor 488 streptavidin nano-gold particles. Scale bar 75 μm.



Supplementary Figure 6 – Representative confocal image of placode stained for CD133 (red) and beta-catenin (green) with derived CTCF values. As an example, four regions were selected for fluorescence intensity analysis (ImageJ) and CTCF value determination (numbers). As can be seen, regions 'a' and 'b' have high beta-catenin and low CD133 whereas 'c' and 'd' show the converse.