nature research

Corresponding author(s): Danelle Devenport

☐ Initial submission ☐ Revised version ☒ Final submission

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Experimental design

1. Sample size

   Describe how sample size was determined.

   All mutant, inhibitor treated or laser ablated phenotypes reported contrast greatly with the controls. Therefore, no calculations were used to determine sample size. Sample size was limited by the number of embryos of a given genotype.

2. Data exclusions

   Describe any data exclusions.

   Only damaged skins were excluded from analysis.

3. Replication

   Describe whether the experimental findings were reliably reproduced.

   All attempts at replication were successful.

4. Randomization

   Describe how samples/organisms/participants were allocated into experimental groups.

   Experimental groups were determined by genotype or experimental condition (inhibitor treated, laser ablated, controls). All mutant, inhibitor treated or laser ablated phenotypes reported contrast greatly with the controls.

5. Blinding

   Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

   Automated cell tracks were produced using Tissue Analyzer software for all relevant genotypes/conditions. Cell masks used to determine Celsr1 polarity using Packing Analyzer software were determined using a membrane channel without visualizing the Celsr1 channel. Sox9 asymmetry was quantified by one researcher while another researcher determined the hair follicle stage using a membrane channel. Blinding was not used for other experiments, but representative images were chosen.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ A statement indicating how many times each experiment was replicated
☐	☒ The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☐	☒ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒ The test results (e.g. *P* values) given as exact values whenever possible and with confidence intervals noted
☐	☒ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒ Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.	Photoshop V10, Tissue Analyzer V2.3 beta 1, Packing Analyzer V2, ImageJ/Fiji V1.51 n, Improvision Velocity V6.3.1, Matlab VR2015b, NIS Elements V4.13, Adobe Illustrator V16.0.3, Prism V5.0f

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.	Mouse anti-Sox9 from Santa Cruz is no longer available. Rabbit anti-Sox9 from Millipore shows the same results.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	All antibodies are commercially available, except for Celsr1 which has previously been used in this tissue (Devenport and Fuchs, 2008). The following primary antibodies were used: guinea pig anti-Celsr 1 (1:1000, D. Devenport), anti-Sox9 (mouse,1:250, Santa Cruz, discontinued; rabbit, 1:1000, Millipore, Cat: AB5535), mouse anti-P-Cadherin (1:250, Clontech, Cat: AB36999), rat anti-E-Cadherin (1:1000, DECMA-1, Thermo Pierce, Cat: MA1-25160), rhodamine phalloidin (1:1000, Cytoskeleton, Inc, Cat: PHDR1) and Acti-stain 488 (1:1000, Cytoskeleton, Inc, Cat: PHDG1). Alexa Fluor-488,-555, and -647 secondary antibodies were used at 1:1000. Hoescht (Invitrogen, Cat: H1399) was used at 1:1000.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.	No eukaryotic cell lines were used.
b. Describe the method of cell line authentication used.	No eukaryotic cell lines were used.
c. Report whether the cell lines were tested for mycoplasma contamination.	No eukaryotic cell lines were used.
d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.	No eukaryotic cell lines were used.

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.	E15.5-17.5 Mouse embryos from C57BL6, C57BL6 mixed background or FVBwere used in this study. All genotypes are listed in the genotypes table. Both sexes were used as sex was not determined in embryos.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.	This study did not involve human research participants.