Expanded View Figures

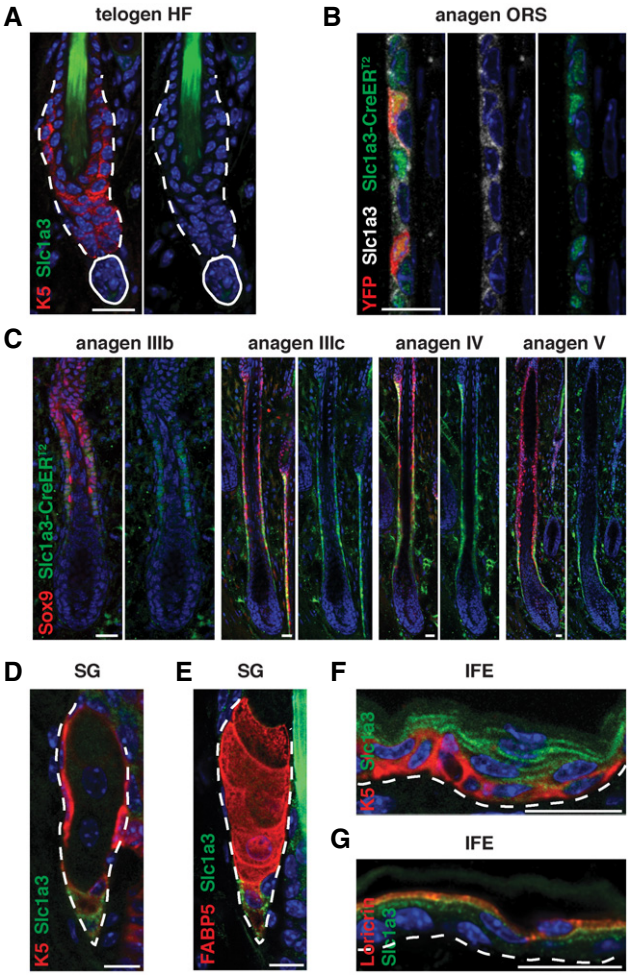

Figure EV1. Slc1a3 is expressed in ORS, SG, and IFE stem cell niches but not in the telogen hair follicle.

A Slc1a3 is not expressed in hair follicle stem cells in telogen.

B Slc1a3 and Slc1a3-CreER^{T2} expression overlap. YFP expression is restricted to Slc1a3-CreER^{T2+} cells immediately after recombination.

C Slc1a3-CreER^{T2} is expressed in the Sox9^{+} ORS and absent from the matrix and inner layers of the growing hair follicle.

D, E Slc1a3 is robustly expressed in a fraction of K5^{+}/K5^{low}FABP5^{-} SG basal cells and a few K5^{+}FABP5^{low} SG cells.

F, G In the IFE, Slc1a3 is expressed in a subset of K5^{+} basal and all Loricrin^{+} suprabasal cells.

Data information: Dashed lines outline bulge (A) and SG (D, E) and indicate epidermal–dermal border (F, G). Continuous lines outline dermal papilla. Scale bars = 20 μm.

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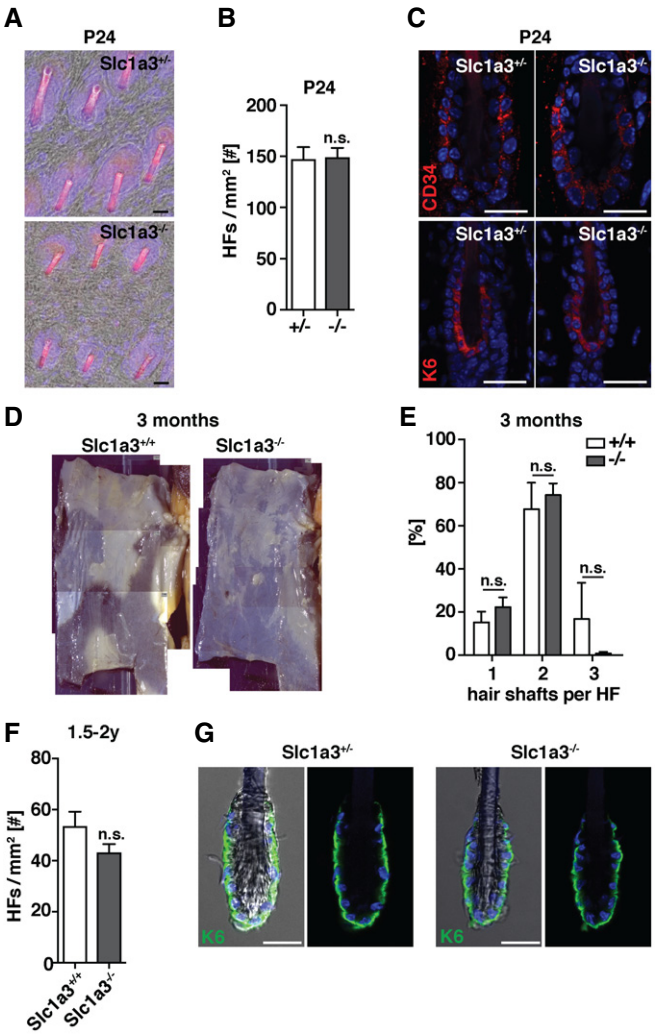

Figure EV2. Slc1a3 deletion does not alter the number of hair follicles and hair anchoring.

A–C Hair follicle density (A, B) and CD34 and K6 stem cell niche marker expression (C) of P24 Slc1a3$^{+/-}$ animals (n = 4, 1,6 mm^2 skin per animal) compared to Slc1a3$^{-/-}$ littermates (n = 4, 1,6 mm^2 skin per animal).

D Tile scan micrographs of back skin indicating pigmented regions in which hair follicles are in anagen of 3-month-old Slc1a3$^{+/+}$ and Slc1a3$^{-/-}$ animals.

E Hair shaft density of 3-month-old Slc1a3$^{+/+}$ (n = 4, 355 HFs) compared to Slc1a3$^{-/-}$ (n = 4, 391 HFs) animals.

F Comparison of hair follicle density of aged Slc1a3$^{+/+}$ (n = 4, 1.6 mm^2 skin per animal) and Slc1a3$^{-/-}$ animals (n = 4, 1.6 mm^2 skin per animal).

G Plucked hair shafts of Slc1a3$^{+/-}$ and Slc1a3$^{-/-}$ mice show no difference in the amount of attached K6+ inner bulge cells.

Data information: Data are mean ± SEM; n.s. = not significant (two-tailed Student's t-test in B and F, Mann–Whitney U-test in E). Scale bars = 20 μm except in (A), scale bar = 30 μm.
Source data are available online for this figure.

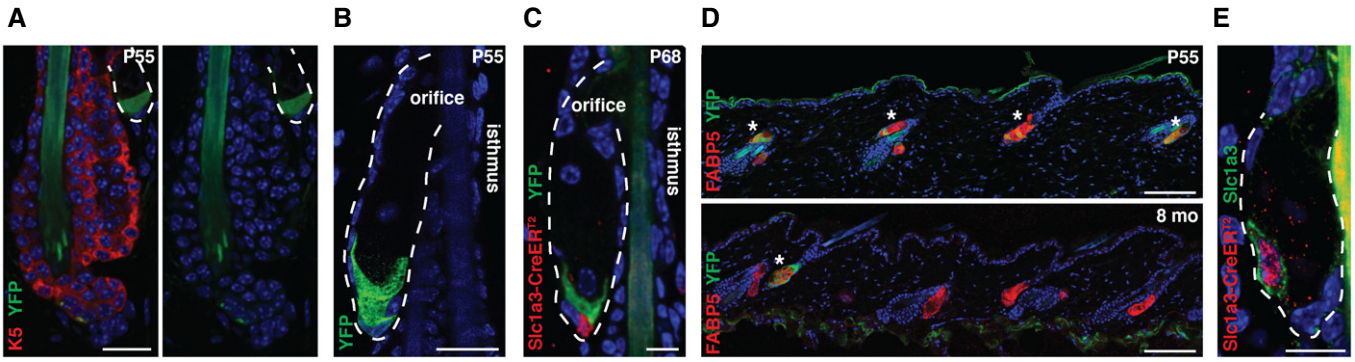

Figure EV3. Slc1a3 is transiently expressed in SG basal cells, which can self-maintain SGs for at least 8 months.

A Recombination induction during telogen (P51-55) labels cells in the sebaceous gland (SG) but not in the hair follicle.

B, C The Slc1a3-CreER^{T2} mouse line does not target cells in the isthmus or around the SG orifice and these regions neither contain YFP$^+$ cells 5 days after recombination induction (B) nor express Slc1a3-CreER^{T2} (C), excluding contribution of YFP$^+$ SG cells from these regions.

D Sections of lineage-traced dorsal back skin directly (P55) or 8 months (8 mo) after 5 consecutive days of tamoxifen injection. Over time, the number of YFP$^+$ cells in YFP$^+$ SGs increases while the number of YFP$^+$ SGs decreases. FABP5$^+$ sebocytes in red mark SGs, and asterisks indicate SGs containing YFP$^+$ cells.

E Following lineage tracing, YFP$^-$ SGs contain CreER^{T2+}Slc1a3$^+$ basal cells.

Data information: Dashed lines outline the SG. Scale bars = 20 μm except in (D) scale bar = 100 μm.

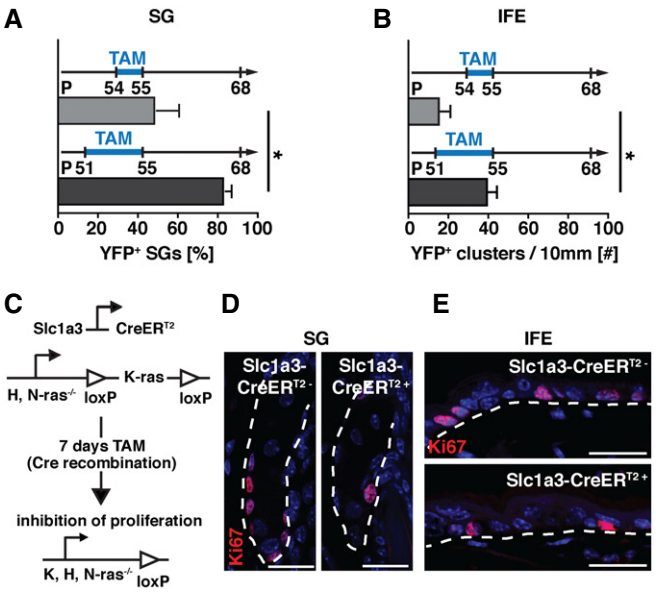

Figure EV4. Slc1a3 is transiently expressed in active cycling SG and IFE cells.

A Percentage of YFP$^+$ SGs after 2 (n = 4, 200 SGs) compared to 5 (n = 4, 200 SGs) consecutive days of tamoxifen injection, assessed at the same time during telogen.

B Number of YFP$^+$ cell clusters in 10 mm IFE after 2 (n = 4, 40 mm) compared to 5 (n = 3, 30 mm) consecutive days of tamoxifen injection, assessed at the same time during telogen.

C Schematic depiction of the genetic strategy to block proliferation of Slc1a3$^+$ cells, by tamoxifen inducible cell-specific deletion of all Ras genes.

D, E Representative micrographs showing proliferating (Ki67$^+$) cells in SG (D) and IFE (E) after recombination-mediated block of proliferation in Slc1a3$^+$ cells (Slc1a3-CreER^{T2+}) compared to control (Slc1a3-CreER^{T2-}) animals.

Data information: Data are mean ± SEM; *P < 0.05 (two-tailed Student's t-test). Dashed lines outline the SG (B) and indicate the epidermal–dermal border (C). Scale bar = 20 μm.
Source data are available online for this figure.

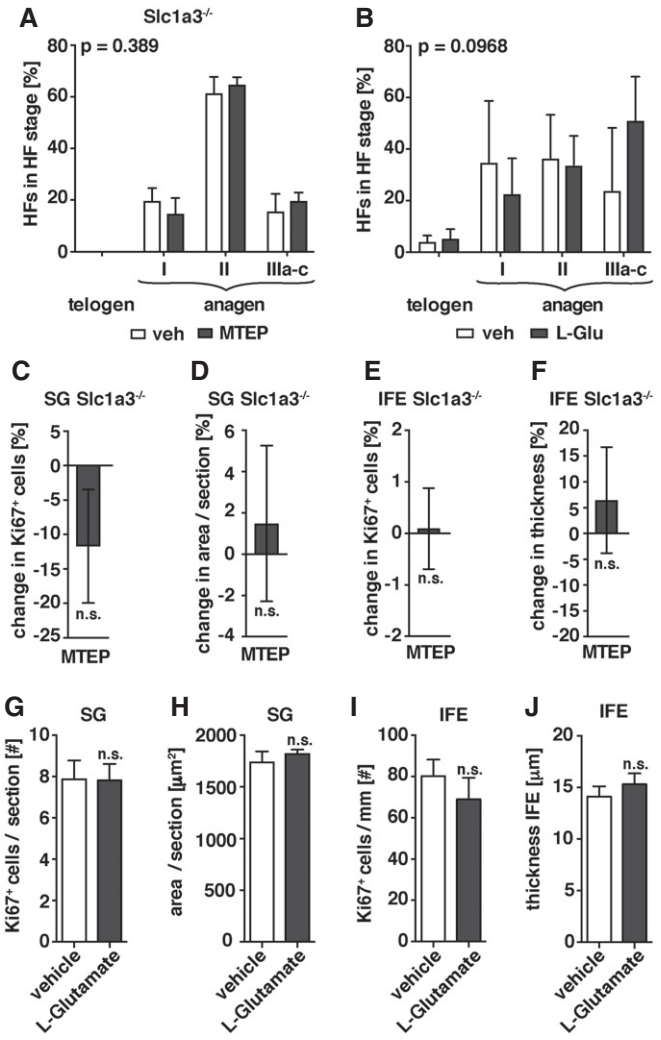

Figure EV5. Slc1a3 and mGluR5 act together.

A Quantification of hair cycle progression assessed by hair follicle staging following topical treatment with mGluR5 antagonist MTEP (n = 4, 120 HFs) or vehicle (n = 4, 120 HFs) in Slc1a3$^{-/-}$ mice. P-value derived from grouped comparison of ranked HF stages in mice treated with vehicle and MTEP.

B Quantification of hair cycle progression assessed by hair follicle staging following cutaneous treatment with L-glutamate (L-Glu; n = 5, 75 HFs) in comparison with vehicle-treated mice (n = 3, 48 HFs). P-value derived from grouped comparison of ranked HF stages in mice treated with vehicle and L-Glu.

C–F Quantification of proliferation (C, E) and growth (D, F) of SG (C, D) and IFE (E, F) following topical treatment with MTEP (n = 4, 79 SGs and 7 mm IFE, respectively) or vehicle (n = 4, 77 SGs and 8 mm IFE, respectively) in Slc1a3$^{-/-}$ mice.

G–J Quantification of proliferation (G, I) and size (H, J) of SG (G, H) and IFE (I, J) following cutaneous treatment with L-glutamate (n = 4, 46 and 48 SGs, 5 mm, respectively) compared to vehicle (n = 3, 32 and 22 SGs, 3 mm, respectively).

Data information: Data are mean ± SEM in (A, B, E–H) and mean ± SEM in (C and D); n.s. = not significant (Mann–Whitney U-test in A and B, two-tailed Student's t-test in C–J).
Source data are available online for this figure.