Figure EV1. Gata6 is up-regulated in differentiating hair germ cells.

A Experimental scheme for doxycycline chased K5TATA × pTRE-H2B-GFP mice used to obtain divided and un-divided cell populations during the differentiating phase—telogen (T)—anagen (A) transition—and self-renewing phase of HFSCs during early anagen, as previously described in Zhang et al (2009). Bulge cells were sorted as divided or un-divided based on H2B-GFP levels and high CD34 and α6-integrin expression; hair germ cells at telogen–anagen transition were the divided cells with low CD34 levels and high expression of α6-integrin.

B qRT-PCR confirmation of Gata6 expression in differentiating (hair germ, divided), self-renewing (bulge, divided), or non-dividing bulge stem cells (average ± SD, n = 3).

C Immunofluorescence image of the skin shows epidermis and infundibulum stained with Hoechst for DNA (blue), basal layer/ORS marker K14 (green), and Gata6 (red). Scale bar: 30 μm.

D Gel image of DNA genotyped for floxed Gata6 allele. Shown are genotypes from three mice: heterozygote (Gata6^{fl/wt}), homozygous WT (Gata6^{wt/wt}), and homozygous floxed (Gata6^{fl/fl}).
Figure EV2. Gata6 is necessary for anagen initiation and maintenance.

A, B Low magnification images of skin with hematoxylin staining for telogen-induced (A) and anagen-induced (B) mice. Scale bars: 30 μm.

C Immunofluorescence images of hair follicles 10 days post-anagen induction stained with bulge marker CD34. Scale bars: 30 μm.

D Quantification of the number of cells in anagen matrix hair follicles post-Gata6 iKO induction (average ± SD, n = 20 follicles from three mice each). Unpaired t-test *P-values: 1 day = 0.86, 2 days = $7 \times 10^{-5}$, 4 days = $4 \times 10^{-10}$. 

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**Figure EV3.** Gata6 is not necessary for terminal differentiation of the hair follicle.

A–D Immunofluorescence staining of skin sections 2 days post-tamoxifen induction for terminally differentiated lineages reveal labeling in the hair bulb, as expected. Specific stains are indicated in corresponding colors. Scale bars: 30 μm.

E Quantification of images like those shown in Fig 3 shows percentage of BrdU+ cells located in the differentiated IRS and hair shaft lineages after the initial 12-h pulse labeling and after the 2 days BrdU chase (average ± SD, n = 3, P-value = 0.7 for 12 h WT versus iKO and 0.4 for 2 days WT versus iKO).
Figure EV4. Gata6 loss does not cause DNA damage or apoptosis in the inter-follicular epidermis or bulge. Skin sections immunostained for caspase-3 and γH2A.X show no detectable signal in the inter-follicular epidermis or bulge HFSCs. Scale bars: 30 μm.
Figure EVS. Overexpression of Edaradd rescues Gata6 iKO keratinocytes.

A qRT-PCR analysis of Gata6 and Edaradd in Gata6 WT and iKO stably transfected with pMock, pGata6, or pEdaradd (average ± SD, n = 4).

B Phase contrast images of keratinocytes from cells stably transfected with empty plasmid, pGata6, or pEdaradd. Scale bars: 20 μm.

C qRT-PCR of selected genes found to be overlapping between Gata6 iKO and Eda transgenic analyzed in Gata6 WT and iKO keratinocytes stably expressing pMock, pGata6, or pEdaradd (average ± SD, n = 4). Unpaired t-test *P-values: 8 × 10^-4 (iKO pGata6 versus pMock), 0.03 (iKO pEdaradd versus pMock). Mann–Whitney U-test P-values: 0.03 (iKO pGata6 versus pMock), 0.03 (iKO pEdaradd versus pMock).