Supplementary Information

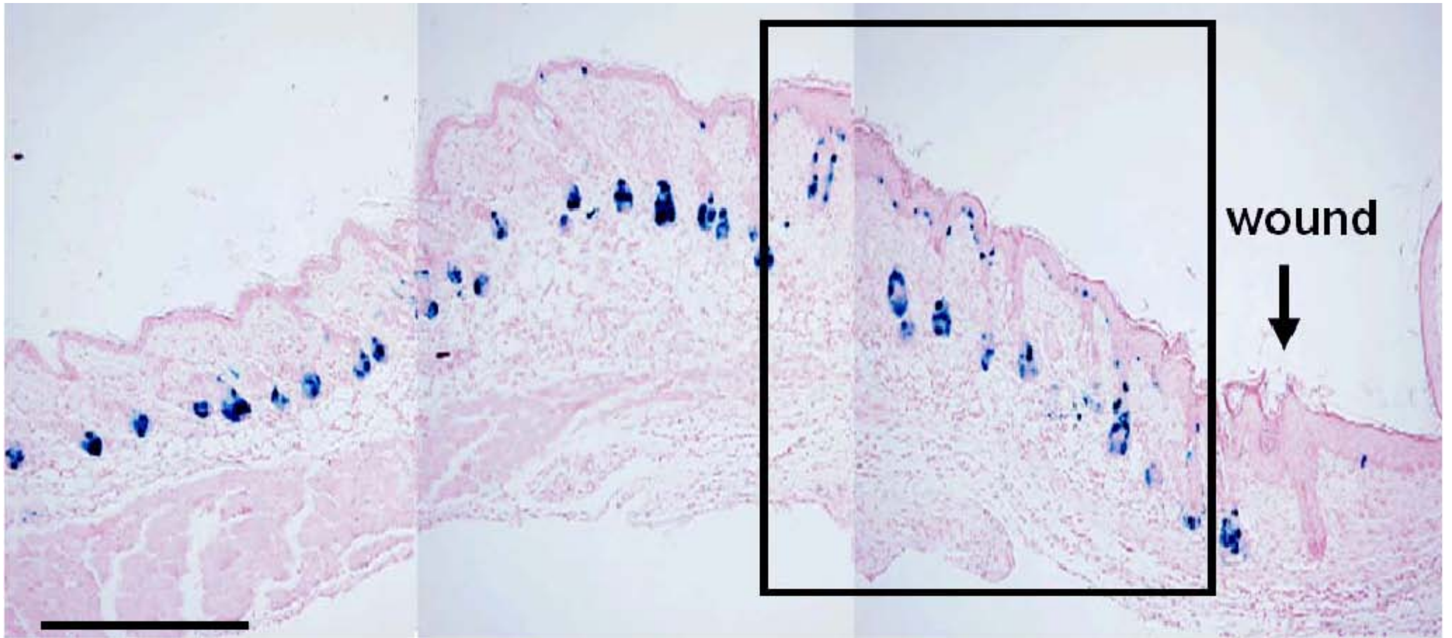


Figure S1: Follicular melanocytes in the wound peripheral area migrate to the epidermis in response to wounding stimuli.
Dorsal skin of Trp2-LacZ mice stained with X-gal and analyzed at 8 days after excisional wounding. Migrating melanocytes in the hair follicles adjacent to the wound is shown in the box. Scale bar, 250 μm.

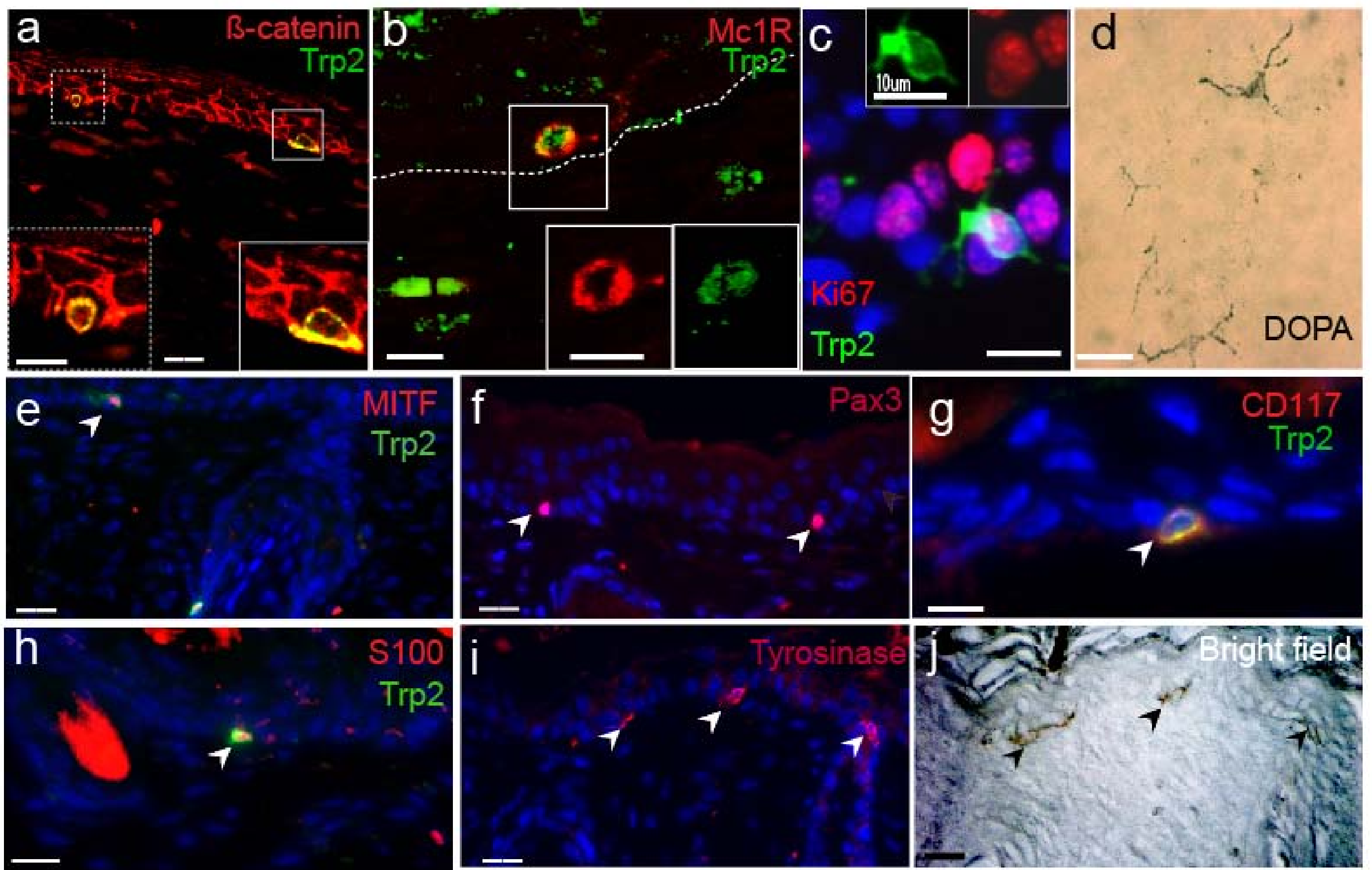


Figure S2: Follicle-derived epidermal melanocytes express differentiation and proliferation markers
(**a-c, e-i**) Expression of nuclear β-catenin, an indicator of active Wnt signaling (a), melanocyte differentiation markers (b, e-i), and proliferation marker (c) was found in epidermal melanocytes at 15 days after wounding. Insets are magnifications of corresponding boxes. (**d**) DOPA staining on whole mount wound epidermis to confirm pigment production in melanocytes. (**j**) Matched bright field image of epidermal melanocytes (arrowhead) shown in i. Scale bar 20 µm (a), 10 µm (inset in a), 10 um (b-d,inset in b-c, g), 20 µm (e-f, h-j)

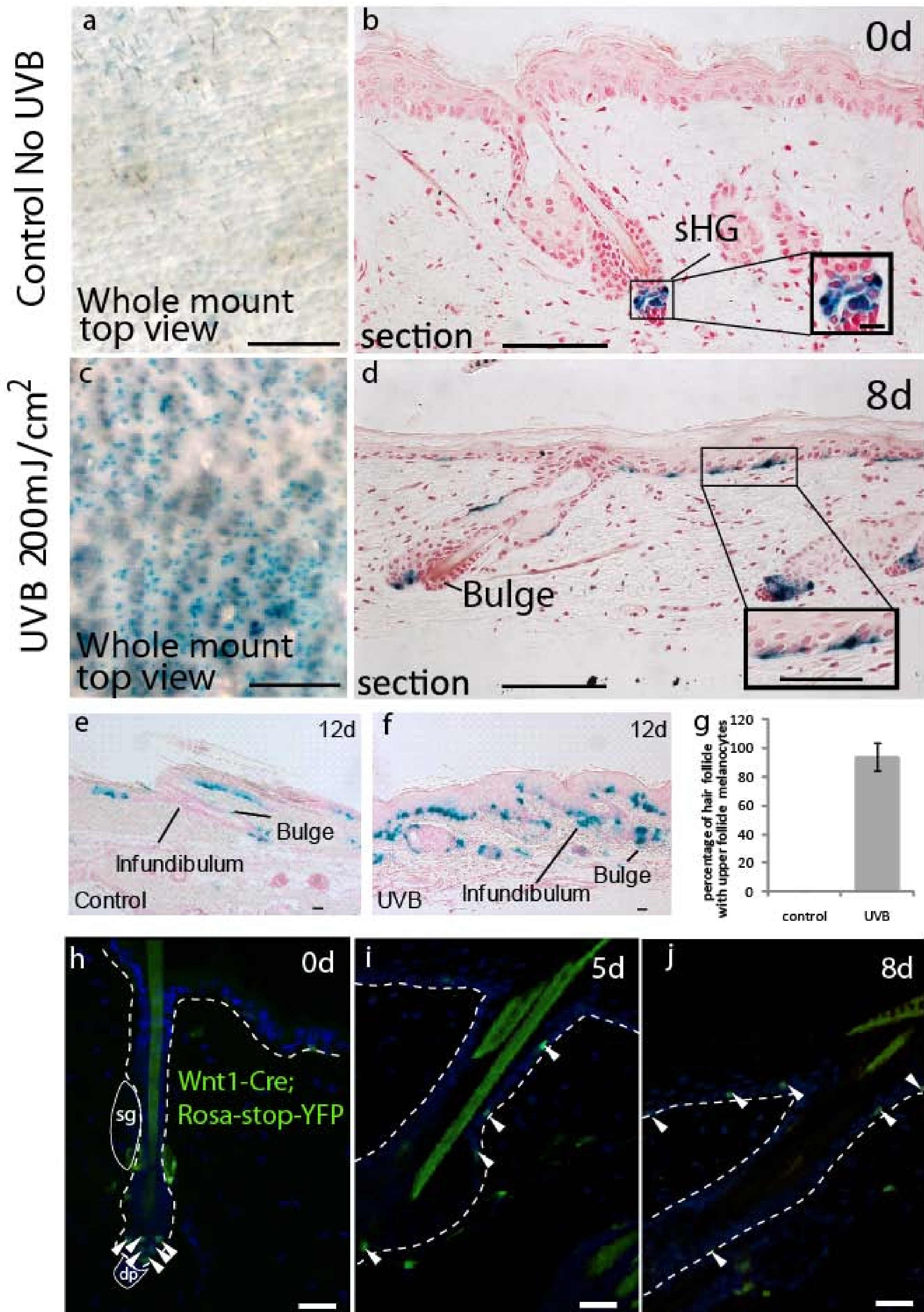
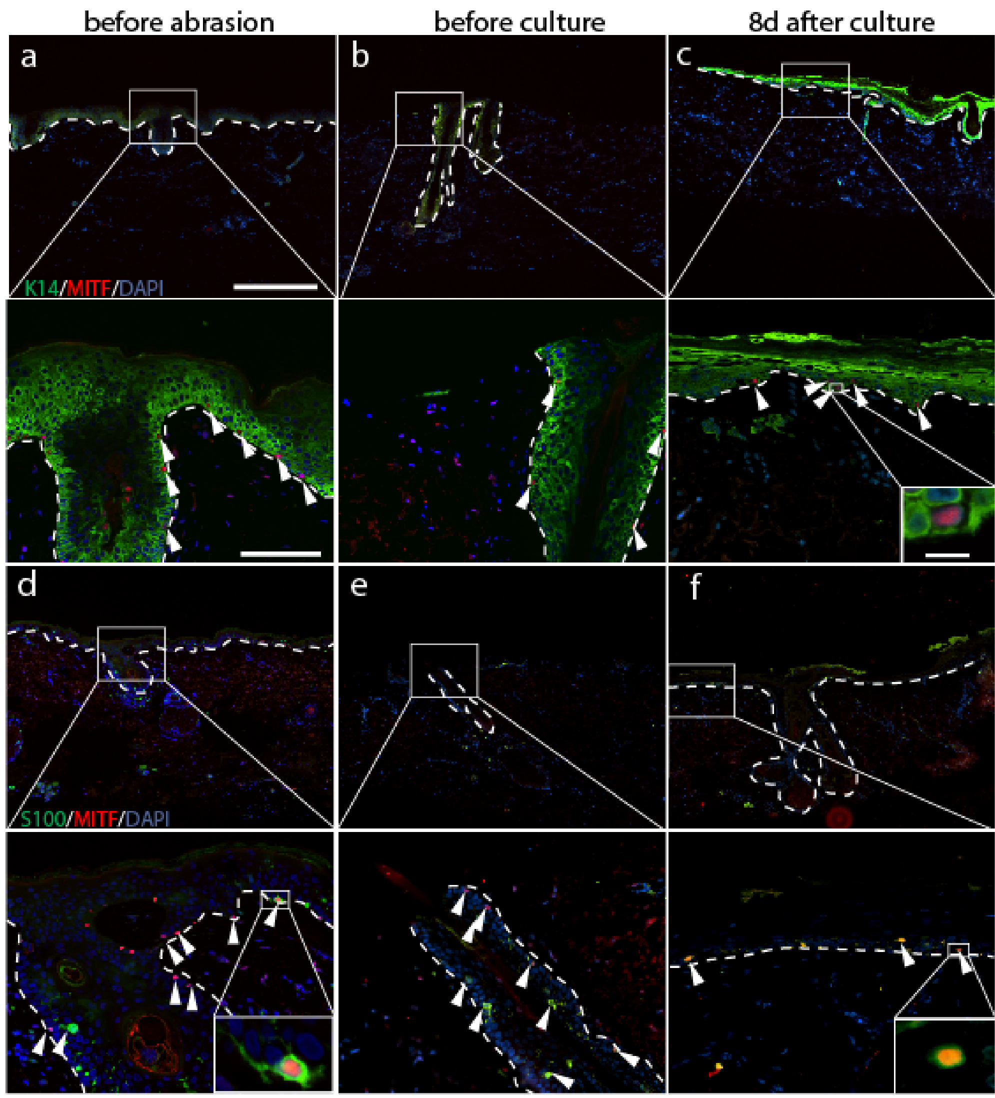
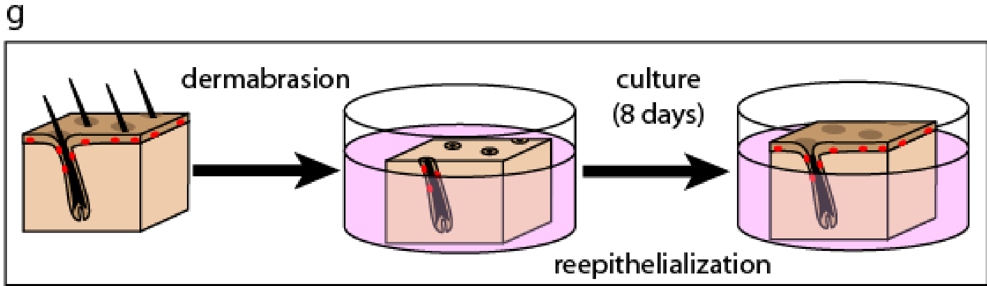

Trp2-LacZ UVB treatment
Control No UVB
UVB 200mJ/cm2
a
Whole mount top view
b
0d
sHG
section
c
Whole mount top view
d
8d
Bulge
section
e
12d
Infundibulum
Bulge
Control
f
12d
Infundibulum
Bulge
UVB
g
percentage of hair follicle with upper follicle melanocytes
120
100
80
60
40
20
0
control
UVB
h
0d
sg
dp
Wnt1-Cre; Rosa-stop-YFP
i
5d
j
8d

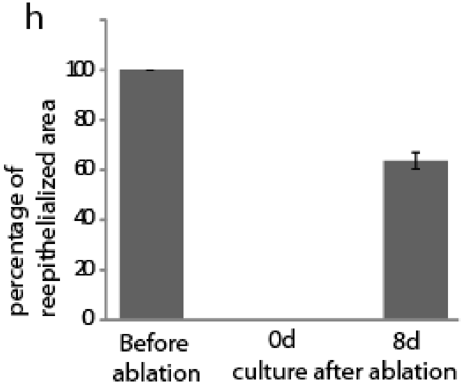
Figure. S3. Follicular melanocytes give rise to epidermal melanocytes after UVB irradiation.
(**a-d**) LacZ staining in bulge/sHG area indicates McSC localization in back skin of adult Trp2-lacZ mice with UVB irradiation and without UVB control. (**e-f**) The effect of epidermal melanocytes on the migration of McSCs was assessed in the tail skin of Trp2-lacZ mice. After UVB irradiation, melanocytes migrate towards the epidermis, as suggested by their presence in the infundibulum (**f**) which is absent in non UVB treated control mice (**e**). (**g**) Melanocytes in the upper follicle of tail skin are indicated as average± standard deviation. (**h-i**) Neural crest derived McSCs are marked with YFP in back skin sections from UVB treated Wnt1-Cre;Rosa-stop-YFP mice[14,15]. Dashed lines indicate epidermis and dermis borders. Arrowheads, McSCs. Sg, sebaceous gland, UF, upper follicle. dp, dermal papilla. Inset in (b,d) is magnification of boxed area. Scale bar, 50 µm (a, c), 100 µm (b,d), 12.5 µm (inset in b), 50 µm (inset in d), 20 µm (e-f, h-j).



g



h



i

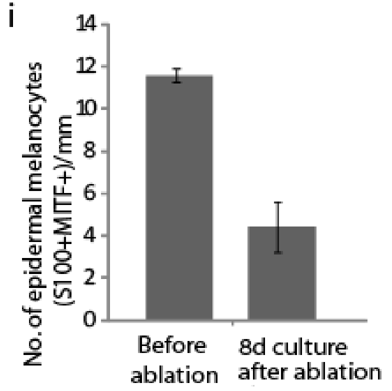


Figure. S4. Follicular melanocytes give rise to epidermal melanocytes in reepithelized human scalp after dermabrasion

(**a-f**) Immunofluorescence staining sections from intact scalp (left column), ablated skin prior to culture (middle column) and re-epithelialized skin (right column). Magnified views of enclosed area were shown below each image. (**g**) Schematic figure of human scalp explant culture experiment (**f**) Quantification of reepithelialized area by skin section analyses. (**i**) Quantification of S100+MITF+ melanocytes in the epidermis of skin explants. Data represent average± standard deviation. Insets within are magnification of area in white box. Scale bars, 500 µm (a-f),100 µm (row below a-f) and 25 µm (insets) Dashed lines indicate epidermis and dermis borders.

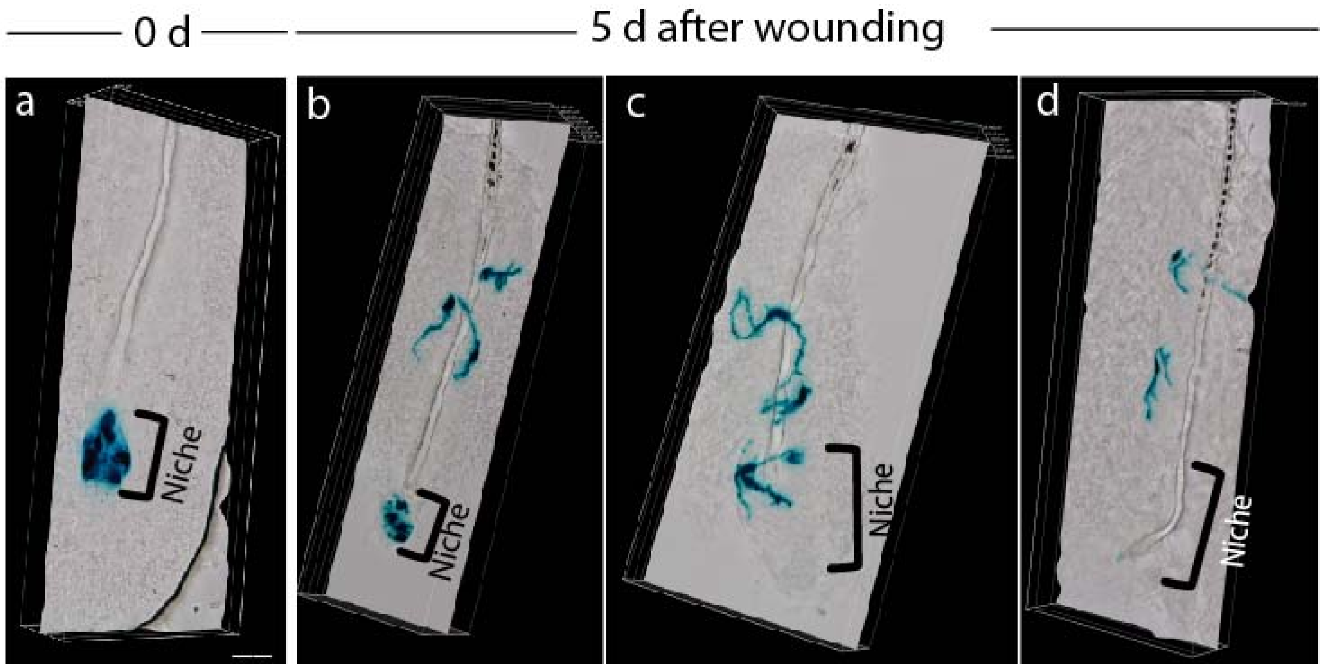


Figure. S5. Whole mount analyses of individual follicles after wounding

(**a-d**) Hair follicles containing migrating melanocytes above the niche were dissected from wound periphery of Trp2-lacZ mice to indicate McSCs distribution (**a**) before and (**b-d**) after wounding. There were rare hair follicles without McSCs in the niche (**d**). Scale bar, 20 µm.

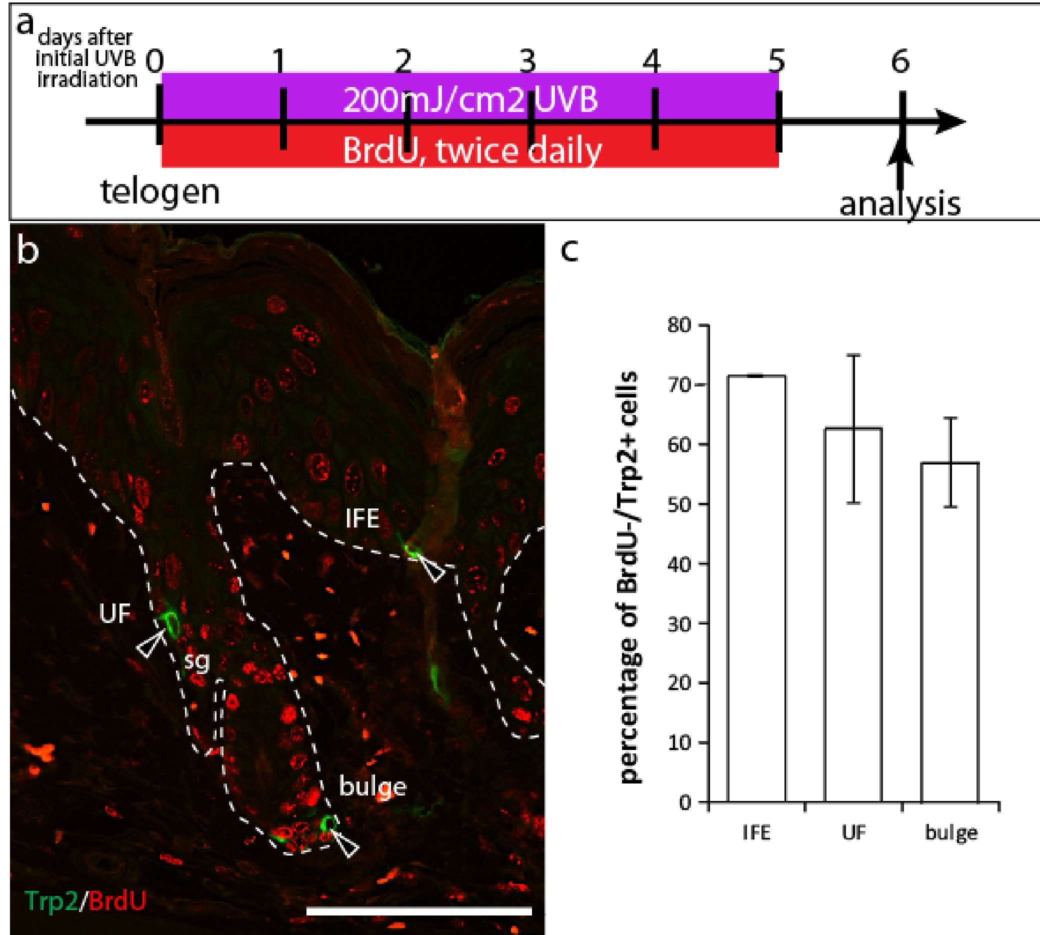


Figure S6. UVB irradiation induces direct migration of follicular melanocytes to the epidermis
(**a**). Experimental scheme for UVB treatment and BrdU injection. (**b**) Double immunofluorescence for BrdU and Trp2 in skin sections 6 d after UVB and BrdU treatment. (**c**) Quantification of BrdU negative melanocytes in IFE, UF and bulge/sHG niche. Data represent average ±standard deviation._Arrowheads, melanocyte. Epidermis and dermis border are indicated by dash lines. Arrowheads, McSCs. Sg, sebaceous gland, UF, upper follicle. IFE, interfollicular epidermis. Scale bar, 100 µm.

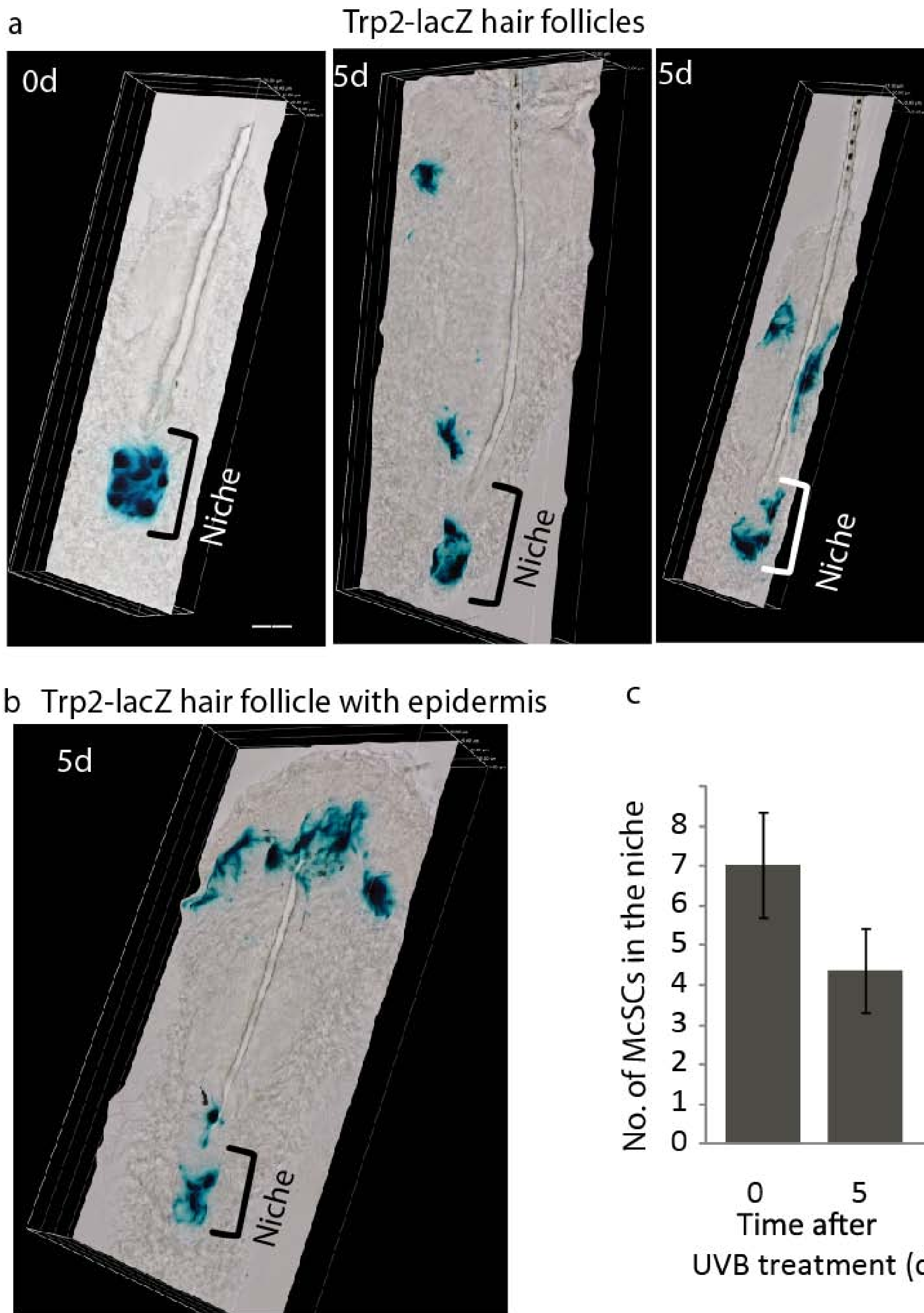


Figure S7. Whole mount analyses of individual follicles after UVB irradiation

(**a-c**) Hair follicles containing migrating melanocytes above the niche were dissected from UVB irradiated Trp2-lacZ mice with (b) or without (a) epidermis, and the number of McSCs in the niche was assessed in whole mount hair follicles (c). Quantification represents average $\pm$ standard deviation. Scale bar, 20 μm.

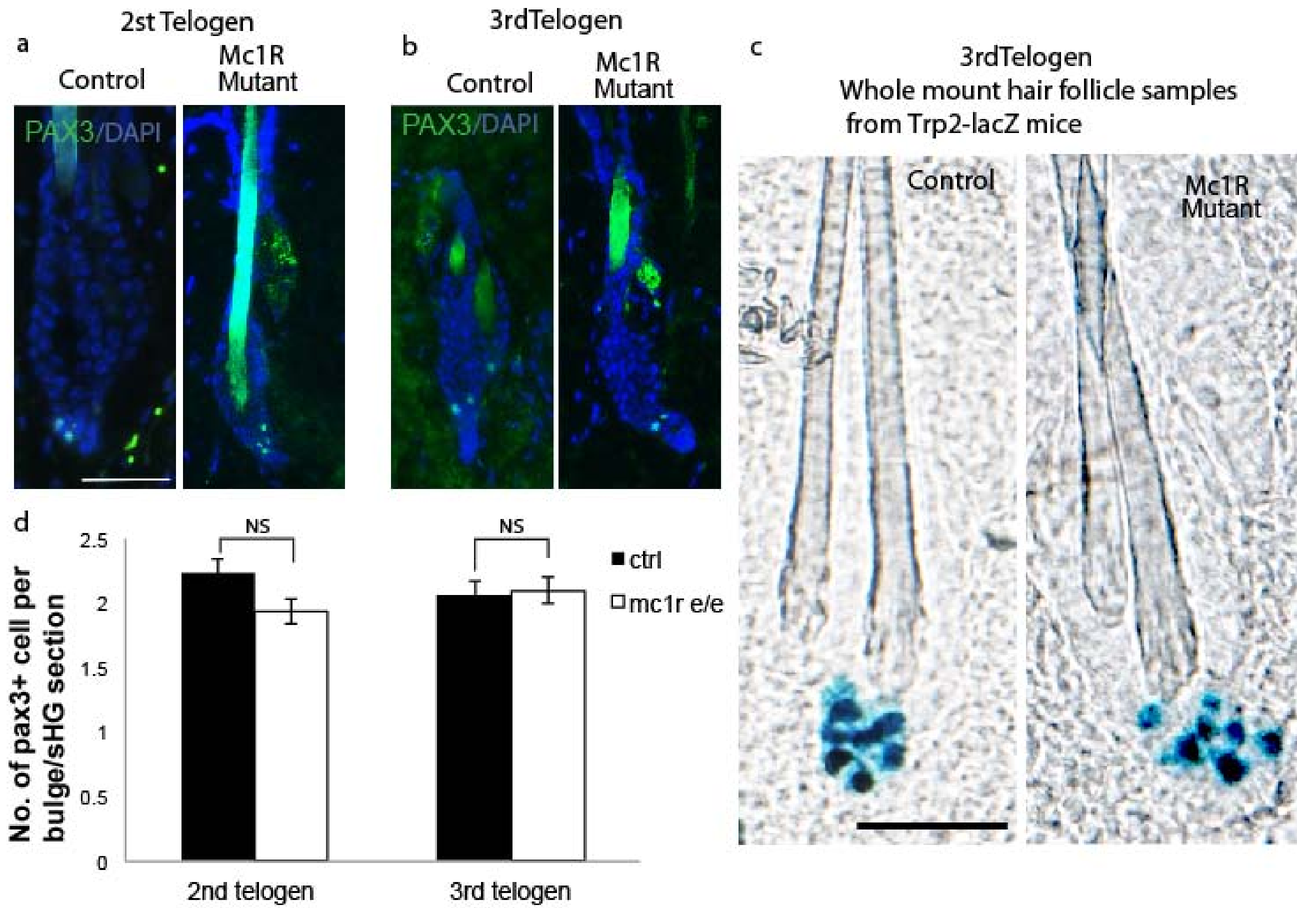


Figure S8. Mc1R is not required for McSC maintenance
(**a,b**) PAX3 staining to indicate follicular McSCs in Mc1R mutant mice (Mc1R$^{e/e}$ mice) and control mice at 2nd and 3rd telogen. (**c**) X-gal stained whole mount hair follicles from Trp2-LacZ control (left) and Mc1R KO;Trp2-LacZ (right) mice. (**d**) Quantification of Pax3 expressing McSCs. Data represented as mean $\pm$standard deviation. scale bar, 100 uM .