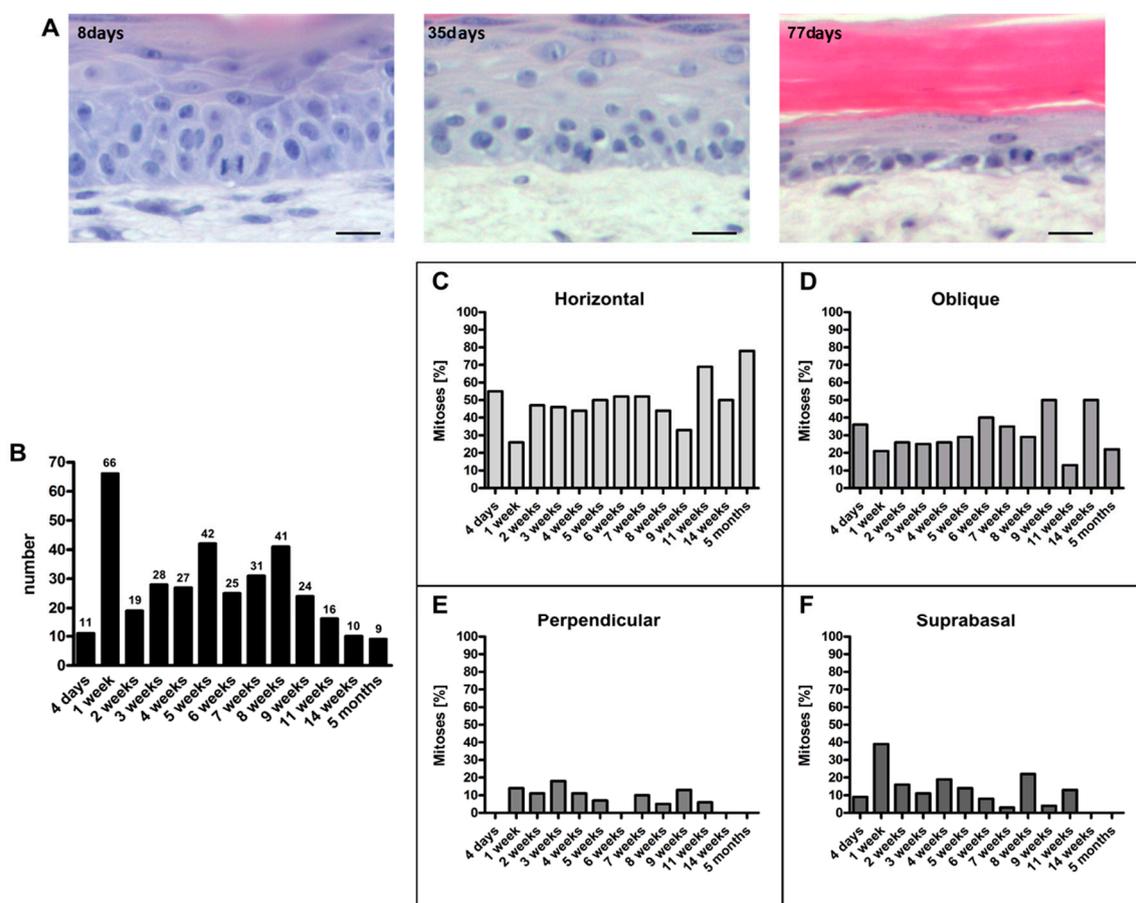
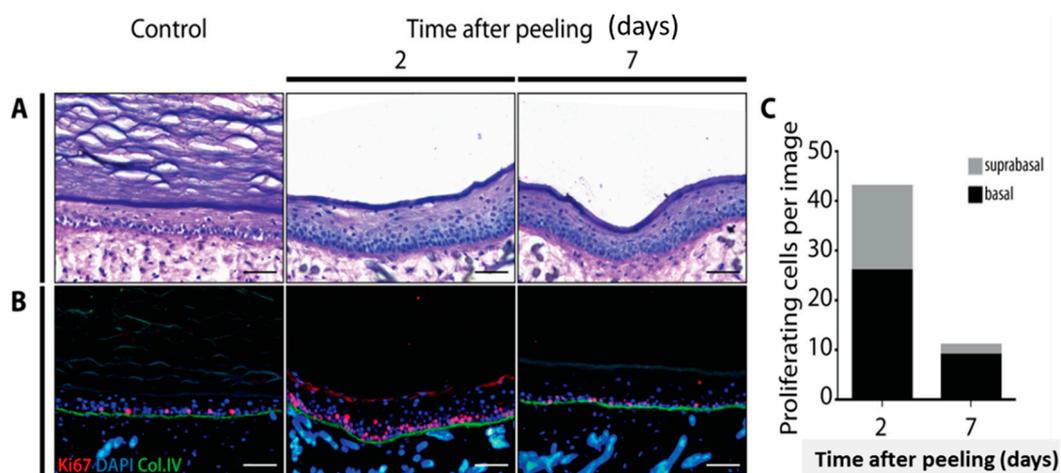


## Supplementary Materials: Mitotic Diversity in Homeostatic Human Interfollicular Epidermis

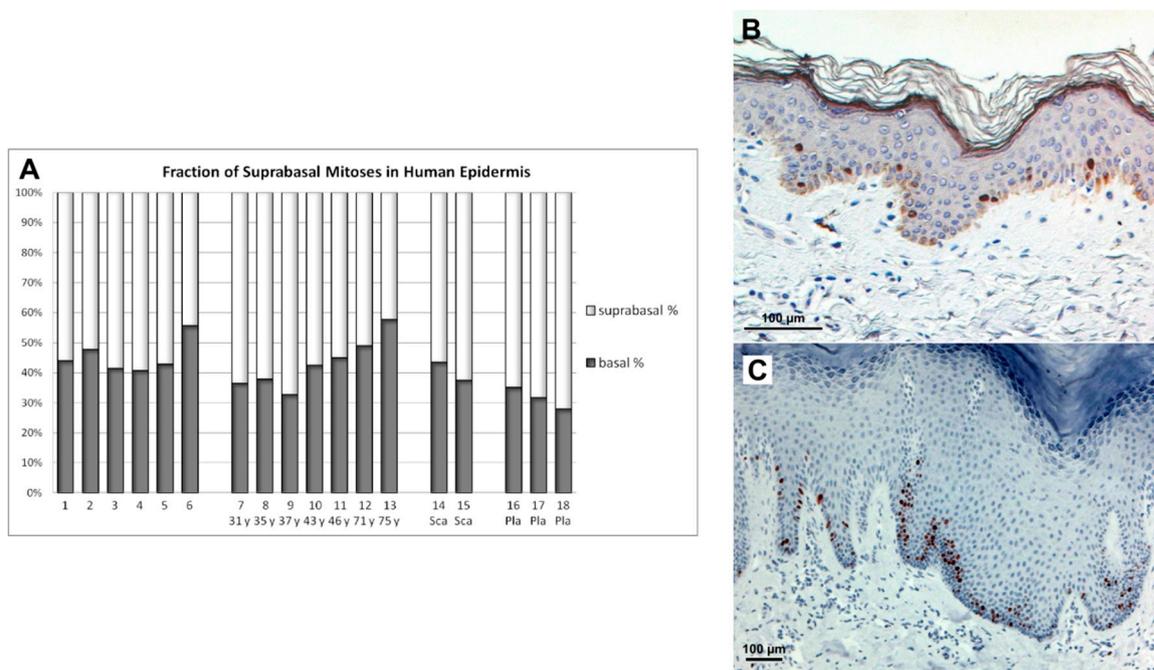
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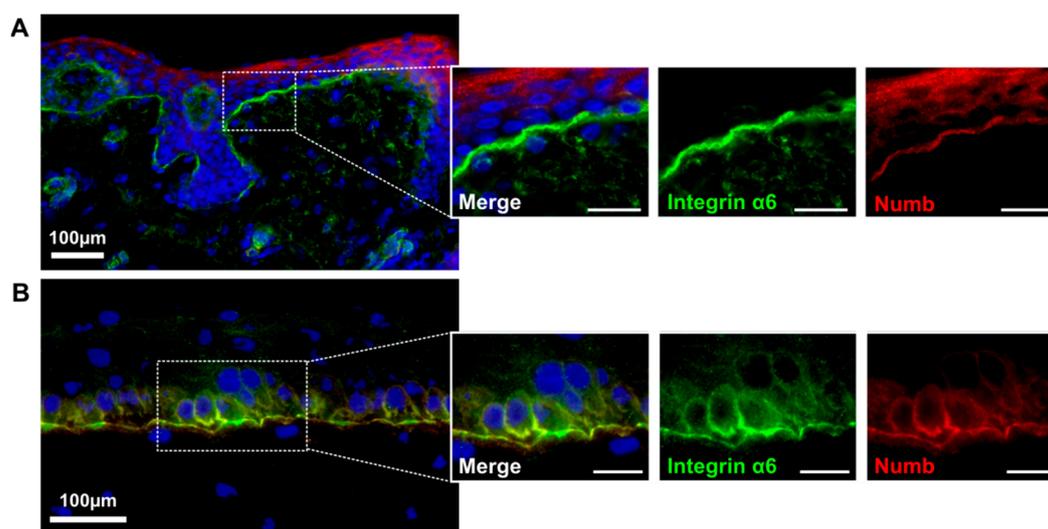
**Figure S1.** Development of tissue architecture in SE and detection of four different types of mitosis in histological sections. **(A)** The initially hyperplastic epidermis of the SE regains reduced stratification (35 days and 77 days), finally resembling homeostatic human epidermis *in situ*; **(B)** 325 epidermal mitotic events were counted over a time course from four days up to five months of SE; **(C)** the majority of cell divisions (20%–80%) occurred in parallel to the BM; **(D)** the second largest proportion was oblique division with 10%–50% of mitoses; **(E)** perpendicular division was found at a frequency up to 20%; **(F)** in addition, a substantial proportion (up to 40%) of dividing cells was located in the suprabasal compartment. Bar = 50  $\mu$ m.



**Figure S2.** Reactivation of human homeostatic epidermis by keratolysis. The effect of stratum corneum removal on tissue architecture and proliferation of human homeostatic epidermis was analyzed by treating 10-week-old SEs (topical application) with 5 M urea for 15 min. In two independent experiments, the stratum corneum was peeled off, and cultures were fixed in duplicates at the indicated time points. **(A)** Cryostat sections were stained with hematoxylin/eosin to demonstrate the regular morphology and the normal degree of stratification in the homeostatic 10-week-old epidermis. After stratum corneum removal, hyperplasia established rapidly and became manifested after 2 days, but did not exceed this level after 7 days; **(B)** Staining for the proliferation marker Ki67 demonstrates the normal low proliferation rate in the homeostatic epidermis (control), which had largely increased 2 days after stratum corneum removal, now showing many dividing cells in the basal and suprabasal position. Already after 7 days, a “rapid” return to the basic proliferation level is evident; **(C)** Quantification of basal and suprabasal Ki67-positive nuclei highlights the prompt induction of both basal and suprabasal proliferation after 2 days and the quick return to the basic distribution in both compartments after 7 days. Bar = 50  $\mu$ m.



**Figure S3.** Quantitation of the proliferation in the basal layer *versus* the suprabasal zone in human epidermis. Proliferating cells were identified by Ki67-staining and enumerated in at least two large microscopic fields per specimen. Eighteen different skin specimens were analyzed (1–18). Specimens 1–13 were from trunk skin, 14 and 15 from scalp (Sca) and 16–18 from foot sole (plantar skin, Pla). Where known, the age of the donor is given below the number of the specimen (7–13) (A). There is a considerable variation in the amount of suprabasal Ki67-positive cells, with 42%–65%. While interfollicular scalp epidermis closely resembles epidermis from trunk skin, the portion of suprabasal Ki67-positive cells is particularly high in plantar skin (Pla, 16–18). On the other hand, with increasing donor age, suprabasal proliferation tends to decline (7–13). Immunohistochemical detection of Ki67-positive cells in trunk skin (B) and plantar skin (C), clearly showing the enrichment of suprabasally-proliferating cells in the latter. Bars = 100 µm.



**Figure S4.** Numb localization is comparable in the SE and human skin. (A) In sections of human skin, Numb (red) was found prominently at the basal pole of basal keratinocytes, colocalizing in part with integrin  $\alpha 6$  (green), as well as in the cytoplasm of some suprabasal cells; (B) A similar distribution of Numb was seen in SE. Blue: DAPI. Scale bars of insets: 50 µm.