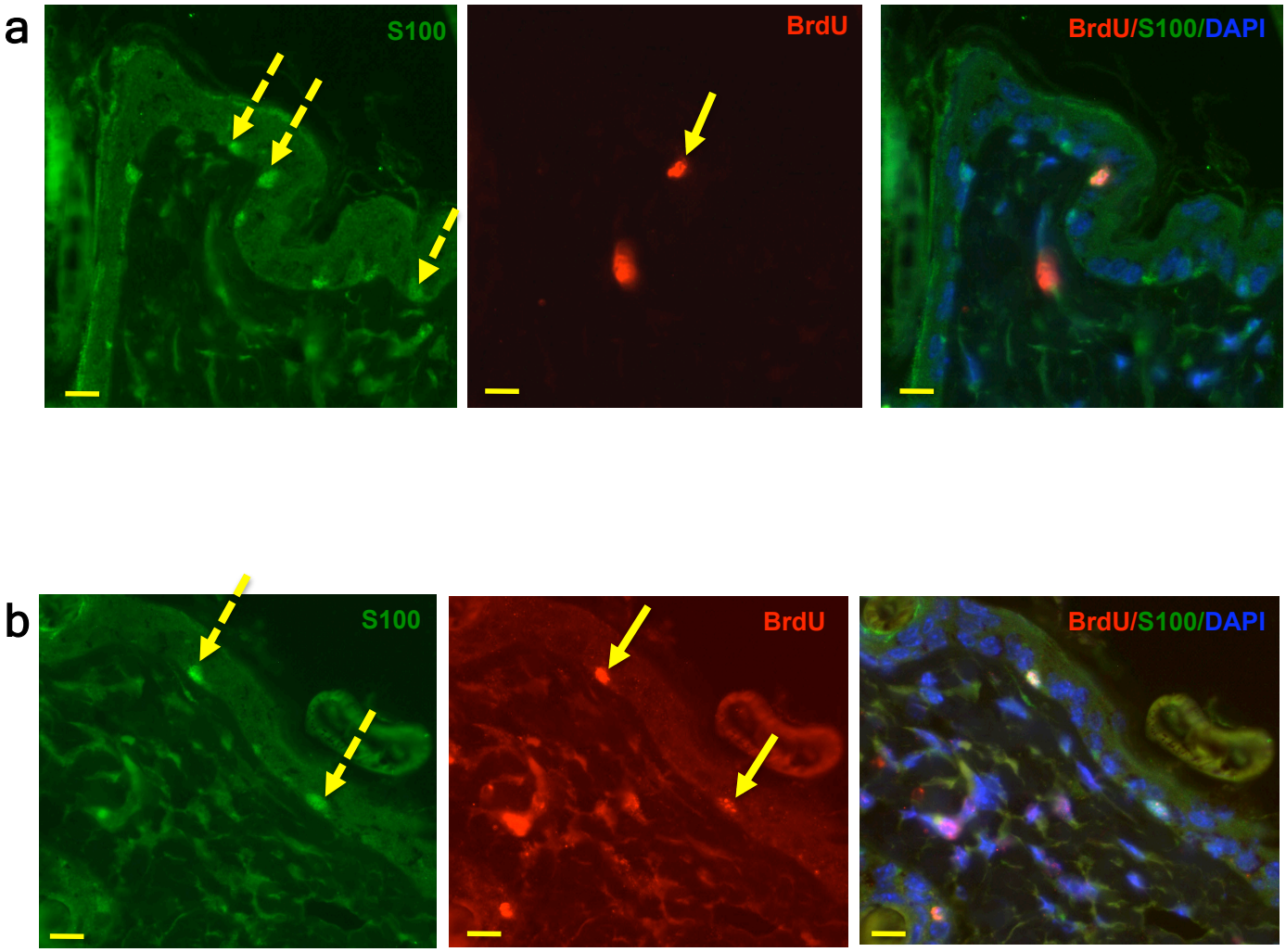

Figure S1. BrdU studies on *K14-Kitl* mouse epidermal melanocytes
(**a**) Double label IF staining on adult *K14-Kitl* mouse skin for BrdU (red) and S100 (green). Dotted yellow arrow indicates S100-positive MCs and solid yellow arrows points to BrdU-positive nuclei. IN the right hand merged image orange/yellow staining is a junctional MC labeling for BrdU. Scale bars = 10μM. (**b**) Another similar section showing two MCs staining for BrdU. Scale bars = 10mM.

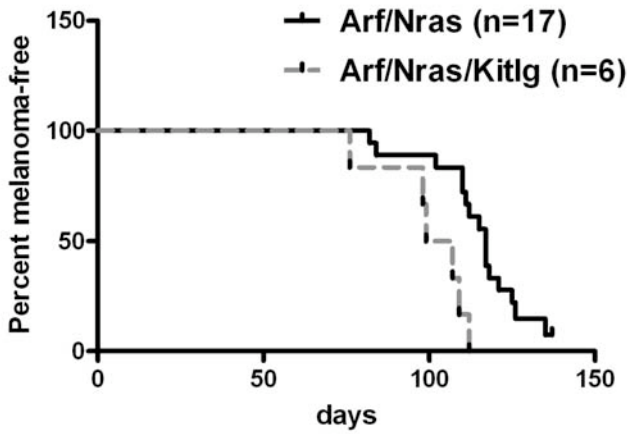

Figure S2. Kaplan Meier curve for melanoma free survival

X-axis denotes the time to the appearance of the first melanoma. The difference between groups was significant ($p<0.01$, log-rank test), but no solid conclusion can be inferred because of strain differences between the two groups.

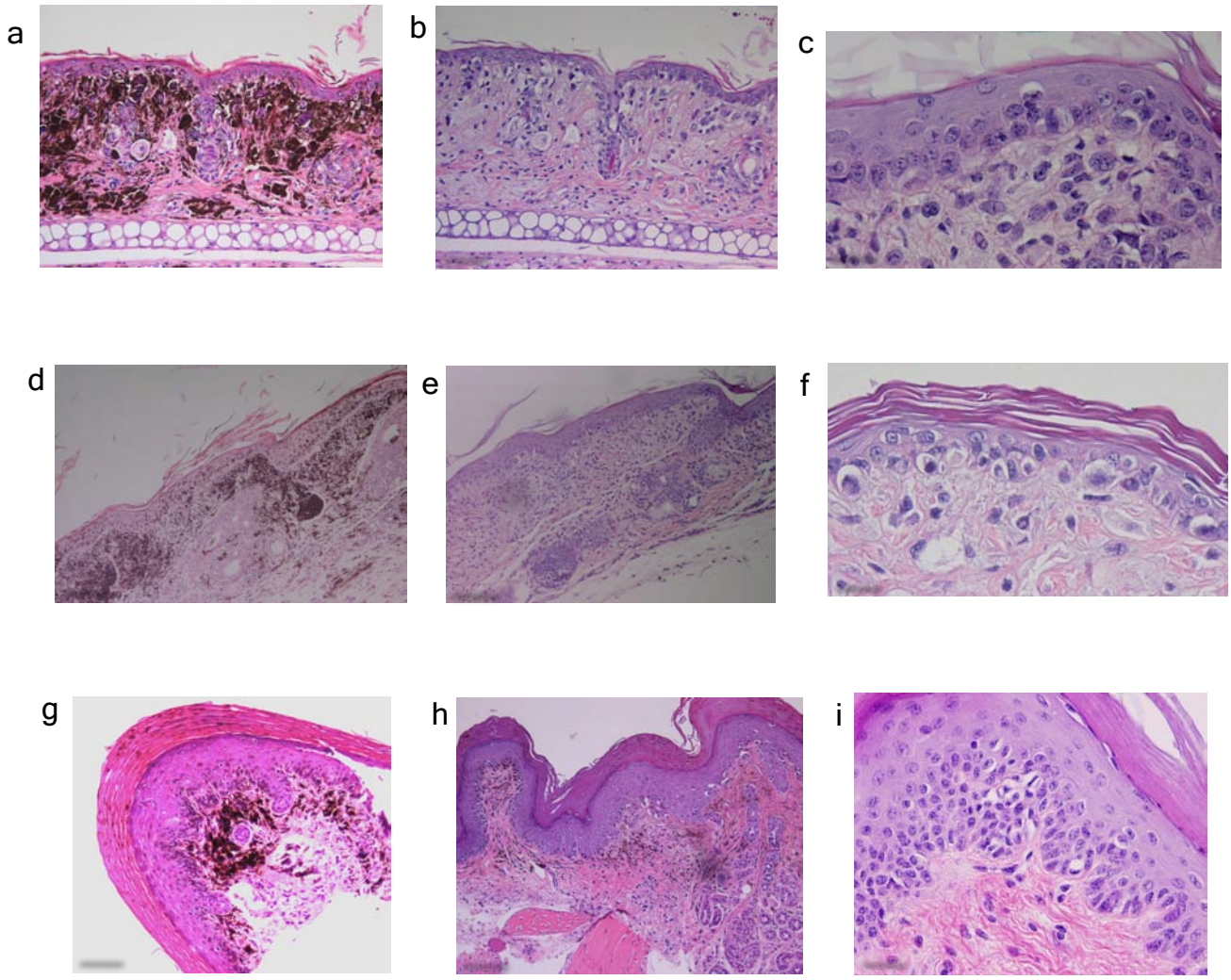

Figure S3. Comparative skin histopathology at different anatomical locations in *Arf*$^{-/-}$*::Tyr-Nras::K14-Kitl* mice.

(**a**). The unbleached H&E section of ear skin reveals heavily pigmented melanocytes at the dermo -epidermal junction and in the adjacent dermis. Scale bar=100μM. (**b**) The bleached sections (b-c) better display the cytomorphologic aspects with atypical melanocytes characterised by pleomorphic nuclei in both skin compartments. Scale bar = 100μM. (c) Scale bar = 20μM. (**d**) The unbleached tail H&E section shows pigmented MCs in the lower epidermis and adjacent dermis. Scale bar = 100mM. (**e**) In the bleached sections (e-f) MCs exhibit cytomorphologic signs of nuclear atypia in both skin compartments. Scale bar = 100μM. (**f**) Scale bar = 20μM. (**g**, **h**) The unbleached H&E sections of the footpad reveal pigmented melanocytes mostly in the superficial dermis. Scale bar = 100μM. (**i**) The bleached section reveals slightly atypical MCs at the dermo-epidermal junctions and to a lesser degree in the adjacent dermis. Scale bar = 20μM.

Supplementary methods

Animals

The *Tyr-Nras*^{Q61K} and *K14-Kitl* mice have been previously described (Ackermann *et al*., 2005, Kunisada *et al*., 1998). *Arf*$^{-/-}$ were obtained from the Mouse Models for Cancer Consortium (http://mouse.ncifcrf.gov). Mice were on a mixed FVB/C57BL6 background (two generations down C57BL6).

UVR treatments

Pups (3-day-old) were given a 10 min exposure to UVB from a bank of 6 Phillips TL100W 12RS UVB lamps (Total UVB dose, 5.9 kJ/m^2, or an erythemally-weighted dose of 1.8 kJ/m^2). UVB dose was measured using a Solar Light (Glenside, PA) PMA2100 radiometer with either a PMA2101 detector to measure biologically-weighted UVB or a PMA2106 detector to measure non-weighted UVB.

Dual label immunofluorescence

4-5 micron sections were dewaxed and antigen retrieval performed using the Dako pH-6 system (Dako, Eli, United Kingdom) at 125^{o}C for 5 min. Sections were blocked with 1% BSA and incubated with rabbit anti-S100 (Dako), or anti-Tyrp1, a gift from Vince Hearing, at 5^{o}C overnight. Antibody dilution as 1:500 for both antibodies. After washing, biotinylated donkey anti-rat (Jackson ImmunoResearch Laboratories, PA, USA) antibody was applied (at 1:500 dilution) for 1 h, followed by washing, and incubation with the secondary AlexFluor 555-labelled donkey anti-rabbit (Jackson Laboratories). After several washes the second primary antibody (anti-K14, Abcam, Cambridge, MA, USA) was applied (1:700) for 1 h, followed with AlexFluor 488-labelled donkey anti-rabbit (Jackson Laboratories). Slides were mounted with Vector Shield (Vector laboratories. Burlingame, CA) containing DAPI. Slides were viewed on a fluorescent microscope. As a control for cross-reaction between the two rabbit antibodies, the second rabbit primary antibody (anti-K14) was left off before secondary (AlexFluor 488-labelled donkey anti-rabbit) was applied. We observed no red MCs that would have indicated cross reactivity between the first primary antibody and final Alexa555-labelled secondary.

Dual label immunofluorescence for melanocyte proliferation.

Sections were dewaxed and antigen retrieval performed as above. Sections were incubated with rat monoclonal anti-Ki-67 (Dako). After washing, biotinylated donkey anti-rat (Jackson ImmunoResearch Laboratories, PA, USA) or biotinylated donkey anti-mouse (Jackson) antibody, was applied (at 1:300 dilution) for 1 h, followed by washing, and incubation with AlexaFluor 488-labelled streptavidin (1:300) for 1 h. For double label studies the second primary antibody (anti-Tyrp1) was applied (1:300) for 1 h. AlexFluor 555-labelled donkey anti-rabbit (Jackson) was added (1:300) for 1h. Slides were mounted with Vector Shield (Vector laboratories) containing DAPI. Slides were viewed on a fluorescent microscope, and positive cells counted. The number of basal MCs (AlexaFluor 555-labelled dendritic cells) per 40X field was counted (when the melanocyte nucleus could clearly be differentiated from adjacent keratinocyte nuclei) along the length of the skin (normally from 15 and 25 fields per skin).

Supplementary methods continued:

Bleaching of pigmented skin sections

Sections were dewaxed as described above, and bleached using 0.5% aqueous potassium permanganate for 5 min followed by 3% aqueous oxalic acid until clear.

BrdU labelling

At post natal day 3 and 5 mice were injected with the pups were injected intraperitoneally with BrdU, at a dose of 150 mg/kg. Mice were then sacrificed at 8 weeks of age. Skin sections were formalin-fixed and paraffin-embedded as above and stained by IF using a Rat Anti-BrdU Monoclonal Antibody (Abcam).

Supplementary references

Ackermann J, Frutschi M, Kaloulis K, McKee T, Trumpp A, Beermann F (2005) Metastasizing melanoma formation caused by expression of activated *NRas*^{Q61K} on an *INK4a*-deficient background. *Cancer Res* 65:4005-11.