Figures and figure supplements

Regulated spindle orientation buffers tissue growth in the epidermis

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Figure 1. Mitotic spindles reorient in response to changes in proliferation. (A) Proliferation of control and K14-rtTA;TRE-Cdkn1b embryonic epidermis measured by pH3 incorporation after treatment with doxycycline from e14.5–16.5. (B–C) Radial histograms of mitotic spindle orientation for e16.5 control (n = 61) and Cdkn1b (n = 59) embryonic epidermis. (D) Proliferation of adult backskin epidermis, control or TPA-treated (10 μl of 0.4 mM TPA, applied daily for 5 days), as measured by BrdU incorporation. n > 150 for each of three mice. (E–F) Radial histograms of mitotic spindle orientation for control (n = 20) and TPA-treated (n = 41) epidermis. Three or more mice were examined for each condition. *p<0.05, ***p<0.001.

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Figure 1—figure supplement 1. Image of a dividing basal epidermal cell demonstrating the method of spindle angle measurement. We measure the angle formed by a line through the spindle poles and one along the basement membrane.

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**Figure 1—figure supplement 2.** Proliferation and spindle orientation differences between back and footpad skin. (A) Proliferation in WT backskin and footpad as measured by BrdU incorporation. n > 300 cells for each of 3 mice. p<0.001. (B,C) H and E images of WT backskin and footpad epidermis, showing their difference in thickness. (D–F) Radial histograms of mitotic spindles in WT backskin (n = 22), footpad (n = 78), and footpad from a K14-rTATRE-Cdkn1b (n = 24) mouse epidermis. Note that the data in (D) is the same as the data presented in Figure 2A.

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**Figure 1—figure supplement 3.** Expansion of keratin 10 (K10) positive cell layers in epidermis treated with TPA. K10 (red), nuclei (blue), and the basement membrane is marked by β4-integrin staining in green.

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**Figure 2.** Oncogenic KRAS alters footpad epidermis spindle orientation in a NuMA-dependent manner. (A,B) Radial histogram of mitotic spindles in adult backskin 21 days after tamoxifen-induced recombination in control (A) and K5CreER; KRASG12D mice (B). (C,D) Radial histogram of mitotic spindles in adult footpad epidermis, 21 days after tamoxifen-induced recombination in control (C) and K5CreER; KRASG12D mice, (n = 78 cells), and (D) (n = 64 cells). (E) Radial histogram of mitotic spindles in adult footpad 21 days after tamoxifen-induced recombination in K5CreER; KRASG12D; NuMAΔMTBD mice (n = 57 cells). Note that the data in 2C is the same as that presented in Figure 1—figure supplement 2E.

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Figure 2—figure supplement 1. Radial histogram of cell division orientation in the footpad epidermis from NuMA mutant mice (K14-Cre;NuMA$^{MTBD/MTBD}$). n = 41 cells.
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Figure 3. Loss of regulated spindle orientation synergizes with oncogenic KRAS to cause tissue overgrowth. (A) Images of KSCreER; KRASG12D and KSCreER; NuMAATMTBD, KRASG12D mice 21 days after recombination with tamoxifen, with inset of footpad and anal-genital region. (B,C) Kaplan-Meier Survival plot of KSCreER; KRASG12D and KSCreER; NuMAATMTBD, KRASG12D mice (B) and KSCreER; p53+−; and KSCreER; p53+−; NuMAATMTBD mice (C); n = 12 mice for KSCreER; NuMAATMTBD, KRASG12D and 10 for other genotypes. (D,E) H and E images of KSCreER; KRASG12D (D) and KSCreER; NuMAATMTBD, KRASG12D (E) footpad epidermis. (F,G) Immunofluorescence images of KSCreER; KRASG12D (F) and KSCreER; NuMAATMTBD, KRASG12D (G) footpad epidermis showing localization of K5/K14+ basal and K10+ suprabasal epidermal layers. Scale bar = 50 μm. (H) Quantitation of basement membrane length divided by tissue length in indicated genotypes (n = 3 mice/condition). (I) Quantitation of proliferation, as assayed by BrdU incorporation, in control and KRASG12D mice. (n > 300 cells, three mice/condition). (J) Image showing co-localization of keratin 10 (red) and histone H2B (green) in a basal cell from a K10-rtTA; TRE-H2B-GFP mouse. (K) Quantitation of Keratin 10 positive basal cells in paw and backskin with indicated genotypes. (n > 300 cells/mouse, three mice/condition). (L) Fluorescence intensity (normalized) of β4-integrin in control and KRASG12D expressing footpad epidermis (n = 3 mice/condition).

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Figure 3—figure supplement 1. Effect of TPA treatment on control and NuMAΔMTBD ear skin. Images show K10 (green) stained ear skin epidermis with the basement membrane noted with a dotted line. These mice were topically treated with TPA for ten days (treatment every second day). The graph indicates the basement membrane length/tissue length of the skin.

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