Defining the clonal dynamics leading to mouse skin tumour initiation

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The changes in cell dynamics after oncogenic mutation that lead to the development of tumours are currently unknown. Here, using skin epidermis as a model, we assessed the effect of oncogenic hedgehog signalling in distinct cell populations and their capacity to induce basal cell carcinoma, the most frequent cancer in humans. We found that only stem cells, and not progenitors, initiated tumour formation upon oncogenic hedgehog signalling. This difference was due to the hierarchical organization of tumour growth in oncogene-targeted stem cells, characterized by an increase in symmetric self-renewing divisions and a higher p53-dependent resistance to apoptosis, leading to rapid clonal expansion and progression into invasive tumours. Our work reveals that the capacity of oncogene-targeted cells to induce tumour formation is dependent not only on their long-term survival and expansion, but also on the specific clonal dynamics of the cancer cell of origin.

Cancer arises through the acquisition of oncogenic mutations¹. How such oncogenic mutations affect the rate of stem and progenitor cell proliferation and the proportion of divisions that result in symmetric and asymmetric fate is currently poorly understood. Recent studies following oncogenic activation in mouse gut before tumour formation showed that intestinal stem cells (SCs) acquire a proliferative advantage over their wild-type neighbours, leading to precocious clonal fixation of mutant crypts^{2,3}. However, the question of whether and how mutant crypts expand and progress into invasive tumours remains unknown.

Basal cell carcinoma (BCC) is the most frequently occurring type of tumour in humans, with more than 5 million new cases diagnosed each year worldwide. BCCs arise from the constitutive activation of the hedgehog (HH) pathway through either Patched (Ptch1) loss of function or Smoothened (Smo) gain of function⁴. Different mouse models of BCC using Ptch1 deletion or oncogenic SmoM2 mutant expression induce the formation of tumours that resemble superficial human BCC⁵. The skin epidermis contains distinct types of SCs that contribute to the homeostasis of discrete regions of epidermis⁶. Interfollicular epidermis (IFE) is maintained by SCs targeted by K14-CreER, that drives the expression of inducible CreER under the control of the Keratin 14 promoter; and committed progenitors (CPs) targeted by Inv-CreER, in which the CreER is expressed under the control of the Involucrin (Inv) promoter in tail, ear, back and ventral skin epidermis^{7,8}. Activation of oncogenic HH signalling through SmoM2 expression or Ptch1 deletion in these different tissues using K14-CreER, which targets both SCs and CPs, induces BCC formation^{7,9–12}. However, the question of whether and how SmoM2 expression in SCs and/or CPs drives BCC formation remains unresolved.

SCs but not CPs initiate BCC formation

To determine whether SCs and CPs can induce BCC, we induced oncogenic SmoM2 expression exclusively in CPs using Inv-CreER, and in both CPs and SCs using K14-CreER⁷ at the same clonal density (Fig. 1a and Extended Data Fig. 1a). As previously reported, activation of SmoM2 expression using K14-CreER induced BCC, characterized by invasion into the dermis and branched morphology, in both tail and ear epidermis⁹⁻¹¹ (Fig. 1b). In sharp contrast, activation of SmoM2 expression in CPs using Inv-CreER lead to pre-neoplastic lesions (including hyperplasia and dysplasia) that did not progress to BCCs (Fig. 1b). These results suggest that only IFE-SCs can induce BCC following activation of SmoM2, whereas IFE-CPs are highly resistant to tumour formation.

We then assessed whether the ability of SCs and CPs to initiate BCC was dependent on the oncogene or tumour suppressor gene used to activate HH signalling. To this end, we induced *Ptch1* deletion using K14-CreER or Inv-CreER (Fig. 1c). *Ptch1* deletion using K14-CreER led to BCCs arising from the IFE and the infundibulum (Fig. 1c). In contrast, *Ptch1* deletion using Inv-CreER, which targets some basal cells in the back and ventral skin epidermis⁸, did not lead to the rapid development of BCC, and only rare and small BCCs were observed 24 weeks after induction (Fig. 1c, d). These results reveal that only IFE/infundibulum SCs can induce BCC formation, whereas CPs are highly resistant, irrespective of the oncogene or tumour suppressor gene used to activate HH signalling and body location (tail, ear, back and ventral skin).

Two distinct self-maintained compartments, scale and interscale, have been described in tail epidermis¹³. To assess whether cells located in these two compartments respond equally to oncogenic activation, we performed immunofluorescence using a scale-specific marker (K31) and SmoM2-YFP to detect the Smoothened oncogene (SmoM2) fused to YFP on whole-mount tail epidermis. Notably, we found that BCCs arose from K14-CreER SmoM2-targeted cells located only in the interscale (Fig. 1e). K14 clones in the interscale progressively lost their normal differentiation program, as evidenced by the loss of spinous-like cells, became hyperplastic, then dysplastic (Fig. 1f and Extended Data Fig. 1b, c). From 4 to 8 weeks after induction, around 15% of clones had progressed into BCC in interscale, increasing to 40% after 24 weeks (Fig. 1e, f). In contrast, K14 clones in scale never progressed to BCC, and maintained a normal differentiation program for an extended period, despite clonal expansion mediated by SmoM2 expression (Fig. 1e, f and Extended Data Fig. 1b, c).

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Figure 1 | SCs but not CPs initiate BCC formation upon HH activation. a, Genetic strategy to activate SmoM2 expression in SCs and CPs. b, Immunostaining of β 4-integrin and SmoM2 in ear and tail skin 24 weeks after SmoM2 activation. c, Immunostaining of β 4-integrin and K14 in ventral skin 24 weeks after *Ptch1* deletion. d, Quantification of tumour burden (total tumour area divided by length of epidermis) following *Ptch1* deletion. Quantification of BCC number per length (mm) after *Ptch1* deletion (n = 4 Inv-CreER/*Ptch1*^{fl/fl} animals and n = 3 K14-CreER/*Ptch1*^{fl/fl} animals). e, Immunostaining of K31 and SmoM2 in wholemount tail skin. f, Quantification of the morphology of SmoM2-expressing clones. Description of number of counted clones can be found in the Methods. Hoechst nuclear staining in blue; scale bars, 100 µm. * $P \le 0.05$, ** $P \le 0.01$. Histograms and error bars represent the mean and the s.e.m.

Together, these data indicate that the fate of oncogene-targeted cells and the ability of these cells to progress into BCC depends both on their location (scale versus interscale) and cellular origin (SC versus CP). This prompted us to investigate whether there are regional differences in SC potential in tail epidermis even under homeostatic conditions.

Homeostasis of the interscale epidermis

To gain quantitative insight into regional variation in SC potential, we performed lineage tracing at homeostasis to determine whether scale and interscale are differentially maintained. To this end, we compared the evolution of K14-CreER/Rosa–YFP-targeted and Inv-CreER/Rosa–YFP-targeted cells at single-cell resolution over a 24-week time course. Interestingly, although both broad, the distributions of clone sizes in the two regions became increasingly divergent (Fig. 2a, b and Extended Data Fig. 2), confirming the importance of regionalization in cellular dynamics (Supplementary Theory).

Consistent with our previous study⁷, the evolution of the mean clone size of progenitors targeted by Inv-CreER in the interscale fits well with the targeting of an equipotent CP population presenting a small but statistically significant imbalance in fate towards terminal differentiation (Fig. 2c, d). Similarly, the evolution of mean clone size for K14-CreER



Figure 2 | **Homeostatic renewal of mouse tail epidermis. a**, **b**, Distribution of basal clone sizes, in K14-CreER/Rosa-YFP (**a**) and Inv-CreER/Rosa-YFP (**b**) epidermis. The number of clones analysed is indicated for each time point and described in the Methods. **c**, Mean basal (top) and suprabasal (bottom) clone size in the interscale. The lines represent the model fit. **d**, Cell fate probabilities of SCs and CPs in the interscale, as extracted from the fits. S, P and D refer to stem, progenitor and differentiated cells. **e**, Clonal persistence (top) and labelled cell fraction (bottom) in the interscale. Description of number of counted clones is in the Methods. The lines are the predictions from the model using only the parameters extracted in **d**. K14-CreER/Rosa-YFP clones display a net expansion, whereas Inv-CreER/Rosa-YFP clones display a net contraction. Histograms and error bars represent the mean and the s.e.m. Shaded areas represent 95% confidence intervals for the model prediction (Supplementary Theory).

cells is consistent with the additional targeting of a long-term selfrenewing SC population that divides more slowly than CPs (Fig. 2c, d). To define quantitatively the dynamics of these two populations (cell-cycle times, relative proportion of SCs and CPs labelled by the K14-CreER and their fate probabilities), we made a joint fit to the basal and suprabasal mean clone sizes, and extracted optimal parameters and confidence intervals (Supplementary Theory).

To verify independently the predictions of the model, the persistence of Inv-CreER- and K14-CreER-targeted clones was used to infer the respective labelled cell fractions. As expected from the labelling of the CP population, for Inv-CreER-targeted clones, we found that the labelled cell fraction decreased over time (Fig. 2e). In contrast, for K14-CreER-targeted clones, the labelled cell fraction increased over time, consistent with the preferential targeting of the SC population (Fig. 2e). Notably, we obtained excellent predictions for the labelled cell fraction for both K14-CreER and Inv-CreER using parameters extracted independently from the fit to the mean clone sizes (Fig. 2e). These results support a SC and CP hierarchy, and rule out the possibility that the differences between K14-CreER- and Inv-CreER-targeted clones are the consequence of differential short-term 'priming' of induced cells (Extended Data Fig. 3a). Importantly, the hierarchical model also predicted accurately the complete distribution of clone sizes at all time points (Extended Data Fig. 3b, c) for both K14-CreER and Inv-CreER.

In sharp contrast, in the scale region of tail epidermis, both basal and suprabasal clone sizes and persistence of K14-CreER- and Inv-CreER-targeted cells were statistically indistinguishable (Extended Data Fig. 4a, c). Notably, the labelled cell fraction did not change significantly between 2 weeks and 24 weeks after labelling (Extended Data Fig. 4c), an indication that K14-CreER and Inv-CreER mark the same balanced CP population¹³. We again validated the model (Extended Data Fig. 4b) by showing that it could quantitatively predict both the



Figure 3 | SmoM2 expression in CPs induces clonal expansion that does not progress into BCC. a, Immunostaining for \(\beta\)4-integrin, YFP and SmoM2 in Inv-CreER/Rosa-YFP and Inv-CreER/Rosa-SmoM2 epidermis at different time points. b, Distribution of Inv-CreER/Rosa-YFP and Inv-CreER/Rosa-SmoM2 basal clone sizes. The number of clones analysed for Inv-CreER/Rosa-SmoM2 is indicated for each time point and for Inv-CreER/Rosa-YFP is indicated in Fig. 2b. c, Quantification of EdU-BrdU double-labelled cells during continuous BrdU administration, at different time points after clonal induction. The lines represent the model fit (Supplementary Theory). d, Quantification of the proportion of apoptotic cells in dysplastic, hyperplastic and normally differentiating Inv-CreER/Rosa-SmoM2 clones 8 weeks after induction (n = 73 clones analysed from 4 independent experiments). **e**, Division rate $(d^{-1}, per day)$ determined from EdU-BrdU double-labelling experiments (data in black, fit in blue dashed line). f, Mean basal and suprabasal clone sizes in the interscale. The lines represent the model fit from which we inferred the cell-fate probabilities displayed in g. g, Cell-fate probabilities of the tumour progenitor expressing SmoM2. h, Basal clone size distribution of Inv-CreER/Rosa-SmoM2 clones (black). Consistent with the hypothesis of a single equipotent progenitor pool, all distributions are well-fit by single exponential. Blue lines represent the model prediction using only the parameters extracted from g. Shaded areas represent 95% confidence intervals for the model prediction. D, dysplasia; H, hyperplasia; N, normal differentiation. Hoechst nuclear staining in blue; scale bars, 10 µm. Histograms and error bars represent the mean and the s.e.m. (b-f) and s.d. (h).

evolution of clonal persistence, as well as the clone size distribution at all time points (Extended Data Fig. 4c, d).

These results show that, during homeostasis, interscale is maintained by two discrete populations; a comparatively slow-cycling SC and a more rapidly dividing CP population, whereas scale is maintained by a single CP population. As well as unifying diverging reports of maintenance hierarchy in tail epidermis^{7,13,14}, these findings raised the question of whether the restriction of BCCs to the interscale correlated with the regional localization of IFE-SCs. To test this hypothesis, we assessed whether the same regionalized lineage hierarchy persisted upon SmoM2 activation.

Oncogene-targeted CPs are frozen into dysplasia

To resolve the cellular dynamics underpinning the differential sensitivity of SCs and CPs to BCC initiation in interscale, we first studied

the dynamics and proliferation kinetics of Inv-CreER/Rosa-SmoM2 clones. Oncogenic activation in Inv-CreER CPs lead to an increase of the average basal clone size, total clone size and clonal persistence compared to homeostatic conditions (Fig. 3a, b and Extended Data Fig. 5a-c), as well as abnormal or decreased differentiation (Fig. 3a and Extended Data Fig. 1 b, c). We assessed the average cell-cycle time of SmoM2 Inv-CreER-targeted cells by first marking proliferating cells using 24 h of EdU administration, followed by variable periods of continuous BrdU administration. From the co-labelling of EdU-BrdU, we found that CPs divided on average every 3.6 ± 0.5 days 4 weeks after SmoM2 expression, 7.2 ± 0.6 days after 8 weeks and 9.8 ± 0.3 days after 12 weeks (Fig. 3c), indicating that the average division rate of SmoM2 CPs decreases with time. Surprisingly, division rates were uncorrelated with clone size at all time points, indicating that the decrease occurs independently of clone size or stage of tumour progression (Extended Data Fig. 5d), and consistent with the Inv-CreER oncogene-targeted cells functioning as a single equipotent population.

As deregulation of apoptosis is also important for cancer formation¹, we assessed whether apoptosis influences the clonal dynamics of oncogene-targeted CPs. In common with their normal counterpart, Inv-CreER-targeted cells did not show evidence of apoptosis over the first 6 weeks after SmoM2 expression (data not shown). However, from 8 weeks on, about 60% of Inv-CreER-targeted clones that presented hyperplasia or dysplasia, contained about 2–4% of apoptotic cells as measured by active caspase-3 immunostaining (Fig. 3d and Extended Data Fig. 5e–i).

Taking these rates (Fig. 3e) as an input, we could obtain an excellent fit to the average clone size (Fig. 3f) with cell fate probabilities that remain constant over time, with the proportions of symmetric renewal (PP) to asymmetric division (PD) and symmetric differentiation (DD) set at 39%, 45% and 16% respectively (Fig. 3g). This result demonstrates that oncogenic expression in CPs leads to enhanced clonal expansion and survival by promoting symmetric proliferation over terminal differentiation. Such an imbalance would lead to exponential clone growth if it were not counteracted by an ever-diminishing effective proliferation rate, leading to a plateau in the mean basal clone size (Fig. 3f). Notably, the model prediction provided a good fit to the clone size distribution at all time points (Fig. 3h).

Finally, to further verify the model, a low short-term dose of EdU was used to mark a minority of dividing cells and their fate outcome was recorded 3 days later by quantifying the basal and suprabasal localization of EdU doublets (Extended Data Fig. 5j). From these results, we could confirm a large imbalance between symmetric division and terminal differentiation (35%).

As the scale is maintained by a single progenitor pool, we investigated whether its response to oncogenic activation was similar to interscale CPs. Notably, after an initial increase, the overall labelled cell fraction remained roughly constant over time in scale between 8 and 24 weeks, at a similar level for both K14-CreER and Inv-CreER (Extended Data Fig. 6a–c), suggesting that, in sharp contrast to interscale, both populations behave identically upon oncogenic activation (Extended Data Fig. 6d). Together, these results show that both interscale and scale CPs are resistant to BCC formation upon oncogenic HH signalling, although interscale clones can persist longer owing to a larger fate imbalance and enhanced differentiation defects, whereas scale clones rapidly converge towards balance. However, as human epidermis does not show scale organization, the absence of BCC formation in the scale region might not have human relevance.

Oncogene-targeted SCs progress into BCC

To gain insight into how SmoM2 expression in SCs promotes BCC formation, we then performed a quantitative analysis of K14-CreER/Rosa-SmoM2 clones. Compared to Inv-targeted clones, SmoM2 expression in K14-targeted cells lead to a more rapid and persistent expansion of a fraction of clones (Fig. 4a, b and Extended Data Fig. 7a–c) that progressed into BCC, as well as the formation of smaller clones that did not





Figure 4 | SmoM2 expression in SCs induces tumour SCs that lead to BCC formation. a, Immunostaining for \(\beta\)4-integrin, YFP and SmoM2 in K14-CreER/Rosa-YFP and K14-CreER/Rosa-SmoM2 epidermis at different time points. b, Distribution of K14-CreER/Rosa-YFP and K14-CreER/Rosa-SmoM2 basal clone sizes. The number of clones analysed for K14-CreER/Rosa-SmoM2 is indicated and for K14-CreER/Rosa-YFP is indicated in Fig. 2a. c, Quantification of EdU-BrdU double-labelled cells following continuous BrdU administration, at 8 weeks after clonal induction for small K14-CreER clones, Inv-CreER clones, and large K14-CreER clones. d, Quantification of the number of apoptotic cells in BCC, dysplastic, hyperplastic and normally differentiating K14-CreER/ Rosa-SmoM2 clones 8 weeks after induction (n = 117 clones analysed from 4 independent experiments). **e**, Division rate $(d^{-1}, per day)$ in large K14 clones determined from double-labelling experiments (data in black, fit in red dashed line). f, Whisker plot of the mean basal clone size in the interscale. The boxes delineate the first and third quartiles of the data and the whiskers delineate the first and last deciles of the data. The thick continuous line is the best fit from the model from which we extract the probability of fate choices in tumour SCs and progenitors displayed in g. The thin lines represent the predicted mean clone sizes of SC- (top thin curve) and CP- (bottom thin curve) derived clones alone. g, Cell-fate probabilities of the tumour SC upon SmoM2 activation. h, Basal clone size distribution of K14-CreER/SmoM2 clones (black). Red lines are the model prediction using only the parameters extracted from g. Shaded areas represent 95% confidence intervals for the model prediction. Hoechst nuclear staining in blue; scale bars, 10 µm. Histograms and error bars represent the mean and the s.e.m. (**b**-**f**) and the s.d. (**h**).

show tumour progression (Figs 1e, 4b). This suggests that, in line with homeostatic conditions, K14-CreER marks a fraction of tumour-like SCs, together with tumour-like CPs, a heterogeneity that we verified using proliferation assays (Fig. 4c). Indeed, we found that one population of K14-CreER-targeted cells consisted of small clones that displayed similar proliferation kinetics as Inv-CreER SmoM2 clones, whereas a second population consisted of larger clones, which re-entered cell cycle significantly faster (Fig. 4c and Extended Data Figs 5d and 7d). The population of small K14-CreER-targeted clones (hyperplasia) also presented higher levels of apoptosis compared to the larger clones (Fig. 4d and Extended Data Fig. 7e–i). As a result, even though the proliferation of the larger clones also decreased with time (Fig. 4e and Extended Data Fig. 7j), their division rate was consistently higher than the Inv-CreERtargeted population (Fig. 4e).

To model BCC initiation, we adapted the hierarchical model obtained during homeostasis and fitted jointly the mean basal and suprabasal clone sizes of all K14-CreER SmoM2 clones, taking as input the division rate as well as the fraction of SCs initially labelled by the K14-CreER-determined from measurements at homeostasis, and used the fate choices of SCs as fitting parameters (Fig. 4f and Extended Data Fig. 7k). In particular, we posited that SCs are imbalanced towards symmetric renewal, whereas CPs derived from these cells remain slightly imbalanced towards symmetric differentiation with the same fate probability as in homeostasis, which gave a good fit to the average basal clone size (Fig. 4g). Notably, the measured clone size distributions from 12 weeks onwards could not be fit with a one-progenitor population model, in contrast to the distributions of Inv-CreER SmoM2 clones. Instead, the K14-CreER SmoM2 clone size distributions displayed a 'double-exponential' decay, consistent with the labelling of two distinct populations, as predicted quantitatively by the model (Fig. 4h and Supplementary Theory). This shows that K14-CreER targets tumour-like SCs making imbalanced stochastic fate choices, in addition to targeting the same tumour-like CP population as Inv-CreER.

As a final consistency check, we addressed a key hallmark of the hierarchical model, that SCs give rise to basal CPs in K14-CreER-targeted clones. This predicts that the fraction of cell divisions resulting in two basal cells should be greater in SC- versus CP-targeted clones. Indeed, short-term EdU pulse-chase experiments revealed that, in BCC, most divisions (77%) lead to two basal cells (Extended Data Fig. 7l). In hyperplasia/dysplasia, the fraction of two EdU⁺ basal cell doublets was intermediate between the BCC and Inv-CreER/Rosa-SmoM2 values (Extended Data Figs 5j, 7l), consistent with a mixture of SC- and CP-targeted clones.

p53 restricts CP progression to BCC

Given the observed differences in apoptosis and division rates between oncogene-targeted SCs and CPs, we assessed whether p53, a tumour suppressor gene frequently mutated in human BCC¹⁵ that controls cell cycle arrest and apoptosis¹⁶, was differentially activated in SCs and CPs upon SmoM2 activation. Immunohistochemistry revealed that p53 was more frequently found in SmoM2 clones arising from Inv-CreER as compared to K14-CreER mice (Extended data Fig. 8a). To determine whether p53 stabilization in oncogene-targeted CPs restricts the potential of these progenitors to generate BCC, we deleted *p53* together with SmoM2 activation and assessed tumour formation. Interestingly, p53 deletion in Inv-CreER targeted CPs leads to BCC in both ear and tail epidermis (Fig. 5a). In the tail, BCCs were restricted to the interscale whereas, in the scale, clones only progressed into dysplasia (Fig. 5b, c and Extended Data Fig. 8b). These results indicate that p53 restricts the competence of SmoM2-targeted CPs of the interscale to progress into BCC.

Although the proportion of clones that progress into BCC continued to be more frequent and more rapid in K14-CreER targeted SCs, at 24 weeks after induction more than half of interscale Inv-CreERtargeted clones had progressed into BCC after *p53* deletion (Fig. 5c). The clonal persistence and clone size were increased upon *p53* deletion in both Inv-CreER/Rosa-SmoM2 and K14-CreER/Rosa-SmoM2 interscale clones, although the clones were still bigger and more persistent in K14-targeted cells (Fig. 5b, d and Extended Data Fig. 8c–e). These results indicate that, upon *p53* deletion, both oncogene-targeted CPs and SCs present an increase in self-renewing divisions allowing CPs to acquire the ability to form BCC upon SmoM2 expression.

We next determined whether the observed increase in clone size in the absence of p53 in CPs and SCs was due to a decrease in apoptosis, an increase in proliferation or both. Immunostaining for active caspase-3 8 weeks after oncogenic activation showed that large Inv-CreER/Rosa-SmoM2/*p53*^{*fl/fl*} dysplastic and BCC clones displayed reduced apoptosis,



Figure 5 | p53 deletion in CPs leads to BCC formation. a,

Immunostaining of ^β4-integrin and SmoM2 in ear and tail skin of K14 and Inv-CreER/Rosa-SmoM2/p53^{fl/fl} mice 24 weeks after tamoxifen administration. b, Whole-mount immunostaining of K31/SmoM2 in tail epidermis over time. c, Quantification of normal, hyperplastic, dysplastic and BCC clones in the interscale region. Description of number of counted clones is found in the Methods section. d, Distribution of basal clone sizes in K14 and Inv-CreER/Rosa-SmoM2/p53^{fl/fl} mice. The number of clones analysed is indicated. Clone merger events were observed 12 weeks after oncogenic activation in K14Cre-ER/Rosa-SmoM2/ $p53^{fl/fl}$ preventing the accurate quantification of clonal persistence and clone size at longer times. e, Quantification of the proportion of apoptotic cells in different clones (K14 n = 82 clones and Inv n = 90 clones from 3 independent experiments). f, Percentage of double-labelled EdU-BrdU SmoM2expressing cells after 6 days of continuous BrdU administration following a 24 h pulse of EdU at 12 weeks after induction. * $P \le 0.05$, ** $P \le 0.01$. g, Mean basal clone size in Inv-CreER/Rosa-SmoM2/p53^{fl/fl} and K14-CreER/ Rosa-SmoM2/p53^{fl/fl} clones. The prediction of the model is indicated by the blue and red lines. Shaded areas represent 95% confidence intervals for the model prediction in g. Hoechst nuclear staining in blue; scale bars, $100\,\mu$ m. Histograms and error bars represent the mean and the s.e.m.

mirroring our observation in K14-CreER/Rosa-SmoM2 (Extended Data Fig. 8f). However, apoptosis was unchanged in Inv-CreER p53-deficient hyperplastic clones, suggesting that p53-dependent and -independent mechanisms control apoptosis in oncogene-targeted cells (Fig. 5e, Extended Data Fig. 8g). EdU–BrdU double-pulse experiments 12 weeks after induction showed that deletion of *p53* increased the rate of proliferation in both Inv-CreER and K14-CreER oncogene-targeted cells (Fig. 5f). According to our model, this increase in the rate of division was sufficient, keeping all other parameters constant, to explain the enhanced tumour growth (Fig. 5g). This provides additional evidence that growth arrest in oncogene-targeted CPs is a key determinant in their inability to mediate BCC progression in the presence of p53.

In summary, our results demonstrate that p53 restricts the ability of CPs to initiate BCC by promoting apoptosis and inducing cell cycle arrest in oncogene-targeted CPs.

Discussion

In this study, we have defined the quantitative dynamics of BCC initiation at single-cell resolution, from the first oncogenic hit to the development of invasive tumours. These results show that the proliferative hierarchical organization of skin epidermis is a key determinant of tumour development, with only IFE-SCs and not CPs able to initiate BCC following oncogenic HH signalling (Extended data Fig. 9). Even though CP-derived clones survive and proliferate for months, they are surprisingly robust to BCC transformation and invasion, becoming 'frozen' in a pre-tumorigenic state. The developmental cerebellar progenitors initiate medulloblastoma upon oncogenic HH signalling^{17,18}, suggesting the developmental stage of progenitors may also dictate competence for tumour initiation. The long-term maintenance of some oncogene-targeted CPs contrasts the classical transient-amplifying cells in other compartments, such as hair matrix in the skin or the non-Lgr5 crypt progenitors in gut, which are resistant to tumour initiation because of their short lifespan^{19–22}.

Our results show that IFE-SCs reside solely in the interscale region, and have the unique and regionalized competence to initiate large and invasive BCCs. Notably, this regionalized hierarchical organization at homeostasis was maintained upon SmoM2 activation. Oncogene expression in SCs lead to a more rapid clonal expansion as compared to CPs for two main reasons: the maintenance of hierarchical organization in early pre-neoplastic lesions, leading to increased symmetric self-renewing divisions; and the combined resistance to apoptosis and enhanced proliferation of SC-derived pre-neoplastic lesions, leading to a larger effective growth rate. These two properties allow SC-targeted tumours to escape the frozen state that characterized CP-targeted pre-neoplastic lesions, and thereby progress to an invasive phenotype.

Finally, our results show that p53 restricts the ability of CPs to undergo BCC initiation by promoting apoptosis and inducing cellcycle arrest in oncogene-targeted CPs. Interestingly, although the division rates of CPs and SCs that are p53-deficient are similar, SC-targeted tumours still grow to larger sizes than CP-targeted tumours, suggesting that the hierarchical organization is at least partially maintained even after two oncogenic hits. By establishing that sustained imbalance towards self-renewing divisions and resistance to p53-mediated apoptosis and cell-cycle arrest are the main drivers of tumorigenesis, our results suggest that therapy promoting differentiation, p53 reactivation and apoptosis could present a promising avenue to promote BCC regression and prevent tumour relapse.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to C.B. (Cedric.Blanpain@ulb.ac.be) and B.D.S (bds10@cam.ac.uk).

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METHODS

Mice. K14-CreER transgenic mice²³ were kindly provided by E. Fuchs, Rockefeller University; Inv-CreER were generated in our laboratory⁸. *Ptch1^{fl/fl}* mice²⁴ and Rosa/ SmoM2-YFP mice²⁵ were obtained from the JAX repository. *p53^{fl/fl}* (ref. 26) mice were obtained from the National Cancer Institute at Frederick.

Mouse colonies were maintained in a certified animal facility in accordance with European guidelines. Experiments involving mice presented in this work were approved by Comité d'Ethique du Bien Être Animal (Université Libre de Bruxelles) under protocol number 483N, that states that animals should be euthanized if they present tumours that exceed 1 cm in diameter. The BCCs observed in this study were microscopic and ranged from 1.5 mm to 100 µm in diameter and in none of the experiments performed, the tumours exceeded the limit (1 cm in diameter) described in protocol 483N. Female and male animals have been used for all experiments and equal animal gender ratios have been respected in the majority of the analysis, analysis of the different mutant mice was not blind and sample size was calculated to reach statistical significance. The experiments were not randomized. Skin tumour induction and clonal YFP expression. For clonal induction 3-months-old mice were used. K14-CreER/Rosa-YFP, K14-CreER/Rosa-SmoM2, K14-CreER/SmoM2/p53^{fl/fl} and K14-CreER/Ptch1^{fl/fl} mice received an intraperitoneal injection of 0.1 mg of tamoxifen and Inv-CreER/Rosa-YFP, Inv-CreER/ Rosa-SmoM2, Inv-CreER/Rosa-SmoM2/p53f1/fl and Inv-CreER/Ptch1f1/fl received a intraperitoneal injection of 2.5 mg of tamoxifen to achieve similar level of recombination in the different models (Extended Data Fig. 1a). Mice were killed and analysed at different time points following tamoxifen administration.

Immunostaining in sections. The tail, ventral skin and ear skin were embedded in optimal cutting temperature compound (OCT, Sakura) and cut into $5-8\,\mu m$ frozen sections using a CM3050S Leica cryostat (Leica Microsystems).

Immunostainings were performed on frozen sections. Owing to the fusion of SmoM2 with YFP, SmoM2-expressing cells were detected using anti-GFP antibody. Frozen sections were dried and then fixed with 4% paraformaldehyde/PBS (PFA) for 10 min at room temperature and blocked with blocking buffer for 1 h (PBS, horse serum 5%, BSA 1%, Triton 0.1%). Skin sections were incubated with primary antibodies diluted in blocking buffer overnight at 4 °C, washed with PBS for 3×5 min, and then incubated with Hoechst solution and secondary antibodies diluted in blocking buffer for 1 h at room temperature. Finally, sections were washed with PBS for 3×5 min at room temperature and mounted in DAKO mounting medium supplemented with 2.5% Dabco (Sigma). Primary antibodies used were the following: anti-GFP (rabbit, 1:1,000, BD, A11122), anti-K14 (Chicken, 1:4,000, Covance, PCK-153P-0100) and anti-B4-integrin (rat, 1:200, BD, 553745). The following secondary antibodies were used: anti-rabbit, anti-rat, anti-chicken, conjugated to AlexaFluor488 (Molecular Probes) and to rhodamine Red-X (JacksonImmunoResearch). Images of immunostaining in sections were acquired using an Axio Imager M1 microscope, an AxioCamMR3 camera and the Axiovision software (Carl Zeiss).

Immunostaining in whole mounts. Whole mounts of tail epidermis were performed as previously described²⁷ and used to quantify the proportion of surviving clones (Extended Data Fig. 2b) as well as the basal suprabasal and total clone size. Specifically, pieces of tail were incubated for 1 h at 37 °C in EDTA 20 mM in PBS in a rocking plate, then using forceps the dermis and epidermis were separated and the epidermis was fixed for 30 min in PFA 4% in agitation at room temperature and washed 3 times with PBS.

For the immunostaining, tail skin pieces were blocked with blocking buffer for 3h (PBS, horse serum 5%, Triton 0.8%) in a rocking plate at room temperature. After, the skin pieces were incubated with primary antibodies diluted in blocking buffer overnight at 4 °C, the next day they were washed with PBS-Tween 0.2% for 3×10 min at room temperature, and then incubated with the secondary antibodies diluted in blocking buffer for 3 h at room temperature, washed 2×10 min with PBS-Tween 0.2% and washed for 10 min in PBS. Finally, they were incubated in Hoechst diluted in PBS for 30 min at room temperature in the rocking plate, washed 3×10 min in PBS and mounted in DAKO mounting medium supplemented with 2.5% Dabco (Sigma). Primary antibodies used were the following: anti-GFP (rabbit, 1:100, BD, A11122), anti-GFP (goat, 1:800, Abcam, Ab6673), anti-active-caspase3 (rabbit, 1:600, R&D, AF835), anti-\u00b34-integrin (rat, 1:200, BD, 553745) and anti-K31 (guinea pig, 1:200, Progen, GP-hHa1). The following secondary antibodies were used: anti-rabbit, anti-rat, anti-chicken, anti-goat and anti-guinea pig, conjugated to AlexaFluor488 (Molecular Probes), to rhodamine Red-X (JacksonImmunoResearch) and to Cy5 (1:400, Jackson ImmunoResearch). Analysis of clone survival, size and apoptosis. Quantification of the proportion of surviving clones, as well as total and basal clone size was determined by counting the number of SmoM2-YFP and YFP-positive cells. in each clone using wholemount tail epidermis. The different clones were imaged using Z-stacks using a confocal microscope LSM 780 (Carl Zeiss) and orthogonal views were used to count the number of basal and total number of SmoM2-YFP or YFP-positive

cells in each clone, as well as the number of active-caspase3-positive cells in each clone. K31 staining was used to classify the clones according to their location in the scale or interscale regions.

Proliferation assays. To measure the kinetics of cell proliferation, a 24 h continuous pulse of EdU followed with a continuous pulse of BrdU were performed. Specifically, mice received at t = 0 an intraperitoneal injection of EdU (1 mg ml⁻¹) and 0.1 mg ml⁻¹ EdU was added to their drinking water for 24 h. The next days the mice received a daily intraperitoneal injection of BrdU (10 mg ml⁻¹) and 1 mg ml⁻¹ of BrdU was added to their drinking water during the 8 days of the continuous BrdU pulse. Mice were killed at different time points and whole-mount stainings for the tail were performed. The pieces of tail were first stained for GFP (following the protocol described in the previous section). Second, EdU staining was performed following the manufacturer's instructions (Invitrogen). The pieces of tail were then washed in PBS and fixed again in PFA 4% for 10 min. After they were washed in PBS, incubated for 20 min in HCl 1 M at 37 °C, washed three times with PBS-Tween 0.2% and incubated overnight with Alexa-647-coupled anti-BrdU antibody (mouse, 1:200, BD). The next day the tail pieces were washed in PBS, incubated in Hoechst for 30 min at room temperature in the rocking plate, washed 3×10 min in PBS and mounted in DAKO mounting medium supplemented with 2.5% Dabco (Sigma). To quantify the number of cells that incorporated EdU and/or BrdU, Z-stacks were acquired for each individual clone and orthogonal views used to count.

Immunohistochemistry. For p53 immunohistochemistry, 4-µm paraffin sections were deparaffinized, rehydrated, followed by antigen unmasking performed for 20 min at 98 °C in citrate buffer (pH 6) using the PT module. Endogenous peroxydase was blocked using 3% H2O2 (Merck) in methanol for 10 min at room temperature. Endogenous avidin and biotin were blocked using the Endogenous Blocking kit (Invitrogen) for 20 min at room temperature. In p53 staining, nonspecific antigen blocking was performed using M.O.M. Basic kit reagent. Mouse anti-p53 antibody (clone 1C12; Cell Signaling) was incubated overnight at 4 °C. Anti-mouse biotinylated with M.O.M. Blocking kit, Standard ABC kit, and ImmPACT DAB (Vector Laboratories) was used for the detection of horseradish peroxidase (HRP) activity. Slides were then dehydrated and mounted using SafeMount (Labonord). Supplementary statistics. For the quantification of the clone morphology of SmoM2-expressing clones in the scale and interscale regions (Fig. 1f), we counted in K14-CreER/Rosa-SmoM2 mice, 128, 109, 76, 195, 168 and 142 clones in the interscale region; 141, 116, 74, 94, 78 and 69 clones in the scale region from 3, 4, 4, 6, 4 and 5 independent experiments at 1, 2, 4, 8, 12 and 24 w respectively. In Inv-CreER/Rosa-SmoM2 mice, 104, 78, 42, 127, 160 and 344 clones were counted in the interscale region; 94, 54, 99, 90, 99 and 39 clones in the scale region from 4, 4, 4, 5, 4 and 8 independent experiments at 1, 2, 4, 8, 12 and 24 weeks, respectively.

For the analysis of the clone size of the K14-CreER/Rosa-YFP mice (Fig. 2a, c and Extended Data Fig. 2), we counted clones (both in scale and interscale) from two independent experiments at 1 week and 2 weeks, five independent experiments at 4 weeks, three independent experiments at 8 weeks, two independent experiments at 12 weeks and four independent experiments at 24 weeks. For the analysis of the clone size of the Inv-CreER/Rosa-YFP mice (Fig. 2b, c, Extended Data Fig. 2), we counted clones (both in scale and interscale) from two independent experiments at 1 weeks, five independent experiments at 4 weeks, three independent experiments at 2 weeks, for independent experiments at 1 week and 2 weeks, five independent experiments at 1 weeks, three independent experiments at 8 weeks, four independent experiments at 12 weeks and three independent experiments at 24 weeks (see raw data in cited figures (Source Data)).

For the clonal persistence of the K14-CreER/Rosa-YFP mice (Fig. 2e and Extended Data Fig. 2), we counted 167, 176, 129, 100, 47 and 246 clones in interscale and 184, 109, 75, 66, 19 and 103 clones in scale from 4, 5, 5, 5, 2 and 4 independent experiments at 1, 2, 4, 8, 12 and 24 w respectively. For 24 weeks, we counted several areas per mice as the number of clones was reduced (see Source Data).

For the clonal persistence of the Inv-CreER/Rosa-YFP mice (Fig. 2e and Extended Data Fig. 2), we counted 138, 95, 25, 31, 76 and 54 clones in interscale and 12, 17, 7, 8, 20 and 10 clones in scale from 2, 4, 2, 3, 4 and 3 independent experiments at 1, 2, 4, 8, 12 and 24 weeks respectively. For 12 and 24 weeks, we counted several areas per mice as the number of clones was low (see Source Data).

For the analysis of the clone size of the Inv-CreER/Rosa-SmoM2 mice (Fig. 3b, f, h, Extended Data Figs 5, 6), we counted clones (both in scale and interscale) from two independent experiments at 1 week and 2 weeks, from four independent experiments at 4 weeks, from six independent experiments at 8 weeks, from six independent experiments at 24 weeks (see Source Data).

For the clonal persistence of the Inv-CreER/Rosa-SmoM2 mice (Extended Data Figs 5, 6), we counted 65, 39, 71, 51, 27, 18 clones in interscale and 67, 27, 47, 31, 12 and 6 clones in scale from 2, 2, 4, 3, 2 and 2 independent experiments at 1, 2, 4, 8, 12 and 24 weeks, respectively (see Source Data).

For the analysis of the clone size of the K14-CreER/Rosa-SmoM2 mice (Fig. 4b, f, h and Extended Data Figs 6, 7), we counted clones (both in scale and

interscale) from three independent experiments at 1 week, from two independent experiments at 2 weeks, 4 weeks, from six independent experiments at 8 weeks, from four independent experiments at 12 weeks and from two independent experiments at 24 weeks (see Source Data).

For the clonal persistence of the K14-CreER/Rosa-SmoM2 mice (Extended Data Figs 6, 7), we counted 122, 63, 81, 79, 74 and 68 clones in interscale and 89, 46, 37, 42, 31 and 16 clones in scale from 4, 3, 4, 4, 4 and 4 independent experiments at 1, 2, 4, 8, 12 and 24 weeks respectively (see Source Data).

For the cell proliferation kinetics experiments in the Inv-CreER/Rosa-SmoM2 mice (Fig. 3c, e): at 4 weeks after induction, we counted 33 clones from 3 independent experiments for 2 days of continuous BrdU, 30 clones from 2 independent experiments for 4 days of continuous BrdU, 33 clones from 2 independent experiments for 6 days of continuous BrdU. At 8 weeks after induction, we counted 41 clones from n = 3 mice for 2 days of continuous BrdU, 16 clones from 2 independent experiments for 6 days of continuous BrdU and 24 clones from 2 independent experiments for 8 days of continuous BrdU and 24 clones from 2 independent experiments for 8 days of continuous BrdU. At 12 weeks after induction, we counted 19 clones from 2 independent experiments for 4 days of continuous BrdU. At 12 weeks after induction, we counted 19 clones from 2 independent experiments for 6 days of continuous BrdU. At 12 weeks after induction, we counted 19 clones from 2 independent experiments for 6 days of continuous BrdU. At 12 weeks after induction, we counted 19 clones from 2 independent experiments for 6 days of continuous BrdU. At 12 weeks after induction, we counted 19 clones from 2 independent experiments for 6 days of continuous BrdU. For the 2 weeks after induction data point, we use solely continuous BrdU incorporation, and counted 54 clones from two independent experiments.

For the cell proliferation kinetics experiments in the K14-CreER/Rosa-SmoM2 mice (Fig. 4c, e): at 4 weeks after induction, we counted 56 clones from 3 independent experiments for 2 days of continuous BrdU, 39 clones from 3 independent experiments for 6 days of continuous BrdU. At 8 weeks after induction, we counted 30 clones from 2 independent experiments for 2 days of continuous BrdU. At 8 weeks after induction, we counted 30 clones from 2 independent experiments for 2 days of continuous BrdU. At 8 weeks after induction, we counted 30 clones from 2 independent experiments for 2 days of continuous BrdU, 63 clones from 3 independent experiments for 6 days of continuous BrdU, and 41 clones from 3 independent experiments for 8 days of continuous BrdU. At 12 weeks after induction, we counted 20 clones from 2 independent experiments for 4 days of continuous BrdU, 21 clones from 2 independent experiments for 4 days of continuous BrdU, 28 clones from 2 independent experiments for 6 days of continuous BrdU, and 26 clones from two independent experiments for 8 days of continuous BrdU.

For the quantification of the clone morphology in absence of p53 interscale (Fig. 5c). For K14-CreER/Rosa-SmoM2/*p53*^{*fU/I*} mice 186, 217, 90, 343, 452 and 543 clones from 3, 3, 2, 3, 5 and 5 independent experiments and for Inv-CreER/Rosa-SmoM2/*p53*^{*fU/I*} 95, 98, 199, 271, 263 and 210 clones from 3, 3, 3, 4, 4 and

4 independent experiments were analysed at 1, 2, 4, 8, 12 and 24 weeks respectively. In the quantification in the scale region (Extended Data Fig. 8b) for K14-CreER/Rosa-SmoM2/ $p53^{fl/fl}$ 178, 204, 100, 132, 232 and 120 clones were counted from 3, 3, 2, 3, 5 and 5 independent experiments 1, 2, 4, 8, 12 and 24 weeks respectively. For Inv-CreER/Rosa-SmoM2 82, 127, 167, 136, 62 and 153 clones were counted from 2, 3, 3, 4, 4 and 5 independent experiments 1, 2, 4, 8, 12 and 24 weeks respectively.

For the analysis of the clone size of K14-CreER/Rosa-SmoM2/*p53*^{fl/fl} mice (Fig. 5d, g, Extended Data Fig 8e, d) we counted clones from the interscale from two independent experiments at 1, 2 and 4 weeks, three independent experiments at 8 weeks, four independent experiments at 12 weeks. For the analysis of the clone size of Inv-CreER/Rosa-SmoM2/*p53*^{fl/fl} mice (Fig. 5d, g and Extended Data Fig 8e, d) we counted clones from the interscale from two independent experiments at 1 and 2 weeks, three independent experiments at 4 and 8 weeks, four independent experiments at 12 weeks.

For the cell proliferation kinetics experiments in the Inv-CreER/Rosa-SmoM2/ *p53*^{fl/fl} mice (Fig. 5f) at 12 weeks after induction 34 clones from 3 independent experiments were counted. For the cell proliferation kinetics experiments in the K14-CreER/Rosa-SmoM2/*p53*^{fl/fl} mice (Fig. 5f) at 12 weeks after induction 44 clones from two independent experiments were counted.

For the clonal persistence experiments in Inv-CreER/Rosa-SmoM2/*p*53^{*fl/fl*}, 132, 78, 68, 58 and 89 clones from 4, 3, 3, 3 and 5 independent experiments were counted at 1, 2, 4, 8, 12 and 24 weeks and in K14-CreER/Rosa-SmoM2/*p*53^{*fl/fl*} mice 124, 82, 53, 76 and 100 clones were counted from 4, 3, 2, 3 and 4 independent experiments at 1, 2, 4, 8 and 12 weeks respectively (Extended Data Fig. 8e) (see Source Data).

Source Data includes the persistence and clone size quantifications of K14-CreER/Rosa-YFP, Inv-CreER/Rosa-YFP, K14-CreER/Rosa-SmoM2, Inv-CreER/Rosa-SmoM2/p53^{fl/fl} and Inv-CreER/Rosa-SmoM2/p53^{fl/fl} animals at different timepoints.

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Extended Data Figure 1 | The fate of oncogene-targeted clones is determined by the initial targeted cell (SC or CP) and their location in scale or interscale regions. a, Orthogonal view used to quantify the number of clones, cells stained with β 4-integrin and SmoM2. (left). Quantification of the number of clones induced 1 week after tamoxifen administration in scale and interscale regions in K14-CreER/Rosa-SmoM2 (n = 4 animals, 0.1 mg tamoxifen) and Inv-CreER/Rosa-SmoM2 (n = 3 animals, 2.5 mg tamoxifen) (right). b, Immunostaining for β 4-integrin and SmoM2 in K14-CreER/Rosa-SmoM2 and Inv-CreER/Rosa-SmoM2 clones located in the scale and interscale regions, 8 weeks after oncogene activation. c, Immunostaining for the differentiation marker keratin-10, K10, and SmoM2 in K14-CreER/Rosa-SmoM2 and Inv-CreER/Rosa-SmoM2 clones 8 weeks after oncogene activation, showing absence of differentiated cells in K14-CreER/Rosa-SmoM2 clones and alteration of the differentiation in Inv-CreER/Rosa-SmoM2 clones. Hoechst nuclear staining is represented in blue; scale bars, $10 \,\mu$ m.



Extended Data Figure 2 | **Evolution of K14-CreER/Rosa-YFP and Inv-CreER/Rosa-YFP clones in scale and interscale regions. a**, Wholemount immunostaining for YFP/K31 in K14-CreER/Rosa-YFP mice and Inv-CreER/Rosa-YFP mice upon tamoxifen administration. **b**, Scheme representing the area of tail epidermis (area comprised by 6 groups of triplets of hair follicles, highlighted in black) that is used to quantify the clone number and persistence. **c**, Distribution of K14-CreER/Rosa-YFP

and Inv-CreER/Rosa-YFP total clone sizes as measured by total cell content of surviving clones, imaged by confocal microscopy on whole-mount tail epidermis from 1 to 24 weeks after tamoxifen administration. The number of analysed clones is indicated for each time point. Hoechst nuclear staining is represented in blue; scale bars, 100 μ m. Histograms and error bars represent the mean and the standard error of the mean (s.e.m.).



Extended Data Figure 3 | The interscale is maintained by two cell populations during homeostasis. a, Evolution in time of the total labelled cell fraction under three hypotheses. For a perfect single population of equipotent balanced progenitors, the labelled cell fraction remains constant. For a single population of equipotent balanced progenitors displaying short-term priming, the labelled cell fraction increases transiently for the cells primed to divide, and decreases transiently for the cells primed to differentiate, but after the priming period, both fractions remain constant at different values. For two populations organized in a hierarchy, the labelled fraction of the progenitors decreases continuously to zero, while the labelled fraction of the stem cells continuously increases to reach a steady state value, corresponding to its average progeny size. **b**, Cumulative basal clone size distribution of Inv-CreER/Rosa-YFP clones at homeostasis in the interscale upon tamoxifen administration. **c**, Cumulative basal clone size distribution of K14-CreER/Rosa-YFP clones at homeostasis in the interscale upon tamoxifen administration. Clonal distributions are plotted in log-plot, error bars indicate s.d., thick lines are the model prediction and shaded areas indicate 95% confidence intervals in the model prediction.



Basal clone size

Extended Data Figure 4 | The scale is maintained by a single population during homeostasis. a, Evolution of mean surviving basal (top) and suprabasal (bottom) clone size in the scale for K14-CreER/Rosa-YFP (red) and Inv-CreER/Rosa-YFP (blue). In contrast to the interscale, in the scale K14-CreER and Inv-CreER clones behave identically, indicative of a single progenitor pool. The lines are the fit from the model from which we extract the fate choices of progenitors displayed in **b**. **b**, Fate choices of the equipotent progenitor pool in the scale, as extracted from the fits. **c**, Clonal persistence (top) and labelled cell fraction (bottom) in the scale for K14-CreER/Rosa-YFP (red) and Inv-CreER/Rosa-YFP (blue). The blue and red lines are the predictions of the model (see Supplementary Notes for details) using only the parameters extracted in **b**. K14- and Inv-CreER clones behave similarly and display near-perfect long-term balance. For the clonal persistence data, we examined in each mouse a randomly chosen area shown in Extended Data Fig. 2b. Error bars represent the s.e.m. **d**, Cumulative basal clone size distribution of K14-CreER/Rosa-YFP clones at homeostasis in the scale upon Tamoxifen administration. One should note that there were too few Involucrin clones in the scale to plot meaningful distributions. Clonal distributions are plotted in log-plot, error bars indicate s.d., thick lines are the model prediction and shaded area indicate 95% confidence intervals in the model prediction.



Extended Data Figure 5 | Clonal dynamics of interscale Inv-SmoM2 clones is consistent with a single imbalanced population of progenitors slowing down in time. a, Distribution of Inv-CreER/Rosa-YFP (black) and Inv-CreER/Rosa-SmoM2 (red) clone sizes as measured by total cell content, imaged by confocal microscopy on whole-mount tail epidermis from 1 weeks to 24 weeks following tamoxifen administration. The number of clones analysed in Inv-CreER/Rosa-SmoM2 is indicated in Fig. 3b. The number of clones counted in Inv-CreER/Rosa-YFP is as indicated in Fig. 2b. b, Evolution of the clonal persistence for interscale Inv-CreER/Rosa-SmoM2 clones. c, Labelled cell fraction for interscale Inv-CreER/Rosa-SmoM2 clones. d. Fraction of EdU-BrdU doublelabelled cells as a function of basal clone size at 8 weeks for Inv-CreER/ Rosa-SmoM2 clones, for 2 (left), 4 (centre) and 6 (right) days of continuous BrdU incorporation. e, Immunostaining for \\beta4-integrin, SmoM2 and active-caspase-3 in Inv-CreER/Rosa-SmoM2 clones at 8 weeks after induction. f, Percentage of dysplastic, hyperplastic and normally differentiating Inv-CreER/Rosa-SmoM2 clones presenting at least one active-caspase positive cell within the clone at 8 weeks after induction (n = 73 clones analysed from 4 independent experiments). g, Quantification of the number (%) of basal and suprabasal apoptotic

cells in dysplastic, hyperplastic and normally differentiating Inv-CreER/ Rosa-SmoM2 clones 8 weeks after SmoM2 activation. h, Percentage of dysplastic, hyperplastic and normally differentiating Inv-CreER/ Rosa-SmoM2 clones presenting apoptosis in basal and suprabasal compartments 8 w after oncogenic activation. i, Cumulative distribution of the fraction of basal apoptosis as a function of basal cell number in an Inv-CreER/Rosa-SmoM2 clone at 8 weeks (data in blue). The green line is the expected theoretical distribution of apoptotic fraction if apoptosis occurred randomly (following a Poisson process), in any clone with the same probability. The data are statistically different from the random theory, showing that apoptosis clusters in certain clones at a given time point. j, Short-term fate outcome of progenitors in Inv-CreER/Rosa-SmoM2 clones at 8 weeks, as assessed by using EdU as a clonal marker. We count only cell doublets and classify them as either basal-basal, basalsuprabasal, or suprabasal–suprabasal (n = 47 clones from 3 independent experiments). Immunostaining for β 4-integrin, EdU and SmoM2 showing the different type of cell fate outcomes found in Inv-CreER/Rosa-SmoM2 clones. Hoechst nuclear staining is represented in blue; scale bars, 10 µm. Histograms and error bars represent the mean and the s.e.m.



Extended Data Figure 6 | Clonal dynamics of Inv-CreER/Rosa-SmoM2 and K14-CreER/Rosa-SmoM2 clones in the scale are similar. a, Evolution of mean surviving basal clone sizes (top) and labelled cell fraction (bottom), for K14-CreER/Rosa-SmoM2, in the scale. b, Evolution of mean surviving basal clone sizes (top) and labelled cell fraction (bottom), for Inv-CreER/Rosa-SmoM2, in the scale. Whereas the interscale clones show net expansion, scale clones, both Inv-CreER and K14-CreER, show near balance at the population level. c, Evolution of the persistence of K14-CreER/Rosa-SmoM2 (green) and Inv-CreER/ Rosa-SmoM2 (purple) clones in the scale. Notably, and in contrast to the interscale, both K14 and Involucrin clones have the same persistence.

d, Mean basal clone size, normalized by the mean clone size at 1 week for both Inv-CreER and K14-CreER clones. Even though one can see on a and b that the final clone size is higher in K14, this is fully explained by short-term differences in fate during the first week indicative of short-term priming for K14. Correspondingly, the evolution of the labelling fraction is very similar for K14 and Involucrin in scale. Therefore, K14-CreER/Rosa-SmoM2 and Inv-CreER/Rosa-SmoM2 in scale display the same long-term kinetics upon oncogenic activation, consistent with the one-population model uncovered at homeostasis. Error bars represent the s.e.m.

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Extended Data Figure 7 | See next page for caption.



Extended Data Figure 7 | Clonal dynamics of interscale K14-CreER/ Rosa-SmoM2 clones is consistent with two populations. a, Distribution of K14-CreER/Rosa-YFP (black) and K14-CreER/Rosa-SmoM2 (red) clone sizes as measured by total cell content, imaged by confocal microscopy on whole mount tail epidermis from 1 week to 24 weeks after induction. The number of clones analysed for K14-CreER/Rosa-SmoM2 is indicated in Fig. 4b; the number of clones counted in K14-CreER/Rosa-YFP is as indicated in Fig. 2a. b, c, Evolution of the clonal persistence (b) and labelled cell fraction (c) for K14-CreER/Rosa-SmoM2 clones in the interscale. d, Fraction of EdU-BrdU double-labelled cells as a function of basal clone size at 8 weeks for K14-CreER/Rosa-SmoM2 clones, for 2 (left), 4 (centre) and 6 (right) days of continuous BrdU incorporation. e, Immunostaining for \beta4-integrin, SmoM2 and active-caspase-3 in K14-CreER/Rosa-SmoM2 clones 8 weeks after SmoM2 activation. f, Percentage of BCC, dysplastic, hyperplastic and normally differentiating clones presenting at least one active-caspase-3 positive cell at 8 weeks after induction (n = 117 clones analysed from 4 independent experiments). g, Quantification of the number (%) of basal and suprabasal apoptotic cells in dysplastic, hyperplastic and normally differentiating Inv-CreER/ Rosa-SmoM2 clones 8 weeks after SmoM2-activation. h, Percentage of dysplastic, hyperplastic and normally differentiating Inv-CreER/Rosa-SmoM2 clones presenting basal and suprabasal apoptosis 8 weeks after oncogenic activation. i, Cumulative distribution of the fraction of basal apoptosis as a function of basal cell number in a K14-CreER/Rosa-SmoM2 clone at 8 weeks (data in red). The green line is the expected theoretical distribution of apoptotic fraction if apoptosis occurred randomly (following a Poisson process), in any clone with the same probability. The data are statistically different from the random theory, showing that apoptosis clusters in certain clones at a given time point. j, Quantification of EdU-BrdU double-labelled cells as a function of the period of continuous BrdU incorporation for large K14 clones at 4 weeks (black), 8 weeks (orange) and 12 weeks (red) after clonal induction. The dashed lines represent the model fit (Supplementary Theory). k, Whisker plot of the suprabasal clone size in the interscale. The boxes delineate the first and third quartiles of the data, and the whiskers delineate the first and last deciles of the data at a given time point. The thick continuous line is the best fit from the model from which we extract the probability of fate choices in tumour SC and progenitors, displayed in Fig. 4g. The thin lines represent the mean clone sizes of SC- (top curve) and CP- (bottom curve) derived clones if they were alone. I, Short-term fate outcome of progenitors in K14-CreER/Rosa-SmoM2 clones at 8 weeks, as assessed by using EdU as a clonal marker. We count only cell doublets and classify them as either basal-basal, basal-suprabasal, or suprabasal-suprabasal (n = 49 clones from 3 independent experiments). Immunostaining for β4-integrin, EdU and SmoM2 in K14-CreER/Rosa-SmoM2 hyperplastic/dysplastic clones (top) and in BCC (bottom panel). SB, suprabasal. Hoechst nuclear staining is represented in blue; scale bars, 10 µm. Error bars represent the s.e.m.

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Extended Data Figure 8 | Effect of p53 deletion in the cellular dynamics of CPs and SCs. a, Immunohistochemistry staining for p53 in Inv-CreER/Rosa-SmoM2 and K14-CreER/Rosa-SmoM2 clones 12 weeks after induction. b, Quantification of normal, hyperplastic, dysplastic and BCC clones in scale region of K14CreER/Rosa-SmoM2/p53^{*li*/*f*¹} and Inv-CreER/Rosa-SmoM2/p53^{*fi*/*f*¹} mice. Description of number of counted clones is found in the Methods section. c, Distribution of clone sizes as measured by total cell content, imaged by confocal microscopy on whole mount tail epidermis. The number of clones analysed is indicated in Fig. 5d. Clone merger events were observed after 12 weeks following oncogenic activation in K14-CreER/Rosa-SmoM2/p53^{*li*/*f*¹} preventing the accurate quantification of clonal persistence and clone size at longer

times. **d**, Comparison of basal clone size distribution of Inv-CreER/Rosa-SmoM2/*p53*^{*fl/fl*} versus Inv-CreER/Rosa-SmoM2 and K14-CreER/Rosa-SmoM2/*p53*^{*fl/fl*} versus K14-CreER/Rosa-SmoM2 at 8 weeks and 12 weeks upon tamoxifen administration. **e**, Evolution of the clonal persistence of Inv-CreER/Rosa-SmoM2/*p53*^{*fl/fl*} and K14-CreER/Rosa-SmoM2/*p53*^{*fl/fl*} clones. **f**, Immunostaining of active-caspase-3 and SmoM2 8 weeks after induction in Inv-CreER/Rosa-SmoM2/*p53*^{*fl/fl*}. **g**, Quantification of the proportion of apoptotic clones in Inv-CreER/Rosa-SmoM2/*p53*^{*fl/fl*} (*n* = 90 clones from 3 independent experiments), and K14-CreER/Rosa-SmoM2/*p53*^{*fl/fl*} (*n* = 82 animals from 3 independent experiments) 8 weeks after induction. Hoechst nuclear staining is represented in blue; scale bars, 10 µm. Error bars represent the s.e.m.



SmoM2 in SCs leads to the generation of BCC owing to an increase in cell proliferation and resistance to apoptosis. However, activation of p53 in

SmoM2-expressing CPs restricts the progression of dysplastic clones to BCC by promoting apoptosis and cell-cycle arrest. Deletion of p53 in CPs allows them to progress into BCC.