1   **Defining the clonal dynamics leading to mouse skin tumour initiation**

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20   **The changes that occur in cell dynamics following oncogenic mutation that lead to**

21   **the development of tumours are currently unknown. Here, using skin epidermis as**

22   **a model, we assessed the impact of oncogenic hedgehog signalling in distinct cell**

23   **populations and their capacity to induce basal cell carcinoma, the most frequent**

24   **cancer in humans. We found that only stem cells, and not progenitors, were**

25   **competent to initiate tumour formation upon oncogenic hedgehog signalling.**

26   **Interestingly, this difference was due to the hierarchical organization of tumour**

27   **growth in oncogene-targeted stem cells, characterized by an increase of symmetric**

28   **self-renewing divisions and a higher p53-dependent resistance to apoptosis, leading**

29   **to rapid clonal expansion and progression into invasive tumours. Our work reveals**

30   **that the capacity of oncogene-targeted cells to induce tumour formation is not only**

31   **dependent on their long-term survival and expansion, but also on the specific**

32   **clonal dynamics of the cancer cell of origin.**

33

34

Introduction

Cancer arises through the acquisition of oncogenic mutations[1]. How such oncogenic mutations impact on 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 prior to tumour formation showed that intestinal stem cells (SCs) acquire a proliferative advantage over their wildtype 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 frequent 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 (Ptch) loss of function or Smoothened (Smo) gain of function[4]. Different mouse models of BCC using Ptch1 deletion or oncogenic SmoM2 mutant expression induce the formation of tumours that resemble superficial human BCC[5]. The skin epidermis contains distinct types of SCs that contribute to the homeostasis of discrete regions of epidermis[6]. Interfollicular epidermis (IFE) is maintained by SCs targeted by K14-CreER and committed progenitors (CPs) targeted by Inv-CreER in tail, ear, back and ventral skin epidermis[7,8]. Activation of oncogenic HH signalling through SmoM2 expression or Patched1 deletion in these different tissues using K14-CreER, which targets both SCs and CPs, induce BCC formation[7,9-12]. However, the question of whether and how SmoM2 expression in SCs and/or CPs drives BCC formation remains unresolved.

60   **Results**

61   **SCs but not CPs initiate BCC formation**

62   To determine whether SCs and CPs are both competent to induce BCC, we induced

63   oncogenic SmoM2 expression exclusively in CPs using Inv-CreER, and in both CPs

64   and SCs using K14-CreER[7] at the same clonal density (Fig.1a and Extended Data

65   Fig.1a). As previously reported, activation of SmoM2 expression using K14-CreER

66   induced BCC, characterized by invasion into the dermis and branched morphology, in

67   both tail and ear epidermis (Fig. 1b)[9-11]. In sharp contrast, activation of SmoM2

68   expression in CPs using Inv-CreER lead to preneoplastic lesions (including hyperplasia

69   and dysplasia) that did not progress into BCCs (Fig. 1b). These results suggest that only

70   IFE-SCs are competent to induce BCC following SmoM2-activation while IFE-CPs are

71   highly resistant.

72

73   We then assessed whether the competence of SCs and CPs to initiate BCC was

74   dependent on the oncogene or tumor suppressor gene used to activate HH signalling. To

75   this end, we induced Ptch1 deletion using K14- or Inv-CreER (Fig. 1c). Ptch1 deletion

76   using K14-CreER lead to BCCs arising from the IFE and the infundibulum (Fig. 1c). In

77   contrast, Ptch1 deletion using Inv-CreER, which targets some basal cells in the back and

78   ventral skin epidermis[8], did not lead to the rapid development of BCC, and only rare

79   and small BCCs were observed 24w post-induction (Fig. 1c-d). These results reveal that

80   only IFE/infundibulum SCs are competent to induce BCC formation whereas CPs are

81   highly resistant, irrespective of the oncogene or tumour suppressor gene used to activate

82   HH signalling and body location (tail, ear, back and ventral skin).

83

84   Two distinct self-maintained compartments, scale and interscale, have been

85   described in tail epidermis[13]. To assess whether cells located in these two compartments

86   respond equally to oncogenic activation, we performed immunofluorescence using a

87   scale-specific marker (K31) and SmoM2-YFP on whole mount tail epidermis.

88   Interestingly, we found that BCCs arose from K14-CreER SmoM2 targeted cells

89   located only in the interscale (Fig. 1e). K14 clones in the interscale progressively lost

90   their normal differentiation program, as evidenced by the loss of spinous-like cells,

91   became hyperplastic, then dysplastic (Fig. 1f and Extended Data Fig. 1b-c). From 4 to 8

92   weeks (w) post-induction, around 15% of clones had progressed into BCC in interscale,

93   increasing to 40% after 24w (Fig. 1e-f). In contrast, K14 clones in scale never

94   progressed to BCC, and maintained a normal differentiation program for an extended

95   period, despite clonal expansion mediated by SmoM2 expression (Fig. 1e-f and

96   Extended Data Fig. 1b-c).

97

98   Together, these data indicate that the fate of oncogene targeted cells and the

99   ability of these cells to progress into BCC depends both on their location (scale versus

100   interscale) and cellular origin (SC versus CP). This prompted us to ask whether there

101   are regional differences in SC potential in tail epidermis even under homeostatic

102   conditions.

103   **Homeostasis of the interscale epidermis**

104   To gain quantitative insight into regional variation in SC potential, we performed

105   lineage tracing at homeostasis to determine whether scale and interscale are

106   differentially maintained. To this end, we compared the evolution of K14-CreER/Rosa-

107   YFP and Inv-CreER/Rosa-YFP targeted cells at single cell resolution over a 24w time-

108   course. Interestingly, although both broad, the distribution of clone sizes in the two

109   regions became increasing divergent (Fig. 2a-b and Extended Data Fig. 2), confirming

110   the importance of regionalization in cellular dynamics (Supplementary Theory).

111

112   Consistent with our previous study[7], the evolution of mean clone size of

113   progenitors targeted by Inv-CreER in the interscale fits well with the targeting of an

114   equipotent CP population presenting a small but statistically significant imbalance in

115   fate towards terminal differentiation (Fig. 2c-d). Similarly, the evolution of mean clone

116   size for K14-CreER cells is consistent with the targeting of a long-term self-renewing

117   SC population that divides more slowly than CPs (Fig. 2c-d). To define quantitatively

118   the dynamics of these two populations (cell cycle times, relative proportion of SCs and

119   CPs labelled by the K14-CreER and their fate probabilities), we made a joint fit to the

120   basal and suprabasal mean clone sizes, and extracted optimal parameters and confidence

121   intervals (Supplementary Theory).

122

123   To independently verify the predictions of the model, the persistence of Inv- and

124   K14-CreER targeted clones was used to infer the respective labelled cell fraction. As

125   expected from the labelling of a CP population, for Inv-CreER targeted clones, we

126   found that the labelled cell fraction decreased over time (Fig. 2e). In contrast, for K14-

127   CreER targeted clones, the labelled cell fraction increased over time, consistent with the

128   preferential targeting of the SC population (Fig. 2e). Strikingly, we obtained excellent

129   predictions for the labelled cell fraction for both K14- and Inv-CreER using parameters

130   extracted independently from a fit to the mean clone sizes (Fig. 2e). These results

131   provide compelling evidence in favour of a SC and CP hierarchy, and rule out the

132   possibility that the differences between K14- and Inv-CreER targeted clones are the

133   consequence of differential short-term "priming" of induced cells (Extended Data Fig.

6

134 3a). Importantly, the hierarchical model also predicted accurately the complete

135 distribution of clone sizes at all time points (Extended Data Fig. 3b-c) for both K14- and

136 Inv-CreER.

137

138 In sharp contrast, in the scale region of tail epidermis, both basal and suprabasal

139 clone size and persistence of K14- and Inv-CreER targeted cells were statistically

140 indistinguishable (Extended Data Fig. 4a and c). Crucially, the labelled cell fraction did

141 not change significantly between 2w and 24w post-labelling (Extended Data Fig.4c), an

142 indication that K14- and Inv-CreER mark the same balanced CP population[13]. We again

143 validated the model (Fig.4b) by showing that it could predict quantitatively both the

144 evolution of clonal persistence, as well the clone size distribution at all time points

145 (Extended Data Fig. 4b-d).

146

147 These results show that, during homeostasis, interscale is maintained by two

148 discrete populations; a comparatively slow-cycling SC and a more rapidly dividing CP

149 population, whereas scale is maintained by a single CP population. As well as unifying

150 diverging reports of maintenance hierarchy in tail epidermis[7,13,14], these findings raised

151 the question of whether the restriction of BCCs to the interscale correlated with the

152 regional localization of IFE SCs. To test this hypothesis, we assessed whether the same

153 regionalized lineage hierarchy persisted upon SmoM2 activation.

154

155 **Oncogene-targeted CPs are frozen into dysplasia**

156 To resolve the cellular dynamics underpinning to the differential sensitivity of SCs and

157 CPs to BCC initiation in interscale, we first studied the dynamics and proliferation

158 kinetics of Inv-CreER/Rosa-SmoM2 clones. Oncogenic activation in Inv-CreER CPs

159   lead to an increase of the average basal clone size, total clone size and clonal

160   persistence compared to homeostatic conditions (Fig. 3a-b and Extended Data Fig.5a-c),

161   as well as abnormal or decreased differentiation (Fig. 3a and Extended Data Fig. 1 b-c).

162   We assessed the average cell cycle time of SmoM2 Inv-CreER-targeted cells by first

163   marking proliferating cells using 24h of EdU administration, followed by variable

164   periods of continuous BrdU administration. From the co-labelling of EdU/BrdU, we

165   found that CPs divided on average every 3.6±0.5 days at 4w following SmoM2

166   expression, 7.2±0.6 days at 8w and 9.8±0.3 days at 12w (Fig. 3c), indicating that the

167   average division rate of SmoM2 CPs decreases with time. Surprisingly, division rates

168   were uncorrelated with clone size at all time points, indicating that the decrease occurs

169   independently of clone size or stage of tumour progression (Extended Data Fig. 5d), and

170   consistent with the Inv-CreER oncogene targeted cells functioning as a single

171   equipotent population.

172

173   Since deregulation of apoptosis is also important for cancer formation[1], we

174   assessed whether apoptosis influences the clonal dynamics of oncogene targeted CPs. In

175   common with their normal counterpart, Inv-CreER targeted cells did not show evidence

176   of apoptosis over the first 6w following SmoM2 expression (data not shown). However,

177   from 8w on, about 60% of Inv-CreER targeted clones that presented hyperplasia or

178   dysplasia contained about 2-4% of apoptotic cells as measured by active caspase-3

179   immunostaining (Fig. 3d, Extended Data Fig. 5e-i).

180

181   Taking these rates (Fig. 3e) as an input, we could obtain an excellent fit to the

182   average clone size (Fig. 3f) with cell fate probabilities that remain constant over time

183   (symmetric renewal (PP): asymmetric division (PD): symmetric differentiation

8

184 (DD)=39%:45%:16%) (Fig. 3g). This result demonstrates that oncogenic expression in

185 CPs leads to enhanced clonal expansion and survival by promoting symmetric

186 proliferation over terminal differentiation. Such an imbalance would lead to exponential

187 clone growth if it were not counteracted by an ever-diminishing effective proliferation

188 rate, leading to a plateau in the mean basal clone size (Fig. 3f). Notably, the model

189 prediction provided a good fit to the clone size distribution at all time points (Fig. 3h).

190

191 Finally, to further verify the model, a low short-term dose of EdU was used to

192 mark a minority of dividing cells and their fate outcome was recorded 3 days later by

193 quantifying the basal and suprabasal localization of EdU doublets (Extended Data Fig.

194 5j). From these results, we could confirm a large imbalance between symmetric division

195 and terminal differentiation (35%).

196

197 Since the scale is maintained by a single progenitor pool, we wished to probe

198 whether its response to oncogenic activation was similar to interscale CPs. Crucially,

199 after an initial increase, the overall labelled cell fraction remained roughly constant over

200 time in scale between 8 and 24w, at a similar level for both K14- and Inv-CreER

201 (Extended Data Fig. 6a-c), suggesting that, in sharp contrast with interscale, both

202 populations behave identically upon oncogenic activation (Extended Data Fig. 6d).

203 Together, these results show that both interscale and scale CPs are resistant to BCC

204 formation upon oncogenic HH signalling, although interscale clones can persist longer

205 due to a larger fate imbalance and enhanced differentiation defects, while scale clones

206 rapidly converge towards balance. However, as human epidermis does not show scale

207 organization, the absence of BCC formation in the scale region might not have human

208 relevance.

209

Oncogene-targeted SCs progress into BCC

211 To gain insight into how SmoM2 expression in SCs promotes BCC formation, we then

212 performed a quantitative analysis of K14-CreER/Rosa-SmoM2 clones. Compared to

213 Inv-targeted clones, SmoM2 expression in K14-targeted cells lead to a more rapid and

214 persistent expansion of a fraction of clones (Fig. 4a-b and Extended Data Fig.7a-c) that

215 progressed into BCC, as well as the formation of smaller clones that did not show

216 tumour progression (Fig. 1e and 4b). This suggests that, in line with homeostatic

217 conditions, K14-CreER marks a fraction of tumour-like SCs, together with tumour-like

218 CPs, a heterogeneity that we verified using proliferation assays (Fig. 4c). Indeed, we

219 found that one population of K14-CreER targeted cells consisted of small clones that

220 displayed similar proliferation kinetics as Inv-CreER SmoM2 clones, while a second

221 population consisted of larger clones, which re-entered cell cycle significantly faster

222 (Fig. 4c, Extended Data Fig. 5d and 7d). The population of small K14-CreER targeted

223 clones (dysplasia and hyperplasia) also presented higher levels of apoptosis compared to

224 the larger clones (Fig. 4d and Extended Data Fig. 7e-i). As a result, even though the

225 proliferation of the larger clones also decreased with time (Fig. 4e and Extended Data

226 Fig.7j), their division rate was consistently higher than the Inv-CreER targeted

227 population (Fig. 4e).

228

229 To model BCC initiation, we adapted the hierarchical model obtained during

230 homeostasis and fitted jointly the mean basal and suprabasal clone sizes of all K14-

231 CreER-SmoM2 clones, taking as input the division rate as well as the fraction of SCs

232 initially labelled by the K14-CreER determined from measurements at homeostasis, and

233 used the fate choices of SCs as fitting parameters (Fig. 4f and Extended Data Fig. 7k).

234 In particular, we posited that SCs are imbalanced towards symmetric renewal, whereas

235 CPs derived from these cells remain slightly imbalanced toward symmetric

236 differentiation with the same fate probability as in homeostasis, which gave a good fit to

237 the average basal clone size (Fig. 4g). Notably, the measured clone size distributions

238 from 12w onwards could not be fit with a one-progenitor population model, in stark

239 contrast to the distributions of Inv-CreER-SmoM2 clones. Instead, the K14-CreER-

240 SmoM2 clone size distributions displayed a "double-exponential" decay, consistent

241 with the labelling of two distinct populations, as predicted quantitatively by the model

242 (Fig. 4h and Supplementary Theory). This shows that K14-CreER targets tumour-like

243 SCs making imbalanced stochastic fate choices, in addition to targeting the same

244 tumour-like CP population as Inv-CreER.

245

246 As a finally consistency check, we addressed a key hallmark of the hierarchical

247 model, that SCs give rise to basal CPs in K14-CreER targeted clones. This predicts that

248 the fraction of cell divisions resulting in two basal cells should be greater in SC versus

249 CP-targeted clones. Indeed, short-term EdU pulse-chase experiments revealed that, in

250 BCC, most divisions (77%) lead to two basal cells (Extended Data Fig. 7l). In dysplasia,

251 the fraction of two EdU+ basal cell doublets was intermediate between the BCC and

252 Inv-CreER/Rosa-SmoM2 values (Extended Data Fig. 5j and 7l), consistent with a

253 mixture of SC and CP-targeted clones.

254

255 **P53 restricts CPs to progress into BCC**

256 Given the observed differences in apoptosis and division rates between oncogene

257 targeted SC and CP, we assessed whether p53, a tumour suppressor gene frequently

258 mutated in human BCC[15] that controls cell cycle arrest and apoptosis[16], was

259   differentially activated in SCs and CPs upon SmoM2 activation. Immunohistochemistry

260   revealed that p53 was more frequently found in SmoM2 clones arising from Inv-CreER

261   as compared to K14-CreER mice (Extended data Fig.8a). To determine whether p53

262   stabilization in oncogene targeted CPs restricts the potential of these progenitors to

263   generate BCC, we deleted p53 together with SmoM2 activation and assessed tumour

264   formation. Interestingly, p53 deletion in Inv-CreER targeted CPs leads to BCC in both

265   ear and tail epidermis (Fig. 5a). In the tail, BCCs were restricted to the interscale

266   whereas, in the scale, clones only progressed into dysplasia (Fig. 5b-c and Extended

267   Data Fig.8b). These results indicate that p53 restricts the competence of SmoM2

268   targeted CPs of the interscale to progress into BCC.

269

270   Although the proportion of clones that progress into BCC continued to be more

271   frequent and more rapid in K14-CreER targeted SCs, at 24w post-induction more than

272   half of interscale Inv-CreER targeted clones had progressed into BCC after p53 deletion

273   (Fig. 5c). The clonal persistence and clone size were increased upon p53 deletion in

274   both   Inv-CreER/Rosa-SmoM2   and   K14-CreER/Rosa-SmoM2   interscale   clones,

275   although the clones were still bigger and more persistent in K14 targeted cells (Fig. 5b

276   and d and Extended Data Fig. 8c-e). These results indicate that, upon p53 deletion, both

277   oncogene targeted CPs and SCs present an increase in self-renewing divisions allowing

278   CPs to acquire the competence to form BCC upon SmoM2 expression.

279

280   We next determined whether the observed increase in clone size in the absence

281   of p53 in CPs and SCs was due to a decrease in apoptosis, an increase in proliferation or

282   both. Immunostaining for active caspase-3 8w after oncogenic activation showed that

283   large Inv-CreER/Rosa-SmoM2/p53fl/fl dysplastic and BCC clones displayed reduced

284   apoptosis, mirroring our observation in K14-CreER/Rosa-SmoM2 (Extended Data Fig.

285   8f). However, apoptosis was unchanged in Inv-CreER p53 deficient hyperplastic clones,

286   suggesting that p53 dependent and independent mechanisms control apoptosis in

287   oncogene-targeted cells (Fig. 5e and Extended Data Fig. 8g). EdU/BrdU double-pulse

288   experiments at 12w post-induction showed that deletion of p53 increased the rate of

289   proliferation in both Inv-CreER and K14-CreER oncogene targeted cells (Fig. 5f).

290   According to our model, this increase in the rate of division was sufficient, keeping all

291   other parameters constant, to explain the enhanced tumour growth (Fig. 5g). This

292   provides additional evidence that growth arrest in oncogene targeted CPs is a key

293   determinant in their inability to mediate BCC progression in the presence of p53.

294

295   In summary, our results demonstrate that p53 restricts the competence of CPs to initiate

296   BCC by promoting apoptosis and inducing cell cycle arrest in oncogene targeted CPs.

297

298   **Discussion**

299   In this study, we have defined the quantitative dynamics of BCC initiation at single cell

300   resolution, from the first oncogenic hit to the development of invasive tumours. These

301   results show that the proliferative hierarchical organization of skin epidermis is a key

302   determinant of tumour development, with only IFE SCs and not CPs competent to

303   initiate BCC following oncogenic HH signalling (Extended data Fig. 9). Even though

304   CP-derived clones survive and proliferate for months, they are surprisingly robust to

305   BCC transformation and invasion, becoming "frozen" in a pre-tumorigenic state. The

306   developmental cerebellar progenitors initiate medulloblastoma upon oncogenic HH

307   signaling[17,18], suggesting the developmental stage of progenitors may also dictate

308   competence for tumour initiation. The apparent long-term maintenance of some

309   oncogene targeted CPs stands in contrast to classical transient-amplifying cells in other

310   compartments, such as hair matrix in the skin or the non-Lgr5 crypt progenitors in gut,

311   which are resistant to tumour initiation because of their short lifespan[19-22].

312

313   　　　　Our results show that IFE SCs reside solely in the interscale region, and have the

314   unique and regionalized competence to initiate large and invasive BCCs. Strikingly, this

315   regionalized hierarchical organization at homeostasis was maintained upon SmoM2

316   activation. Oncogene expression in SCs lead to a more rapid clonal expansion as

317   compared to CPs for two main reasons: the maintenance of hierarchical organization in

318   early pre-neoplastic lesions, leading to increased symmetric self-renewing divisions;

319   and the combined resistance to apoptosis and enhanced proliferation of SC-derived pre-

320   neoplastic lesions, leading to a larger effective growth rate. These two properties allow

321   SC-targeted tumours to escape the frozen-state that characterized CP-targeted pre-

322   neoplastic lesions, and thereby progress to an invasive phenotype.

323

324   　　　　Finally, our results show that p53 restricts the competence of CPs to undergo

325   BCC initiation by promoting apoptosis and inducing cell cycle arrest in oncogene

326   targeted CPs. Interestingly, although the division rates of CPs and SCs deficient for p53

327   are similar, SC-targeted tumours still grow to larger sizes than CP-targeted tumours,

328   suggesting that the hierarchical organization is at least partially maintained even after

329   two oncogenic hits. By establishing that sustained imbalance towards self-renewing

330   divisions and resistance to p53 mediated apoptosis and cell cycle arrest are the main

331   drivers of tumorigenesis, this study suggests that therapy promoting differentiation, p53

332   reactivation and apoptosis could present a promising avenue to promote BCC regression

333   and prevent tumour relapse.

334

344

345   **AUTHOR CONTRIBUTION**

346   A.S-D., C.B., E.H., B.D.S. designed the experiments, performed data analysis and wrote

347   the manuscript; A.S-D performed all of the biological experiments; E.H. performed all

348   the mathematical modelling. J.C.L and M.L. provided technical support. K.K.Y. made

349   initial observations pertinent to the study.

350

351   **AUTHOR INFORMATION**

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419
420

FIGURE LEGENDS

Figure 1. SCs but not CPs are competent to initiate BCC formation upon HH

activation

424   (a) Genetic strategy to activate SmoM2 expression in SCs and CPs. (b) Immunostaining

425   of β4-integrin/SmoM2 in ear and tail skin 24w after SmoM2 activation. (c)

426   Immunostaining of β4-integrin/K14 in ventral skin 24w after Ptch1 deletion. (d)

427   Quantification of tumour burden (total tumour area divided by length of epidermis)

428   following Ptch1 deletion. Quantification of BCC number per length (mm) following

429   Ptch1 deletion. (n=4 Inv-CreER/Ptch1KO animals and n=3 K14CreER/Ptch1KO

430   animals (e) Immunostaining of K31/ SmoM2 in whole mount tail skin. (f)

431   Quantification of the morphology of SmoM2-expressing clones. Description of number

432   of counted clones is found in the method section. Hoechst nuclear staining in blue; scale

433   bars, 100μm. *P≤0,05, **P≤0,01, ***P≤0,001. Histograms and error bars represent the

434   mean and the standard error of the mean (s.e.m).

435

436

437   **Figure 2: Homeostatic renewal of mouse tail epidermis.**

438   (a, b) Distribution of basal clone sizes, in K14-CreER/Rosa-YFP (a) and Inv-

439   CreER/Rosa-YFP (b) epidermis. The number of clones analysed is indicated for each

440   time point and in the method section. (c) Mean basal (top) and suprabasal (bottom)

441   clone size in the interscale. The lines represent the model fit. (d) Cell fate probabilities

442   of SCs and CPs in the interscale, as extracted from the fits. (e) Clonal persistence (top)

443   and labelled cell fraction (bottom) in the interscale. Description of number of counted

444   clones is found in the method section. The lines are the predictions from the model

445   using only the parameters extracted in d. K14-CreER/Rosa-YFP clones display a net

446   expansion, whereas Inv-CreER/Rosa-YFP clones display a net contraction. Histograms

447 and error bars represent the mean and the s.e.m. Shaded areas represent 95% confidence

448 intervals for the model prediction (Supplementary Theory).

449

450

451 **Figure 3: SmoM2 expression in CPs induces clonal expansion that does not**

452 **progress into BCC.**

453 (a) Immunostaining for β -integrin, YFP and SmoM2 in Inv-CreER/Rosa-YFP and

454 Inv-CreER/Rosa-SmoM2 epidermis at different time points.(b) Distribution of Inv-

455 CreER/Rosa-YFP and Inv-CreER/Rosa-SmoM2 basal clone sizes. The number of

456 clones analysed in Inv-CreER/Rosa-SmoM2 is indicated for each time point and for

457 Inv-CreER/Rosa-YFP indicated in Fig. 2b. (c) Quantification of EdU/BrdU double-

458 labelled cells during continuous BrdU administration, at different time points post

459 clonal induction. The lines represent the model fit (Supplementary Theory). (d)

460 Quantification of the proportion of apoptotic cells in dysplastic, hyperplastic and

461 normally differentiating Inv-CreER/Rosa-SmoM2 clones 8w post-induction (n= 73

462 clones analysed from 4 independent experiments). (e) Division rate determined from

463 EdU/BrdU double-labelling experiments (data in black, fit in blue dashed line). (f)

464 Mean basal and suprabasal clone size in the interscale. The lines represent the model fit

465 from which we inferred the cell fate probabilities displayed in g. (g) Cell fate

466 probabilities of the tumour progenitor expressing SmoM2. (h) Basal clone size

467 distribution of Inv-CreER/Rosa-SmoM2 clones (black). Consistent with the hypothesis

468 of a single equipotent progenitor pool, all distributions are well-fit by single

469 exponential. Blue lines represent the model prediction using only the parameters

470 extracted from g. Shaded areas represent 95% confidence intervals for the model

471 prediction. D: dysplasia; H: hyperplasia; N: normal differentiation. Hoechst nuclear

472 staining in blue; scale bars, 10μm. Histograms and error bars represent the mean and the

473 s.e.m .

474

475

Figure 4: SmoM2 expression in SCs induces tumour SCs that lead to BCC
formation.

478 (a) Immunostaining for β -integrin, YFP and SmoM2 in K14-CreER/Rosa-YFP and

479 K14-CreER/Rosa-SmoM2 epidermis at different time points. (b) Distribution of K14-

480 CreER/Rosa-YFP and K14-CreER/Rosa-SmoM2 basal clone sizes. The number of

481 clones analysed for K14-CreER/Rosa-SmoM2 is indicated and for K14-CreER/Rosa-

482 YFP indicated in Fig. 2a. (c) Quantification of EdU/BrdU double-labelled cells

483 following continuous BrdU administration, at 8w post clonal induction for small K14-

484 CreER clones, Inv-CreER clones, and large K14-CreER clones. (d) Quantification of

485 the number of apoptotic cells in BCC, dysplastic, hyperplastic and normally

486 differentiating K14-CreER/Rosa-SmoM2 clones 8w post-induction (n= 117 clones

487 analysed from 4 independent experiments). (e) Division rate in large K14 clones

488 determined from double-labelling experiments (data in black, fit in red dashed line). (f)

489 Whisker plot of the mean basal clone size in the interscale. The boxes delineate the first

490 and third quartiles of the data, while the whiskers delineate the first and last decile of

491 the data. The thick continuous line is the best fit from the model from which we extract

492 the probability of fate choices in tumour SCs and progenitors displayed in g. The thin

493 dashed lines represent the predicted mean clone sizes of SCs- (top thin curve) and

494 progenitors- (bottom thin curve) derived clones alone. (g) Cell fate probabilities of the

495 tumour SC upon SmoM2 activation. (h) Basal clone size distribution of

496 K14CreER/SmoM2 clones (black). Red lines are the model prediction using only the

497   parameters extracted from g. Shaded areas represent 95% confidence intervals for the

498   model prediction. BCC: basal cell carcinoma; D: dysplasia; H: hyperplasia; N: normal

499   differentiation. Hoechst nuclear staining in blue; scale bars, 10μm. Histograms and error

500   bars represent the mean and the s.e.m..

501

502

503   **Figure 5: p53 deletion in CPs leads to BCC formation.**

504   (a) Immunostaining of β4-integrin/SmoM2 in ear and tail skin of K14 and Inv-

505   CreER/Rosa-SmoM2/p53fl/fl mice 24w after Tamoxifen administration (b) Whole

506   mount immunostaining of K31/SmoM2 in tail epidermis overtime. (c) Quantification of

507   normal, hyperplastic, dysplastic and BCC clones in the interscale region. Description of

508   number of counted clones is found in the method section. (d) Distribution of basal clone

509   sizes in K14 and Inv-CreER/Rosa-SmoM2/p53fl/fl mice. The number of clones

510   analysed is indicated. Clone merger events were observed after 12w following

511   oncogenic activation in K14Cre-ER/Rosa-SmoM2/p53fl/fl preventing the accurate

512   quantification of clonal persistence and clone size at longer times. (e) Quantification of

513   the proportion of apoptotic cells in different clones (K14 n= 82 clones and Inv n=90

514   clones from 3 independent experiments). (f) Percentage of double-labelled EdU/BrdU

515   SmoM2-expressing cells after 6 days of continuous BrdU administration following a

516   24h pulse of EdU at 12w post-induction. *P≤0,05, **P≤0,01. (i) Mean basal clone size

517   in Inv-CreER/Rosa-SmoM2/p53fl/fl and K14-CreER/Rosa-SmoM2/p53fl/fl clones. The

518   prediction of the model is indicated by the blue and red lines. Histograms and error bars

519   represent the mean and s.e.m. Shaded areas represent 95% confidence intervals for the

520   model prediction in i. Scale bars, 100μm.

521

METHODS

Mice

K14CREER transgenic mice[23] were kindly provided by Elaine Fuchs, The Rockefeller University; INVCREER were generated in our laboratory[8]. Ptch1 flox/flox mice [24] and Rosa–SmoM2–YFP mice[25] were obtained from the JAX repository. p53fl/fl[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 1cm in diameter. The BCCs observed in this study were microscopic and ranged from 1.5 mm to 100μm in diameter and in none of the experiments performed, the tumours exceeded the limit 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.

Skin tumour induction and clonal YFP expression

For clonal induction 3-months-old mice were used. K14CreER/Rosa-YFP, K14CreER/Rosa-SmoM2, K14CreER/SmoM2/p53fl/fl and K14CreER-Ptch1fl/fl mice received an intraperitoneal injection of 0,1mg of Tamoxifen and Inv-CreER/Rosa-YFP, Inv-CreER/Rosa-SmoM2, Inv-CreER/Rosa-SmoM2/p53fl/fl and INVCreER-Ptch1fl/fl received a intraperitoneal injection of 2.5mg of Tamoxifen to achieve similar level of recombination in the different models (Extended Data Figure 1a). Mice were sacrificed 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 5–8μm 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 10min at

556　room temperature and blocked with blocking buffer for 1h (PBS, horse serum 5%, BSA

557　1%, Triton 0.1%). Skin sections were incubated with primary antibodies diluted in

558　blocking buffer overnight at 4 ° C, washed with PBS for 3 × 5 min, and then incubated

559　with Hoechst solution and secondary antibodies diluted in blocking buffer for 1h at

560　room temperature. Finally, sections were washed with PBS for 3 × 5 min at room

561　temperature and mounted in DAKO mounting medium supplemented with 2,5% Dabco

562　(Sigma). Primary antibodies used were the following: anti- GFP (Rabbit, 1/1000, BD,

563　ref. A11122), anti-K14 (Chicken, 1/4000, Covance, ref. PCK-153P-0100) and anti-B4-

564　integrin(Rat, 1:200, BD, ref.553745). The following secondary antibodies were used:

565　anti-rabbit, anti-rat, anti-chicken, conjugated to AlexaFluor488 (Molecular Probes) and

566　to rhodamine Red-X (JacksonImmunoResearch). Images of the immunostainings in

567　sections were acquired using an Axio Imager M1 microscope, an AxioCamMR3 camera

568　and the Axiovision software (Carl Zeiss).

569

570　**Immunostaining in whole mounts**

571　Whole mounts of tail epidermis were performed as previously described[27] and used to

572　quantify the proportion of surviving clones (Extended Data Fig.2b) as well as the basal

573　suprabasal and total clone size. Specifically, pieces of tail were incubated for 1h 37°C

574　in EDTA 20mM in PBS in rocking plate, then using forceps the dermis and epidermis

575　were separated and the epidermis was fixed for 30 minutes in PFA 4% in agitation at

576　room temperature and washed 3 times with PBS.

577　For the immunostaining: tail skin pieces were blocked with blocking buffer for 3h

578　(PBS, horse serum 5%, Triton 0.8%) in a rocking plate at room temperature. After, the

579　skin pieces were incubated with primary antibodies diluted in blocking buffer overnight

580　at 4 ° C, the next day they were washed with PBS-Tween 0.2% for 3 × 10 min at room

581　temperature, and then incubated with the secondary antibodies diluted in blocking

582　buffer for 3h at room temperature, washed 2x10 min with PBS-Tween 0.2% and washed

583　for 10min in PBS. Finally, they were incubated in Hoechst diluted in PBS for 30

584　minutes at room temperature in the rocking plate, washed 3x10 min in PBS and

585　mounted in DAKO mounting medium supplemented with 2,5% Dabco (Sigma).

586　Primary antibodies used were the following: anti-GFP (Rabbit, 1/100, BD, ref.

587　A11122), anti-GFP (Goat, 1:800,Abcam, ref. Ab6673), anti-active-caspase3 (Rabbit,

588　1/600,R&D, ref. AF835), anti-β4-integrin(Rat, 1:200, BD, ref. 553745) and anti-K31

589　(Guinea Pig, 1:200, Progen, ref. GP-hHa1). The following secondary antibodies were

590 used: anti-rabbit, anti-rat, anti-chicken, anti-goat and anti-guinea pig, conjugated to

591 AlexaFluor488 (Molecular Probes), to rhodamine Red-X (JacksonImmunoResearch)

592 and to Cy5 (1:400, Jackson ImmunoResearch).

593

Analysis of clone survival, size and apoptosis

595 Quantification of the proportion of surviving clones, as well as, total and basal clone

596 size was determined by counting the number of SmoM2-YFP and YFP-positive cells in

597 each clone using whole-mount tail epidermis. The different clones were imaged using

598 Z-stacks using a confocal microscope LSM 780 (Carl Zeiss) and orthogonal views were

599 used to count the number of basal and total number of SmoM2-YFP or YFP-positive

600 cells in each clone, as well as, the number of active-caspase3 positive cells in each

601 clone. K31 staining was used to classify the clones according to their location in the

602 scale or interscale regions.

603

Proliferation assays

605 To measure the kinetics of cell proliferation, a 24h continuous pulse of EdU followed

606 with a continuous pulse of BrdU were performed. Specifically, mice received at t=0 an

607 intraperitoneal injection of EdU (1mg/ml) and 0.1mg/ml EdU was added to their

608 drinking water for 24h. The next days the mice received a daily intraperitoneal

609 injection of BrdU (10mg/ml) and 1mg/ml of BrdU was added to their drinking water

610 during the 8 days of the continuous BrdU pulse. Mice were sacrificed at different time

611 points and whole mount stainings for the tail were performed. The pieces of tail were

612 first stained for anti-GFP (following the protocol described in the previous section).

613 Secondly, EdU staining was performed following the manufacturer's instructions

614 (Invitrogen). The pieces of tail were then washed in PBS and fixed again in PAF 4% for

615 10 minutes. After they were washed in PBS, blocked for 3h in the blocking buffer and

616 incubated for 20 min in HCl 1 N at 37 °C, washed three times with PBS-Tween 0.2%

617 and incubated overnight with Alexa 647-coupled anti-BrdU antibody (mouse, 1:200,

618 BD). The next day the tail pieces were washed in PBS, incubated in Hoechst for 30

619 minutes at room temperature in the rocking plate, washed 3x10 min in PBS and

620 mounted in DAKO mounting medium supplemented with 2,5% Dabco (Sigma). To

621 quantify the number of cells that incorporated EdU and/or BrdU, Z-stacks were

622 acquired for each individual clone and orthogonal views used to count.

623

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 in M.O.M. Blocking kit, Stan- dard ABC kit, and ImmPACT DAB (Vector Laboratories) was used for the detection of HRP activity. Slides were then dehydrated and mounted using SafeMount (Labonord).

Supplemental 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 1,2,4,8,12,24 w 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 1w and 2w, five independent experiments at 4w, three independent experiments at 8w, two independent experiments at 12w and four independent experiments at 24 w. 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 1w and 2w, five independent experiments at 4w, three independent experiments at 8w, four independent experiments at 12w and three independent experiments at 24 w (see Data Source) .

For the clonal persistence of the K14-CreER/Rosa-YFP mice (Fig. 2e and Extended Data Fig.2), we counted 167,176,129,100,47,246 clones in interscale and

658   184,109,75,66,19,103 clones in scale from 4,5,5,5,2,4, independent experiments at

659   1,2,4,8,12,and 24 w respectively. For 24w, we counted several areas per mice as the

660   number of clones was reduced (see Data Source).

661   For the clonal persistence of the Inv-CreER/Rosa-YFP mice (Fig. 2e and Extended Data

662   Fig.2), we counted 138,95,25,31,76,54 clones in interscale and 12,17,7,8,20,10 clones

663   in scale from 2,4,2,3,4,and 3 independent experiments at 1,2,4,8,12 and 24 w

664   respectively. For 12 and 24w, we counted several areas per mice as the number of

665   clones was low (see Data Source)

666

667   For the analysis of the clone size of the Inv-CreER/Rosa-SmoM2 mice (Fig. 3b,f, h,

668   Extended Data Fig. 5-6), we counted clones (both in scale and interscale) from two

669   independent experiments at 1w and 2w, from 4 independent experiments at 4w, from 6

670   independent experiments at 8w, from 6 independent experiments at 12w and from 4

671   independent experiments at 24 w.

672   For the clonal persistence of the Inv-CreER/Rosa-SmoM2 mice (Extended Data Fig 5-

673   6), we counted 65,39,71,51,27,18 clones in interscale and 67,27,47,31,12 and 6 clones

674   in scale from 2,2,4,3,2 and 2 independent experiments at 1,2,4,8,12 and 24w

675   respectively.

676   For the analysis of the clone size of the K14-CreER/Rosa-SmoM2 mice (Fig. 4b,f, h,

677   Extended Data Fig 6- 7), we counted clones (both in scale and interscale) from 3

678   independent experiments at 1w, from 2 independent experiments at 2w, 4w, from 6

679   independent experiments at 8w, from 4 independent experiments at 12w and from 2

680   independent experiments at 24w.

681   For the clonal persistence of the K14-CreER/Rosa-SmoM2 mice (Extended Data Fig.6-

682   7), we counted 122,63,81,79,74 and 68 clones in interscale and 89,46,37,42,31 and 16

683   clones in scale from 4,3,4,4,4 and 4 independent experiments at 1,2,4,8,12 and 24w

684   respectively.

685

686

687   For the cell proliferation kinetics experiments in the Inv-CreER/Rosa-SmoM2 mice

688   (Fig. 3c, e): At 4w post-induction, we counted 33 clones from 3 independent

689   experiments for 2 days of continuous BrdU, 30 clones from 2 independent experiments

690   for 4 days of continuous BrdU, 33 clones from 2 independent experiments for 6 days of

691   continuous BrdU. At 8w post-induction, we counted 41 clones from n=3 mice for 2 day

692 of continuous BrdU, 16 clones from 2 independent experiments for 4 day of continuous
693 BrdU, 30 clones from 2 independent experiments for 6 day of continuous BrdU and 24
694 clones from 2 independent experiments for 8 day of continuous BrdU. At 12w post-
695 induction, we counted 19 clones from 2 independent experiments for 2 day of
696 continuous BrdU, 26 clones from 2 independent experiments for 4 day of continuous
697 BrdU, 27 clones from 2 independent experiments for 6 day of continuous BrdU and 31
698 clones from 2 independent experiments mice for 8 day of continuous BrdU. For the 2w
699 post-induction data point, we use solely continuous BrdU incorporation, and counted 54
700 clones from 2 independent experiments.

701 For the cell proliferation kinetics experiments in the K14-CreER/Rosa-SmoM2 mice
702 (Fig. 4c,e): At 4w post-induction, we counted 56 clones from 3 independent
703 experiments for 2 days of continuous BrdU, 39 clones from 3 independent experiments
704 for 4 days of continuous BrdU, 29 clones from 3 independent experiments for 6 days of
705 continuous BrdU. At 8w post-induction, we counted 30 clones from 2 independent
706 experiments for 2 days of continuous BrdU, 25 clones from 2 independent experiments
707 for 4 days of continuous BrdU, 63 clones from 3 independent experiments for 6 days of
708 continuous BrdU and 41 clones from 3 independent experiments for 8 days of
709 continuous BrdU. At 12w post-induction, we counted 20 clones from 2 independent
710 experiments for 2 days of continuous BrdU, 21 clones from 2 independent experiments
711 for 4 days of continuous BrdU, 28 clones from 2 independent experiments for 6 days of
712 continuous BrdU and 26 clones from 2 independent experiments for 8 days of
713 continuous BrdU.

714

715 For the quantification of the clone morphology in absence of p53 interscale (Fig.5c).
716 For K14-CreER/Rosa-SmoM2/p53fl/fl mice   186,217,90,343,452 and 543 clones from
717 3,3,2,3,5 and 5 independent experiments and for Inv-CreER/Rosa-SmoM2/p53fl/fl
718 95,98,199,271,263 and 210 clones from 3,3,3,4,4 and 4 independent experiments were
719 analysed at 1,2,4,8,12 and 24 w respectively. In the quantification in the scale region
720 (Extended Data Fig.8b) for K14-CreER/Rosa-SmoM2/p53fl/fl 178,204,100,132,232
721 and 120 clones were counted from 3,3,2,3,5,and 5 independent experiments 1,2,4,8,12
722 and 24 w respectively. For Inv-CreER/Rosa-SmoM2 82,127,167,136,62 and 153 clones
723 were counted from 2,3,3,4,4 and 5 independent experiments 1,2,4,8,12 and 24 w
724 respectively.

725

726

For the cell proliferation kinetics experiments in the Inv-CreER/Rosa-SmoM2/p53fl/fl mice (Fig. 5f) at 12w post-induction 34 clones from 3 independent experiments were counted. For the cell proliferation kinetics experiments in the K14-CreER/Rosa-SmoM2/p53fl/fl mice (Fig. 5f) at 12w post-induction 44 clones from two independent experiments were counted.

For the clonal persistence experiments in Inv-CreER/Rosa-SmoM2/p53fl/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 w and in K14-CreER/Rosa-SmoM2/p53fl/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 12w respectively (Extended Data Fig.8e). (See Data Source)

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Extended Data Figure legends

764

765 **Extended data Figure 1. The fate of oncogene targeted clones is determined by the**

766 **initial targeted cell (SC or CP) and their location in scale or interscale regions**

767 (a) Orthogonal view used to quantify the number of clones, cells stained with 4-

768 integrin/SmoM2. (left). Quantification of the number of clones induced 1 week after

769 Tamoxifen administration in scale and interscale regions in K14-CreER/Rosa-SmoM2

770 (n=4 animals, 0.1mg Tamoxifen) and Inv-CreER/ Rosa-SmoM2 (n=3 animals, 2.5 mg

771 Tamoxifen) (right). (b) Immunostaining for β4-integrin and SmoM2 in K14-

772 CreER/Rosa-SmoM2 and Inv-CreER/Rosa-SmoM2 clones located in the scale and

773 interscale regions, 8w after oncogene activation. (c) Immunostaining for the

774 differentiation marker keratin-10, K10, and SmoM2 in K14-CreER/Rosa-SmoM2 and

775 Inv-CreER/Rosa-SmoM2 clones 8w after oncogene activation, showing absence of

776 differentiated cells in K14-CreER/Rosa-SmoM2 clones and alteration of the

777 differentiation in Inv-CreER/Rosa-SmoM2 clones. Hoechst nuclear staining is

778 represented in blue; Scale bars, 10μm.

779

780 **Extended data Figure 2. Evolution of K14-CreER/Rosa-YFP and Inv-**

781 **CreER/Rosa-YFP clones in scale and interscale regions**

782 (a) Whole mount immunostaining for YFP/K31 in K14-CreER/Rosa-YFP mice and Inv-

783 CreER/Rosa-YFP mice upon Tamoxifen administration. (b) Scheme representing the

784 area of tail epidermis (area comprised by 6 groups of triplets of hair follicles,

785 highlighted in black) that is used to quantify the clone number and persistence. (c)

786 Distribution of K14-CreER/Rosa-YFP and Inv-CreER/Rosa-YFP total clone sizes as

787 measured by total cell content of surviving clones, imaged by confocal microscopy on

788   whole-mount tail epidermis from 1 to 24 weeks following Tamoxifen administration.

789   The number of analyzed clones is indicated for each time point. Hoechst nuclear

790   staining is represented in blue; scale bars, 100µm. Histograms and error bars represent

791   the mean and the standard error of the mean (s.e.m)

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796   **Extended data Figure 3. The interscale is maintained by two cell populations**

797   **during homeostasis.**

798   (a) Evolution in time of the total labelled cell fraction under three hypotheses. For a

799   perfect single population of equipotent balanced progenitors, the labelled cell fraction

800   remains constant. For a single population of equipotent balanced progenitors displaying

801   short-term priming, the labelled cell fraction increases transiently for the cells primed to

802   divide, and decreases transiently for the cells primed to differentiate, but after the

803   priming period, both fractions remain constant at different values. For two populations

804   organised in a hierarchy, the labelled fraction of the progenitors decreases continuously

805   to zero, while the labelled fraction of the stem cells continuous increases to reach a

806   steady state value, corresponding to its average progeny size. (b) Cumulative basal

807   clone size distribution of Inv-CreER/Rosa-YFP clones at homeostasis in the interscale

808   upon Tamoxifen administration. (c) Cumulative basal clone size distribution of K14-

809   CreER/Rosa-YFP clones at homeostasis in the interscale upon Tamoxifen

810   administration. Clonal distributions are plotted in log-plot, error bars indicate S.D.,

811   thick lines are the model prediction and shaded area indicate 95% confidence intervals

812   in the model prediction.

813

814

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816

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- 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.

836

Extended data Figure 5. Clonal dynamics of interscale InvSmoM2 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 1w to 24w 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 double-labelled cells as a function of basal clone size at 8w for Inv-CreER/Rosa-SmoM2 clones, for 2 (left), 4 (centre) and 6 (right) days of continuous BrdU incorporation. (e) Immunostaining for β4-integrin, SmoM2 and active-caspase-3 in Inv-CreER/Rosa-SmoM2 clones at 8w post-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 8w post-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 8w after SmoM2-activation. (h) Percentage of dysplastic, hyperplastic and normally differentiating Inv-CreER/Rosa-SmoM2 clones presenting apoptosis in basal and suprabasal compartments 8w 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 8w (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 is statistically different from the random theory, showing that

32

863   apoptosis clusters in certain clones at a given time point. (j) Short-term fate outcome of

864   progenitors in Inv-CreER/Rosa-SmoM2 clones at 8w, as assessed by using EdU as a

865   clonal marker. We count only cell doublets and classify them as either basal-basal,

866   basal-suprabasal, or suprabasal-suprabasal. (n=47 clones from 3 independent

867   experiments). Immunostaining for β4-integrin, EdU and SmoM2 showing the different

868   type of cell fate outcomes found in Inv-CreER/Rosa-SmoM2 clones. D: dysplasia; H:

869   hyperplasia; N: normal differentiation. Hoechst nuclear staining is represented in blue;

870   Scale bars, 10μm. Histograms and error bars represent the mean and the s.e.m.

871

872   **Extended data Figure 6. Clonal dynamics of Inv-CreER/Rosa-SmoM2 and K14-**

873   **CreER/Rosa-SmoM2 clones in the scale are similar**

874   (a) Evolution of mean surviving basal clone sizes (top) and labelled cell fraction

875   (bottom), for K14-CreER/Rosa-SmoM2, in the scale. (b) Evolution of mean surviving

876   basal clone sizes (top) and labelled cell fraction (bottom), for Inv-CreER/Rosa-SmoM2,

877   in the scale. Whereas the interscale clones show net expansion, scale clones, both Inv-

878   CreER and K14-CreER, show near balance at the population level. (c) Evolution of the

879   persistence of K14-CreER/Rosa-SmoM2 (green) and Inv-CreER/Rosa-SmoM2 (purple)

880   clones in the scale. Strikingly, and in contrast to the interscale, both K14 and Involucrin

881   clones have the same persistence. (d) Mean basal clone size, normalised by the mean

882   clone size at 1w for both Inv-CreER and K14-CreER clones. Even though one can see

883   on panel (a) and (b) that the final clone size is higher in K14, this is fully explained by

884   short-term differences in fate during the first week indicative of short-term priming for

885   K14. Correspondingly, the evolution of the labelling fraction is very similar for K14

886   and Involucrin in scale. Therefore, K14-CreER/Rosa-SmoM2 and Inv-CreER/Rosa-

887   SmoM2 in scale display the same long-term kinetics upon oncogenic activation,

888 consistent with the one-population model uncovered at homeostasis. Error bars

889 represent the s.e.m.

890

891 **Extended data Figure 7. Clonal dynamics of interscale K14-CreER/Rosa-SmoM2**

892 **clones is consistent with two populations**

893 (a) Distribution of K14-CreER/Rosa-YFP (black) and K14-CreER/Rosa-SmoM2 (red)

894 clone sizes as measured by total cell content, imaged by confocal microscopy on whole

895 mount tail epidermis from 1w to 24w post-induction. The number of clones analyzed

896 for K14-CreER/Rosa-SmoM2 is indicated in Fig 4b; the number of clones counted in

897 K14-CreER/Rosa-YFP is as indicated in Fig. 2a. (b) Evolution of the clonal persistence

898 and (c) labelled cell fraction for K14-CreER/Rosa-SmoM2 clones in the interscale. (d)

899 Fraction of EdU/BrdU double-labelled cells as a function of basal clone size at 8w for

900 K14-CreER/Rosa-SmoM2 clones, for 2 (left), 4 (centre) and 6 (right) days of

901 continuous BrdU incorporation. (e) Immunostaining for β4-integrin, SmoM2 and

902 active-caspase-3 in K14-CreER/Rosa-SmoM2 clones 8w after SmoM2 activation. (f)

903 Percentage of BCC, dysplastic, hyperplastic and normally differentiating clones

904 presenting at least one active-caspase-3 positive cell at 8w post-induction (n=117 clones

905 analysed from 4 independent experiments). (g) Quantification of the number (%) of

906 basal and suprabasal apoptotic cells in dysplastic, hyperplastic and normally

907 differentiating Inv-CreER/Rosa-SmoM2 clones 8w after SmoM2-activation. (h)

908 Percentage of dysplastic, hyperplastic and normally differentiating Inv-CreER/Rosa-

909 SmoM2 clones presenting basal and suprabasal apoptosis 8w after oncogenic activation.

910 (i) Cumulative distribution of the fraction of basal apoptosis as a function of basal cell

911 number in a K14-CreER/Rosa-SmoM2 clone at 8w (data in red). The green line is the

912 expected theoretical distribution of apoptotic fraction if apoptosis occurred randomly

913   (following a Poisson process), in any clone with the same probability. The data is

914   statistically different from the random theory, showing that apoptosis clusters in certain

915   clones at a given time point. (j) Quantification of EdU/BrdU double-labelled cells as a

916   function of the period of continuous BrdU incorporation for large K14 clones at 4w

917   (black), 8w (orange) and 12w (red) post clonal induction. The dashed lines represent the

918   model fit (Supplementary Theory). (k) Whisker plot of the suprabasal clone size in the

919   interscale. The boxes delineate the first and third quartiles of the data, while the

920   whiskers delineate the first and last decile of the data at a given time point. The thick

921   continuous line is the best fit from the model from which we extract the probability of

922   fate choices in tumor SC and progenitors, displayed in Fig.4g. The thin lines represent

923   the mean clone sizes of stem cells (top curve) and progenitors (bottom curve) if they

924   were alone. (l) Short-term fate outcome of progenitors in K14-CreER/Rosa-SmoM2

925   clones at 8w, as assessed by using EdU as a clonal marker. We count only cell doublets

926   and classify them as either basal-basal, basal-suprabasal, or suprabasal-suprabasal.

927   (n=49 clones from 3 independent experiments) Immunostaining for β4-integrin, EdU

928   and SmoM2 in K14-CreER/Rosa-SmoM2 hyperplastic/dysplastic clones (top) and in

929   BCC (bottom panel). BCC: basal cell carcinoma; D: dysplasia; H: hyperplasia; N:

930   normal differentiation; SB: suprabasal. Hoechst nuclear staining is represented in blue;

931   Scale bars, 10μm. Error bars represent the s.e.m.

932

933   **Extended data Figure 8. Effect of p53 deletion in the cellular dynamics of CPs and**

934   **SCs**

935   (a) IHC staining for p53 in Inv-CreER/Rosa-SmoM2 and K14-CreER/Rosa-SmoM2

936   clones 12 w post-induction (b) Quantification of normal, hyperplastic, dysplastic and

937   BCC clones in scale region of K14CreER/Rosa-SmoM2/p53fl/fl and Inv-CreER/Rosa-

938   SmoM2/p53fl/fl mice. Description of number of counted clones is found in the method

939   section.(c) Distribution of clone sizes as measured by total cell content, imaged by

940   confocal microscopy on whole mount tail epidermis. The number of clones analysed is

941   indicated in Fig 5d. Clone merger events were observed after 12w following oncogenic

942   activation in K14Cre-ER/Rosa-SmoM2/p53fl/fl preventing the accurate quantification

943   of clonal persistence and clone size at long times. (d) Comparison of basal clone size

944   distribution of Inv-CreER/Rosa-SmoM2/p53fl/fl vs. Inv-CreER/Rosa-SmoM2 and K14-

945   CreER/Rosa-SmoM2/p53fl/fl vs. K14-CreER/Rosa-SmoM2 at 8w and 12w upon

946   Tamoxifen administration. (e) Evolution of the clonal persistence of Inv-CreER/Rosa-

947   SmoM2/p53fl/fl and K14-CreER/Rosa-SmoM2/p53fl/fl clones. (f) Immunostaining of

948   active-caspase-3 and SmoM2 8w post-induction in Inv-CreER/Rosa-SmoM2/p53fl/fl

949   (g) Quantification of the proportion of apoptotic clones in Inv-CreER/Rosa-

950   SmoM2/p53fl/fl (n=90 clones from 3 independent experiments), and K14-CreER/Rosa-

951   SmoM2/p53fl/fl (n=82 animals from 3 independent experiments) 8w post-induction.

952   Hoechst nuclear staining is represented in blue; Scale bars, 10μm. Error bars represent

953   the s.e.m.

954

955   **Extended data Figure 9. Model of BCC initiation**

956   Activation of SmoM2 in SCs leads to the generation of BCC due to an increase in cell

957   proliferation and resistance to apoptosis. However, activation of p53 in SmoM2-

958   expressing CPs restricts the progression of dysplastic clones to BCC by promoting

959   apoptosis and cell cycle arrest. Deletion of p53 in CPs allows them to progress into

960   BCC.

961

a

K14-CreER /Rosa-SmoM2

K14 × CREER

STOP × SmoM2 YFP

Inv-CreER/Rosa-SmoM2

Involucrin × CREER

STOP × SmoM2 YFP

b

K14-CreER /Rosa-SmoM2   Inv-CreER/Rosa-SmoM2

Tail — β4 SmoM2

Ear — β4 SmoM2

c

K14-CreER/ Ptch1KO   Inv-CreER/ Ptch1KO

K14 β4

d

Tumor burden

K14CreER Ptch1 KO   InvCreER Ptch1 KO   *

BCC number/ length (mm)

K14CreER Ptch1 KO   InvCreER Ptch1 KO   **

e

2w   8w   12w   24w

K31 SmoM2 — K14-CreER/ Rosa- SmoM2

Inv-CreER/ Rosa- SmoM2

f

K14-CreER /Rosa-SmoM2

Clone morphology (%)

Interscale

Scale

Inv-CreER/Rosa-SmoM2

Interscale

Scale

Time post-induction (weeks)

■ Normal differentiation ■ Hyperplasia ■ Dysplasia ■ BCC

Sánchez-Danés et al. Figure 1

a K14-CreER/Rosa-YFP
Interscale
Scale

b Inv-CreER/Rosa-YFP
Interscale
Scale

Interscale: K14-CreER/Rosa-YFP and Inv-CreER/Rosa-YFP

c Fits

d every 15d $^{+5d}_{-2d}$

S → S S 3% $^{+4\%}_{-3\%}$
S → S P 94% $^{+6\%}_{-8\%}$
S → P P 3% $^{+4\%}_{-3\%}$

every 4d

P → P P 17% $^{+10\%}_{-8\%}$
P → P D 62% $^{+18\%}_{-22\%}$
P → D D 21% $^{+13\%}_{-11\%}$

every 4.5d $^{+1d}_{-1d}$

D → ○ shedding

e Predictions

Sánchez-Danés et al. Figure 2

a

Inv-CreER/Rosa-YFP   Inv-CreER/Rosa-SmoM2

1w   β4 YFP   β4 SmoM2

2w

4w

8w

b

Basal clone size (cells)

Inv-CreER/Rosa-YFP

Inv-CreER/Rosa-SmoM2

86   139   177   192

65   39

1w 2w 4w 8w 12w 24w

Basal clone size (cells)

c

Fraction of EdU+/BrdU+

Inv 4w
Inv 8w
Inv 12w

Continuous Brdu injection (days)

d

% Apoptotic cells/clone

D   H   N

e

Division rate (d⁻1)

Time post-induction (weeks)

f

Mean clone size

basal
suprabasal

Time post-induction (weeks)

g

P → P P   39%   +4% −5%

P → P D   45%   +11% −8%

P → D D   16%   +4% −7%

h

Cumulative distribution (%)

1w   2w   4w

8w   12w   24w

Basal clone size

Sánchez-Danés et al. Figure 3

a

K14-CreER/Rosa-YFP   K14-CreER/Rosa-SmoM2

1w   β4 YFP   β4 SmoM2

2w

4w

8w

b

K14-CreER /Rosa-SmoM2
K14-CreER/Rosa-YFP

Basal clone size (cells)   Basal clone size (cells)

48   28   40   149   97   79

1w   2w   4w   8w   12w   24w

c

Fraction of Edu⁺/Brdu⁺

K14 large 8w
K14 small 8w
Inv 8w

Continuous Brdu injection (days)

d

Apoptotic cells/clone (%)

BCC   D   H   N

e

Division rate (d⁻¹)

Time post-induction (weeks)

f

Mean basal clone size

Time post-induction (weeks)

g

S → S S   34% +10% -10%

S → S P   49% +21% -23%

S → P P   17% +12% -11%

h

Cumulative distribution (%)

1w   2w   4w
8w   12w   24w

Basal clone size

Sánchez-Danés et al. Figure 4

a

K14-CreER/Rosa SmoM2 /P53 fl/fl   Inv-CreER/Rosa SmoM2/P53fl/fl

Tail β4 SmoM2

Ear β4 SmoM2

b

8w   12w   24w

K14-CreER/Rosa SmoM2 /P53 fl/fl

Inv-CreER/Rosa SmoM2/P53fl/fl

c

K14-CreER / Rosa-SmoM2/P53fl/fl

Inv-CreER / Rosa-SmoM2/P53fl/fl

Clone morphology(%)

1w 2w 4w 8w 12w 24w

■ Normal differentiation ■ Hyperplasia ■ Dysplasia ■ BCC

d

K14 Inv

Basal clone size (cells)

Basal clone size (cells)

64 139 83 136 102 84 56 61 93 91

1w 2w 4w 8w 12w

e

K14 Inv

Apoptotic cells/clone (%)

BCC D H

f

Fraction double labelled EdU+/BrdU+ (%)

** *

Inv-CreER Rosa-SmoM2 p53 fl/fl
Inv-CreER Rosa-SmoM2
K14-CreER Rosa-SmoM2 p53 fl/fl
K14-CreER Rosa-SmoM2

g

K14 Inv

Mean clone size

0 4 8 12

Time post-induction (weeks)

Sánchez-Danés et al. Figure 5