

SOX2 confers tumour permissiveness in a specific skin progenitor population

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1 TITLE

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16 ABSTRACT

17 The continuous renewal of the skin relies on stem and progenitor cells, yet their differential
18 susceptibility to oncogenic mutations in cutaneous squamous cell carcinoma (cSCC) remains unclear.
19 Rapid cSCC develops in melanoma patients on BRAF inhibitors due to paradoxical MAPK activation. To
20 model this in mice, we use two complementary approaches: HRAS^{G12V} with a BRAF inhibitor to mimic
21 paradoxical MAPK activation, and BRAF^{V600E}, which drives MAPK hyperactivation without further
22 treatment. We target these mutations to the interfollicular stem and differentiation-committed
23 progenitors of the basal epidermis. While stem cells rapidly form tumours, progenitors exhibit long-
24 latency resistance despite retaining mutations and repopulating the basal layer. Ultimately, both
25 populations produce similar tumours, showing a shared transformation process. However, SOX2
26 uniquely upregulated in progenitor-derived tumours and is expressed in 20% of human cSCC,
27 indicating it might mark tumours arising from committed progenitors. Here, we show that SOX2
28 overexpression, along with MAPK activation, in progenitors induces a stem-like state and renders this
29 otherwise resistant population permissive to rapid transformation.

30 INTRODUCTION

31 Adult stem and progenitor cells located in the basal layer continuously regenerate to maintain the
32 mammalian interfollicular epidermis, ensuring its structural integrity and protection against the
33 external environment. Within this compartment, a stem cell population expressing K14/K5+ coexists
34 with a differentiation-committed progenitor population that additionally expresses differentiation
35 markers, such as IVL+. Two models have been proposed to explain the basal layer heterogeneity. One
36 describes the existence of two distinct basal progenitors that contribute differentially to epidermal
37 homeostasis, wound healing^{1,2}, and mechanical stretching³. The other proposes that the basal layer
38 heterogeneity arises from a continuum of cells at different levels of commitment towards
39 differentiation in response to cues from the surrounding environment⁴. These cell populations also
40 differ in their ability to initiate basal cell carcinoma^{5,6}, but whether they exhibit similar functional
41 divergence in cutaneous squamous cell carcinoma (cSCC) remains unclear.

42 Spontaneous human cSCC exhibits high mutational burden, heterogeneity and copy number
43 alterations accumulated with age and UV exposure⁷, including mutations in known drivers of cSCC⁸.
44 The skin hierarchy and spatial compartmentalisation typically restrict the proliferation of mutated
45 cells. However, external stressors and promoter events, such as wounding, inflammation, UV
46 exposure, and certain oncogenic mutations, can overcome these constraints, leading to
47 tumorigenesis^{3,9-13}. The most frequently observed mutations in cSCC accumulate in Notch (79.5%) and
48 TP53 (71%) signalling pathways¹⁰. Single mutations within these pathways alone are insufficient to
49 induce cSCC in mice, and additional genetic alterations involving the TGF β and MAPK signalling
50 pathways are typically required^{7,14-16}. Murine models have demonstrated the tumour's ability to arise
51 from different epidermal compartments, including the hair follicle, the sebaceous glands, and the
52 interfollicular epidermis^{9,14,15,17}. The complexity and slow tumour progression of these models have
53 limited mechanistic insights into skin carcinogenesis.

54 The most widely used model for cSCC is the well-established two-stage chemical carcinogen 7,12-
55 dimethylbenz[a]anthracene (DMBA), followed by the promoting agent 12-O-tetradecanoylphorbol-
56 13-acetate (TPA)^{9,18}. This leads to rapid tumorigenesis from the *Lgr6+* hair follicle stem cells,
57 predominantly driven by *Hras*^{Q61L} and *Trp53* mutations^{9,19}. While *HRAS* mutations are relatively
58 uncommon in spontaneous human cSCC (9-16%), they are frequent in healthy skin as passenger
59 mutations^{8,14,20}. Besides, a rapid onset of HRAS-driven cSCC is commonly found in ~60% of melanoma
60 patients treated with BRAF^{V600E} inhibitors (BRAFi)²⁰⁻²². This is attributed to the paradoxical activation
61 of the MAPK signalling pathway driven by BRAFi, which promotes BRAF-CRAF dimers to synergise with
62 pre-existing *HRAS* mutations in the skin^{22,23}.

63 Here, we assess the mechanisms that confer permissiveness to initiate cSCC in the interfollicular stem
64 and progenitor basal populations. We develop a suite of genetically engineered mouse models to
65 study the aetiology of rapidly growing cSCC driven by hyperactivation of the MAPK signalling pathway.
66 We define a tumour-primed and a tumour-resistant population and uncover a shared transcriptional
67 programme responsible for transformation. Our findings demonstrate the different susceptibility of
68 basal populations to cSCC transformation and reveal that SOX2 renders the otherwise tumour-
69 resistant progenitor population susceptible to oncogenic transformation.

70 RESULTS

71 PARADOXICAL MAPK SIGNALLING ACTIVATION IS A TUMOUR PROMOTER IN CSCC

72 To study the susceptibility of the two different basal populations to transformation and tumour
73 formation, we set out to generate genetically engineered mouse models of cSCC that resembled
74 human disease. We drove the expression of *Hras*^{G12V} in the basal stem and progenitor populations by
75 using tamoxifen-inducible Cre recombinase expressed under the control of the *Krt14* promoter (*Krt14*-
76 CreERT2; hereafter K14) or the *Ivl* promoter (*Ivl*-CreERT; hereafter Ivl), respectively (Fig. 1a).

77 Expression of one copy of the *Hras*^{G12V} allele (hereafter HRAS^{G12V}) in the K14+ or the IVL+ population
78 did not result in tumorigenesis (Fig. 1b and 1c). This result is consistent with human studies
79 demonstrating that normal-looking skin carries multiple genetic mutations, including oncogenes, and
80 that a promotion event is required to trigger transformation^{8,9}. Therefore, we applied topical TPA, a
81 well-known agent that promotes inflammation and stimulates epidermal proliferation, to our models
82 three times a week. However, this treatment was also insufficient for transformation, and only basal
83 MAPK signalling activation was seen in epidermis carrying one copy of HRAS^{G12V}, either with or without
84 TPA treatment (Fig. 1d). Two copies of the *Hras*^{G12V} were sufficient to drive higher levels of MAPK
85 activation in both models (Fig. 1d). However, when expressed in the K14+ population, mice became
86 unwell, likely due to the essential role of RAS proteins in the oesophagus and forestomach
87 gastrointestinal epithelium before any skin-related malignancies developed. Nevertheless,
88 K14:HRAS^{G12V/G12V} mice showed hyperproliferation of the skin basal layer, as evidenced by H&E and
89 increased Ki67 expression (Supplementary Fig. 1a). In the IVL+ basal population, two copies of the
90 allele drove tumorigenesis with a tumour-free survival of 24 days (Fig. 1c; Supplementary Fig. 1a).

91 Interestingly, melanoma patients treated with BRAFi, such as Dabrafenib, commonly develop fast-
92 growing cSCC within weeks of treatment, driven by preexisting *Hras* mutations and paradoxical
93 activation of the MAPK signalling pathway²⁰⁻²². Thus, we treated Ivl:HRAS^{G12V/+} and K14:HRAS^{G12V/+}
94 mice daily with the BRAFi Dabrafenib to mimic the patient dosing regimen. Notably, we confirmed

95 sustained MAPK activation in both models (Fig. 1d), and onset of tumour formation with a tumour-
96 free survival rate of 22 days for the K14:HRAS^{G12V/+} model and 58 days for the Ivl:HRAS^{G12V/+} model (Fig.
97 1b, c; Supplementary Fig. 1b). Hereafter, the tumour-free survival rate refers to the time from
98 oncogene induction to the first macroscopically change observed in the skin. BRAFi-promoted tumours
99 exhibited the classic cSCC hallmarks, including keratin pearls, parakeratosis, and nuclear dysplasia,
100 together with increased proliferation (Fig. 1e). Moreover, we also treated non-tumour-bearing mice
101 carrying one copy of the HRAS^{G12V} in the K14+ or Ivl+ populations at 180±5 days post-oncogene
102 induction with the BRAFi. Following treatment, these aged-treated mice quickly developed tumours
103 within days, which highlights the strong tumour-promoting effect of BRAFi in *Hras* mutant skin and
104 models the development of cSCC in melanoma patients with *Hras* mutations (Fig. 1f).

105 Overall, we have shown that MAPK activation drives SCC from stem and committed progenitor
106 epidermal populations. While one copy of *Hras*^{G12V}, combined with TPA, causes only basal MAPK
107 activation, two copies of the mutated gene are needed to achieve sufficient activation for
108 transformation. In contrast, BRAFi acts as a potent tumour promoter able to drive MAPK paradoxical
109 activation from a single copy of HRAS-mutated skin (Fig. 1g).

110 TUMOUR-PRIMED AND TUMOUR-RESISTANT POPULATIONS COEXIST IN THE SKIN

111 To gain further insights into the tumorigenic capacity of these distinct pools of progenitors, we used
112 the potent oncogenic mutation BRAF^{V600E/+} (hereafter BRAF^{V600E}), which encodes a different
113 downstream oncogenic effector of the pro-proliferative MAPK signalling pathway (Fig. 2a). We
114 generated a suite of mouse models expressing BRAF^{V600E} under the control of K14 and *Krt5* (K5-CreERT;
115 hereafter K5) promoters, targeting the stem-cell basal population, and the Ivl promoter, targeting the
116 committed basal and suprabasal populations (Fig. 2b; Supplementary Fig. 2a).

117 Upon BRAF^{V600E} induction in the stem-cell population (K5/K14+), cells rapidly activated the MAPK
118 signalling pathway without the need for further promotion (Supplementary Fig. 2b), resulting in fast-
119 growing tumours (9 and 10 days to tumour onset) (Fig. 2c, d). Reducing the dose and duration of the
120 tamoxifen-induction regimen in the K5:BRAF^{V600E} and K14:BRAF^{V600E} models delayed tumour onset, but
121 tumours still grew rapidly (Supplementary Fig. 2c). Transplantation of subcutaneous pieces from
122 K5:BRAF^{V600E}-derived tumours into C57BL/6J and NSG-II2 immunodeficient mice revealed cancer stem
123 cell potential and an ability to form secondary tumours (Supplementary Fig. 2d). Thus, this population
124 is permissive to tumour development and primed for oncogenic transformation. Therefore, we
125 termed it tumour-primed.

126 In contrast, despite BRAF^{V600E}-driven activation of MAPK signalling reaching similar levels in the
127 progenitor population (Ivl+) (Supplementary Fig. 2e), tumours exhibited a longer onset latency (125

128 days to tumour onset) and grew slowly (Supplementary Fig. 2c, e). This was consistent with tumours
129 showing an increased number of apoptotic regions, marked by caspase-3 (CASP3) and cPARP cleavage,
130 compared to tumours derived from the K14/K5+ population (Supplementary Fig. 2f). The longer
131 latency and increased experimental time allowed this model to develop multiple lesions at various
132 sites, including the back, paws, ears, and lips, while the stem-cell models developed only one tumour
133 on their back at the precise area of tamoxifen application at clinical end point (Fig. 2f). The longer
134 latency of tumours arising from the progenitor population suggests that this population requires
135 additional cooperating events to facilitate tumour onset, so we termed this population tumour-
136 resistant.

137 To promote cSCC in the IVL+ tumour-resistant population, we exposed the back of the *Ivl:BRAF^{V600E}*
138 mice to mild UV [four weekly doses, six standard erythema doses (SED)]. This did not accelerate
139 tumour onset nor increase tumour burden, despite UV being a risk factor for cSCC (Fig. 2c, f). Similarly,
140 topical treatment with the inflammation-promoting agent TPA (three times per week) did not
141 accelerate tumorigenesis (Supplementary Fig. 2g). No statistical differences in tumour onset were
142 seen between *K14:BRAF^{V600E}* cohorts housed in two different establishments or by sex (Supplementary
143 Fig. 2h, i). However, the *Ivl:BRAF^{V600E}* model showed a slightly shorter tumour onset in the cohort
144 housed at the CRUK SI compared to CRUK MI, while no differences were seen between sexes
145 (Supplementary Fig. 2j, k).

146 TUMOURS SHARE HISTOPATHOLOGICAL FEATURES

147 We characterised the histopathological features of tumours originating in the *K5:BRAF^{V600E}*,
148 *K14:BRAF^{V600E}* and *Ivl:BRAF^{V600E}* models and found remarkable similarities among them. Tumours were
149 formed by vertical columns of atypical keratinocytes, including hyperchromatic and pleomorphic
150 nuclei (Fig. 2g-i; Supplementary Fig. 3a). Detailed histopathological analysis showed distinctive
151 features of cSCC. These included enlarged interfollicular epidermis and cornified layers with an
152 aberrant accumulation of extracellular keratin (keratin pearls), nuclear atypia, and incomplete
153 maturation of keratinocytes (parakeratosis) as the keratinocytes in the cornified layer retain their
154 nuclei (Fig. 2g-i).

155 Regardless of the cell of origin, tumours presented dyskeratosis and abnormal expression of
156 differentiation markers. They show an increase in the number of layers expressing the basal markers
157 KRT5 and KRT14, as well as the suprabasal markers KRT1 and IVL (Supplementary Fig. 3b-d). In the
158 most advanced cases, all markers were expressed throughout the entire epidermal layer, indicating a
159 complete loss of cellular hierarchy and disruption of multi-layer organisation. Moreover, the increase
160 in Ki67 expression denotes epidermal hyperproliferation and hyperplasia of the interfollicular

161 epidermis (Supplementary Fig. 3e). These lesions were highly proliferative, recapitulating the rapid
162 onset and histopathology observed in human cSCC.

163 Overall, despite both populations residing in the basal layer and sharing the microenvironment, they
164 show profound differences in susceptibility to transformation upon oncogene induction. Notably,
165 BRAF^{V600E} mimics the MAPK hyperactivation seen in cSCC from patients treated with BRAFi and
166 harbouring *Hras* mutations in normal-looking skin, making it a valuable model for studying the disease.

167 THE IVL+ PROGENITOR POPULATION IS TUMOUR-RESISTANT

168 In homeostasis, IVL+ marks the suprabasal layer and a minority population in the basal layer that is
169 committed to differentiation, although its differentiation does not prevent cell cycle entry^{1,4}. We
170 aimed to understand how this population resists tumorigenic transformation after oncogene
171 expression.

172 We followed IVL+ and KRT14+ expressing cells by co-expressing the *Rosa26* LSL-tdTomato fluorescent
173 protein (hereafter tdRFP) reporter in the presence and absence of BRAF^{V600E} (Fig. 3a). We validated
174 the fidelity of our lineage tracing models by co-immunofluorescence imaging of RFP and KRT14,
175 followed by confocal microscopy analysis. In the absence of oncogene activation, the K14:tdRFP model
176 labelled all basal layer cells (Supplementary Fig. 4a), whereas the Ivl:tdRFP model labelled only a few
177 basal cells but most of the suprabasal cells (Fig. 3b). However, when BRAF^{V600E} was expressed in the
178 IVL+ population (Ivl:BRAF^{V600E}-tdRFP), we observed a progressive expansion of this population in the
179 basal layer, with RFP and IVL proteins colocalising, further validating the model's fidelity (Fig. 3c).

180 We then performed lineage-tracing experiments at various time points to compare the RFP-labelled
181 populations between control and oncogene-expressing skin in K14 and Ivl-driven models. These time
182 points, marked by tdRFP and oncogene induction, span from before visible skin changes to day 160 or
183 the clinical endpoint (Fig. 3d).

184 In the Ivl-tdRFP control model (no oncogene), a ~50% reduction in the label-retaining population in
185 the basal layer was observed from day 8, consistent with committed cell differentiation and
186 delamination (Fig. 3e, f). Besides, suprabasal cells that were not located above an RFP label basal
187 cluster turned over, and those regions progressively lost the tdRFP label. After that initial drop, the
188 labelled population remained stable for up to 160 days, marking a long-residing, stable population of
189 the basal layer that was able to self-renew and contribute with its progeny to the suprabasal layers,
190 consistent with previous data^{1,4}.

191 In contrast, upon oncogene activation in the Ivl:BRAF^{V600E}-tdRFP model, we saw a rapid ~40% increase
192 in the clonal persistence of labelled basal cells, which quickly outcompeted the unlabelled population

193 (Fig. 3e, f). At day 15, the entire basal layer was labelled to an equivalent level to the K14+ labelled
194 population, and tumours sampled at end point retained tdRFP (Fig. 3e; Supplementary Fig. 4a). IVL
195 protein IHC staining also confirmed that IVL+ cells became the majority population in the basal layer
196 in the presence of BRAF^{V600E}, whilst retaining the expression of this differentiation commitment
197 marker (Supplementary Fig. 4b). Moreover, in the Ivl:BRAF^{V600E}-tdRFP model, there was an increase in
198 epidermal thickness compared to the control, which decreased over time but remained significant
199 throughout the study (Fig. 3g). Notably, despite most basal cells carrying the oncogenic mutation,
200 constraints remained in place, resisting transformation.

201 In the K14-driven models, the standard induction regimen (4 times over 7 days) labelled all cells in the
202 basal layer and their progeny in the suprabasal layer to saturation (Supplementary Fig. 4c). Therefore,
203 we repeated the study at the lower induction dose regimen (single application), allowing us to study
204 the dynamics of single clones. By day 8, quantification of RFP+ cells revealed an almost two-fold
205 increase in the basal population in the oncogene-carrying group, which translated into rapidly growing
206 tumours by day 15 (Fig. 3h, i). Tumours and hyperplastic lesions also exhibited increased expression
207 of the IVL-suprabasal marker throughout all layers, indicating a previously observed breakdown of the
208 skin hierarchy from early timepoints (Supplementary Fig. 4d).

209 Varying levels of MAPK signalling activation could not explain the difference in tumour permissivity
210 observed between these populations, as high levels of pERK were observed in both models from early
211 time points (Supplementary Fig. 4e).

212 Together, these data demonstrate that oncogene expression in the committed IVL+ population led to
213 a clonal proliferation and expansion similar to the stem-cell K14+ population. However, it stays
214 tumour-restrictive for a longer period. This indicates that transformation constraints are still active in
215 the IVL+ population, even though oncogene-bearing cells have colonised the basal layer.

216 CSCC SHARED TRANSCRIPTIONAL PROFILE

217 Given the histological similarities, we next conducted transcriptional profiling of the total tumour
218 tissue to identify potential differences and vulnerabilities in these tumours. After batch correction
219 (Supplementary Fig. 5a), we merged the K5:BRAF^{V600E} and K14:BRAF^{V600E} tumour samples as they
220 clustered independently of the model, and our analysis returned no differentially expressed genes
221 (Supplementary Fig. 5b, c). Note that these tumours arose from the same cell populations
222 simultaneously expressing KRT5 and KRT14. Then, we compared K14/K5:BRAF^{V600E}- and Ivl:BRAF^{V600E}-
223 derived tumours against normal skin from control littermates. Unsupervised clustering and principal
224 component analysis (PCA) showed that the tumours separated from the normal skin samples (Fig. 4a).

225 Both tumour groups revealed a large number of transcriptionally upregulated genes when compared
226 to normal skin (Fig. 4b, c). Notably, most upregulated genes (82.8%) were shared between the
227 K14/K5:BRAF^{V600E} and Ivl:BRAF^{V600E} tumours (Fig. 4d), of which 253 were expressed significantly higher
228 in tumours compared to normal skin (log fold change >3 and padj <0.001; Supplementary Data 1).
229 Digital sorting of the bulk transcriptome and deconvolution into previously defined skin populations²⁴,
230 revealed that most cells (~70%) were keratinocytes belonging to the permanent part of the epidermis,
231 with some samples also containing keratinocytes derived from hair follicles (Supplementary Fig. 5d).
232 Fibroblasts made up to 10% of the total transcriptome analysed, and overall tumour composition
233 across K14, K15, or Ivl-derived tumours was similar.

234 Gene set enrichment analysis highlighted commonly upregulated signatures, including those
235 associated with the *Myc*, *Lgr5* and *Lgr6* wound healing response²⁵, embryonic epidermis²⁶, and the
236 human-derived cSCC tumour-specific keratinocyte population²⁷ among others (Fig. 4e; Supplementary
237 Fig. 5e). Importantly, no major differences were seen in pathway enrichment between models. It is
238 possible that these results may reflect variations in epithelial/stroma ratios. Therefore, to validate the
239 transcriptional findings in the epithelial cells, we assessed protein levels using IHC directly in the tissue.

240 We examined the expression of individually selected differentially expressed genes that were enriched
241 in the tumours compared to normal skin, including *Myc*, the cSCC markers *Krt6a* and *Krt6b*²⁸, along
242 with *Ly6a* and *Anxa1* ligand (Fig. 4f). ANXA1 is normally secreted by wounded epithelia during tissue
243 regeneration²⁵ and is enriched in the tumour-specific keratinocyte signature²⁷. Interestingly, a model
244 for tissue injury-sensing uncovered the role of IL24, produced at wound sites in response to hypoxia,
245 in promoting epithelial proliferation and re-epithelialization via pSTAT3 activation²⁹. This pathway may
246 also extend to cSCC, as evidenced by increased expression of the hypoxia sensor HIF1 α , IL24, and
247 pSTAT3 in our models (Fig. 4f and 4g). We also observed elevated *Il6*, which stimulates the release of
248 proinflammatory cytokines from the cutaneous microenvironment in response to wounding,
249 activating TGF β receptor signalling and the STAT3 signal transduction pathway^{29,30}.

250 These transcriptomic results were validated by RNA *in situ* hybridization for *Ly6a*, and IHC for MYC,
251 ANXA1 and pSTAT3 in our models driven by BRAF^{V600E} and HRAS^{G12V} in combination with BRAFi (Fig.
252 4g, h; Supplementary Fig. 6a). Similar findings were seen in tumours originating from the hair follicle
253 stem cells upon MAPK signalling hyperactivation and deletion of the TGF β receptor 1 (*Alk5*)
254 (Lgr5:BRAF^{V600E}-ALK5^{fl/fl}, Lgr5:HRAS^{G12V/G12V}-ALK5^{fl/fl}, Lgr5:KRAS^{G12D/+}-ALK5^{fl/fl})¹⁴ and deletion of *Trp53*
255 and *Notch2* (Lgr5:TRP53^{fl/fl}-NOTCH2^{fl/fl}) (Supplementary Fig. 6b).

256 Interestingly, oncogene expression in the tumour-primed population (K14-BRAF^{V600E}-tdRFP) exhibited
257 strong pSTAT3 and MYC activation from day 8 in hyperplastic lesions, which persisted later in the
258 tumours (Supplementary Fig. 6c). In contrast, oncogene expression in the tumour-resistant population
259 (Ivl-BRAF^{V600E}-tdRFP) exhibited high pSTAT3 levels at early time points, but these diminished and
260 became limited to small clones by day 28, with only weak MYC activation observed across the time
261 course (Supplementary Fig. 6c).

262 Overall, these results reveal shared molecular and transcriptional hallmarks of epidermal
263 transformation that rely on MYC and pSTAT3 activation, independent of the cell of origin, tumour
264 latency, or oncogenic driver mutations.

265 SOX2 IS EXPRESSED IN TUMOURS DERIVED FROM THE IVL+ POPULATION

266 We next assessed whether transcriptional profiling could identify potential markers to distinguish
267 tumours arising from the different cell populations. Thus, we compared the transcriptomic profile of
268 tumours from K5/K14:BRAF^{V600E} and Ivl:BRAF^{V600E} model, which revealed only a handful of
269 differentially expressed genes (Fig. 5a). Notably, SOX2 emerged as a key transcriptional difference,
270 being highly enriched in Ivl:BRAF^{V600E} tumours, relative to K5/K14:BRAF^{V600E} tumours. SOX2 is a super
271 pioneer transcription factor capable of binding to closed chromatin through its ability to interfere with
272 the maintenance of DNA methylation³¹. Furthermore, SOX2 is commonly used as a marker of cancer
273 stem cells and is upregulated in ~20% of cSCC patients, where it rewires cells for tumour initiation and
274 growth^{32,33}.

275 We confirmed SOX2 upregulation in Ivl:BRAF^{V600E} tumours compared to K5:BRAF^{V600E} or K14:BRAF^{V600E}
276 tumours by IHC (Fig. 5b). Similar results were obtained in HRAS-driven tumours, where SOX2
277 expression was only seen in those arising from the IVL+ population (Fig. 5c). SOX2 expression was
278 negligible in normal skin during homeostasis or in TPA-treated skin, suggesting that its expression is
279 not linked to inflammation (Fig. 5d). SOX2 in combination with CD34 have been proposed to mark
280 “tumour initiating cells” in cSCC³³. Thus, we stained K14 and Ivl-derived tumours taken at clinical
281 endpoint for CD34 but found no expression (Supplementary Fig. 7a). We did not detect SOX2
282 expression or CD34 in either of the models at earlier time points, while the skin remained histologically
283 normal (Supplementary Fig. 7b, c).

284 We extended our investigation to a broader suite of models. We found SOX2 expressed in DMBA/TPA-
285 induced tumours, as previously shown³³, but not in tumours originating from *Lgr5+* cells (hair follicle
286 bulge) driven by different oncogene combinations (Supplementary Fig. 7d, e). We also assessed the
287 expression of SOX9, a distinct regulator of chromatin accessibility that controls hair follicle stem cell
288 fate and is activated during tumorigenesis³⁴. In contrast to SOX2, SOX9 show not cell specificity, as it

289 expression expanded from hair follicles in normal skin to the basal layer in tumours across all models,
290 regardless of cell of origin (Supplementary Fig. 8a). The suprabasal markers KRT4 and KRT13,
291 commonly expressed in skin homeostasis, were also differentially upregulated at RNA level in the
292 tumours derived from the *Ivl:BRAF^{V600E}* model (Fig. 5a). However, we found no substantial differences
293 at protein level across models (Supplementary Fig. 8b).

294 To investigate how these data translate to human cSCC, we reanalysed a human transcriptional
295 dataset with different stages of disease progression⁷. We observed that *SOX2* levels were increased in
296 27% of premalignant actinic keratosis (AK) and 35% of cSCC lesions, whereas *SOX2* expression levels
297 were low in normal skin (NS) (Fig. 5e, f). *SOX2* levels did not correlate with the patient's age or tumour
298 aggressiveness (tumour depth or diameter) (Supplementary Fig. 8c). We then examined *SOX2* protein
299 expression in a tissue microarray containing 250 human cSCC³⁵, which ranged from no expression, in
300 most of the samples, to mid and high in 21.7% of the histocores (Fig. 5g). These results are consistent
301 with previous studies that reported that ~25% of human cSCC express high or medium levels of
302 *SOX2*^{32,33}. These data underscore a diverse cell origin for cSCC, where *SOX2* could mark a subset of
303 tumours emerging from the IVL+ committed tumour-resistant population.

304 **SOX2 RENDERS THE IVL+ POPULATION SUSCEPTIBLE TO TUMORIGENESIS**

305 Given the potential role of *SOX2* activation in skin tumorigenesis, we assessed whether it is required
306 for cSCC development in a cell-specific manner. We crossed the *K14:BRAF^{V600E}* and *Ivl:BRAF^{V600E}* models
307 with mice harbouring a conditional *Sox2* knockout allele (hereafter *SOX2^{fl/fl}*) (Fig. 6a). Whilst conditional
308 *Sox2* deletion did not affect tumorigenesis or tumour growth in the *K14:BRAF^{V600E}-SOX2^{fl/fl}* model, it
309 significantly delayed tumour onset and tumour growth in the *Ivl:BRAF^{V600E}-SOX2^{fl/fl}* model (Fig. 6b-e).
310 Loss of *Sox2* on its own had no impact on skin homeostasis in either population, consistent with its
311 lack of expression in normal skin.

312 The histopathological features of *K14:BRAF^{V600E}-SOX2^{fl/fl}* and *Ivl:BRAF^{V600E}-SOX2^{fl/fl}* tumours were
313 similar to those expressing the wild-type *Sox2* allele, including an enlarged epidermis displaying
314 vertical columns of keratinocytes, incomplete maturation of keratinocytes, increased proliferation,
315 and MYC and pSTAT3 activation (Supplementary Fig. 9a). Interestingly, the slow-growing *Ivl:BRAF^{V600E}-*
316 *SOX2^{fl/fl}* tumours show patches escaping recombination and retaining *SOX2* protein expression, as
317 demonstrated by IHC, which highlights its critical role in tumorigenesis in this model (Supplementary
318 Fig. 9b). Expression of *SOX2* was not observed in the *K14:BRAF^{V600E}-SOX2^{fl/fl}* tumours (Supplementary
319 Fig. 9b).

320 Next, we assessed whether the overexpression of *SOX2* could render IVL+ cells competent for
321 tumorigenesis when coupled to a BRAF mutation. To this end, we interbred an allele that allows the

322 inducible overexpression of SOX2 from the Rosa26 locus [*Rosa26LSL-SOX2-IRES-eGFP* (hereafter
323 *SOX2^{LSL}*)] into the IVL+ tumour-resistant population. The resulting *lvl:BRAF^{V600E}-SOX2^{LSL}* model gave rise
324 to fast-growing tumours, and significantly accelerated tumour onset from 65 to 31 days (Fig. 6c, f).
325 Meanwhile, SOX2 overexpression alone showed no phenotype (Fig. 6c).

326 The *lvl:BRAF^{V600E}-SOX2^{LSL}* tumours exhibited advanced histological features of cSCC, including poorly
327 differentiated epithelial, keratin pearls, parakeratosis and invasion. These tumours showed enhanced
328 and disorganised proliferation beyond the basal layer, indicated by Ki67 expression, and activated the
329 previously described shared transcriptional profile, which includes pSTAT3, ANXA1, and MYC, along
330 with high SOX2 expression (Fig. 6g).

331 Importantly, overexpression of SOX2 in the IVL+ tumour-resistant population overrode the activation
332 of the apoptotic programme, marked by the expression of activated cleaved CASP3 and PARP seen
333 before in the *lvl:BRAF^{V600E}* model (Fig. 6g; Supplementary Fig. 2f). Tumours derived from the
334 *lvl:BRAF^{V600E}-SOX2^{LSL}* model show reduced expression of apoptotic markers, similar to those found in
335 tumours derived from the K14 and K5 models (Fig. 6h). This reduction of apoptosis was accompanied
336 by an increased expression of the stem cell marker CD34, marking tumour-initiating cells³³ (Fig. 6i).

337 To understand the kinetics and mechanisms by which SOX2 promotes tumorigenesis, we sampled skin
338 at different times post-induction in the presence and absence of *BRAF^{V600E}* (Fig. 6j). In the absence of
339 the oncogene, the *lvl:SOX2^{LSL}* expressed SOX2 mainly in the suprabasal layer with some basal layer
340 cells (Fig. 6k). By day 28, the remaining SOX2-labelled cells were limited to a few clones in the basal
341 layer, as the suprabasal cells overexpressing this protein had undergone turnover. In the presence of
342 the oncogene (*lvl:BRAF^{V600E}-SOX2^{LSL}*), we initially observed a high number of cells expressing SOX2 in
343 the suprabasal layer at day 8; however, from day 15, clones expanding in the basal population became
344 more prominent (Fig. 6k). CD34 expression was also seen from day 15 and maintained in tumours
345 consistent with a population of tumour initiating cells³³ (Supplementary Fig. 9c). These cells did not
346 follow the normal differentiation route towards delamination and remained proliferating and
347 anchored to the basal layer. By day 28, some of the basal clones have expanded, and the skin showed
348 dysplastic regions that later progress to tumours. At this time, we could already see *BRAF^{V600E}-SOX2+*
349 keratinocytes invading the dermis (Fig. 6k). Histological analysis of the skin at different timepoints
350 revealed increased MAPK signalling activation, as evidenced by high levels of DUSP6, along with
351 increased MYC and pSTAT3 expression levels starting from day 8 (Supplementary Fig. 9c).

352 Transcriptome analysis of bulk tumours derived from *lvl:BRAF^{V600E}-SOX2^{LSL}* showed a large number of
353 differentially expressed genes compared to tumours derived from *lvl:BRAF^{V600E}* taken at the clinical

354 endpoint (Supplementary Fig. 10a-d). Ivl:BRAF^{V600E}-SOX2^{LSL} transcriptome further activated pathways
355 such as pSTAT3, epithelial-mesenchymal transition, angiogenesis, and immune and inflammation-
356 related responses, among others, to support the faster progression of tumour growth (Supplementary
357 Fig. 10e). From those, we derived a “SOX2_tumorigenesis” signature with the top 150 differentially
358 expressed genes (Log₂ Fold Change >2 and padj <0.01) of which 139 had direct human orthologs
359 (Supplementary Data 2), and conducted single-sample gene set enrichment in a cSCC human dataset⁷.
360 Consistent with our murine-derived data, human cSCC samples enriched for our
361 “SOX2_tumorigenesis” signature showed lower enrichment in the apoptosis hallmark (Supplementary
362 Fig. 10f).

363 Overall, these data suggest that SOX2 is a cell-specific requirement for the IVL+ tumour-resistant
364 progenitor population to initiate cSCC. SOX2 activation in this population, combined with an
365 oncogene, results in a clonal behaviour similar to the K14/K5+ tumour-primed population and
366 promotes the re-acquisition of stemness and tumour-initiating capabilities in the skin's basal
367 compartment. The resulting IVL+ derived tumours shared most histological features and a core
368 transcriptional transformation programme with those originating from a K14/K5+ stem cell population
369 (Fig. 7).

370 DISCUSSION

371 We generated genetically engineered mouse models that recapitulate the rapid cSCC development
372 seen in melanoma patients treated with BRAFi, due to paradoxical MAPK signalling activation²⁰⁻²². High
373 levels of MAPK signalling activation in the epidermis were critical for transformation. While a single
374 copy of HRAS^{G12V} was insufficient, treatment with BRAFi rapidly promoted tumours. Similarly,
375 BRAF^{V600E} stimulated MAPK signalling hyperactivation, also promoting tumour development. This data
376 suggests that the skin can tolerate oncogenic mutations with minimal histological changes, but can
377 rapidly progress to tumorigenesis when exposed to a specific stimulus or promoter.

378 Notably, we obtained rapidly developing tumours within days of oncogene induction in the K14/K5+
379 basal stem cell population; hence, we called this population tumour-primed. However, the IVL+
380 differentiation-committed progenitor population remained normal despite the oncogene-bearing
381 cells colonising the basal epithelium within a week. Thus, we termed this population tumour-resistant.
382 Oncogene-expressing IVL+ cells remained in the basal layer, where the proliferative environment is
383 confined, challenging their differentiation commitment. This allowed for a longer latency period
384 before tumour development.

385 Tumours from both basal populations were histologically indistinguishable and showed the features
386 of cSCC, including keratin pearl formation, dermal invasion, nuclear atypia, parakeratosis and loss of
387 skin epidermal hierarchy. They also shared transcriptional programmes enriched for wound-healing²⁵
388 and embryonic²⁶ signatures, and for a tumour-specific keratinocyte population seen in human cSCC²⁷.
389 In addition, we observed strong MYC activation, which has been proposed as a mechanism to confer
390 stem cell properties and dedifferentiation in the *Gata6*+ epidermal populations¹². We also identified
391 evidence of hijacking a tissue injury-sensing and wound repair pathway activated by hypoxia and
392 driven by IL24 and pSTAT3²⁹. These results highlight the close interplay between proliferation during
393 tumorigenesis and re-epithelialisation during wound healing.

394 Most of our understanding of cSCC in the mouse has come from seminal work using DMBA/TPA^{9,18} or,
395 more recently, mouse models carrying different combinations of oncogenes, including KRAS or other
396 MAPK mutations in combination with TP53^{7,14,15}. Despite the diversity of oncogenic events across
397 these models, they also converge on the core set of skin transformation pathways described above
398 and including MYC and hypoxia/STAT3 activation.

399 One of our key findings was that SOX2 activation in the IVL+ tumour-resistant population is sufficient
400 to render this population susceptible to oncogenic transformation. SOX2 overexpression in
401 combination with oncogene expression rapidly accelerated tumorigenesis in this population *in vivo*
402 and reduced apoptosis. Moreover, these tumours expressed the stem-cell marker CD34, which
403 together with SOX2 have been proposed to mark a population of “tumour initiating cells”³³.
404 Conversely, loss of SOX2 in the IVL+ tumour-resistant population further prevented tumour onset and
405 growth upon oncogene expression, while it did not affect the K5/K14+ tumour-primed population.

406 This SOX2-driven stemness state also appears to be activated in tumours derived from the LGR6+
407 population in the DMBA/TPA model⁹. Indeed, ablation of SOX2 in the K14+ lineage delayed tumour
408 growth in this model. Since the LGR6+ hair follicle population also expresses K14, we hypothesise that
409 this LGR6+/K14+ population requires SOX2 for transformation, similar to the IVL+ population, which
410 also expresses K14+ (IVL+/K14+). Meanwhile, the interfollicular stem cell basal K14+ (IVL-/LGR6-)
411 population would not rely on SOX2-driven stemness for transformation.

412 Although the role of SOX2 in cSCC initiation has been previously described^{32,33}, our findings clarify that
413 its requirement is cell-specific. As a pioneer factor, SOX2 can bind closed chromatin and rewire the
414 epigenome to drive stem cell features^{31,36}. In the context of SCC, a bi-stable transcriptional network,
415 involving SOX2, TRP63 and PITX1, has been proposed to promote cell proliferation and to inhibit
416 differentiation by acting on KLF4³⁷. This network may enable tumour cells to switch between

417 proliferation and self-renewal or differentiation and keratin pearl formation. Building on this model,
418 we propose that SOX2 expression enables IVL+ progenitors to escape their differentiation programme
419 and adopt a more plastic, transformation-permissive state. This is consistent with the recent model of
420 fluid and gradual cell differentiation⁴, in which committed cells residing in the basal layer have not
421 necessarily exited the cell cycle and thus remain susceptible to reprogramming and oncogenic
422 transformation.

423 Importantly, SOX2 activation occurs in ~20% of cSCC patients^{32,33}. This suggests that a differentiation-
424 committed cell that has regained stemness potential, such as basal IVL+ or follicular LGR6+, could be
425 the cell of origin in these tumours. Unfortunately, although there is genetic data available for cSCC
426 from melanoma patients treated with BRAFi, and these have high levels of HRAS mutations²², there is
427 no transcriptomic data available to explore whether the proportion of tumours expressing SOX2 is
428 increased.

429 The IVL+ basal population's bias towards asymmetric division and upward differentiation is part of its
430 transcriptome-engrained commitment towards differentiation^{4,4}, likely contributing to its tumour-
431 resistant phenotype. This resistance is also seen in basal cell carcinoma, where only the stem-cell
432 population and not the progenitors have been proposed to initiate tumorigenesis⁶. However,
433 committed progenitors can trigger skin tumorigenesis once they re-acquire stemness features,
434 induced by SURVIVIN expression in basal cell carcinoma⁶ or by SOX2 in cSCC, as shown here.

435 The mechanism by which oncogenic signals activate SOX2 or related stemness programmes remains
436 unclear, but MAPK or MYC-dependent pathways are likely candidates. Understanding not only the
437 tissue-specific mechanisms that drive transformation but also the cell-specific vulnerabilities that
438 govern progenitor state and maintenance in homeostasis and tumorigenesis will be critical for
439 developing therapeutic strategies aimed at halting tumour initiation at its cellular origin.

440 In summary, we introduce genetically driven *in vivo* models of cSCC to dissect tumour susceptibility in
441 basal epidermal populations. Oncogene expression in the K14/K5+ stem cell population led to rapid
442 tumour development, whereas IVL+ committed progenitors remained resistant to transformation.
443 Nevertheless, SOX2 rendered this population permissive to tumorigenesis, preventing delamination
444 and reducing apoptosis^{32,33}, by inducing a stem-like transcriptional state that mirrors the tumour-
445 primed K14/K5+ population. Despite differences in cell of origin or oncogenic driver, we uncovered a
446 shared transcriptional programme underpinning transformation, also extensible to other cSCC
447 models. These findings highlight SOX2 as a critical switch that unlocks the tumorigenic potential of
448 IVL+ differentiation-committed basal progenitors.

449 **METHODS**450 **GENETICALLY ENGINEERED MOUSE MODELS AND HUSBANDRY**

451 Animal experiments conducted at the CRUK Scotland Institute Animal Facility were in accordance with
452 the UK Home Office guidelines, under project licence PP3908577, and were reviewed and approved
453 by the University of Glasgow Animal Welfare and Ethical Review Board (AWERB). Experiments
454 conducted at the CRUK Manchester Institute were performed at Alderley Park Animal Research Unit,
455 with breeding at the University of Manchester incubator breeding facility, in accordance with UK
456 Home Office regulations, under project licences PE4369EDB and P671A5B06, and reviewed and
457 approved by the CRUK Manchester Institute's AWERB. Mice were housed in accordance with UK
458 Home Office Regulations, maintained in a pathogen-free facility under a 12-h light-dark cycle at
459 a constant temperature between 19–23°C, and 55 ± 10% humidity, and given drinking water and fed
460 standard chow diet *ad libitum*. Male and female mice were used throughout the study. Animals were
461 monitored regularly until terminally euthanised by schedule 1, cervical dislocation, at clinical
462 endpoint: either when the cumulative tumour burden had reached a maximum volume of 1500 mm³
463 or when a single tumour exceeded 15 mm in diameter, weight loss >20%, or any other signs of ill
464 health and distress. The aforementioned limits were not exceeded. For all mouse studies, no formal
465 randomisation was performed, and researchers were not blinded to the mouse genotypes. No
466 exclusions were performed and all mice included in experimental cohorts were included in the
467 analysis.

468 Genotyping was performed by Transnetyx according to the previously published protocols provided
469 in the references for each allele. Mice were maintained on a C57BL/6J background, carrying the
470 following alleles/transgenes: *Ivl-CreERT2*¹⁵, *Krt5-CreERT2*³⁸, *Krt14-CreERT2*³⁹, *BRAF*^{V600E}^{40,41}, *HRAS*^{G12V}
471 ⁴², *Rosa26LSL-tdRFP*⁴³, *Sox2*^{fl}⁴⁴ and *Rosa26LSL-SOX2-IRES-eGFP*⁴⁵. Archived tumour blocks [from¹⁴]
472 included the additional alleles: *Lgr5-CreERT2*⁴⁶, *Kras*^{G12D}⁴⁷, *Notch2*^{fl}⁴⁸, and *Trp53*^{fl}⁴⁹.

473 Genetic recombination was induced in mice of either sex 8-12 weeks of age by topical application to
474 the shaved backs of the mice of 100 µL tamoxifen (10 mg/ml in ethanol; T5648, Sigma) in models
475 driven by *Ivl-CreERT2*, or 4 µl 4-hydroxytamoxifen (20 mg/ml in ethanol; H6278, Sigma) in models
476 driven by *Krt5-CreERT2* and *Krt14-CreERT*, unless otherwise stated. This was repeated three more
477 times over 8 days.

478 **UV IRRADIATION**

479 UV irradiation experiments were conducted at the CRUK Manchester Institute husbandry facility
480 following the UK Home Office regulations under project licences PE4369EDB and P671A5B06. In

481 cohorts exposed to UVR, mice were subjected to UVR exposure once per week for 4 weeks, beginning
482 4 weeks after the final tamoxifen application. For this process, mice were anaesthetised by
483 intraperitoneal injection with 1 mg/kg Domitor and 100 mg/kg ketamine, with 5 mg/kg Antisedan
484 anaesthetic reversal. The backs were shaved, and a black cloth was used to cover regions that were to
485 remain unexposed. The Waldmann UV181 unit with UV6 broad wavelength (280-380 nm) lamp was
486 used for UVR exposure and the intensity was tested regularly with a USB2000+ spectroradiometer
487 (Ocean Optics). The UV dose used was 0.6 kJ/m², which equated to 3 minutes and 14 seconds of
488 exposure. Following irradiation, E45 moisturising cream was topically applied to the back.

489 TUMOUR FRAGMENT IMPLANTATION

490 Tumour implantation experiments were conducted at the CRUK Manchester Institute husbandry
491 facility following the UK Home Office regulations under project licences PE4369EDB and P671A5B06.
492 A tumour derived from a K5:BRAF^{V600E} mouse was resected upon clinical endpoint under a laminar
493 flow hood aseptically. The tumour was cut into 2-3 mm³ pieces for immediate implantation on
494 anaesthetised 6-8 week-old C57/6J littermates and NSGII2 mice. Briefly, recipient animals were
495 anaesthetised with isoflurane and kept on a heated stage. A tumour piece was implanted
496 subcutaneously between the skin and the peritoneal wall. The surgical incision was closed using a
497 surgical clip. Rymadyl/Carprofen analgesic was given at 4mg/kg. Mice were monitored until
498 consciousness was regained and then monitored daily. The surgical clip was removed 7 days post-
499 surgery.

500 *IN VIVO* DRUG TREATMENTS

501 *In vivo* drug treatments were conducted at the CRUK Scotland Institute Animal Facility following the
502 UK Home Office guidelines, under project licence PP3908577. Skin inflammation was induced by
503 topically applying 150 µl of TPA (31.25 µg/ml) in acetone (Sigma-Aldrich 16561-29-8) to shaved dorsal
504 skin three times a week until tumour signs appeared. The BRAFi Dabrafenib was administered by daily
505 oral gavage (30 mg/kg in 100 µl) during the experiment.

506 IMMUNOHISTOCHEMISTRY AND IMMUNOFLUORESCENCE

507 Organs collected in 10% neutral buffered formalin were stored at room temperature for 20-28 hours,
508 followed by transfer to 70% ethanol and storage at 4°C until processing. All haematoxylin and eosin
509 (H&E), immunohistochemistry, co-immunofluorescence and *in situ* hybridisation staining were
510 performed on 4-µm formalin-fixed paraffin-embedded sections (FFPE) which had previously been
511 heated at 60°C for 2 hours.

512 The following antibodies were used on a Leica Bond Rx autostainer: KRT5 (905501, Biolegend), Ki67
513 (12202, Cell Signalling), SOX2 (14962, Cell Signalling), cleaved CASP3 (9661, Cell Signalling) and pSTAT3

514 (9131, Cell Signalling). All FFPE sections underwent onboard dewaxing (AR9222, Leica) and epitope
515 retrieval using ER2 solution (AR9640, Leica) for 20 minutes at 95°C. Sections were rinsed with Leica
516 wash buffer (AR9590, Leica) before peroxidase block was performed using an Intense R kit (DS9263,
517 Leica) for 5 minutes. Sections were rinsed with wash buffer before primary antibody application at an
518 optimal dilution (KRT5, 1/1500; Ki67, 1/1000; pSTAT3, 1/100; SOX2, 1/200; CASP3, 1/500). The
519 sections were rinsed with wash buffer before the application of anti-rabbit EnVision HRP-conjugated
520 secondary antibody (K4003, Agilent) for 30 minutes. The sections were rinsed with wash buffer,
521 visualised using DAB, and counterstained with haematoxylin from the Intense R kit.

522 FFPE sections for KRT14 (ab7800, Abcam), c-MYC (ab32072, Abcam), RFP (600-401-379, Rockland),
523 CD34 (119302, Biolegend), cPARP (ab32064, Abcam) and SOX9 (AB5535, Millipore) staining were
524 stained on an Agilent autostainer Link48. FFPE sections were loaded into an Agilent pre-treatment
525 module to be dewaxed and undergo heat-induced epitope retrieval (HIER) using a High pH target
526 retrieval solution (K8004, Agilent). After HIER, the sections were rinsed in FLEX wash buffer (K8007,
527 Agilent) before being loaded onto the Agilent autostainer. The sections underwent peroxidase
528 blocking (S2023, Agilent) for 5 minutes and were rinsed with FLEX buffer. The primary antibody
529 application was at an optimised dilution (KRT14, 1/300; c-MYC, 1/800; RFP, 1/1000; SOX9, 1/500;
530 CD34, 1/100; cPARP 1/1000). Sections were washed with FLEX buffer before application of anti-rabbit
531 EnVision secondary antibody for 30 minutes. Sections were rinsed with FLEX wash buffer before
532 applying Liquid DAB (K3468, Agilent) for 10 minutes. Sections were washed in water and
533 counterstained with haematoxylin 'Z' (RBA-4201-00A, CellPath).

534 In-situ hybridisation detection for *Anxa1* (509298), *IvI* (422538), *Ly6a* (427578), PPIB (313918; positive
535 control) and *dapB* (312038; negative control) (all Bio-Techne) mRNA was performed using RNAScope
536 2.5 LSx (Brown) detection kit (322700; Bio-Techne) according to the manufacturer's instructions. H&E
537 staining was performed on a Leica autostainer (ST5020). Sections were dewaxed in xylene, taken
538 through graded ethanol solutions and stained with haematoxylin 'Z' (RBA-4201-00A, CellPath) for 13
539 minutes. Sections were washed in water, differentiated in 1% acid alcohol, washed and the nuclei
540 stained blue in Scott's tap water substitute (in-house). After washing with tap water, sections were
541 placed in Putt's eosin (in-house) for 3 minutes. To complete H&E, IHC & ISH staining, sections were
542 rinsed in tap water, dehydrated through a series of graded alcohols, and placed in xylene. The stained
543 sections were coverslipped in xylene using DPX mountant (SEA-1300-00A, CellPath).

544 Sections for KRT14 and RFP co-IF staining were loaded onto a Leica Bond Rx autostainer. The FFPE
545 sections underwent on-board dewaxing and epitope retrieval using ER2 solution for 20 minutes at
546 95°C. Sections were rinsed with Leica wash buffer (AR9590, Leica) before application of 10% normal

547 goat serum (X090710-8, Agilent) for 30 minutes. The sections were rinsed with Leica wash buffer
548 before application of anti-RFP antibody at 1/1000 dilution for 1 hour. Sections were rinsed with Leica
549 wash buffer and goat anti-rabbit IgG 488 (A11034, Invitrogen) secondary antibody diluted 1/250 for
550 30 minutes. After rinsing with Leica wash buffer KRT14 antibody was applied at 1/750 dilution for 1
551 hour. Sections were rinsed with Leica wash buffer and goat anti-mouse IgG 647 secondary antibody
552 (A21236, Invitrogen diluted) 1/250 for 30 minutes before application of DAPI (MBD0015, Sigma-
553 Aldrich). To complete the staining, sections were mounted using ProLong Diamond antifade mountant
554 (P36970, Thermo Fisher Scientific).

555 Immunohistochemistry images were acquired using a SCN400F slide scanner (Leica Microsystems) at
556 $\times 20$ or $\times 40$ magnification. Confocal images were collected on a Zeiss 710 point-scanning confocal
557 microscope, built on an inverted Zeiss Axio Imager.Z2 stand. Images were acquired using an EC Plan-
558 Neofluar 40x/1.30 Oil and a confocal pinhole diameter of 107 μm . Multi-channel images were
559 captured sequentially: DAPI (nuclear marker) using 405 nm excitation and 410-481 nm emission
560 bandwidth, IgG 488 using 488 nm excitation and 514-582 nm emission, and IgG 647 using 633 nm
561 excitation and 638-747 nm emission. Images were collected with a 1x zoom with an image size of 2320
562 \times 2320 pixels, yielding a pixel size of 92 \times 92 nm, and a 1.38 μs pixel dwell time. Z-stacks were collected
563 using a step size of 2 μm . Images were acquired using the software Zen LSM 2.1 Black (Zeiss).

564 Quantification of RFP+ and CASP3+ cells for IHC-stained samples was performed using the HALO Image
565 Analysis Platform version 3.6.4134 (Indica Labs, Inc.) to quantify the optical density of cellular staining
566 after manual annotation of the epidermal layer and training to detect skin epithelial cells.

567 RNASEQ SEQUENCING AND ANALYSIS

568 RNA was extracted from fresh frozen tumour samples using an AllPrep DNA/RNA kit (74104, Qiagen)
569 according to the manufacturer's instructions. Indexed poly(A) libraries were prepared using 200 ng of
570 total RNA and 14 cycles of amplification with the Agilent SureSelect Strand-Specific RNA Library
571 Preparation Kit for Illumina Sequencing (G9691B, Agilent). RNA polyA libraries were sequenced using
572 NovaSeq 6000 XP SP, 200 cycles, paired-end reads (2 \times 101 bp), 20-30 million reads. RNA preparation
573 and sequencing of mouse samples were performed at the CRUK Manchester Institute histology core
574 and sequencing facility.

575 RNA preparation and sequencing of *lvi*:BRAF^{V600E} and *lvi*:BRAF^{V600E}-SOX2^{LSL} tumours (Fig. S10) were
576 performed by GENEWIZ Azenta. rRNA depletion was performed before library preparation. \sim 30 million
577 paired-end reads (2 \times 150bp) per sample.

578 For all samples raw sequence quality control was performed using FastQC (v0.11.8) before and after
579 removing adapters and low-quality base calls (Phred score <20) using TrimGalore with default options
580 (v0.6.6). Trimmed reads were aligned to GRCm38, release 100 using Hisat2 (v2.1.0). Gene counts were
581 subsequently estimated using featurecounts (subread/1.6.3). Sva::ComBat-seq was used for batch
582 correction (v3.52.0). For all samples, after removing transcripts without a minimum of 5 reads in at
583 least one sample, the differential expression analysis between mouse tumours and skin was
584 performed using the R package DESeq2 (v1.44.0). The resultant p-values were corrected for multiple
585 comparisons using the Benjamini-Hochberg approach. The following additional R packages were used
586 for downstream analysis: pheatmap (v1.0.8), fgsea (v1.30.0), GSVA (v4.5), enhancedVolcano (v1.22.0),
587 clusterProfiler (v4.12.6), dplyr (v1.1.4), tidyr (v1.3.1), AnnotationHub (v3.12.0), ggvenn (0.1.10),
588 AnnotationDbi (v1.66.0), mixOmics (v6.26.0).

589 Digital sorting and transcriptome deconvolution was performed using CIBERSORTx⁵⁰ using a signature
590 matrix generated from the single-cell RNAseq dataset from Joost et al., 2020 (GEO: GSE129218).
591 CIBERSORTx was run online in relative mode to compare relative cellular fractions, using B-mode for
592 batch correction, and 100 permutations.

593 The “cSCC MAPK signalling shared” signature derived from this work was generated by selecting the
594 commonly upregulated and differentially expressed genes (Log_2 Fold Change >3 and padj <0.01)
595 obtained from Ivl:BRAF^{V600E}, K5:BRAF^{V600E} and K14:BRAF^{V600E} tumours compared to normal skin from
596 control litter mates (Supplementary Data 1). The “SOX2_tumorigenesis” signature derived from this
597 work was generated by selecting the top 150 differentially expressed genes (Log_2 Fold Change >2 and
598 padj <0.01) between Ivl:BRAF^{V600E} and Ivl:BRAF^{V600E}-SOX2^{LSL}, of which 139 genes were converted to
599 human orthologs (Supplementary Data 2).

600 STATISTICS AND REPRODUCIBILITY

601 Statistical analysis was performed using GraphPad Prism (v.10.0.1) for Windows. Normality and
602 lognormality tests were used to establish the appropriate significance test, followed by a statistical
603 test to compare the means. To compare the means of two groups, a two-tailed Student’s t-test was
604 performed. All error bars shown are Mean \pm standard error of the mean. Micrographs showing
605 representative images refer to at least three independent mice unless stated otherwise. No statistical
606 method was used to predetermine sample size. No data were excluded from the analyses,
607 experiments were not randomised, and the investigators were not blinded to allocation during
608 experiments and outcome assessment. Mice allocation into experimental groups was random.
609 Differences were considered statistically significant at * p <0.05, ** p <0.01, *** p < 0.001 and **** p <
610 0.0001.

611 DATA AVAILABILITY

612 The data discussed in this publication have been deposited in NCBI's Gene Expression Omnibus. The
613 accession numbers for the RNA-seq data reported in this paper are NCBI GEO [GSE280236](#) and
614 [GSE303703](#). Source Data are provided with this paper. The remaining data are available within the
615 Article, Supplementary Information or Source Data file

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630 AUTHOR CONTRIBUTIONS STATEMENT

631 P.P.C., A.C., R.M. and O.J.S. designed experiments and interpreted results. P.P.C, C.C., G.K, C.A.F.,
632 R.A.R., T.J.S. and P.C. performed experiments and analysed results. P.P.C. analysed publicly available
633 human cancer data sets. P.P.C. processed and analysed the RNA sequencing data. G.J.I. contributed to
634 discussions and analysed results. P.P.C., A.C. and O.J.S. wrote the paper, and reviewed and discussed
635 the drafted manuscript. All authors contributed to the manuscript.

636 COMPETING INTEREST STATEMENT

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639 and CEO of MyT Bioscience Ltd and the founder, Director and CSO of Oncodrug Ltd. The remaining
640 authors declare no competing interests. Other authors declare no competing interests.

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792 **FIGURE LEGENDS**

793

794 Fig. 1. Paradoxical MAPK signalling activation is a tumour promoter in cSCC.

795 a. Schematic representation of the mouse models used (top) and the epidermis (bottom)
 796 formed by the suprabasal layer, made by IVL+ cells, and the basal layer, which includes a
 797 mixture of K14+/K5+ and IVL+ cells. Created in BioRender. Centeno, P. (2025)
 798 <https://BioRender.com/zzh4ohx>

799 b-c. Kaplan-Meier tumour-free survival [time of the first lesion or macroscopic change in the skin]
 800 plot for K14:HRAS^{G12V/+} (n=9) and those treated with the BRAFi Dabrafenib (n=8) and TPA
 801 (n=7) (b), and IvI:HRAS^{G12V/+} (n=12), IvI:HRAS^{G12V/G12V} (n=9) and those treated with BRAFi
 802 Dabrafenib (n=8) and TPA (n=7) (c). P-values were determined using the log-rank (Mantel-Cox)
 803 test and HRAS^{G12V/+} are used as controls. ***p<0.001.

804 d. Representative histological images showing downstream MAPK signalling activation via
 805 pERK and DUSP6 IHC in normal skin carrying HRAS^{G12V} mutations, untreated and TPA and
 806 BRAFi treated, collected at the end of the experiment. Images representative of four
 807 animals per genotype. Scale bar is 200 µm.

808 e. Representative histological images including H&E (scale bar 200µm and 800µm) and
 809 Ki67 (scale bar 300µm and 400µm) IHC in tumours derived from K14:HRAS^{G12V/+} and
 810 IvI:HRAS^{G12V/+} mice treated with the BRAFi Dabrafenib at the clinical endpoint. Images
 811 representative of four animals per genotype.

812 f. Kaplan-Meier tumour-free survival plots for K14:HRAS^{G12V/+} (n=4) and those treated with
 813 BRAFi Dabrafenib (n=5) at 182 days after oncogene induction (left) and IvI:HRAS^{G12V/+} (n=7)
 814 and those treated with BRAFi Dabrafenib (n=5) at 189 days after oncogene induction (right).
 815 P-values were determined using the log-rank (Mantel-Cox) test and untreated HRAS^{G12V/+} are used
 816 as controls.

817 g. Schematic representation of the different levels of MAPK signalling pathway activation
 818 observed in the skin of HRAS^{G12V} mice, with heterozygous mutation and TPA treatment,
 819 homozygous mutation, and heterozygous mutation and BRAFi treatment. A single copy of
 820 Hras^{G12V} combined with TPA is insufficient to reach the threshold of MAPK signalling activation in the
 821 basal layer for tumour formation, and two copies are necessary. In contrast, BRAFi acts as a potent
 822 tumour promoter, driving MAPK paradoxical activation from a single copy of HRAS-mutated skin.
 823 pERK is at the end of the MAPK signalling cascade, and is used throughout the study as a direct
 824 readout for activation. DUSP6 is activated through a negative feedback loop in response to increased
 825 pERK levels. Created in BioRender. Centeno, P. (2025) <https://BioRender.com/zzh4ohx>

826 Source data are provided as a Source Data file.

827

828 Fig. 2. Tumour-primed and tumour-resistant populations coexist in the basal layer.

829 a. Schematic representation of the activation of the MAPK signalling pathway observed in
830 the skin of BRAF^{V600E} mice. Created in BioRender. Centeno, P. (2025)
831 <https://BioRender.com/zzh4ohx>

832 b. Schematic representation of the mouse models used for skin tumorigenesis driven from
833 K5, K4 and Ivl promoters.

834 c. Kaplan-Meier plot displaying tumour-free survival for K5:BRAF^{V600E}(n=8),
835 K14:BRAF^{V600E}(n=5), Ivl:BRAF^{V600E} (n=12), and Ivl:BRAF^{V600E} UV-treated (n=12) female mice
836 aged until clinical endpoint. P-values were determined using the log-rank (Mantel-Cox) test. NS not
837 significant.

838 d,e. Total tumour growth in K5:BRAF^{V600E} (n=8) and K14:BRAF^{V600E} (n=5) (d) and in Ivl:BRAF^{V600E} (n=7)
839 and UV-treated Ivl:BRAF^{V600E} (n=6) female mice (e).

840 f. Total tumour burden (Mean and SEM) in K5:BRAF^{V600E} (n=8), K14:BRAF^{V600E} (n=5), Ivl:BRAF^{V600E} (n=7)
841 and UV-treated Ivl:BRAF^{V600E} (n=7) female mice at clinical endpoint.

842 g-i. Representative H&E of K14:BRAF^{V600E} (g), K5:BRAF^{V600E} (h), and Ivl:BRAF^{V600E} (i) tumours at clinical
843 endpoint showing keratin pearls (KP) and nuclear atypia (arrows). Images representative of five
844 animals per genotype. Scale bar is 200 µm unless otherwise stated in the image.

845 Source data are provided as a Source Data file.

846

847

848 Fig. 3. Oncogene-expressing IVL+ tumour-resistant population takes over the entire skin but remains
849 tumour-restrictive.

850 a. Schematic representation of the mouse models used for lineage tracing of the KRT14+
851 and IVL+ populations.

852 b,c. Representative Z-stack co-immunofluorescence (IF) of KRT14 and RFP of skin derived from
853 Ivl:tdRFP (control) at day 28 post-induction (b; Scale bar is 21 µm) and IVL and RFP of skin derived
854 from Ivl:BRAF^{V600E}-tdRFP at day 15 and 28 post-induction (c; images taken on a 50x objective).
855 Images representative of three animals per genotype.

- 856 d. Schematic representation of the experimental design used for lineage tracing.
- 857 e. Representative RFP IHC at different time points post-induction in Ivl:tdRFP and Ivl:BRAF^{V600E}-tdRFP.
858 Scale bar is 50 µm unless otherwise stated in the image. Images representative of three
859 animals per genotype and time point.
- 860 f. Quantification of the clonal persistence (%RFP) in the basal epidermis region of the skin from
861 Ivl:tdRFP and Ivl:BRAF^{V600E}-tdRFP mice at different times post-induction. Individual measurements
862 averaged from three different skin stripes per animal. N=3 independent mice per timepoint in each
863 arm. Data are presented as mean values +/- SEM.
- 864 g. Epidermis thickness quantification at different time points post-induction in Ivl:tdRFP and
865 Ivl:BRAF^{V600E}-tdRFP. Ten measurements from three different skin stripes per animal. Points represent
866 individual animals, three animals per time point. Data are presented as mean values +/- SEM. Unpaired
867 two-tail T-test. NS not significant P>0.05, ** p<0.01 and *** p<0.001.
- 868 h. Representative RFP IHC at different time points post-induction in K14:tdRFP and K14:BRAF^{V600E}-
869 tdRFP skin and tumours. Data representative of two independent animals and three skin regions per
870 group and time point. Scale bar is 200 µm unless otherwise stated in the image.
- 871 i. Quantification of the clonal persistence (%RFP) in the basal epidermis region of the skin from
872 K14:tdRFP and K14:BRAF^{V600E}-tdRFP mice at day 8 post-induction. N=4 from 2 independent mice per
873 timepoint in each arm.
- 874 Source data are provided as a Source Data file.
- 875
- 876 Fig. 4. Shared transcriptional profile independent of the cell-of-origin or oncogene.
- 877 a. Principal component analysis (PCA) of normalised expression values showing the correlation
878 between the transcriptional profiles of normal skin (NS, n=4), Ivl:BRAF^{V600E} (Ivl, n=6) and
879 K5/K14:BRAF^{V600E} (K5_K14, n=9).
- 880 b,c. Volcano plots showing differentially expressed genes in Ivl tumours (n=6) (b) and K5_K14 tumours
881 (n=9) (c) compared with normal skin (n=4) using the Wald test (two-tailed).
- 882 d. Venn diagram showing upregulated genes shared between Ivl:BRAF^{V600E} (n=6; Ivl) and
883 K5/K14:BRAF^{V600E} (n=9; K5_K14) tumours vs normal skin (n=4).

884 e. Gene set enrichment analysis of Hallmarks and indicated pathways in K5_K14 (n=9) tumours and IvI
 885 (n=6) tumours. Showing pathways significantly enriched ($p_{adj} < 0.01$ and $NES > 2$ based on an adaptive
 886 multi-level split Monte-Carlo).

887 f. Hierarchical clustering heatmap of selected genes showing normalised expression and correlation
 888 between normal skin (NS; n=4), IvI:BRF^{V600E} (IvI; n=6) and K5/K14:BRF^{V600E} (K5_K14; n=9).

889 g,h. Representative images of IHC validation of selected targets in K14:BRF^{V600E} and IvI:BRF^{V600E},
 890 including MYC, pSTAT3, Ly6A and ANXA1 at clinical endpoint. Images representative of four
 891 animals per genotype. Scale bar is 200 μ m.

892

893 Fig. 5. SOX2 is specifically expressed in tumours derived from the IVL+ -resistant population.

894 a. Volcano plot showing differentially expressed genes between K5/K14:BRF^{V600E} (n=9) and
 895 IvI:BRF^{V600E} (n=6) tumours using Wald test (two-tailed).

896 b,c. Representative images of IHC validation of SOX2 in IvI:BRF^{V600E}, K5:BRF^{V600E}, and K14:BRF^{V600E}
 897 (b) and BRAFi treated K14:HRAS^{G12D/+} and IvI:HRAS^{G12D/+} tumours (c) at clinical endpoint. Images
 898 representative of four animals per genotype. Scale bars are 200 μ m, 300 μ m and 700 μ m.

899 d. Representative images of IHC of SOX2 in normal skin (left) and normal skin treated with TPA (right).
 900 Images representative of three animals per genotype. Scale bar is 200 μ m.

901 e. Normalised RNA expression levels (\log_2 counts) of SOX2 from a cSCC human dataset ⁷ including
 902 normal skin (NS, n=17), and different levels of disease progression [actinic keratosis (AK, n=14) and
 903 cutaneous squamous cell carcinoma (SCC, n=66)]. Dotted lines mark the mean SOX2 normalised
 904 expression in normal skin and two standard deviations from the mean. Pie charts showing the
 905 percentage of samples with high and low SOX2 normalised expression for cSCC and AK groups. Two
 906 standard deviations from the normal skin mean were used as a threshold.

907 f. Heatmap showing individual SOX2 normalised expression levels range in AK (n=5 high vs n=9 low)
 908 and cSCC (n=32 high vs n=30 low) groups from a human dataset⁷.

909 g. Representative IHC of SOX2 conducted in a human tissue microarray containing 250 cSCC histocores
 910 described in³⁵. 196 (78.4%) of the samples were negative, and 54 (21.6%) were positive for SOX2
 911 protein expression. The scale bar is 50 μ m.

912

913 Fig. 6. SOX2 overexpression in combination with MAPK activation renders the Ivl+ tumour-resistant
914 population permissive to cSCC.

915 a. Schematic representation of the mouse models used for tumorigenesis.

916 b,c. Kaplan-Meier tumour-free survival plot for K14:BRAF^{V600E} (n=5), K14:BRAF^{V600E}-SOX2^{fl/fl} (n=6) and
917 K14:SOX2^{fl/fl} (n=4) (b), and for Ivl:BRAF^{V600E} (n=12), Ivl:BRAF^{V600E}-SOX2^{fl/fl} (n=10), Ivl:SOX2^{fl/fl} (n=7),
918 Ivl:BRAF^{V600E}-SOX2^{LSL} (n=5), and Ivl:SOX2^{LSL} (n=4) (c). P-values were determined using the log-rank
919 (Mantel-Cox) test. NS not significant P>0.05, **p<0.01, ***p<0.001. Note that K14:BRAF^{V600E} and
920 Ivl:BRAF^{V600E} cohorts correspond to CRUK-SI cohorts also shown in Supplementary Fig. 2, and are
921 shown here for comparison.

922 d,e. Total tumour burden growth curves for K14:BRAF^{V600E} (n=5) and K14:BRAF^{V600E}-SOX2^{fl/fl} (n=6) (d),
923 and for Ivl:BRAF^{V600E} (n=12) and Ivl:BRAF^{V600E}-SOX2^{fl/fl} (n=10) (e).

924 f. Representative picture showing Ivl:BRAF^{V600E} and Ivl:BRAF^{V600E}-SOX2^{LSL} littermates at day 30 post
925 oncogene induction. Representative of Ivl:BRAF^{V600E} (n=12) and Ivl:BRAF^{V600E}-SOX2^{LSL} (n=5) cohorts.

926 g. Representative H&E and IHC of Ki67, SOX2, MYC, pSTAT3, CASP3, cPARP and ANXA1 in Ivl:BRAF^{V600E}-
927 SOX2^{LSL} tumour at clinical endpoint. Images representative of four animals per genotype. Scale
928 bar is 200 µm unless otherwise stated in the image.

929 h. Apoptosis quantification (% cleaved CASP3 and cPARP of positive cells) in tumours driven by
930 BRAF^{V600E} from different populations (CASP3; K14 n=3, K5 n=2, Ivl n=3, Ivl-SOX2^{LSL} n=4, and cPAPR; K14
931 n=4, Ivl n=6, Ivl-SOX2^{LSL} n=4 independent tumours). Quantified area from a total of 15mm², including
932 an average of 23240.73 cells. Two-way ANOVA multiple comparison. *** p<0.001 and **** p<0.0001.
933 Data are presented as mean values +/- SEM.

934 i. Representative IHC of CD34 in Ivl:BRAF^{V600E}-SOX2^{LSL} tumour at clinical endpoint. Images
935 representative of four animals per genotype. Scale bar is 200 µm.

936 j. Schematic representation of the mouse models used for tumorigenesis and experimental design.

937 k. Representative IHC of SOX2 in Ivl:SOX2^{LSL} and Ivl:BRAF^{V600E}-SOX2^{LSL} at different time points. Images
938 representative of three animals per genotype and per time point. Scale bar is 70 µm unless
939 otherwise stated in the image.

940 Source data are provided as a Source Data file.

941

942 Fig. 7. SOX2 confers tumour permissiveness to the IVL+ tumour-resistant population.

943 Graphic abstract of the model arising from this study. cSCC can be initiated from a stem cell or
944 differentiation-committed progenitor. The latter will require SOX2 activation and will take longer to
945 escape constraints and trigger tumorigenesis. SOX2 activates stem-like features and reduces
946 apoptosis, which is critical for tumorigenesis. During this process, actively dividing IVL+ progenitors
947 expand through the entire epithelium with little histological change, preparing for tumour initiation
948 and progression and leading to tumorigenesis. We propose a common oncogenic programme, seen
949 in human cSCC²⁷ and induced by inflammation, injury or paradoxical activation of MAPK, which
950 allows the transformation of “normal” oncogene-bearing epithelial cells into a tumour without
951 additional oncogenic events. Created in BioRender. Centeno, P. (2025)

952 <https://BioRender.com/zzh4ohx>

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