Distinct modes of cell competition shape mammalian tissue morphogenesis

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Cell competition—the sensing and elimination of less fit ‘loser’ cells by neighbouring ‘winner’ cells—was first described in Drosophila. Although cell competition has been proposed as a selection mechanism to optimize tissue and organ development, its evolutionary generality remains unclear. Here, by using live imaging, lineage tracing, single-cell transcriptomics and genetics, we identify two cell competition mechanisms that sequentially shape and maintain the architecture of stratified tissue during skin development in mice. In the single-layered epithelium of the early embryonic epidermis, winner progenitors kill and subsequently clear neighbouring loser cells by engulfment. Later, as the tissue begins to stratify, the basal layer instead expels losers through upward flux of differentiating progeny. This cell competition switch is physiologically relevant: when it is perturbed, so too is barrier formation. Our findings show that cell competition is a selective force that optimizes vertebrate tissue function, and illuminate how a tissue dynamically adjusts cell competition strategies to preserve fitness as its architectural complexity increases during morphogenesis.

Not all cells that arise during development contribute to adult tissues, as exemplified by studies of cell competition studies in Drosophila wing epithelial development and germ line stem cell niches1–3. To date, most vertebrate cell competition studies have been limited to mouse epiblast and cancerous tissues10,12–17. Classically, cell competition is defined by three features: (1) differences in growth rates among cell populations within a mosaic tissue; (2) active removal of more slowly growing, less fit loser cells that depends on contact with more fit winner cells; and (3) relativity of winner/loser fates that change depending on the fitness of neighbouring cells.

Increasing attention has been paid to cell competition in mammalian systems. An elegant description has emerged from studies of cultured embryonic stem cells and early post-implantation epiblasts12–14. However, the functional importance of cell competition is not yet clear, and we do not know whether cell competition functions in mammals as it does in Drosophila to govern tissue fitness during growth. This question is particularly interesting for surface epithelia. During evolution from exo- to endo-skeletons, these tissues became stratified to produce protective barriers that constantly rejuvenate from an inner layer of proliferative progenitors.

During mouse embryogenesis, following specification from surface ectoderm, the epidermis expands its surface area by around thirty times to accommodate rapid body-plan growth. The initial progenitor monolayer also stratifies and differentiates to yield a functional, multilayered permeability barrier at birth. To determine whether cell competition operates during this process, we exploited the earlier finding that mosaic variation in the Myc proto-oncogene triggers cell competition across a range of proliferative Drosophila epithelia6,18 as well as in mouse epiblasts12.

A model of cell competition in skin development

At embryonic day 10.5 (E10.5), mouse epidermis expresses Myc and its related isoform, Mycn19 (Extended Data Fig. 1a). Given the higher expression of Mycn, we selected floxed Myn mice (Mycnfl/fl) to generate a mosaic ‘loser’ model. Using in utero ultrasound-guided delivery20, we co-injected the amniotic sacs of E9.5 Mycnfl/+ or control (Mycnfl/fl) mouse embryos with two equally low-titre lentiviruses harbouring either Pgr-creRFP or Pgr-GFP (LV-CreRFP or LV-GFP). By E12.5, the lentivirus-packaged genes were integrated and thereafter stably propagated to epithelial progenitor offspring20 (Extended Data Fig. 1b, c), providing the necessary mosaic embryonic skin for us to investigate whether cell competition is operative and triggered when surrounding epidermal progenitors encounter neighbours that lack a Mycn allele.

To test for differences in proliferative capacities, we used comparative growth assays combined with quantitative whole-mount imaging analyses (Fig. 1a, b). By E17.5, RFP Mycn+/− cells were diminished relative to their initial representation at E12.5 (Fig. 1c). This difference was rooted in a growth disadvantage caused by loss of one Mycn allele, as GFP+ epidermal cell representation was unchanged between E12.5 and E17.5. Similarly, in Mycnfl/fl embryos in which RFP+ cells were Mycn+/−, the RFP:GFP ratio was low compared to that in Mycn+/+ embryos where RFP+ cells were wild-type (Fig. 1d). Incorporation of 5-ethyl-2′-deoxyuridine (EdU) confirmed that Mycn+/− cells have a proliferative disadvantage (Fig. 1e), thereby fulfilling the first criterion for cell competition.

To assess the second cell competition criterion, that elimination of loser cells from tissue depends on contact with fitter neighbours, we used time-lapse imaging of E12.5 R26tmTomG embryos (Mycn+/+ or Mycnfl/fl) transduced with LV-Cre at E9.5 (Fig. 1f). This approach enabled us to count cell death events (Fig. 1g; Extended Data Fig. 2a–d; Supplementary Videos 1–4) for both GFP+ (either Mycn+/+ or Mycnfl/fl) and Tomato+ cells (always Mycn+/+). We tested whether neighbours of dying cells were the same colour (and thus the same genotype as the dead cell), or whether dying cells contacted neighbours of both colours.

Quantifications revealed that GFP+ Mycn+/+ cells in contact with one or more Tomato+ Mycn+/+ neighbour(s) died about four times more often than either Tomato+ wild-type cells in contact with only Tomato+ wild-type neighbours, or GFP+ Mycn+/+ cells in contact with only GFP+ Mycn+/+ cells (Fig. 1h). Dying Mycn+/+ cells also displayed fragmented DNA and active caspase-3 (Fig. 1i–k). These results were not an artefact of LV-Cre infection, as in wild-type embryos transduced with LV-Cre, GFP+ Mycn+/+ cells died no more often than their Tomato+ Mycn+/+ neighbours. Moreover, live imaging showed
representative death of a Mycn+/– cell (asterisk) in contact with wild-type neighbours. Bottom, segmented image traces. h, Quantification of death events. i, TUNEL+ fragments (white) accumulate along boundaries of wild-type (red) and Mycn+/– (green) cells; image representative of five independent experiments. j, Activated caspase-3 expression (green) captured within a dying E12.5 RFP+ Mycn+/– cell (white dashed outline); image representative of two independent experiments. k, TUNEL+ RFP+ corpses within three cell lengths of CreRFP+ cells at E12.5. l, m, Quantification and representative images of neighbouring TUNEL+ corpses (data obtained from two litters, two to four measurements were made per embryo, n = number of images analysed; WT/WT n = 18, WT/Mycnfl/+ n = 13, Mycnfl/+Mycnfl/+ n = 12, Mycnfl/+Mycnfl/+; shMycn n = 14; Kruskal–Wallis test with Dunn’s multiple comparisons). All arrowheads denote apoptotic bodies. Scale bars, 50 μm with the following exceptions: b, bottom, 500 μm; g, i–k, 10 μm. All data shown as mean ± s.e.m. NS, not significant.

Elimination and clearance of loser cells

To elucidate the mechanisms that underlie elimination of loser cells, we investigated whether apoptosis is required for cell competition. We blocked apoptosis throughout the mosaic Mycn+/+ epidermis by co-transducing K14-rtTA;Mycnfl/+ embryos with low-titre LV-CreRFP and higher-titre LV-TRE-Bcl2l1, which harbours a doxycycline-inducible transgene encoding the cell survival protein BCL-XL (Extended Data Fig. 4a, b). Upon induction, most Mycn+/+ cells (RFP+) were also BCL-XL+ and were surrounded mostly by BCL-XL+/Mycn+/+ neighbours. TUNEL assays confirmed that elevation of BCL-XL prevented the cell competition-induced apoptosis that otherwise arises in Mycn+/+ mosaic tissue, and, strikingly, the proliferative capacity of Mycn+/+ cells no longer differed from that of their neighbours (Fig. 2a, Extended Data Fig. 4e). Accordingly, the overall growth disadvantage of Mycn+/+ cells at E17.5 was rescued by BCL-XL elevation (Fig. 2a). Thus, cell death signalling appears to be required for Mycn+/+ winner
progenitors to exert their competitive advantage over Mycn+/– losers for limited space within the basal epidermal layer. We next investigated how dying loser cells are cleared from the embryonic epidermal sheet. Macrophages did not seem to be responsible, as at E12.5 they were in the dermis, where they remained after mosaic manipulation to induce cell competition (Extended Data Fig. 5a, b). However, adult epidermal cells are known to phagocytose dying cells during the hair cycle22, and as embryonic epidermal progenitors express engulfment machinery genes23, we hypothesized that they might eliminate dying neighbours. Therefore, using high-titre shRNA transduction (50–80% of basal cells receive the hairpin), we knocked down Gulp1, a key component of the engulfment complex, and induced Mycn-dependent cell competition. Compared to scramble shRNA-transduced embryos, the epidermis of Gulp1 knockdown embryos contained more TUNEL+ corpses adjacent to CreGFP+/Mycn+/– loser cells (Fig. 2b). Thus, although engulfment was not required to induce cell competition, its machinery appears to be necessary for neighbours to clear loser cell corpses that arise from cell competition-induced apoptosis in developing skin.

**Evidence for endogenous cell competition**

We next investigated whether cell competition is endogenous to the developing mammalian epidermis. Although TUNEL assays of wild-type skin rarely identified corpses, we surmised that rapidly cleared death events may be difficult to detect. We therefore dampened the engulfment machinery by transducing wild-type epidermis with Gulp1-shRNA at a high titre. This perturbation resulted in the presence of numerous TUNEL+ epidermal corpses, plausibly representing loser cells that died as a result of endogenous cell competition (Fig. 2c). Most TUNEL+ corpses were RFP+, suggesting that Gulp1 knockdown did not cell-autonomously affect viability (Extended Data Fig. 5c). These findings imply that cell competition is a natural phenomenon, and that winner cells clear dead or dying loser neighbours from the progenitor monolayer through engulfment.

To bolster evidence for endogenous cell competition, we devised a strategy to identify and characterize emerging winners and losers in wild-type embryos. We hypothesized that winner/loser status might correlate to distinct transcriptional signatures, and reasoned that we could exploit the behaviour of Mycn+/– cells as losers to uncover the signatures of loser cell identity. As our Gulp1 knockdown data further suggested that in wild-type epidermis, apoptotic cells represent putative losers (Fig. 2c), additional isolation and analysis of pre-apoptotic AnnexinV+ cells from otherwise wild-type embryos offered the opportunity to broaden the scope of our transcriptional analyses beyond Mycn-dependent cell competition.

We performed single-cell RNA sequencing (scRNA-seq) on AnnexinV+/DAPI (live putative losers), AnnexinV−DAPI (putative winners) and Mycn+/– epidermal cells (established losers) at E12.5 (Extended Data Fig. 6a–c). We grouped AnnexinV+ and Mycn+/– cells as losers and compared them to AnnexinV− cells (winners) to define a transcriptional signature associated with cell competition (Fig. 3a; Extended Data Fig. 6d). Notably, many genes that were expressed at low levels in loser cells encoded ribosomal genes.

Armed with our cell competition signature, we next performed scRNA-seq on individual K14-H2BGFP+ epidermal cells from wild-type embryos at E12.5 and E17.5 (Extended Data Fig. 7a–e). Each time-point clustered distinctly (Fig. 3b). The data are consistent with a single population of unspecified progenitors at E12.5, whereas at E17.5, distinct sub-clusters emerged that encompassed interfollicular epidermis (IFE), differentiating suprabasal layers and hair follicle progenitors (Extended Data Fig. 7f).

To identify putative losers during embryonic development, we assigned each sequenced cell two scores23 based on expression of our winner or loser score genes. We then plotted each cell's score and performed a LOESS regression. Cells outside one standard deviation...
from the mean were considered winners (high winner scores, x axis) or losers (high loser scores, y axis; Fig. 3c, Extended Data Fig. 6e). Consistent with cell competition acting to increase fitness during development, there were fewer losers at E17.5 than E12.5 (Fig. 3c, middle). Moreover, and in contrast to E12.5, loser cells within E17.5 epidermis more often harboured a transcriptional identity associated with differentiation (Fig. 3c, right; Extended Data Fig. 7f).

**Developmentally regulated cell competition mechanisms**

Given these data, we wondered whether maturing epidermis might have cleared out most of its loser cells and therefore no longer require cell competition. To address this possibility, we first inspected mosaic CreRFP-transduced clonal patches of Mycn+/− epidermis across a developmental time-course. Between E12.5 and E14.5, levels of TUNEL+ neighbours were comparable, indicating that during rapid expansion of the progenitor monolayer, physical contact with wild-type winners led to loser death and engulfment (Fig. 4a). From E15.5 onwards, however, basal epidermal progenitors displayed a decline in expression of engulfment-related genes (Fig. 4b). Concomitantly, basal loser cells showed reduced proliferation (Extended Data Fig. 8a) but no signs of apoptosis. This result suggests that, at late embryonic time points, either cell competition is attenuated or cell competition clears losers by different mechanisms.

In mature epidermis, the innermost (basal) layer maintains its proliferative potential and fuels a steady outward cellular flux of differentiating cells. Because E17.5 cells that were transcriptionally classified as losers showed a trend towards differentiation, we investigated the possibility that late-stage losers are cleared by differentiation and upward efflux from the progenitor layer. Notably, however, fewer Mycn+/− loser cells were retained as K14+/− basal progenitors, more losers were found in the K10+ and FLG+ terminally differentiating layers of MycnR/+ epidermis (Fig. 4c; Extended Data Fig. 8b–d). Critically, tissue-wide depletion of Mycn did not accelerate epidermal differentiation (Extended Data Fig. 8e).

To further address whether this new mode of clearance entails bona fide cell competition, we analysed cell division angles. During normal epidermal development, the formation of stratified, differentiating layers is partially fuelled by orienting more than 60% of basal progenitor spindles perpendicularly to the basement membrane, such that one daughter cell remains within the progenitor pool while the other becomes suprabasal and fated to terminally differentiate. Notably, in E15.5 mosaic skin, spindle orientations became skewed towards perpendicularly divisions within loser Mycn+/− clones compared to wild-type counterparts (Fig. 4d; Extended Data Fig. 8f). In addition, knockdown of Lgn, which is required for asymmetric cell division (ACD) and differentiation in developing skin, resulted in increased retention of Mycn+/− cells in the basal layer at E17.5 (Fig. 4c).
Fig. 5 | Consequences of cell competition for epidermal barrier function and clonal dynamics. a, b. Representative images (a) and quantification (b) of recruitment of tight junction components claudin-1 (wild-type n = 6, BCL-XL+ n = 4) and ZO-1 (wild-type n = 8, BCL-XL+ n = 7) in control and BCL-XL+ embryos at E17.5 (n denotes number of images from two different mice per genotype, two-tailed Mann–Whitney test). c. TEWL measurements at E17.5 for control or BCL-XL+ embryos with or without Mycn+/− losers compared to controls (n denotes number of TEWL measurements obtained from all embryos in two litters per genotype; Kruskal–Wallis Test with Dunn’s multiple comparisons; bottom, two-tailed Mann–Whitney Test). d, Recruitment of tight junction components and TEWL measurements at P0 (two-tailed Mann–Whitney test). e, f. Quantification at E17.5 of basal cells per clone (e) and extent of differentiation (per cent basal cells within clone; f). Scatter plots show raw data; bar graphs show binned distributions. Differences from wild-type distributions assessed using Kruskal–Wallis test; n denotes number of clones counted from three litters per genotype; wild-type n = 44. Mycn+/− n = 28, BCL-XL+ n = 53. Scale bar, 50 μm. TEWL data shown as median ± interquartile range (box), minima/maxima (whiskers); all other data shown as mean ± s.e.m.

Most revealing, and in line with roles for both ACD and cell competition in promoting loser basal cell clearance, perpendicular spindle angling was higher in Mycn+/− progenitors that were surrounded by wild-type neighbours at E15.5 than in those that were surrounded by Mycn+/− neighbours (Fig. 4e). Although the correlation was modest, it was not found with control-labelled clones, suggesting that this mode of eliminating less fit cells may also depend on direct contact between winner and loser cells, one of the hallmarks of cell competition.

Functional consequences of cell competition
Finally, we tackled the physiological relevance of cell competition. To this end, we exploited the fact that apoptotic or engulfment-mediated cell competition was efficiently blocked in BCL-XL+ embryos and tested these embryos for epidermal barrier function, which in wild-type embryos is operative by E17.527. Tight junctions, which are critical barrier components of the upper epidermal layers, showed signs of compromise: cortical localization of the tight junction components claudin-1 and ZO-1 was diminished in E17.5 BCL-XL+ embryos compared to control embryos (Fig. 5a, b). In addition, as judged by trans-epidermal water loss (TEWL)28 assays, blocking apoptotic-mediated cell competition in BCL-XL+ embryos led to greater and more variable TEWL compared to control embryos (Fig. 5c, top; Extended Data Fig. 9a, b). When we enhanced cell competition through mosaic manipulation of Mycn expression, TEWL was even further increased in BCL-XL+ embryos; this increase was not observed when apoptotic cell competition was unperturbed (Fig. 5c, bottom). Although we cannot unequivocally exclude the possibility that BCL-XL has a role(s) other than cell competition during the emergence of barrier function, our results suggest that BCL-XL acts directly on cell competition (Extended Data Fig. 4e), and that when apoptotic cell competition is abrogated, basal progenitors cannot establish the skin’s barrier as efficiently as when cell competition is intact.

Notably, and consistent with a developmental switch in cell competition mechanisms, differences in tight junctions TEWL between BCL-XL+ embryos and wild-type embryos disappeared by birth (Fig. 5d). Together, these data suggest that sequential mechanisms for clearance of loser cells in the epidermis (progressing from death to differentiation) ensure that, if the first mechanism is defective, an effective barrier can still be established by birth. To further test this possibility, we used an R26Confetti-based lineage tracing strategy to investigate how cell competition impinges on clonal expansion dynamics during epidermal growth and differentiation (Extended Data Fig. 9c, d). We looked at clones that were wild-type, BCL-XL+ or Mycn+/− and measured the impact on growth and differentiation when cell competition was either accentuated (Mycn+/−) or intercepted (BCL-XL+).

Compared to wild-type clones, both Mycn+/− (pink) and BCL-XL+ (teal) clones showed substantial reductions in clone size (Extended Data Fig. 9e); this is not unexpected, given the slight decrease in proliferation we observed for both genotypes early in skin development (Fig. 1e; Extended Data Fig. 4d). However, closer inspection revealed differences in basal–suprabasal distributions between Mycn+/− and BCLX-L clones (Fig. 5c, f). Extended Data Fig. 9f). In line with their loser status, Mycn+/− clones were smaller and more differentiated than their wild-type counterparts, but by E17.5, most clones still had basal cells. By contrast, many BCL-XL+ clones consisted of fully differentiated cells, corroborating our assertion that in the absence of apoptotic cell competition, differentiation becomes a more prominent mechanism to rid the basal layer of less fit progenitors.

Discussion
In summary, we describe a physiologically relevant cell competition circuit that operates in a mammalian tissue. In the early stages of mouse skin development, when the epidermis is single-layered, arising loser cells trigger fitter neighbouring cells to kill and engulf them. This process depends upon loser–winner contact and occurs even in

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wild-type epidermis, as revealed by our transcriptomic studies and by the accumulation of corpses when engulfment is blocked. Thus, the early embryonic mouse epidermis appears to have retained evolutionary cell competition mechanisms similar to those in the fly.

The enrichment of ribosomal genes in our cell competition transcriptional signature provides a further striking parallel with classic Drosophila studies\(^{1-3,29}\), and fuels the idea that regulatory control at the level of translation or protein synthesis is a determining feature of cell fitness during growth. This notion might also explain why our study does not include genes from pathways that have been implicated in cell competition that may be regulated post-transcriptionally. The developmental switch from apoptosis- or engulfment-mediated cell competition to ACD or differentiation-mediated cell competition (Extended Data Fig. 10) sets the mammalian epidermis apart from other cell competition systems studied so far. The ability to remove loser progenitors from the basal layer via differentiation provides a backup mechanism to eliminate losers not cleared earlier by apoptosis. Thus, the stratified, differentiating epidermis of mammalian skin is endowed with an evolutionary advantage that single-layered epithelia lack.

As evidence that cell competition is a tumour-suppressive mechanism mounts\(^{15,16,30}\), our findings have implications for the control of growth not only in healthy tissues but also in disease. It has been proposed that spatial relationships and competitive behaviours amongst clones may act to impede malignant progression in human skin\(^{31}\), especially in light of the large mutational burden that accumulates in phenotypically normal tissue during postnatal life\(^{22}\). Indeed, in mouse models, wild-type epidermal cells can promote the regression of pre-cancerous overgrowths\(^{32}\). It is tempting to speculate that elimination of unfit cells in these contexts occurs through one or both of the mechanisms of cell competition that we describe. As we learn more about fitness-sensing mechanisms in tissues, it should become clearer whether cell competition could be harnessed for future therapeutic applications.

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Methods

Mouse lines and lentiviral constructs. Mice were housed in the Association for Accreditation and Certification of Laboratory Animal Care International (AAALAC)-accredited Comparative Bioscience Center at the Rockefeller University. All procedures were performed using Institutional Animal Care and Use Committee (IACUC)-approved protocols and in accordance with the procedures outlined in the Guide for the Care and Use of Laboratory Animals. The following previously generated mouse lines were used in this study: Rosa26MrtGlo (ref. 34; lacZ stock 007018), Rosa26Rluc1prb (ref. 35; lacZ stock 013731), Rosa26GFP (ref. 36), Keratin14rtTA (Fuchs lab), Mexo2Cre (ref. 21; lacZ stock 007755) and Rosa262TFP (ref. 37).

For most experiments, B6/CD1 mixed background heterozygous Mycn+/− females were bred to either CD1 wild-type males to generate Mycn+/− mice and Mycn−/− littermates. Embryos were injected with lentivirus at 9.5 days post coitum (d.p.c.) as previously described30. To induce recombination of transgenic cassettes, the following lentiviruses were injected: LV-Cre, LV-CreERT226, LV-nls-iCreH2BRFP2, or LV-nls-iCre-nlsGFP. To activate LV-CreERT2 activity at E14.5 in R26mtmgMycn+−, lentiviruses were injected into the basal epidermis from LV-CreRFP and LV-GFP at both E12.5 and E17.5. Images were segmented with ImageJ. Image processing and analysis. Cell proliferation assays. In most cases (except Extended Data Fig. 8a, for which mitotic events were measured by looking through chromosome condensation visible by DAPI staining), proliferation was inferred from the incorporation of labelled nucleotide analogues following a 45-min EdU pulse. To assess subtle differences in proliferation that occurred as a result of cell competition (as in Figs 1e, 2a), measurements were paired, and the incorporation of EdU by CreRFP+ cells was compared directly to that by their CreRFP− neighbours in the same region. This is especially important because, distinct from mosaic approaches in Drosophila, the lentiviral technique does not allow twin-spot analyses. However, in cases where we more generally assessed for changes in proliferation, we pooled EdU incorporation measurements between multiple animals of the same genotype, and performed unpaired analyses (as in Extended Data Figs. 3b, 4d).

Live imaging processing and analyses. In ImageJ, videos and single time-point images were processed by averaging three adjacent z-stack images and then applying a Gaussian blur of radius 1 pixel (0.27 μm). Death events were manually scored. For spindle orientation analysis, the spindle angle was calculated using the CellProliferation package, which was developed by the contributing author. Spindle orientation was defined relative to the basal plane of the epidermis from LV-CreRFP+ transduced embryos, which were imaged using the TUNEL assay.

Microscopy and live imaging. Live imaging was carried out based on a published protocol34. E12.5 mouse embryos were placed on their sides in a 35-mm Lumo-bottom dish (Sarstedt). Each embryo was immobilized with a custom-built holder and further stabilized in an agarose solution composed of 2% low-melting SeaPlaque Agarose (Cambrex) in a solution of epidermal culture medium. After equilibrating at 37 °C and 5% CO2 for ~2 h, imaging was performed on a customized modified invertedepinning disk confocal system (Andor) at 12-min intervals for up to 24 h (488 nm laser beams, 20× air objective (NA = 0.75). Time-lapse images were acquired with a Zyla CMOS camera (Andor). Three or four regions were filmed from each embryo. During imaging, the embryos were maintained at 37 °C and 5% CO2. Acquisition was controlled through ip software (Andor). All whole-mount immunofluorescence images were acquired using a Zen-software-driven Zeiss LSM 780 inverted laser scanning confocal microscope and a 20× air objective (NA = 0.8), a 40× water immersion objective (NA = 1.2), or a 63× oil immersion objective (NA = 1.4). Images of epidermal cryosections were acquired using a Zeiss Axio Observer.Z1 epifluorescent/brightfield microscope with a Hamamatsu ORCA-ER camera and an ApoTome.2 slider (to reduce light scatter in z), and controlled via Zen software. All images were assembled and processed using ImageJ.

Cell proliferation assays. In most cases (except Extended Data Fig. 8a, for which mitotic events were measured by looking through chromosome condensation visible by DAPI staining), proliferation was inferred from the incorporation of labelled nucleotide analogues following a 45-min EdU pulse. To assess subtle differences in proliferation that occurred as a result of cell competition (as in Figs 1e, 2a), measurements were paired, and the incorporation of EdU by CreRFP+ cells was compared directly to that by their CreRFP− neighbours in the same region. This is especially important because, distinct from mosaic approaches in Drosophila, the lentiviral technique does not allow twin-spot analyses. However, in cases where we more generally assessed for changes in proliferation, we pooled EdU incorporation measurements between multiple animals of the same genotype, and performed unpaired analyses (as in Extended Data Figs. 3b, 4d).

Live imaging processing and analyses. In ImageJ, videos and single time-point images were processed by averaging three adjacent z-stack images and then applying a Gaussian blur of radius 1 pixel (0.27 μm). Death events were manually scored. For spindle orientation analysis, the spindle angle was calculated using the CellProliferation package, which was developed by the contributing author. Spindle orientation was defined relative to the basal plane of the epidermis from LV-CreRFP+ transduced embryos, which were imaged using the TUNEL assay.

Tight junction analyses. To quantify the recruitment of tight junction markers, ImageJ was used to measure the integrated density (a well-established method of measuring fluorescence intensity that accounts for differences in area) of either claudin-1 or ZO-1 signal in the suprabasal layer of the skin (labelled by F-actin staining).

Transepidermal water loss assay. A Tewatometer TM300 (Courage + Khazaka electronic GmbH) was used to take measurements from the back skin of E17.5 embryos and P0 neonates. To adjust for the relatively small size of these mice, an adaptor was used on the probe head such that the area of measurement was ~2 mm2. We selected the shoulder region (just posterior to the ear) for analysis so that two measurements could be performed on each mouse in areas infected with either RFP+ or RFP−, which was also recorded for future correlation analysis, which was performed using Prism8 (Graphpad).
between the five measurements is minimized. These stable values were recorded as data points. The absolute value of the difference between the two values recorded from the left and right sides of each animal was used as an index of variability. Genotyping was performed following the experiment such that we were blinded to genotypes during measurements.

**Lineage tracing strategy for assessment of clonal growth dynamics.** To induce CreDD2 activity for R26Cre experiments, pregnant mothers were treated with 100 μl 2% tamoxifen (Sigma) dissolved in corn oil (Sigma) by intraperitoneal injection at 11.5 d.p.c. Embryos were dissected from the mother after euthanasia at 14.5 and 17.5 d.p.c. Dissected E17.5 back skins were stained for GFP, RFP, and P-cadherin (to label basal cells) and/or E-cadherin and mounted dermis side down, and entire pieces of back skin were imaged such that all labelled clones in the population were captured via laser scanning confocal microscopy. Overall clone size was manually counted at E17.5 (Extended Data Fig. 9e). The basal layer was labelled with P-cadherin, allowing us to identify cells within each clone that remained basal versus suprabasal (Fig. 5e, f; Extended Data Fig. 9f). E-cadherin could also be used to distinguish basal cells from all other cells owing to their distinctive morphology compared to all epidermal cells at E17.5. Induction efficiency was low so that clonal labelling density was sparse, affording us confidence that we were always observing single clones rather than merged clones of the same colour that may have intermixed as a result of clonal dispersion. Any clones of the same colour that were found within ~50 μm of one another were excluded from analysis. Membrane-bound CFP and cytoplasmic YFP could be detected with a GFP antibody and distinguished from one another by their subcellular localization. Consistent with prior reports, we rarely observed GFP-labelled clones. YFP, CFP, and RFP were found in roughly equal proportions throughout the back skin (Extended Data Fig. 9d).

**FACS and quantitative real-time PCR.** For scRNA-seq, single cell suspensions were obtained from either E12.5 or E17.5 K14-H2B-GFP/+ or K14-H2B-GFP/+; mice; single epidermal cells were sorted into 96-well plates sorting for lineage negative (‘lin’) GFP+ cells using a BD FACSariaII. CD45 (biotinylated rat anti CD45, BD Biocytener, 1:200), CD117 (biotintlated rat anti CD117/c-kit, Biocytener, 1:200), CD31 (biotinylated rat anti-CD31/PECAM, Bioscience, 1:200), and CD140a (biotinylated rat anti-CD140a, Biocytener, 1:200) were used as lineage negative markers (to exclude immune cells, melanoblasts, endothelium, and fibroblasts, respectively). All lin− cells were detected with a strepavidin-conjugated PeCy7 secondary antibody (Biocytener, 1:500). To obtain samples required to generate our cell competition signature, K14-H2B-GFP cells were further stained for annexinV (Alexo Fluor 568 conjugated; ThermoFisher Scientific, 1:200); two 96-well plates of cells were obtained for lin−/GFP+/annexinV− (losers) and two plates for lin−/GFP+/annexinV+ (winners). Mycn−/− epidermal cells (losers) were obtained from E12.5 K14-H2B-GFP/+;Mycn−/− embryos transduced at low titre with LV-CreH2B-RFP and sorted for lin−/GFP+/RFP−. Each sample comprised single cells from at least three embryos.

To measure Mycn levels in Mycn+/− cells, E12.5 basal epidermal cells were isolated from embryos infected with LV-nsCreH2B-RFP. Single-cell suspensions were stained with anti-CD49f/α4b8-PE/Cy7 (Biocytener, 1:1,000) and/or annexinV (Alexo Fluor 568 conjugated, Life Technologies, 1:200) and sorted using a BD FACSAriaII. RNA was isolated in Trizol from α4b8−, RFP+, lin− cells using the Direct-zol RNA Miniprep Plus kit (Zymo Research). CD45 (biotinylated rat anti CD45, BD Biocytener, 1:200), CD117 (biotinylated rat anti CD117/c-kit, Biocytener, 1:200), CD31 (biotinylated rat anti-CD31/PECAM, Bioscience, 1:200), and CD140a (biotinylated rat anti-CD140a, Biocytener, 1:200) were used as lineage negative markers (to exclude immune cells, melanoblasts, endothelium, and fibroblasts, respectively). All lin− cells were detected with a strepavidin-conjugated PeCy7 secondary antibody (Biocytener, 1:500). To obtain samples required to generate our cell competition signature, K14-H2B-GFP cells were further stained for annexinV (Alexo Fluor 568 conjugated; ThermoFisher Scientific, 1:200); two 96-well plates of cells were obtained for lin−/GFP+/annexinV− (losers) and two plates for lin−/GFP+/annexinV+ (winners). Mycn−/− epidermal cells (losers) were obtained from E12.5 K14-H2B-GFP/+;Mycn−/− embryos transduced at low titre with LV-CreH2B-RFP and sorted for lin−/GFP−/RFP−. Each sample comprised single cells from at least three embryos.

**Data availability** Single cell RNA sequencing data that support the findings of this study have been deposited in the Gene Expression Omnibus under accession number GSE128241. RNA sequencing data shown in Fig. 4b and Extended Data Fig. 1a were extracted from ref. 19 and were previously deposited in GEO with the accession number GSE75931. All other data are in the manuscript, Supplementary Materials and Source Data or are available from the corresponding author upon reasonable request.

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Extended Data Fig. 1 | Mycn is expressed in single-layered embryonic epidermis and its expression is halved in both Mycn\textsuperscript{fl/+};LV-CreRFP and Mycn\textsuperscript{fl/+};Meox2\textsuperscript{Cre/loxp} mice with no compensatory upregulation of Myc. 

\textbf{a}, Mycn and Myc expression in E10.5 epidermis by RNA-seq\textsuperscript{19}. Mycn is expressed at higher levels than Myc. 

\textbf{b}, Fluorescence activated cell sorting (FACS) strategy to isolate LV-CreRFP-transduced wild-type and Mycn\textsuperscript{+/-} basal keratinocytes from E12.5 embryonic skin. 

\textbf{c}, d, Quantitative PCR revealed that transcript levels were halved in Mycn\textsuperscript{+/-} cells compared to wild-type at E12.5 using both LV-Cre (n = 2) and Meox2\textsuperscript{Cre/loxp} (n = 4, unpaired two-tailed t-test) samples. No significant compensatory change in levels of Myc mRNA was observed. Embryos of each genotype within a single litter were pooled for cDNA collection. This was repeated to generate two biological replicates per genotype. Data shown as mean ± s.e.m.
Extended Data Fig. 2 | Live imaging reveals that loser cell death is preceded by increased contact with wild-type cells and suggests that engulfment mediates clearance of loser cell corpses. 

**a.** Schematic showing planes imaged in time-lapse microscopy experiments. 

**b–d.** Extracted images showing frames from Supplementary Videos 2–4. Dying GFP⁺/Mycn⁻ cells are marked with an arrowhead in the first frame of fluorescence images (top two rows) and by an asterisk in the traced panels (bottom). Wild-type cells are RFP⁺. 

**e.** Images from the suprabasal plane extracted from the first and last time points of Supplementary Video 1, and corresponding to the still images shown in the first and last panels of Fig. 1g. The asterisk marks the position of the dying cell 11 μm below. No new cells enter the suprabasal plane through the time course of the video, supporting the conclusion that the cell dies and is eliminated within the plane of the epidermis and not extruded to the suprabasal plane. Scale bars, 10 μm.
Extended Data Fig. 3 | A genetic model to switch the fate of Mycn\(^{+/−}\) epidermal cells from losers to winners. a, R26\(^{YFP}\) reporter for Cre activity shows undesired mosaicism in some embryos, which were prescreened and excluded from further analysis. All Meox2\(^{Cre}\) embryos in our study were subjected to this analysis. b, TUNEL stain of Mycn\(^{+/−}\); R26\(^{YFP}\) embryos revealed neither TUNEL labelling in regions of ubiquitous Cre activity (as indicated by YFP expression), nor any marked differences in proliferation as assayed by EdU incorporation (\(n\) denotes number of images analysed from each genotype; control \(n = 4\), Meox2\(^{Cre}\) \(n = 7\); two-tailed unpaired t-test). There also was no discernable effect on postnatal viability or growth (P15 males shown in bottom panels, genotypes as indicated). c, d, Extended fields of view (c) from images shown in Fig. 1m of whole-mount E12.5 back skins stained for DAPI (nuclei, blue), RFP (transduced cells, red) and TUNEL (dying cell corpses, white; arrowheads) for each of the indicated genotypes. An additional representative image and quantification (d) is also shown for a second Mycn shRNA (\(n\) denotes number of images quantified from two litters per genotype; Mycn\(^{+/−}\)/Mycn\(^{−/−}\) \(n = 13\), Mycn\(^{+/−}\)/Mycn\(^{−/−}\)/shMycn 2 \(n = 12\); two-sided Mann–Whitney test). Data show mean ± s.e.m, scale bar, 50 μm.
Extended Data Fig. 4 | Elevated BCL-XL expression in early embryonic epidermis is sufficient to block Mycn-dependent apoptotic cell competition and has no long-term consequence for skin development.  
a, Doxycycline-inducible activation of K14rtTA by lentivirally delivered TRE-driven transgenes at E12.5 (embryo transduced with TRE-GFP is shown, representative of results obtained in two independent litters).  
b, Experimental strategy for elevating BCL-XL expression in the early embryonic epidermis. TRE-Bcl2l1 was transduced at a relatively high titre, whereas CreRFP was transduced at a low titre (n = 4 embryos).  
c, Whole-mount images of E12.5 transduced embryos show that >50% of basal epidermal cells express BCL-XL and surround a small population of cells that express both BCL-XL (green) and CreRFP (red). TUNEL (white, signal inverted in right-most image) was quantified (Fig. 2a) relative to the position of CreRFP-expressing Mycn−/− loser cells that also express BCL-XL. Note that appreciable TUNEL labelling was not observed. Images are representative of data obtained from two litters.  
d, Proliferation in WT embryos at E12.5 (n = 4 per genotype) was lower in BCL-XL+ cells than in controls. At E17.5 there was no difference in proliferation between controls (n = 11) and BCL-XL+ cells (n = 14; n denotes number of images analysed from embryos from two litters for each genotype; two-sided Mann–Whitney test).  
e, Induction of BCL-XL expression from E9.5 to E15.5 had no appreciable consequence for epidermal differentiation or thickness at E17.5 (control n = 54; BCL-XL+ n = 68; n denotes number of thickness measurements taken from images of back skin cryosections from two mice per genotype). Representative images of back skin sections are shown immunolabelled for K14 (green) to mark the basal epidermis, and K10, Loricrin, or Filaggrin (red) to mark the differentiating spinous and granular layers. Dashed lines denote epidermal–dermal border; solid line demarcates the skin surface. Scale bars, 50 μm. Data are mean ± s.e.m.
Extended Data Fig. 5 | Epidermal cells, and not phagocytic immune cells, mediate clearance of corpses at E12.5. a, b, CD45^+ immune cells do not infiltrate the epidermis at E12.5. Wild-type and Mycn^fl/+ embryos were infected with LV-Cre at E9.5 and analysed at E12.5. Whole-mount immunofluorescence and confocal microscopy showed that CD45^+ immune cells (mostly macrophages at this time) were confined primarily to the deeper dermis. Immune cells were quantified over image tiles that encompassed a region of 425 μm × 425 μm (n denotes number of images analysed from two embryos per genotype, wild-type n = 5, Mycn^fl/+ n = 6; one-way ANOVA with Tukey's multiple comparisons test). Representative images from quantifications in a are shown in b at subsequent z positions within the tissue as indicated. c, Few uncleared cell corpses in Gulp1 shRNA-treated epidermis are RFP^+, suggesting that corpses do not accumulate simply because of the consequences of Gulp1 knockdown on cell viability. Left, quantification of RFP status of TUNEL^+ corpses in Gulp1 knockdown epidermis (n = 9 images from 4 embryos, two-tailed students t-test). Scale bar, 50 μm in b; 10 μm in c. Data are mean ± s.e.m.
Extended Data Fig. 6 | Generation of a cell competition signature and validation of method to classify wild-type epidermal cells via scRNA-seq. a, Experimental strategy to identify endogenous winners and losers in the epidermis. b, c, FACS-based sorting strategies to isolate winner and loser populations from the epidermis. d, Differential expression comparing transcriptomes of 39 winner (annexinV–) cells with those of 104 loser (66 Mycn+/– and 38 annexinV+) cells uncovers genes that define the cell competition signature (P values generated from Wilcoxon rank sum test). Top, loser genes; bottom, winner genes. e, Putative winners (annexinV+), putative losers (annexinV–), and Mycn+/– losers fall where expected relative to the wild-type distribution based on expression of cell competition signature genes.
Extended Data Fig. 7 | scRNA-seq of wild-type epidermal cells at E12.5 and E17.5. **a**, Sorting strategy to isolate wild-type epidermal cells. **b–f**, Principal component analysis (b, via the Jackstraw Method, see Methods) and t-SNE clustering (c). Analysis was performed to confirm that neither cell cycle differences (d) nor batch effects (e) account for the clustering. **f**, Further subclustering of E17.5 cells reveals distinct lineage populations: interfollicular epidermis (IFE), hair follicle (HF), and differentiating suprabasal cells (SB). Analysis was performed on 227 cells (111 E17.5 cells and 116 E12.5 cells). Heatmap shows the top 30 enriched genes that define each cluster.
Extended Data Fig. 8 | See next page for caption.
Extended Data Fig. 8 | Differentiation mediates the context-dependent exit of Mycn\(^{+/−}\) loser cells from the basal layer during cell competition in late epidermal development. a, Fewer Mycn\(^{+/−}\) proliferating basal cells than proliferating WT neighbours were observed at E15.5 (\(n = 15\) regions measured, two-sided Mann–Whitney test). b, c, Mycn\(^{+/−}\) loser cells were found more often than wild-type cells in the FLG\(^{+}\) layer of the epidermis at E17.5 (\(n > 10\) clones, two-sided Mann–Whitney test). d, Binned distributions extracted from data shown in Fig. 4c of GFP\(^{+}\)K14\(^{+}\) clones (left) and GFP\(^{+}\)K10\(^{+}\) clones (right) for each of three genotypes at E17.5: wild-type (top, grey), Mycn\(^{+/−}\) (middle, pink), and Mycn\(^{+/−}\);shLgn (bottom, teal). e, Tissue-wide loss of one allele of Mycn yields no epidermal differentiation phenotypes or evidence of accelerated differentiation. The thickness of the region labelled with three markers of differentiation in the skin (keratin-10, involucrin, and filaggrin) was measured and no significant difference was found between control and Meox2\(^{Cre}\);Mycn\(^{flo}\) embryos (\(n\) denotes number of regions measured from cryosections generated from two different animals per genotype; unpaired two-tailed \(t\)-test). R26\(^{YFP}\) was used as a marker of Cre expression and is shown in the inset. f, Spindle angle data from rose diagrams in Fig. 4d, plotted as a scatter plot for statistical comparison. Wild-type \(n = 32\), Mycn\(^{+/−}\) \(n = 25\); two-sided Mann–Whitney test. Scale bars, 50 \(\mu\)m. Data are mean ± s.e.m.
Extended Data Fig. 9 | Barrier assays and R26<sup>Confetti</sup> labelling experiments uncover functional consequences of disrupting cell competition during epidermal development. a, Strategy to block apoptotic cell competition and measure barrier function. b, Asymmetry score compares TEWL values from embryo’s left and right sides (n denotes number of embryos, F-test to compare variance). c, Experimental strategy for confetti lineage tracing. d, Representative whole-mount images of confetti-marked clones from each genotype at E17.5 (left, examples of clones from three litters of embryos analysed for each genotype). Examples of maximum projections and optical sections of individual epidermal layers are shown for each analysed fluorescent protein. SB, suprabasal. Right, quantification in wild-type embryos shows that approximately equal labelling efficiency is obtained at E17.5 with each of the three confetti fluorescent proteins (RFP n = 9 clones, YFP n = 11 clones, CFP n = 12 clones; Kruskal–Wallis test). e, f, Total clone size (e) and suprabasal clone size (f) dynamics for the genotypes indicated. All data are mean ± s.e.m. except for bar graphs, which show binned distributions.
Extended Data Fig. 10 | A transition in cell competition mechanisms during skin morphogenesis. Less fit loser cells are initially cleared from the developing epidermis by apoptosis and subsequent engulfment by epidermal neighbours. As the tissue begins to stratify and differentiate, at E15.5, losers are instead removed from the basal layer of epidermal progenitors via asymmetric cell division and differentiation into the suprabasal layers of the developing skin. Although mechanistically distinct from one another, both phases of cell competition require cell–cell contact between winners and losers to trigger loser cell elimination.
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- An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
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- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted. Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated
- Clearly defined error bars
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Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

**Data collection**

For microscopy, Zen Pro 2.0 (Zeiss) was used for acquisition of most imaging data. iQ3 (Andor) was used for live imaging. BD FACSDiva software was used for FACS.

**Data analysis**

Data was analysed using ImageJ (NIH), Microsoft Excel, Prism 7 (Graphpad) and Prism 8 (Graphpad). Single cell sequencing data was analysed using R (version 3.5.2), salmon (version 0.11.3), Seurat (version 2.3.4) and msir (version 1.3.2).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Single cell RNA sequencing data that support the findings of this study are deposited in Gene Expression Omnibus with the accession number GSE128241. RNA
sequencing data shown in Figure 4b and Extended Data Figure 1a were extracted from the study by Asare et al. and were previously deposited in GEO with the accession number GSE75931. All other data are in the manuscript, supplementary materials and source data or available from the corresponding author upon reasonable request.

Field-specific reporting

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

**Sample size**

Preliminary experiments were performed when possible to determine requirements for sample size, taking into account resources available and ethical, reductionist animal use. Sample size was somewhat limited by the litter size and low Mendelian ratios of complex genotypes but this was overcome by collecting at least 2 litters per experiment.

**Data exclusions**

No data were excluded, with the exception of the Meox2-Cre experiments, which is well known in the mouse field to be somewhat mosaic. Embryos were excluded in the case that the YFP reporter expression was notably mosaic.

**Replication**

Every experiment was performed on at least 2 embryos, and preferably at least 5 embryos, of each genotype from at least 2 independent litters. All attempts at replication were successful.

**Randomization**

No randomization was used beyond the random nature of Mendelian genetics.

**Blinding**

Investigators were not blinded to experimental groups/genotypes because in general this would preclude knowing what genotypes are associated with each sample post-analysis and would make it difficult to make sure there were enough n associated with each genotype. However, the TEWL experiments were blinded because it was possible to genotype following data acquisition.

Reporting for specific materials, systems and methods

**Materials & experimental systems**

| n/a | Involved in the study |
|-----|-----------------------|
|     | Unique biological materials |
|     | Antibodies |
|     | Eukaryotic cell lines |
|     | Palaeontology |
|     | Animals and other organisms |
|     | Human research participants |

**Methods**

| n/a | Involved in the study |
|-----|-----------------------|
|     | ChIP-seq |
|     | Flow cytometry |
|     | MRI-based neuroimaging |

**Unique biological materials**

Policy information about availability of materials

Obtaining unique materials

All unique materials are available from the authors upon request.

**Antibodies**

Antibodies used

Antibodies used were as follows: Rat anti-RFP (Chromotek, 5F8, lot#60706002AB, 1:1000), Rabbit anti-RFP (MBL, PM005, lot number 044, 1:1000), Chicken anti-GFP (Abcam, ab13970, Lot#GR3190550-6, 1:1000), Rat anti-E-cadherin (M. Takeichi, 1:50), Rabbit anti-keratin14 (Fuchs lab, 1:200), Rabbit anti-keratin10 (Covance, poly19054, Lot#D15LF02452, 1:500), Rabbit anti-Cleaved-Caspase3 (Cell Signalling cat#95795 lot 1, 1:200), rabbit anti-myc epitope [71D10] (Cell Signalling, cat#2278, lot#5, 1:200), rat biotinylated anti-CD45 (BD Bioscience, cat# 553078, lot#629869, 1:1000), rabbit anti-survivin (Cell Signalling, 71G4B7, 1:200), rabbit anti-Involucrin (Biolegend, PRB-140C, 1:1000), rabbit anti-Filaggrin (Fuchs lab, 1:500), rabbit anti-Loricrin (Fuchs lab, 1:500), and goat anti-P-cadherin (R&D, AF761, 1:200), rat
anti-CD104 (beta4-integrin; BD Biosciences, 1:200), rabbit anti-Claudin 1 (Cell Signaling, DSH1D, 1:100), rabbit anti-ZO-1 (Zymed, 2533938, 1:200), and Alexafluor568-conjugated AnnexinV (Life Technologies, A13202, Lot#1848438). Secondary antibodies used for immunofluorescence: Alexafluor488-conjugated donkey anti-rabbit (Jackson ImmunoResearch Lab, 711-545-152, 1:500), Alexafluor488-conjugated donkey anti-rat (Jackson ImmunoResearch Lab, 712-295-150, 1:500), Rhodamine Red-conjugated donkey anti-rat (Jackson ImmunoResearch Lab, 712-295-150, 1:500), Rhodamine Red-conjugated donkey anti-rabbit (Jackson ImmunoResearch Lab, 711-295-152, 1:500), Alexafluor647-conjugated donkey anti-rabbit (Jackson ImmunoResearch Lab, 712-605-150, 1:500) and Alexafluor647-conjugated donkey anti goat (Jackson ImmunoResearch Lab, 705-605-003, 1:500). Secondary antibodies used for flow cytometry were FITC-conjugated streptavidin (Biolegend, 405202, lot# B231737, 1:500), APC-Cy7-conjugated streptavidin (BD Pharmingen, 554066, lot#7067781, 1:500).

Validation

Rb anti-keratin14, anti-Filaggrin, and anti-Loricrin antibodies were generated in the Fuchs lab and the Rat anti E-cadherin antibody was a gift of M. Takeichi. These antibodies were validated by immunofluorescence on control and knock-out tissue. All other antibodies are commercially available and validated as noted on the manufacturers website. They were also subject to additional validation in the Fuchs lab by immunofluorescence and/or flow cytometry.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals

The following previously generated mouse (Mus musculus) lines were used in this study: Rosa26mTmG, Rosa26Brainbow2.1, Mycnfl/fl, Keratin14rtTA, Meox2Cre, Rosa26EYFP, K14-H2B-GFP. All strains were maintained in a mixed CD1 background. Ages of embryos analysed ranged from E12.5 – E17.5, as well as P0 neonates. Both sexes were used as sex was not determined in embryos.

Wild animals

The study did not involve wild animals.

Field-collected samples

The study did not involve samples collected from the field.

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a ‘group’ is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

E12.5 embryos were incubated overnight at 4C in 1:1 PBS/Dispase to detach epidermis from the dermis. Skin was dissected and incubated in trypsin for 20min at 37C, and then filtered through a 40um cell strainer (VWR) to generate a single cell suspension.

Instrument

BD FACSAriaII, 85um nozzle for experiments shown Extended Data Figure 1; 100um nozzle for single cell isolation associated with single-cell RNA-sequencing

Software

BD FACSDiva Software

Cell population abundance

Lin-K14-H2B-GFP cell populations were about 5-10% at E12.5 and 30-40% at E17.5. Mycn+/- cell population is ~1-5% of parent cell population from E12.5 dissected backskins.
Gating strategy

For cell isolation underlying qPCR experiments, cells were gated on DAPI-negative (for live/dead) FSC/SSC singlets. Lineage-negative cells were identified by staining with biotinylated primary antibodies against CD45 (to exclude immune cells), CD117/c-kit (to exclude the melanoblast lineage), CD31 (to exclude endothelial lineage), and CD140a (to exclude fibroblasts). All lineage-negative cells were labelled with a streptavidin-FITC conjugated secondary antibody. CD49f/alpha6high cells (a marker of the basal epidermis) that double labeled with RFP (as a marker of LV-Cre transduction) were isolated to identify WT LV-Cre transfected cells (control genotype) and Mycnfl/+ LV transfected cells (Nmyc+/- experimental genotype). For cell isolation underlying single-cell RNA-sequencing, single cell suspensions were obtained from either E12.5 or E17.5 K14-H2B-GFP/+ mice; single epidermal cells were sorted into 96-well plates sorting for lineage negative (“lin−”) GFP+ cells using a BD FACSariaII. CD45 (biotinylated rat anti CD45, BD Biolegend, 1:200), CD117 (biotinylated rat anti CD117/c-kit, Biolegend, 1:200), CD31 (biotinylated rat anti-CD31/PECAM, Bioscience, 1:200), and CD140a (biotinylated rat anti-CD140a, Biolegend, 1:200) were used as lineage negative markers (to exclude immune cells, melanoblasts, endothelium, and fibroblasts, respectively). All lineage-negative cells were detected with a strepavidin-conjugated PeCy7 secondary (Biolegend, 1:500). To obtain samples required to generate our cell competition signature, K14-H2B-GFP cells were further stained for AnnexinV (Alexa Fluor 568 conjugated; ThermoFisher Scientific, 1:200); 2 96-well plates of single cells were obtained for each lin−/GFP+/AnnexinV+ (losers) and 2 plates worth of lin−/GFP+/AnnexinV− (winners). Mycn+/− epidermal cells (losers) were obtained from E12.5 K14-H2B-GFP/+; Mycnfl/+ embryos transduced at low titer with LV-CreH2B-RFP and sorted for lin−/GFP+/RFP+. Each sample comprised single cells from at least 3 embryos per genotype.

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.