Supplementary Note

Quantitative mathematical modelling

Modelling of H2B-GFP fluorescence dilution data implies two independent populations with different proliferation rates and primarily coupled division and transport for the rapidly dividing cells

The BL, SL, and GL cells are labeled with the same maximal amount of H2B-GFP at the beginning of the doxycycline-induced chase. As the chase proceeds, BL stem cells (SCs) and progenitors divide, diluting the H2B-GFP per cell by a factor of two at each division, so the number of times that a cell has divided since the start of the chase, d, can be determined from the 2^d-fold reduction in its H2B-GFP fluorescence. Therefore, H2B-GFP fluorescence intensity histograms of BL cells indicate the relative proportions of cells that have divided $d = 0, 1, 2, \ldots$ times[1,2]. This d-distribution depends primarily on the rate of cell division, but is also affected by the rate at which the undivided and divided cells are transported from the BL to the SL. Transport of divided cells into the SL and GL replaces the $d = 0$ cells in these compartments, so their H2B-GFP histograms provide insight into the transport mechanism and rates. (Since CL cells are not included in the FACS analysis, GL→CL transport is not analyzed here.)

In these experiments, tdTomato, α6-integrin, and CD34 markers were used to identify BL, SL, and GL cells from the back skin of mice that had been sacrificed after 3-, 7-, and 21-day chases (Fig. 3a). Their H2B-GFP histograms (Fig. 3b) were deconvolved (Fig. 3d) as previously described[3] to determine the d-distributions for all 9 compartment/chase duration combinations (Fig. 3e, green). (See Theoretical Methods, below, for further details.) A maximum of eight peaks could be resolved in the FACS data, which limited the analysis to $d \leq 7$.

As discussed in the main text, two observations are striking: First, most of the BL cells are dividing rapidly—85% of them divided at least once by 3 days. Second, all the SL and GL cells are replaced at similar fast rates. The second observation is only partly explained by the rapid division and the homeostatic requirement that the density of cells in each compartment remain constant. Homeostasis implies that the BL→SL transport rate must equal the mean BL division rate, λ^{BL}, and that the SL→GL and GL→CL transport rates are fixed by λ^{BL} and the ratios between the layer cell densities (ρ^{BL}, ρ^{SL}, and ρ^{GL}); the transport rates are not independent parameters. The cell density ratios were determined by microscopy and cell counting of the BL, SL, and GL in back skin sections to be $\rho^{\mathsf{SL}}/\rho^{\mathsf{BL}} = 0.78 \pm 0.07$ and $\rho^{\mathsf{GL}}/\rho^{\mathsf{BL}} = 0.52 \pm 0.05$. Since the ratios are less than one, we expect the SL→GL and GL→CL upward cell transport rates to be faster than the mean division rate.

However, even these fast transport rates would not be adequate to account for the very rapid replacement of undivided SL and GL cells with divided cells from the BL if division were uncorrelated with transport. (We call this "uncoupled division".) In that case, the transported cells would be randomly selected from, and have the same d-distribution as, the BL cells. Then, because of the time required to displace the bulk of the SL and GL cells, the SL and GL d-distributions would lag the BL distribution. On the other hand, the rapid reduction in the number of undivided SL and GL cells can be explained by the hypotheses that rapid division is tightly "coupled" to transport. In that case, every transported cell will have just divided, and at each instant the mean number of divisions of the transported cells will be one greater than the mean number of divisions of the BL cells. This will accelerate the rate of change of the d-distributions in both SL and GL.

We quantitatively tested this hypothesis by dynamically modelling the division and transport processes. We tested both previously analyzed[4,5] and new models that differ in the numbers of subpopulations within the BL, SL, and GL compartments, the lineal relationships between them, and the extent of coupling between division and BL→SL transport. All the models allow stochastic variation of both division and transport intervals, which are parameterized by the mean rates. The best-fit parameters were determined by minimiz-

ing the total discrepancy Δ between the experimental and predicted d-distributions summed over the nine compartment/chase-duration measurements (Figs. 3e and Supplementary Fig. 3b; The bars are normalized so that the sums of densities over the displayed ranges are unity). $\Delta = 0$ for a perfect fit and increases as the fit deteriorates; the best-fit parameters and Δ for the different models are given in Fig. 3c and Supplementary Fig. 3a. Adding the number of parameters to Δ approximates half the Akaike Information Criterion[6] and can be used to compare models with different numbers of parameters.

To challenge the inference that the fast division must be coupled to BL→SL transport, we considered three models in which division and transport are *un*coupled. The simplest such model is one in which the BL contains a single population of stem cells (SCs) that symmetrically divide to SCs at rate λ^{S} while also undergoing uncoupled, stochastic differentiation and transport from BL→SL, and then from SL→GL→CL (Supplementary Fig. 3a, blue). As in all the models considered, the transport rates are not independent parameters; rather, they are determined from λ^{S} by the homeostatic requirement that the density of cells in each layer be constant in time. While this model with the best-fit value of $\lambda^{\mathsf{S}} = 0.29/\text{day}$ fits the BL division data reasonably well (Supplementary Fig. 3b, blue), the transport rate is much too slow and does not fit the SL and GL data. In this model complete replacement of the undivided $d = 0$ cells in SL and GL is not predicted until 3 weeks, while the data show that most SL and GL cells are replaced by 3 days and that all cells are replaced by 1 week (Supplementary Fig. 3b, green).

We next tested the single-progenitor "P-D" (or "committed progenitor") model that was proposed by Clayton et al.[4] to explain their lineage-tracing experiments. In this model the BL contains a population of self-renewing, dividing progenitor (P) cells that generate a second population of non-proliferating differentiated cells (Ds). Only the differentiated cells are transported to the SL, so transport and division are uncoupled (Fig. 3c, gray). Both division and transport are modeled as random processes governed by mean rate constants. The predictions of the model for a fluorescence dilution experiment are governed by two independent parameters: the fraction of differentiated cells in BL and λ^{P}, the mean progenitor division rate. (The mean transport rate is fixed by the homeostatic requirement of constant cell density. The symmetry parameter r that was used in applying the P-D model to lineage tracing data is not relevant for fluorescence dilution analysis; therefore, we do not distinguish between symmetric and asymmetric divisions in Fig. 3c.) Clayton et al. found that this model provided a good fit to their lineage tracing data with 78% of the BL comprised of differentiated cells and $\lambda^{\mathsf{P}} \approx 0.16/\text{day}$. The model with these values clearly does not fit the d distributions from our H2B-GFP dilution data since it implies that only half of the BL cells would have divided by day 3. An improved, but still poor, fit to the BL data can be obtained if we use the best-fit division rate of $\lambda^{\mathsf{P}} = 1.7/\text{day}$ ($\Delta = 204$; Supplementary Fig. 3b, gray); but this very fast rate is biologically improbable, and the predicted transport rates are still much too small to explain the SL and GL H2B-GFP dilution data. This problem can be ameliorated if the proportion of differentiated cells in the BL is decreased to 50%, but even then the best fit that can be obtained (with $\lambda^{\mathsf{P}} = 0.58/\text{day}$) does not predict the observed fast replacement of the undivided cells in SL and GL ($\Delta = 70$; Fig. 3e, gray).

If both P-D model parameters are allowed to vary without constraint, the best-fit model contains only 8% differentiated cells in the BL and $\lambda^{\mathsf{P}} = 0.47/\text{day}$. Because the fraction of differentiated cells is so small, to maintain constant BL cell density the differentiated cells must be transported from BL→SL almost as soon as they are created. Therefore, the model with these parameters is degenerate and is effectively equivalent to a single-population model in which division is coupled to transport: it generates almost the same d-distribution and discrepancy ($\Delta = 35$) as the single-population coupled SC model (see below and Supplementary Fig. 3b, dark blue). As with that model, the fit to the SL and GL data is improved, but the fit to the day 7 BL distribution is degraded. For example, the p-values for the $d = 0$ and $d = 1$ probabilities under the best-fit model are 10^{-7} and 5×10^{-7}, respectively. No good balance between the requirements imposed by the SL and GL data and those imposed by the BL data could be found. Moreover, the P-D model requires an additional adjustable parameter to do this, so it is not of interest. We conclude that the P-D model is poor for these data.

We also considered the stem cell/progenitor "SC-P-D" model that was introduced by Mascre et al.[5] This makes both quantitative and qualitative changes to the P-D model: (1) The fraction of differentiated cells in the BL is decreased to 20%. (2) The model adds a SC population that undergoes stochastic division both for self-renewal and the generation of Ps (Fig. 3c, blue). This introduces two additional parameters that are relevant to analyzing a H2B-GFP dilution experiment: the proportion of the BL composed of SCs and λ^{S}, their mean division rate. This model with the Mascre et al. parameters (5% SCs, $\lambda^{\mathsf{S}} \sim 5$/year, $\lambda^{\mathsf{P}} = 0.18/\text{day}$) also failed to fit the data.

For an extended test of the possibility that hierarchically combining a SC population with a descendant progenitor population could improve performance, we tested the model allowing all parameters except for the fraction of differentiated cells to vary. An improved best-fit was obtained ($\lambda^{\mathsf{S}} = 0.30$/day, $\lambda^{\mathsf{P}} = 0.48$/day; $\Delta = 37$; Fig. 3e, blue), but it contains no SCs and so is equivalent to the P-D model, albeit with a reduced fraction of differentiated BL cells. When all four SC-P-D parameters were allowed to vary without constraint, the best-fit model was the same as the unconstrained P-D model: it has no SCs and only a small number of differentiated cells that are almost immediately transported from BL→SL after creation. Thus, like the P-D model, the SC-P-D model cannot fit all the data even though two additional adjustable parameters are included. We conclude that allowing for a SC population that generates the progenitor population does not improve the fit to the data.

Having demonstrated that models in which division and BL→SL transport are uncoupled cannot well-model all the data, we tested the simple single-population SC model in which these two processes are tightly coupled (Supplementary Fig. 3a, dark blue; red arrow denotes coupled transport). This could model, for example, the biological situation in which the mitotic plane is perpendicular to the basement membrane and division is physically asymmetric: a SC attached to the basement membrane divides into a SC that remains membrane-attached and a descendant that is transported to the SL and differentiates[7]. As a result, divided cells are more rapidly transported from BL→SL. (However, physical asymmetry is not required and the model is consistent with any mechanism that couples division to BL→SL transport.) Like the uncoupled SC model, there is only one adjustable parameter—the SC division rate λ^{S}, which had a best-fit value of 0.47/day ($\Delta = 36$). As hypothesized, the coupling greatly improves the fit to the SL and GL data (Supplementary Fig. 3b, dark blue). The fit obtained with this simple model is very close to that obtained with the more complicated unconstrained P-D and SC-P-D models. We conclude that the data strongly suggest that most fast division is tightly coupled to BL→SL transport and that adding multiple lineally related components to the BL does not improve the fit to the data.

However, as discussed above, none of these models provides a good fit to the day 7 BL d-distribution. This suggests that the BL contains an additional population that divides at a slower rate. The most economical way to model this is by including a second, slowly dividing SC population. This gives a model with two independent proliferating populations that we call the "two-SC model" ("2×SC"; Fig. 3c, yellow). It has three independent parameters: the division rates of the fast- and slow-dividing populations, λ^{S_1} and λ^{S_2}, and the fraction of fast SCs in BL, f^{S_1}. We considered variants of the model having different degrees of coupling between division and BL→SL transport. For simplicity, we first consider a semi-coupled model in which division of the fast population is completely coupled with transport (red arrow in Fig. 3c, yellow) while division of the slow population is completely uncoupled, since this is adequate to well-model the H2B-GFP dilution data. (In the sequel we discuss a refinement of the model for analyzing the lineage tracing data.) This model well-fits the data if cells in the fast population divide on average once every two days ($\lambda^{\mathsf{S}_1} \approx 0.51/\text{day}$), cells in the slow population divide on average every ~ 5 days ($\lambda^{\mathsf{S}_2} \approx 0.19/\text{day}$), and the fast cells comprise about two-thirds of the BL ($f^{\mathsf{S}_1} \approx 0.70$; $\Delta = 27$; Fig. 3e, yellow).

We conclude that dynamic modelling of the H2B-GFP fluorescence dilution suggests that the BL contains at least two independently dividing populations and that transport of at least most of the rapidly dividing cells is coupled to cell division. The slowly and rapidly dividing SC populations identified here probably correspond to the LRC and non-LRC populations identified from the independent experimental data. The

quantitative modelling implies that the non-LRC's divide ~ 2.5 times faster than the LRCs. The correspondence is further supported by the agreement between the fraction of fast SCs in the model (f^{S_1}) and the fraction of non-LRCs estimated by examining the areas of the spatially separated, non-LRC and LRC regions in the tail: photomicrography suggests that they are present in a 65:35 ratio (Fig. 1h and Supplementary Fig. 5a), which is close to the 70:30 ratio computed here.

The tail epidermis short-term H2B-GFP fluorescence dilution data reported by Mascre et al.[5] for the BL compartment at 6 and 9 days (their Fig. 3j and Supplemental Fig. 6d) is consistent with the 3- and 7-day back skin BL data reported here (Fig. 3e, green). However, their 3-week data (their Fig. 3j) is quite different from ours (Fig. 3e, green). While our data show much larger proportions of highly divided cells at 3 weeks relative to 7 days, their 3-week proportions are only increased slightly beyond their 9-day proportions. This may reflect differences in the experimental selection procedures, mouse age during the chase, or the type of skin (tail vs back) that was analyzed. For example, it may be relevant that we used an additional selection to isolate cells for H2B-GFP FACS analysis: in addition to the $\alpha 6$-integrin$^+$/CD34$^-$ selection used in both sets of experiments, the cells analyzed here were also selected for K14$^{\mathrm{CreER}}$-induced recombination and expression of the tdTomato marker (Figs. 2a and 3a). At the low doses of tamoxifen used, this selection excluded other epidermal skin compartments such as infundibulum, isthmus, hair follicle outer root sheath, sebaceous gland, and hair germ that could contribute to the data. In any case, their modelling[5] may not have revealed the fast-dividing population because, as shown above, the identification depends critically on including the SL and GL data, which was not available for their analysis.

Turnover and division rates in the tail scale (enriched in non-LRCs) and interscale (enriched in LRCs) epidermal regions

The ability to morphologically distinguish the scale and interscale epidermal regions in the tail provided an opportunity to compare the division rates within these regions with the values calculated from the H2B-GFP dilution data. (This could not be done for back skin, because its non-LRC and LRC regions are not morphologically distinct in tissue sections prior to doxycycline chase.) K5-tTA/pTRE-H2B-GFP mice were chased with doxycycline (Supplementary Fig. 3c), and the H2B-GFP signals within the BL and SL (scale) or the BL and SL+GL (interscale) regions were measured by fluorescence microscopic image analysis of transverse sections (Supplementary Fig. 3d). The initial (i.e., day 0) H2B-GFP fluorescence signals varied by only $\sim 5\%$ between mice, so the temporal reduction of fluorescence could be determined by comparing the values from mice chased for different intervals (Supplementary Fig. 3e). By interpolation we see that the turnover half-lives for the BL plus analyzed suprabasal region are ~ 3.5 days (non-LRC) and ~ 6.2 days (LRC).

The least-square best-fit non-LRC and LRC division rates were determined from the BL data using the fast (S_1) and slow (S_2) 2×SC subpopulation models, respectively. This gave $\lambda^{S_1} = 0.40$/day and $\lambda^{S_2} = 0.14$/day. These values are 20–25% lower than the best-fit values from the back skin H2B-GFP dilution experiments, while the ratio between them, $\lambda^{S_1}/\lambda^{S_2} = 2.9$, is very close to the back skin ratio, 2.7. (The division rate ratio differs somewhat from the inverse of the turnover half-life ratio because the BL H2B-GFP turnover depends on the extent to which division is coupled to transport.)

This excellent agreement, allowing for overall slightly slower cell division in the tail than in the back, supports the two stem cell model and the hypothesis that the back and tail LRC and the back and tail non-LRC populations are closely related.

Lineage tracing implies a mixture of coupled and uncoupled division/transport

Mixed (hybrid) coupling and uncoupling of non-LRC and LRC division to BL→SL transport. SCs that divide with complete division/transport coupling, such as the fast SCs in the minimal 2×SC model described above, cannot form BL clones since the descendants are transported as quickly as they are gen-

erated. However, lineage tracing showed that the non-LRCs as well as the LRCs form clones in the BL (Fig. 4f). To accommodate this observation, the 2×SC model must be extended to allow at least some uncoupled division of the fast (i.e., non-LRC) population as well. A mixture of coupled and uncoupled division/transport might result from differing microenvironments or from stochastic behavior within a single environment with probabilities $1 - u^{S1}$ for coupled- and u^{S1} for uncoupled-division/transport (Supplementary Fig. 3a, yellow; red arrow denotes coupled transport). u^{S1} cannot be determined from the dilution data, but we found that the stochastic hybrid model provides a better fit than all the other models as long as $u^{S1} \leq 30\%$. For example, Supplementary Fig. 3b, yellow, displays the best-fit of the 2×SC hybrid model ($f^{S1} = 0.74$, $\lambda^{S1} = 0.47$, $\lambda^{S2} = 0.19$, $u^{S1} = 0.20$), in which 20% of the rapidly dividing cells divide symmetrically and retain both descendants in the BL, thus allowing for clone formation in the BL.

Conversely, while the H2B-GFP dilution data was well-fit by the minimal model in which slow population division was completely uncoupled from BL→SL transport, that data does not exclude the possibility that the slow population also divides with partial coupling to transport.

Neutral drift and clone number evolution in the tail interscale. In many, if not most, cases, homeostatic tissue replacement involves neutral competition between clones, which predicts a neutral drift of individual clone sizes and numbers with time in lineage tracing experiments: marked stem cells form a decreasing number of clones having increasing mean size over time[8]. The rate of change depends on the probability that a cell division changes the number of proliferating cells in the BL: "asymmetric divisions" do not change the number while "symmetric divisions" decrease or increase the number by one. In this context these terms do not imply physical asymmetry or symmetry of the division process. For example, a division that is tightly coupled to BL→SL transport in the 2×SC model is asymmetric because the number of SCs does not change, whether or not this results from physical asymmetry[7] or another biological mechanism. Conversely, in this context an uncoupled division is symmetric regardless of the physical symmetry of the process. The hybrid 2×SC model is consistent with neutral drift; therefore, quantitative analysis of lineage tracing experiments can be used to estimate the fraction of uncoupled divisions in the non-LRCs (u^{S1}) and LRCs (u^{S2}).

Since Dlx1 is preferentially expressed in the LRC cells (Figs. 4c and 5c), we used it in lineage tracing to estimate u^{S2} in the tail epidermal interscale regions: Dlx1^{CreER}/Rosa-tdTomato mice were injected with tamoxifen to induce low-frequency Dlx1^{CreER}-induced recombination and expression of tdTomato. This permitted the change in the number and total vertically projected size (i.e., fractional area) of the tdTomato^{+} clones within the line and non-line interscale substructures to be measured for periods up to one year. Consistent with neutral drift, the number of clones decreased (Supplementary Fig. 5f) even as their fractional area increased due to clone spread into the suprabasal region (Fig. 5f). Combining this information with the interscale LRC division rate determined from the H2B-GFP reduction measurements ($\lambda^{S2} = 0.14$/day; Supplementary Fig. 3e) implies that the probability of uncoupled division is similar in both interscale regions (Supplementary Fig. 5g, solid lines): $u^{S2} = 0.18$ (non-line) and 0.12 (line). Accounting for the 2.6:1 ratio of non-line to line area (Supplementary Fig. 5a), we estimate that $u^{S2} \approx 0.16$ in interscale overall. This value for the LRCs is within the range $u^{S1} \leq 0.3$ that is consistent with the H2B-GFP dilution data for the non-LRCs, so it is possible that both populations divide with the same degree of coupling.

The data exclude the alternative model that the extent of coupling between division and transport is determined by microenvironments within the interscale and scale subregions as long as the Dlx1^{CreER}-marked cells are representative of the complete LRC population. However, while it seems unlikely, we cannot exclude the possibility that Dlx1 marks a subset of the LRCs that divide with a slower rate and with different values of u^{S2}. The slowest rate consistent with the data is $\lambda^{S2} \sim 0.02 - 0.03$/day, which would fit the data if division were completely uncoupled (i.e, $u^{S2} = 1$; Supplementary Fig. 5f, dotted lines).

The lineage of the small number of Dlx1^{CreER}-marked cells in the scale region (which mainly contains non-LRCs) is unclear since Dlx1 is only a preferential, not absolute, marker of LRCs. The number of

clones formed by these cells decreased with time at about the same rate as the Dlx1$^{\text{CreER}}$-marked cells in the interscale (Supplementary Fig. 5f). However, these clones were almost twice as small as those in the non-line interscale (Supplementary Fig. 5e), indicating decreased proliferation and differentiation of the Dlx1$^{\text{CreER}}$-marked cells in the scale region.

Similar analyses were not performed to determine u^{S_1} for the non-LRC cells because the rapid growth and overlap of the Slc1a3$^{\text{CreER}}$-marked clones prevented counting clone number at the later times.

In summary, the H2B-GFP dilution data and lineage tracing data together imply that the non-LRCs divide at similar fast rates (0.4–0.5 divisions/day) in both the back and tail with most, but not all, divisions tightly coupled to BL→SL transport. The back and tail LRCs divide at slower rates (0.14–0.19 divisions/day) and it is likely, but not unequivocal, that most of their divisions are also coupled to transport.

Comparison to previous quantitative models of the mouse epidermis

The hybrid 2×SC model is quite different from the P-D and SC-P-D models previously proposed by Clayton et al.[4] and Mascre et al.[5] Both SC populations in our hybrid 2×SC model are independent and have two distinct populations of differentiated descendants that travel independently through the SL and GL. In contrast, the P-D Clayton et al. model differs from a single-population model only in that differentiated cells are retained in the BL before transport to the SL; only a single type of differentiated cells travels through the SL and GL in this model. In the same vein, while the SC-P-D Mascre et al. model introduces another population, K14$^{\text{CreER}}$ SCs, into the BL, these do not comprise an independent population. Rather, these SCs are precursors of the Inv$^{\text{CreER}}$ cells. Moreover, they are not spatially segregated and only one type of differentiated cell is generated. These differences are displayed in the subdivisions of the bars in Fig. 3e and Supplementary Fig. 3b, which show the individual subpopulation contributions.

The Clayton et al., Mascre et al., and hybrid 2×SC models all imply that neutral drift of clone number and size will be observed in lineage tracing experiments, and such data can be used to estimate the mixture of "asymmetric" and "symmetric" division that is occurring[8]. Interestingly, the best-fit division rate (~ 0.17/day) and asymmetric division fraction ($\sim 80\%$) computed using those models for the mouse tail Ah$^{\text{CreER}^+}$ and Inv$^{\text{CreER}^+}$ committed progenitor populations are both similar to the corresponding best-fit values (~ 0.14/day and $\sim 84\%$, respectively) computed from the 2×SC model for our tail skin LRC population. However, the biological processes that are assumed to explain the mixture of symmetric and asymmetric divisions in the neutral drift process are different: in the previous models, asymmetric division generates one differentiated and one committed progenitor cell in the basal layer, and symmetric division generates either zero or two differentiated cells in the basal layer; all three types of division are uncoupled from transport. In the hybrid 2×SC model, asymmetric (i.e., coupled) division involves the closely coincident transport of one descendant to the SL during a division, while neither descendant is immediately transported during a symmetric (i.e., uncoupled) division; there is no need to posit differentiation within the BL. However, the possibility of some small fraction of differentiated cells in the BL or additional minor subpopulations is not excluded.

Relationship to the mouse epidermis turnover rate

Potten et al.[9] injected mice with [^{14}C]thymidine and showed that the time to peak ^{14}C in the GL was ~ 4.5 days. This includes the time required for cell division in the BL (during which the thymidine is incorporated) and BL→SL→GL transport, and so represents the turnover time of the nucleated epidermal layers. Since transport itself requires on average about three days [9], this implies that a large amount of BL cell division must occur with two days. This is consistent with our H2B-GFP data, which implies that $\sim 70\%$ of the BL cells divide on average every two days. We quantitatively tested this by simulating the thymidine-labelling experiments using the 2×SC model and, in remarkable agreement, found that the peak of the transit-time distribution of a radioactive label from the BL to the GL was also ~ 4.5 days.

Summary of modelling results

The simultaneous collection of H2B-GFP dilution data for the SL and GL layers along with the BL layer permitted a more detailed and accurate analysis of the kinetics of mouse epidermal division and transport than has previously been possible. Mathematical modelling of this data allowed us to infer, independently of the molecular analysis, that the back skin BL contains at least two independent dividing populations of cells. The rapidly dividing population, which comprises about two-thirds of the BL, divides on average about once every two days; this is almost three times faster than the slow population, which comprises about one-third of the BL and divides on average once every five days. The relative proportions inferred from modelling agree with the relative proportions of non-LRC and LRC cells measured by photomicrography, supporting the hypothesis that the mathematically inferred populations correspond to the experimentally identified types. In addition, by analyzing the temporal reduction of total H2B-GFP fluorescence in the morphologically distinct tail scale (enriched in non-LRCs) and interscale (enriched in LRCs) regions, we were able to estimate the tail skin non-LRC and LRC division rates and nucleated layer turnover times. The $\sim 3:1$ ratio between the division rates in tail skin was in excellent agreement with the $\sim 2.7:1$ ratio of the back skin rates computed from the H2B-GFP dilution analysis, although both tail rates were $\sim 20-25\%$ slower than those of their back counterparts.

Since essentially all nucleated epidermal cells expressed the H2B-GFP transgene, we can be certain that the fast and slow populations identified by the dilution data analysis comprise the bulk of the granulated epidermis. This assurance is an important complement to the data utilizing molecular expression patterns. Using the best-fit 2×SC model, we compute that a FACS-selected population of BL cells having $d \leq 3$ after a 14 day chase will contain 85% LRCs, and that the population having $d \geq 6$ will contain 80% non-LRCs. Therefore, these populations provided good reagents for identifying mRNA expression differences between the LRC and non-LRC populations (Fig. 2).

Theoretical methods

Deconvolution of the H2B-GFP fluorescence dilution data

Cells from the back skin of two mice each were analyzed at days 3 and 7, and from five mice at day 21. Compartment-gated FACS data (e.g., tdTomato$^+$, α6-integrin$^+$, CD34$^-$ for BL; Fig. 3b) were exported as scale values from FlowJo 6.5.7. Histograms of these data were deconvolved by variational Bayesian Gaussian mixture modeling as previously described[3]. In a few cases where peaks could not be resolved by this method, the relative d-proportions were determined by dissecting the relevant histogram region using the dilution relationship $\log_2 I(d) = \text{const} - d$, where $I(d)$ is the fluorescence intensity corresponding to the value of d.

The complete histograms were resolved for day 3, but only the peaks for $d \leq d_{\max}(7) = 5$ and $d \leq d_{\max}(21) = 7$ could be resolved for days 7 and 21, respectively. In those cases, the proportions were renormalized so that their sum for $0 \leq d \leq d_{\max}(t)$ equalled one. [To permit uniform notation in which all d-summations are bounded by $d_{\max}(t)$, we define $d_{\max}(3) = \infty$.]

Mathematical modeling

Notation.

x**:** Cell type/compartment (*component*) indicator, which can denote a stem cell (SC, S), progenitor (P), or differentiated (D) cell in the BL, a differentiated cell in the SL (SL), or a differentiated cell in the GL (GL).

c**:** Compartment indicator, which can be BL, SL, or GL.

ρ^x, ρ^c**:** Area density of cells of component x or compartment c; the ratios of the ρ^c are determined experimentally.

λ^x**:** Division rate of cells of component x.

$k^{x\to x'}$: Stochastic transfer rate of x to x' cells.

$\eta_d^x(t)$**:** Area d-density of cells of component x that have divided d times by time t.

$\pi_d^c(t)$**:** Proportion of cells in compartment c that have divided d times by time t.

A general formulation for H2B-GFP fluorescence dilution modeling. The dynamical models that describe the time evolution of the $\eta_d^x(t)$ in a H2B-GFP dilution experiment are described by linear differential equations. To facilitate consideration of a large number of models, we develop a general form that can be applied in all cases.

The uncoupled, single SC model. First consider a simple model for SCs that divide symmetrically and undergo uncoupled, stochastic transport (Supplementary Fig. 3a, blue). When the division half-life is long compared with the shortest possible time between divisions, division and transport can be modeled as a Poisson processes[3,4,5]. The differential equations and boundary conditions for the (area) cell d-densities are

$$\begin{aligned} D_t\eta_d^{\mathsf{S}}(t) &= 2\lambda^{\mathsf{S}}\eta_{d-1}^{\mathsf{S}}(t) - (\lambda^{\mathsf{S}} + k^{\mathsf{S}\to\mathsf{SL}})\eta_d^{\mathsf{S}}(t) & \eta_d^{\mathsf{S}}(0) &= \delta_{d0}\,\rho^{\mathsf{BL}} \\ D_t\eta_d^{\mathsf{SL}}(t) &= k^{\mathsf{S}\to\mathsf{SL}}\eta_d^{\mathsf{S}}(t) - k^{\mathsf{SL}\to\mathsf{GL}}\eta_d^{\mathsf{SL}}(t) & \eta_d^{\mathsf{SL}}(0) &= \delta_{d0}\,\rho^{\mathsf{SL}} \\ D_t\eta_d^{\mathsf{GL}}(t) &= k^{\mathsf{SL}\to\mathsf{GL}}\eta_d^{\mathsf{SL}}(t) - k^{\mathsf{GL}\to\mathsf{CL}}\eta_d^{\mathsf{GL}}(t) & \eta_d^{\mathsf{GL}}(0) &= \delta_{d0}\,\rho^{\mathsf{GL}}\ . \end{aligned} \tag{1}$$

where λ^{S} is the SC division rate, the $k^{x\to x'}$ are the transport rates, and, since there is only one population in BL in this model, $\rho^{\mathsf{S}} = \rho^{\mathsf{BL}}$. The terms in the first equation correspond to the creation of two d-cells from one $(d-1)$-cell, and all the other terms correspond to stochastic transport between compartments.

The total density of each component is

$$\sum_{d=0}^{\infty}\eta_d^x(t) = \rho^x\ , \tag{2}$$

where, in this model, $x \in \{\mathsf{S}, \mathsf{SL}, \mathsf{GL}\}$. Homeostasis requires that, as indicated, the ρ^x must be constant. This constrains the transport rate constants: summing the differential equations over d, applying Eq. (2), and setting the derivatives equal to zero gives linear equations that imply

$$\begin{aligned} k^{\mathsf{S}\to\mathsf{SL}} &= \lambda^{\mathsf{S}} \\ k^{\mathsf{SL}\to\mathsf{GL}} &= k^{\mathsf{S}\to\mathsf{SL}}\rho^{\mathsf{S}}/\rho^{\mathsf{SL}} = \lambda^{\mathsf{S}}\rho^{\mathsf{S}}/\rho^{\mathsf{SL}} \\ k^{\mathsf{GL}\to\mathsf{CL}} &= k^{\mathsf{SL}\to\mathsf{GL}}\rho^{\mathsf{SL}}/\rho^{\mathsf{GL}} = \lambda^{\mathsf{S}}\rho^{\mathsf{S}}/\rho^{\mathsf{GL}}\ . \end{aligned} \tag{3}$$

Since $\rho^{\mathsf{S}} = \rho^{\mathsf{BL}}$, only the ratios of the compartment densities are relevant, and these were fixed at the experimentally determined values $\rho^{\mathsf{SL}}/\rho^{\mathsf{BL}} = 0.78 \pm 0.07$ and $\rho^{\mathsf{GL}}/\rho^{\mathsf{BL}} = 0.52 \pm 0.06$. Therefore, the only independent parameter in this model is λ^{S}.

We can solve the equations analytically using generating functions to get the component d-densities. We sum the d-densities for all the components (i.e., populations) within a compartment to get the d-densities for compartment c, $\eta_d^c(t)$. In this case there is only one component in each compartment, so this is not

necessary. Because we experimentally determine the relative proportions only for $d \leq d_{\max}$, we similarly renormalize the model-predicted values with this bound:

$$\pi_d^c(t) = \frac{\eta_d^c(t)}{\sum_{d'=0}^{d_{\max}} \eta_{d'}^c(t)} \ ,$$

where c is any compartment.

Vector generating functions. The above equations can be compacted using a vector format. We begin by defining the N_x-vector $\mathbf{x}$, which identifies the N_x components, and the d-density vectors over the component space:

$$\mathbf{x} = (x_1, x_2, \ldots, x_{N_x}) \tag{4a}$$
$$\boldsymbol{\eta}_d(t) = [\eta_d^{x_1}(t), \eta_d^{x_2}(t), \ldots, \eta_d^{x_{N_x}}(t)] \tag{4b}$$
$$\boldsymbol{\rho} = (\rho^{x_1}, \rho^{x_1}, \ldots, \rho^{x_{N_x}}) \tag{4c}$$
$$\boldsymbol{\eta}_d(0) = \delta_{d0}\, \boldsymbol{\rho} \ . \tag{4d}$$

In this case,

$$\begin{aligned} N_x &= 3 \\ \mathbf{x} &= (\mathsf{S}, \mathsf{SL}, \mathsf{GL}) \\ \boldsymbol{\eta}_d(t) &= [\eta_d^{\mathsf{S}}(t), \eta_d^{\mathsf{SL}}(t), \eta_d^{\mathsf{GL}}(t)] \\ \boldsymbol{\rho} &= (\rho^{\mathsf{BL}}, \rho^{\mathsf{SL}}, \rho^{\mathsf{GL}}) \ . \end{aligned}$$

The predicted d-proportion vectors for comparison with the experimental data,

$$\boldsymbol{\pi}_d(t) = [\pi_d^{\mathsf{BL}}(t), \pi_d^{\mathsf{SL}}(t), \pi_d^{\mathsf{GL}}(t)] \ , \tag{5}$$

are determined by projection using the $3 \times N_x$-matrix C and renormalization:

$$\boldsymbol{\pi}_d(t) = \mathrm{diag}\left[\sum_{d'=0}^{d_{\max}} C \cdot \boldsymbol{\eta}_{d'}(t)\right]^{-1} \cdot C \cdot \boldsymbol{\eta}_d(t) \quad (d \leq d_{\max}) \ . \tag{6}$$

where $\mathrm{diag}(\cdot)$ is the diagonal matrix with elements specified by the argument. In this simple model, C equals the identity matrix I, but this will not be so when there is more than one component per compartment. In that case, C will be a rectangular, not square, matrix.

Defining the vector generating function,

$$\boldsymbol{\eta}(\xi, t) = \sum_{d=0}^{\infty} \xi^d\, \boldsymbol{\eta}_d(t) \ ,$$

Eqs. (1) and (2) can be rewritten as

$$\partial_t \boldsymbol{\eta}(\xi, t) = -\Gamma(\xi) \cdot \boldsymbol{\eta}(\xi, t) \tag{7a}$$
$$\boldsymbol{\eta}(\xi, 0) = \boldsymbol{\eta}(1, t) = \boldsymbol{\rho} \ , \tag{7b}$$

where, using the transport rate constraints, the transition matrix for this model is

$$\Gamma(\xi) = -\begin{bmatrix} 2\lambda^{\mathsf{S}}(\xi - 1) & 0 & 0 \\ \lambda^{\mathsf{S}} & -\lambda^{\mathsf{S}}\rho^{\mathsf{S}}/\rho^{\mathsf{SL}} & 0 \\ 0 & \lambda^{\mathsf{S}}\rho^{\mathsf{S}}/\rho^{\mathsf{SL}} & -\lambda^{\mathsf{S}}\rho^{\mathsf{S}}/\rho^{\mathsf{GL}} \end{bmatrix} \ .$$

These equations have the solution

$$\boldsymbol{\eta}(\xi,t) = e^{-\Gamma(\xi)t}\cdot\boldsymbol{\rho}\ . \tag{8}$$

The matrix exponential can be evaluated analytically and the individual d-components can be obtained by differentiation

$$\boldsymbol{\eta}_d(t) = \frac{1}{d!}\left.\frac{\partial^d\boldsymbol{\eta}(\xi,t)}{\partial\xi^d}\right|_{\xi=0}\ . \tag{9}$$

Factoring the transition matrix. Rewriting Eqs. (1) with each d-density divided by its respective total component density and applying the transport rate constraints gives

$$\begin{aligned} D_t\eta_d^{\mathsf{S}}(t) &= 2\lambda^{\mathsf{S}}\rho^{\mathsf{S}}[\eta_{d-1}^{\mathsf{S}}(t)/\rho^{\mathsf{S}} - \eta_d^{\mathsf{S}}(t)/\rho^{\mathsf{S}}] \\ D_t\eta_d^{\mathsf{SL}}(t) &= \lambda^{\mathsf{S}}\rho^{\mathsf{S}}[\eta_d^{\mathsf{S}}(t)/\rho^{\mathsf{S}} - \eta_d^{\mathsf{SL}}(t)/\rho^{\mathsf{SL}}] \\ D_t\eta_d^{\mathsf{GL}}(t) &= \lambda^{\mathsf{S}}\rho^{\mathsf{S}}[\eta_d^{\mathsf{SL}}(t)/\rho^{\mathsf{SL}} - \eta_d^{\mathsf{GL}}(t)/\rho^{\mathsf{GL}}]\ . \end{aligned} \tag{10}$$

This form reveals a structure that is common to all the models that we consider: The equations, with the transport rates set to their constrained values, are simplified when expressed in terms of the fractional d-densities η_d^x/ρ^x. The remaining (division) rates appears as pre-factors. (In this simple case there is only one.) To illuminate and exploit this inherent structure, we factor the transition matrix as

$$\Gamma(\xi) = -\mathrm{diag}(\mathbf{F})\cdot T(\xi)\cdot\mathrm{diag}(\boldsymbol{\rho})^{-1}\ , \tag{11}$$

where $\mathbf{F}$ is a *stochastic flow N_x-vector* that contains the division rates and the constrained transport rates expressed in terms of the division rates. $T(\xi)$ is a $N_x\times N_x$ *transfer matrix*. In this case F^{S} is the rate of density creation, and F^{SL} and F^{GL} are the flow rates of density transport. Each of these is $\lambda^{\mathsf{S}}\rho^{\mathsf{S}}$, so $\mathbf{F} = \lambda^{\mathsf{S}}\rho^{\mathsf{S}}(1,1,1)$. The transfer matrix mirrors the structure of the fractional d-density terms within brackets in Eqs. (10)

$$T(\xi) = \begin{bmatrix} 2(\xi-1) & 0 & 0 \\ 1 & -1 & 0 \\ 0 & 1 & -1 \end{bmatrix}\ .$$

[We present below the definitions of $\mathbf{F}$ and $T(\xi)$ in the general case.]

Generalization of the vector generating function form (VGFF) to more complex models. This representation, using vector generating functions and factored $\Gamma(\xi)$, generalizes to all the models that we consider and can be used to facilitate their analysis. It automatically incorporates the homeostatic constraints of Eq. (7)b to fix the stochastic transport rate constants. In addition, $T(\xi)$, which is dimensionless and free of most model parameters, elucidates the underlying structure of the division and transport processes in the model.

To understand how this works in general, note that since each component initiates at most one stochastic transport process, we can associate each transport rate with a unique component. Therefore, as in the example above, summing the differential equations for these components over d and setting the derivatives to zero gives one linear equation per transport rate. (We do not include in this count density transfers that are coupled to division, and therefore have no independent rate constant.) Because there are no closed transport cycles, the complete set of transport rate linear equations is triangular, and therefore has a unique solution for the rates.

Eqs. (7) imply that the constant-density constraints are equivalent to

$$\Gamma(1)\cdot\boldsymbol{\rho} = 0\ .$$

The right-factorization of $\mathrm{diag}(\boldsymbol{\rho})^{-1}$ reduces this equation to

$$T(1)\cdot\mathbf{1} = 0\ , \tag{12}$$

where $\mathbf{1}$ is the constant-unity vector (i.e., with all components equal one). Therefore, with the $\mathrm{diag}(\boldsymbol{\rho})^{-1}$ factorization, we know that the transport rates have been correctly fixed if Eq. (12) is satisfied.

A further simplification is provided by the left-factorization of $\mathrm{diag}(\mathbf{F})$, which removes the remaining (i.e., the division) rates from $T(\xi)$ leaving it dimensionless. In general, we set F^x equal to the rate of density creation for the dividing (S and P) components, or to the density outflow rate for the non-dividing (D, SL, and GL) components.

Left-factoring these terms from $\Gamma(\xi)$, as in Eq. (11), leaves $T(\xi)$ with a simple structure: the diagonal elements of the non-dividing components are always -1 because transport is their only stochastic process and the outflow rate has been factored into the flow vector. The diagonal elements of the dividing components always include a -1 corresponding to stochastic division, and may include additional terms corresponding to the immediate retention of one (ξ) or both (2ξ) descendants after division. They will also contain terms corresponding to stochastic transport out of the dividing compartment if transport is uncoupled from division. The off-diagonal terms represent inter-component transfers. These will be multiplied by ξ if transport is coupled to division (i.e., the d-value increases), but not if it is an uncoupled stochastic process.

Using these rules, $\Gamma(\xi)$ can be constructed for most models without explicitly solving the homeostatic constraints or writing out the differential equations and boundary conditions. (The hybrid 2×SC model that we discuss below is the only exception considered here.) Nonetheless, for clarity we also list the explicit differential equations and boundary conditions for each model below.

$T(1)$ also satisfies the weighted column summation condition

$$\mathbf{F} \cdot T(1) = \boldsymbol{\Delta} \,, \tag{13}$$

where $\boldsymbol{\Delta}$ is the N_x-vector with components Δ^x, where Δ^x is the rate of density change contributed by component x to the system:

$$\Delta^x = \begin{cases} \lambda^x \rho^x & (x \in \{\mathsf{S}, \mathsf{P}\}) \\ 0 & (x \in \{\mathsf{SL}, \mathsf{D}\}) \\ -k^{\mathsf{GL}\to\mathsf{SL}} \rho^{\mathsf{GL}} = -\sum_{x \in \{S,P\}} \lambda^x \rho^x & (x = \mathsf{GL}) \end{cases}$$

$$\boldsymbol{\Delta} \cdot \mathbf{1} = 0 \,. \tag{14}$$

[P (progenitor) and D (differentiated cell) are BL components in models that we consider below.] Eq. (13) reflects the steady-state, density-conserving flow of the cells: all the density generated in BL must flow out through GL. Eq. (14) reflects the steady-state flow of total density within the system.

Most of the models that we consider below have multiple components per compartment. The N_x–vector $\boldsymbol{\rho}$ of component total densities is constrained by the 3-vector $\boldsymbol{\rho}^{\mathsf{exp}}$ of experimental compartment densities:

$$\boldsymbol{\rho}^{\mathsf{exp}} = C \cdot \boldsymbol{\rho} \,. \tag{15}$$

Since the ratios between the three compartment densities are fixed at their experimental values and only ratios of the ρ^x affect the solution, there are only $N_x - 3$ independent density parameters. The component d-densities are combined into compartment d-densities by Eq. (6) for comparison with the experimental compartment d-densities, but the individual component d-densities are used for subdividing the bars in the charts in Fig. 3e and Supplementary Fig. 3b.

Using this representation, each model is completely specified by $\mathbf{x}$, C, $\boldsymbol{\rho}$, $\mathbf{F}$, and T; Eqs. (4)–(9) and (11)–(15) always hold true.

Computation in the $x \times d$ vector-product space. Because $T(\xi)$ is usually a sparse matrix, Eqs. (8) and (9) can be solved analytically in (at least most of) the cases we consider. However, the high-order differentiations needed to determine the $\boldsymbol{\eta}_d(t)$ from $\boldsymbol{\eta}(\xi, t)$ for $d \gg 1$ in the models with many components give

complicated collections of terms that are numerically difficult to sum with adequate accuracy. Therefore, we extend the formulation to a numerically stable variant in which the $\eta_d^x(t)$ are reinterpreted as components of doubly-indexed vectors in the $x \otimes d$ product space, $\boldsymbol{\lambda}$ and $\boldsymbol{\rho}$ are reinterpreted as outer products of the corresponding vectors in the x-space and the constant-unity vector in the d-space, ξ-independent terms in $T(\xi)$ are reinterpreted as multiples of the identity matrix in the d-space, and ξ in $T(\xi)$ is reinterpreted as the raising operator in the d-space [i.e., which have matrix elements $\delta_{d,d-1}$ $(1 \leq d \leq d_{\max})$]. With this understanding, the VGFF provides a convenient method for structuring the computational algorithm. $x \otimes d$ vectors are encoded as $N_x \times (d_{\max}+1)$-vectors and, since $\Gamma(\xi)$ is very sparse in this representation, the matrix exponentiation appearing in Eq. (8) can be computed accurately.

The coupled, single SC model. Division and transport are coupled in this model; one descendant of a divided SC remains in the BL while the other is immediately transported to the SL (Supplementary Fig. 3a, dark blue). The dynamic equations and boundary conditions are

$$\begin{aligned}
D_t\eta_d^{\mathsf{S}}(t) &= \lambda^{\mathsf{S}}[\eta_{d-1}^{\mathsf{S}}(t) - \eta_d^{\mathsf{S}}(t)] & \eta_d^{\mathsf{S}}(0) &= \delta_{d0}\rho^{\mathsf{S}} \\
D_t\eta_d^{\mathsf{SL}}(t) &= \lambda^{\mathsf{S}}\eta_{d-1}^{\mathsf{S}}(t) - k^{\mathsf{SL}\to\mathsf{GL}}\eta_d^{\mathsf{SL}}(t) & \eta_d^{\mathsf{SL}}(0) &= \delta_{d0}\rho^{\mathsf{SL}} \\
D_t\eta_d^{\mathsf{GL}}(t) &= k^{\mathsf{SL}\to\mathsf{GL}}\eta_d^{\mathsf{SL}}(t) - k^{\mathsf{GL}\to\mathsf{CL}}\eta_d^{\mathsf{GL}}(t) & \eta_d^{\mathsf{GL}}(0) &= \delta_{d0}\rho^{\mathsf{GL}}\,.
\end{aligned}$$

The VGFF is specified by the same $\mathbf{x}$, $\boldsymbol{\eta}_d(t)$, C, $\boldsymbol{\rho}$, and $\mathbf{F}$ as the uncoupled model; the only difference is that

$$T(\xi) = \begin{bmatrix} \xi - 1 & 0 & 0 \\ \xi & -1 & 0 \\ 0 & 1 & -1 \end{bmatrix}\,.$$

The differences arise from the coupling of division and transport in this case: $T_{\mathsf{SL},\mathsf{S}}(\xi)$ is changed from 1 in the uncoupled model to ξ here because BL→SL transport is coupled to division, so the division number of the transported cell in SL is one greater than that of the parental cell in BL. The 2ξ and -2 terms in the uncoupled $T_{\mathsf{S},\mathsf{S}}(\xi)$ are changed to ξ and -1 because only one descendant remains in the BL immediately after division and because there is no stochastic BL→SL transport. Because of this, λ^{S} must be twice as large in this model as in the uncoupled, single SC model to yield the same S d-distribution. Concomitantly, the predicted rates-of-change of the SL and GL d-distributions relative to that of the BL d-distribution are twice as fast in this coupled model than in the uncoupled model.

The single progenitor, P-D model. This model, proposed by Clayton et al.[4], has both progenitor and differentiated cells in the BL. The progenitors divide to self-renew and generate differentiated cells that are stochastically transported to SL (Fig. 3c, gray). The dynamic equations and boundary conditions are

$$\begin{aligned}
D_t\eta_d^{\mathsf{P}}(t) &= \lambda^{\mathsf{P}}[\eta_{d-1}^{\mathsf{P}}(t) - \eta_d^{\mathsf{P}}(t)] & \eta_d^{\mathsf{P}}(0) &= \delta_{d0}\rho^{\mathsf{P}} \\
D_t\eta_d^{\mathsf{D}}(t) &= \lambda^{\mathsf{P}}\eta_{d-1}^{\mathsf{P}}(t) - k^{\mathsf{D}\to\mathsf{SL}}\eta_d^{\mathsf{D}}(t) & \eta_d^{\mathsf{D}}(0) &= \delta_{d0}\rho^{\mathsf{D}} \\
D_t\eta_d^{\mathsf{SL}}(t) &= k^{\mathsf{D}\to\mathsf{SL}}\eta_d^{\mathsf{D}}(t) - k^{\mathsf{SL}\to\mathsf{GL}}\eta_d^{\mathsf{SL}}(t) & \eta_d^{\mathsf{SL}}(0) &= \delta_{d0}\rho^{\mathsf{SL}} \\
D_t\eta_d^{\mathsf{GL}}(t) &= k^{\mathsf{SL}\to\mathsf{GL}}\eta_d^{\mathsf{SL}}(t) - k^{\mathsf{GL}\to\mathsf{CL}}\eta_d^{\mathsf{GL}}(t) & \eta_d^{\mathsf{GL}}(0) &= \delta_{d0}\rho^{\mathsf{GL}}\,,
\end{aligned}$$

where, in accord with Eq. (15), $\rho^{\mathsf{P}} + \rho^{\mathsf{D}} = \rho^{\mathsf{BL}}$. The VGFF is

$$\begin{aligned}
\mathbf{x} &= (\mathsf{P}, \mathsf{D}, \mathsf{SL}, \mathsf{GL}) \\
C &= \begin{bmatrix} 1 & 1 & 0 & 0 \\ 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 \end{bmatrix} \\
\boldsymbol{\rho} &= (\rho^{\mathsf{P}}, \rho^{\mathsf{D}}, \rho^{\mathsf{SL}}, \rho^{\mathsf{GL}}) \\
\mathbf{F} &= \lambda^{\mathsf{P}} \rho^{\mathsf{P}} (1, 1, 1, 1) \\
T &= \begin{bmatrix} \xi - 1 & 0 & 0 & 0 \\ \xi & -1 & 0 & 0 \\ 0 & 1 & -1 & 0 \\ 0 & 0 & 1 & -1 \end{bmatrix} .
\end{aligned}$$

There are two independent parameters, the progenitor division rate, λ^{P}, and the fraction of BL cells that are progenitors, $f^{\mathsf{P}} = \rho^{\mathsf{P}}/\rho^{\mathsf{BL}}$.

The stem cell-progenitor, SC-P-D model. This model, proposed by Mascre et al.[5], adds self-renewing SCs to the P-D model (Fig. 3c, blue). The equations used by Mascre et al. (their Supplementary Information Sec. 4.1) do not preserve cell densities, because the SCs are constantly adding cells to the independently self-renewing progenitor population. Nonetheless, those equations provided an adequate approximation for their analyses since only a very small fraction, $f^{\mathsf{S}} = \rho^{\mathsf{S}}/(\rho^{\mathsf{S}} + \rho^{\mathsf{P}} + \rho^{\mathsf{D}}) = 0.05$, of SCs were included in their modeling. However, in this case we explore the complete range $0 < f^{\mathsf{S}} < 1$, so it is necessary to amend the equations to ensure homeostasis.

A number of biological mechanisms could be involved in homeostasis; e.g., progenitor cell death, occasional differentiation of both progenitor cell division descendants, or differentiation independent of progenitor division. Each mechanism introduces an additional parameter into the differential equations, which is then fixed by the constant-density requirement. For example, some progenitor cell death combined with homeostatic feedback to the SC division rate gives the amended differential equations and boundary conditions

$$\begin{aligned}
D_t \eta_d^{\mathsf{S}}(t) &= \lambda^{\mathsf{S}} [\eta_{d-1}^{\mathsf{S}}(t) - \eta_d^{\mathsf{S}}(t)] & \eta_d^{\mathsf{S}}(0) &= \delta_{d0} \rho^{\mathsf{S}} \\
D_t \eta_d^{\mathsf{P}}(t) &= \lambda^{\mathsf{S}} \eta_{d-1}^{\mathsf{S}}(t) + \lambda^{\mathsf{P}} \eta_{d-1}^{\mathsf{P}}(t) - (\lambda^{\mathsf{P}} + k^d) \eta_d^{\mathsf{P}}(t) & \eta_d^{\mathsf{P}}(0) &= \delta_{d0} \rho^{\mathsf{P}} \\
D_t \eta_d^{\mathsf{D}}(t) &= \lambda^{\mathsf{P}} \eta_{d-1}^{\mathsf{P}}(t) - k^{\mathsf{D}\to\mathsf{SL}} \eta_d^{\mathsf{D}}(t) & \eta_d^{\mathsf{D}}(0) &= \delta_{d0} \rho^{\mathsf{D}} \\
D_t \eta_d^{\mathsf{SL}}(t) &= k^{\mathsf{D}\to\mathsf{SL}} \eta_d^{\mathsf{D}}(t) - k^{\mathsf{SL}\to\mathsf{GL}} \eta_d^{\mathsf{SL}}(t) & \eta_d^{\mathsf{SL}}(0) &= \delta_{d0} \rho^{\mathsf{SL}} \\
D_t \eta_d^{\mathsf{GL}}(t) &= k^{\mathsf{SL}\to\mathsf{GL}} \eta_d^{\mathsf{SL}}(t) - k^{\mathsf{GL}\to\mathsf{CL}} \eta_d^{\mathsf{GL}}(t) & \eta_d^{\mathsf{GL}}(0) &= \delta_{d0} \rho^{\mathsf{GL}} ,
\end{aligned}$$

where λ^{P} is the mean net rate of successful divisions, and $\rho^{\mathsf{S}} + \rho^{\mathsf{P}} + \rho^{\mathsf{D}} = \rho^{\mathsf{BL}}$. The addition to the original formulation of the term $-k^d \, \eta_d^{\mathsf{P}}(t)$ in the second equation represents the rate of division-coupled cell death; it is constrained by the constant-density requirement to be $k^d = \lambda^{\mathsf{S}} \rho^{\mathsf{S}} / \rho^{\mathsf{P}}$. (While mechanistically this is a feedback constraint on λ^{S}, for convenience we treat λ^{S} as an independent parameter and k^d as a constrained parameter.) We used this form because it is biologically plausible.

The corresponding VGFF is

$$\begin{aligned}
\mathbf{x} &= (\mathsf{S}, \mathsf{P}, \mathsf{D}, \mathsf{SL}, \mathsf{GL}) \\
C &= \begin{bmatrix} 1 & 1 & 1 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 1 \end{bmatrix}
\end{aligned}$$

$$\boldsymbol{\rho} = (\rho^{\mathsf{S}}, \rho^{\mathsf{P}}, \rho^{\mathsf{D}}, \rho^{\mathsf{SL}}, \rho^{\mathsf{GL}})$$
$$\mathbf{F} = (\lambda^{\mathsf{S}}\rho^{\mathsf{S}}, \lambda^{\mathsf{P}}\rho^{\mathsf{P}}, \lambda^{\mathsf{P}}\rho^{\mathsf{P}}, \lambda^{\mathsf{P}}\rho^{\mathsf{P}}, \lambda^{\mathsf{P}}\rho^{\mathsf{P}})$$
$$T = \begin{bmatrix} \xi - 1 & 0 & 0 & 0 & 0 \\ \gamma\xi & \xi - 1 - \gamma & 0 & 0 & 0 \\ 0 & \xi & -1 & 0 & 0 \\ 0 & 0 & 1 & -1 & 0 \\ 0 & 0 & 0 & 1 & -1 \end{bmatrix},$$

where $\gamma = \lambda^{\mathsf{S}}\rho^{\mathsf{S}}/(\lambda^{\mathsf{P}}\rho^{\mathsf{P}})$. There are four independent parameters: λ^{S}, λ^{P}, and the fractions of BL cells that are SCs and progenitors, $f^{\mathsf{S}} = \rho^{\mathsf{S}}/(\rho^{\mathsf{S}} + \rho^{\mathsf{P}} + \rho^{\mathsf{D}})$ and $f^{\mathsf{P}} = \rho^{\mathsf{P}}/(\rho^{\mathsf{S}} + \rho^{\mathsf{P}} + \rho^{\mathsf{D}})$.

The two-SC, 2×SC models. These models have independent self-renewing, fast- (S_1) and slow- (S_2) dividing populations. We assume that both populations differentiate into a combined pool of cells in the SL that are subsequently transported to the GL. We focus on two closely related variants: the semi-coupled variant in which division of S_1 is coupled to transport and division of S_2 is uncoupled (Fig. 3c, yellow), and a hybrid variant in which S_1 divisions are either uncoupled or coupled to transport with probabilities u^{S_1} or $1 - u^{\mathsf{S}_1}$, respectively, while division of S_2 remains completely uncoupled (Supplementary Fig. 3a, yellow). Although the semi-coupled model is a special case ($u^{\mathsf{S}_1} = 0$) of the hybrid model, for clarity we discuss it separately. (The doubly hybrid variant, in which both the S_1 and S_2 populations undergo a mixture of uncoupled and coupled divisions could be analyzed similarly. However, the dependence on the S_2 uncoupling probability u^{S_2} is not detectable in the dilution experiments, so we fix $u^{\mathsf{S}_2} = 1$ here.)

If we were to assume that all the stochastic processes were Poisson processes, the differential equations and boundary conditions for the semi-coupled variant would be

$$D_t\eta_d^{\mathsf{S}_1}(t) = \lambda^{\mathsf{S}_1}[\eta_{d-1}^{\mathsf{S}_1}(t) - \eta_d^{\mathsf{S}_1}(t)] \qquad \eta_d^{\mathsf{S}_1}(0) = \delta_{d0}\,\rho^{\mathsf{S}_1} \tag{16a}$$
$$D_t\eta_d^{\mathsf{SL}_1}(t) = \lambda^{\mathsf{S}_1}\eta_{d-1}^{\mathsf{S}_1}(t) - k^{\mathsf{SL}\to\mathsf{GL}}\eta_d^{\mathsf{SL}_1}(t) \qquad \eta_d^{\mathsf{SL}_1}(0) = \delta_{d0}\,\rho_1^{\mathsf{SL}} \tag{16b}$$
$$D_t\eta_d^{\mathsf{GL}_1}(t) = k^{\mathsf{SL}\to\mathsf{GL}}\eta_d^{\mathsf{SL}_1}(t) - k^{\mathsf{GL}\to\mathsf{CL}}\eta_d^{\mathsf{GL}_1}(t) \qquad \eta_d^{\mathsf{GL}_1}(0) = \delta_{d0}\,\rho_1^{\mathsf{GL}} \qquad (\textit{not used}) \tag{16c}$$
$$D_t\eta_d^{\mathsf{S}_2}(t) = 2\lambda^{\mathsf{S}_2}\eta_{d-1}^{\mathsf{S}_2}(t) - (\lambda^{\mathsf{S}_2} + k^{\mathsf{S}_2\to\mathsf{SL}})\eta_d^{\mathsf{S}_2}(t) \qquad \eta_d^{\mathsf{S}_2}(0) = \delta_{d0}\,\rho^{\mathsf{S}_2} \tag{16d}$$
$$D_t\eta_d^{\mathsf{SL}_2}(t) = k^{\mathsf{S}_2\to\mathsf{SL}}\eta_d^{\mathsf{S}_2}(t) - k^{\mathsf{SL}\to\mathsf{GL}}\eta_d^{\mathsf{SL}_2}(t) \qquad \eta_d^{\mathsf{SL}_2}(0) = \delta_{d0}\,\rho_2^{\mathsf{SL}} \tag{16e}$$
$$D_t\eta_d^{\mathsf{GL}_2}(t) = k^{\mathsf{SL}\to\mathsf{GL}}\eta_d^{\mathsf{SL}_2}(t) - k^{\mathsf{GL}\to\mathsf{CL}}\eta_d^{\mathsf{GL}_2}(t) \qquad \eta_d^{\mathsf{GL}_2}(0) = \delta_{d0}\,\rho_2^{\mathsf{GL}}\,, \tag{16f}$$

where the mass conservation and homeostatic constraints imply that $\rho^{\mathsf{S}_1} + \rho^{\mathsf{S}_2} = \rho^{\mathsf{BL}}$, $\rho^{\mathsf{SL}_1} = \gamma_1\rho^{\mathsf{SL}}$, $\rho^{\mathsf{SL}_2} = \gamma_2\,\rho^{\mathsf{SL}}$, $\rho^{\mathsf{GL}_1} = \gamma_1\,\rho^{\mathsf{GL}}$, $\rho^{\mathsf{GL}_2} = \gamma_2\,\rho^{\mathsf{GL}}$, $\gamma_1 = \lambda^{\mathsf{S}_1}\rho^{\mathsf{S}_1}/(\lambda^{\mathsf{S}_1}\rho^{\mathsf{S}_1} + \lambda^{\mathsf{S}_2}\rho^{\mathsf{S}_2})$, and $\gamma_2 = \lambda^{\mathsf{S}_2}\rho^{\mathsf{S}_2}/(\lambda^{\mathsf{S}_1}\rho^{\mathsf{S}_1} + \lambda^{\mathsf{S}_2}\rho^{\mathsf{S}_2})$. These equations are just the union of the coupled and uncoupled SC equations. Writing them in this form emphasizes that the two populations are independent in all three layers. However, since the SL_1 and SL_2 cells are transported by the same stochastic processes, we can add Eqs. (16)b and d together to get a single equation for $\eta_d^{\mathsf{SL}}(t) = \eta_d^{\mathsf{SL}_1}(t) + \eta_d^{\mathsf{SL}_2}(t)$. The same holds for the GL_1 and GL_2 cells and Eqs. (16)c and f. Doing this, we get the more compact form

$$D_t\eta_d^{\mathsf{S}_1}(t) = \lambda^{\mathsf{S}_1}[\eta_{d-1}^{\mathsf{S}_1}(t) - \eta_d^{\mathsf{S}_1}(t)] \qquad \eta_d^{\mathsf{S}_1}(0) = \delta_{d0}\,\rho^{\mathsf{S}_1}$$
$$D_t\eta_d^{\mathsf{S}_2}(t) = 2\lambda^{\mathsf{S}_2}\eta_{d-1}^{\mathsf{S}_2}(t) - (\lambda^{\mathsf{S}_2} + k^{\mathsf{S}_2\to\mathsf{SL}})\,\eta_d^{\mathsf{S}_2}(t) \qquad \eta_d^{\mathsf{S}_2}(0) = \delta_{d0}\,\rho^{\mathsf{S}_2} \qquad (\textit{not used})$$
$$D_t\eta_d^{\mathsf{SL}}(t) = \lambda^{\mathsf{S}_1}\eta_{d-1}^{\mathsf{S}_1}(t) + k^{\mathsf{S}_2\to\mathsf{SL}}\eta_d^{\mathsf{S}_2}(t) - k^{\mathsf{SL}\to\mathsf{GL}}\,\eta_d^{\mathsf{SL}}(t) \qquad \eta_d^{\mathsf{SL}}(0) = \delta_{d0}\,\rho^{\mathsf{SL}}$$
$$D_t\eta_d^{\mathsf{GL}}(t) = k^{\mathsf{SL}\to\mathsf{GL}}\,\eta_d^{\mathsf{SL}}(t) - k^{\mathsf{GL}\to\mathsf{CL}}\,\eta_d^{\mathsf{GL}}(t) \qquad \eta_d^{\mathsf{GL}}(0) = \delta_{d0}\,\rho^{\mathsf{GL}}\,.$$

The hybrid equations would be

$$
\begin{aligned}
D_t\eta_d^{\mathsf{S}_1}(t) =&(1+u^{\mathsf{S}_1})\,\lambda^{\mathsf{S}_1}\eta_{d-1}^{\mathsf{S}_1}(t) - (\lambda^{\mathsf{S}_1}+u^{\mathsf{S}_1}\,k^{\mathsf{S}_1\to\mathsf{SL}})\,\eta_d^{\mathsf{S}_1}(t)\\
D_t\eta_d^{\mathsf{S}_2}(t) =&2\lambda^{\mathsf{S}_2}\eta_{d-1}^{\mathsf{S}_2}(t) - (\lambda^{\mathsf{S}_2}+k^{\mathsf{S}_2\to\mathsf{SL}})\,\eta_d^{\mathsf{S}_2}(t) \qquad (\textit{not used})\\
D_t\eta_d^{\mathsf{SL}}(t) =&u^{\mathsf{S}_1}\,k^{\mathsf{S}_1\to\mathsf{SL}}\eta_d^{\mathsf{S}_1}(t) + (1-u^{\mathsf{S}_1})\,\lambda^{\mathsf{S}_1}\eta_{d-1}^{\mathsf{S}_1}(t) + k^{\mathsf{S}_2\to\mathsf{SL}}\eta_d^{\mathsf{S}_2}(t) - k^{\mathsf{SL}\to\mathsf{GL}}\eta_d^{\mathsf{SL}}(t)\\
D_t\eta_d^{\mathsf{GL}}(t) =&k^{\mathsf{SL}\to\mathsf{GL}}\eta_d^{\mathsf{SL}}(t) - k^{\mathsf{GL}\to\mathsf{CL}}\eta_d^{\mathsf{GL}}(t)\,,
\end{aligned}
$$

with the same boundary conditions as those of the semi-coupled model.

Gamma distribution waiting-time processes. Preliminary studies using the equations above indicated that the 2×SC model provides the best fit to the data of all the models tested. To further improve its biological significance, we replaced the Poisson process with a process having a more realistic waiting-time distribution for the fast division process. The Poisson process, although often used for modeling because of its mathematical simplicity, has an exponential waiting-time (τ) distribution that is largest at $\tau = 0$. This corresponds to biologically impossible instantaneous re-division, but the approximation is acceptable when the division rate is slow compared to the minimum time required for division (i.e., allowing for DNA synthesis and mitosis), as was the case in the analyses of Refs. 3, 4, and 5. However, it is not appropriate for modelling the fast division of the non-LRC cells.

To fix this, we replaced the Poisson process with a process having a waiting-time distribution equal to a Gamma distribution with shape parameter $\alpha = 2$. This vanishes for $\tau \to 0$, thereby providing a more realistic model. In the same vein, if rapid asymmetric replication is "pushing" cells along, it seems unlikely that a cell transported into one compartment would immediately be transported to the next. Therefore, we used the Gamma distribution process for transport as well. We continued to use a Poisson process to model S_2 division since it is slow. While the Gamma distribution process could also be used with the SC, P-D, and SC-P-D models, in contrast with the 2×SC model, this degraded their best-fits to the data, so this was not done.

The Gamma ($\alpha = 2$) waiting-time distribution is achieved simply by replacing the S_1, SL, and GL Poisson processes by two-step Poisson processes involving two subpopulations, each having half the mean waiting-time (i.e., twice the rate) of the original process. For example, S_1 is split into S_1^a and S_1^b subpopulations that undergo the two-step division process $(\mathsf{S}_1^a)_d \to (\mathsf{S}_1^b)_d$ and $(\mathsf{S}_1^b)_d \to (\mathsf{S}_1^a)_{d+1}$. The $(\mathsf{S}_1^b)_d \to \mathsf{SL}_{d+1}^a$ process models BL→SL transport when division and transport are coupled. The additional $(\mathsf{S}_1^a)_d \to \mathsf{SL}_d^a$ and $(\mathsf{S}_1^b)_d \to \mathsf{SL}_d^a$ processes model the additional uncoupled transport in the hybrid variant. Analogously, the $\mathsf{SL}^a \to \mathsf{SL}^b$, $\mathsf{SL}^b \to \mathsf{GL}^a$, $\mathsf{GL}^a \to \mathsf{GL}^b$, and $\mathsf{GL}^b \to \mathsf{CL}$ processes together model SL→GL→CL transport.

The differential equations and boundary conditions for the semi-coupled model are

$$
\begin{aligned}
D_t\eta_d^{\mathsf{S}_1^a}(t) &= 2\lambda^{\mathsf{S}_1}[\eta_{d-1}^{\mathsf{S}_1^b}(t) - \eta_d^{\mathsf{S}_1^a}(t)] && \eta_d^{\mathsf{S}_1^a}(0) = \delta_{d0}\,\rho^{\mathsf{S}_1}/2 && \text{(17a)}\\
D_t\eta_d^{\mathsf{S}_1^b}(t) &= 2\lambda^{\mathsf{S}_1}[\eta_d^{\mathsf{S}_1^a}(t) - \eta_d^{\mathsf{S}_1^b}(t)] && \eta_d^{\mathsf{S}_1^b}(0) = \delta_{d0}\,\rho^{\mathsf{S}_1}/2 && \text{(17b)}\\
D_t\eta_d^{\mathsf{S}_2}(t) &= 2\lambda^{\mathsf{S}_2}\eta_{d-1}^{\mathsf{S}_2}(t) - (\lambda^{\mathsf{S}_2}+k^{\mathsf{S}_2\to\mathsf{SL}})\,\eta_d^{\mathsf{S}_2}(t) && \eta_d^{\mathsf{S}_2}(0) = \delta_{d0}\,\rho^{\mathsf{S}_2} && \text{(17c)}\\
D_t\eta_d^{\mathsf{SL}^a}(t) &= 2\lambda^{\mathsf{S}_1}\eta_{d-1}^{\mathsf{S}_1^b}(t) + k^{\mathsf{S}_2\to\mathsf{SL}}\eta_d^{\mathsf{S}_2}(t) - 2k^{\mathsf{SL}\to\mathsf{GL}}\eta_d^{\mathsf{SL}^a}(t) && \eta_d^{\mathsf{SL}^a}(0) = \delta_{d0}\,\rho^{\mathsf{SL}}/2 && \text{(17d)}\\
D_t\eta_d^{\mathsf{SL}^b}(t) &= 2k^{\mathsf{SL}\to\mathsf{GL}}\,[\eta_d^{\mathsf{SL}^a}(t) - \eta_d^{\mathsf{SL}^b}(t)] && \eta_d^{\mathsf{SL}^b}(0) = \delta_{d0}\,\rho^{\mathsf{SL}}/2 && \text{(17e)}\\
D_t\eta_d^{\mathsf{GL}^a}(t) &= 2k^{\mathsf{SL}\to\mathsf{GL}}\,\eta_d^{\mathsf{SL}^b}(t) - 2k^{\mathsf{GL}\to\mathsf{CL}}\eta_d^{\mathsf{GL}^a}(t) && \eta_d^{\mathsf{GL}^a}(0) = \delta_{d0}\,\rho^{\mathsf{GL}}/2 && \text{(17f)}\\
D_t\eta_d^{\mathsf{GL}^b}(t) &= 2k^{\mathsf{GL}\to\mathsf{CL}}\,[\eta_d^{\mathsf{GL}^a}(t) - \eta_d^{\mathsf{GL}^b}(t)] && \eta_d^{\mathsf{GL}^b}(0) = \delta_{d0}\,\rho^{\mathsf{GL}}/2\,. && \text{(17g)}
\end{aligned}
$$

The VGFF is

$$
\begin{aligned}
\mathbf{x} &= (\mathsf{S}_1^{\mathsf{S}_1^a}, \mathsf{S}_1^{\mathsf{S}_1^b}, \mathsf{S}_2, \mathsf{SL}^a, \mathsf{SL}^b, \mathsf{GL}^a, \mathsf{GL}^b) \\
C &= \begin{bmatrix} 1 & 1 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & 1 \end{bmatrix} \\
\boldsymbol{\rho} &= (\rho^{\mathsf{S}_1}/2, \rho^{\mathsf{S}_1}/2, \rho^{\mathsf{S}_2}, \rho^{\mathsf{SL}}/2, \rho^{\mathsf{SL}}/2, \rho^{\mathsf{GL}}/2, \rho^{\mathsf{GL}}/2) \\
\mathbf{F} &= (\lambda^{\mathsf{S}_1}\rho^{\mathsf{S}_1}, \lambda^{\mathsf{S}_1}\rho^{\mathsf{S}_1}, \lambda^{\mathsf{S}_2}\rho^{\mathsf{S}_2}, F_\Sigma, F_\Sigma, F_\Sigma, F_\Sigma)
\end{aligned}
$$

$$
T(\xi) = \begin{bmatrix} -1 & \xi & 0 & 0 & 0 & 0 & 0 \\ 1 & -1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 2(\xi-1) & 0 & 0 & 0 & 0 \\ 0 & \gamma_1\xi & \gamma_2 & -1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & -1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & -1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & -1 \end{bmatrix},
$$

where $F_\Sigma = \lambda^{\mathsf{S}_1}\rho^{\mathsf{S}_1} + \lambda^{\mathsf{S}_2}\rho^{\mathsf{S}_2}$, $\gamma_1 = \lambda^{\mathsf{S}_1}\rho^{\mathsf{S}_1}/F_\Sigma$, $\gamma_2 = \lambda^{\mathsf{S}_2}\rho^{\mathsf{S}_2}/F_\Sigma$, and $\rho^{\mathsf{S}_1} + \rho^{\mathsf{S}_2} = \rho^{\mathsf{BL}}$.

Because their transition rates are the same, the S_1 subcomponent densities are equal, $\rho^{\mathsf{S}_1^a} = \rho^{\mathsf{S}_1^b} = \rho^{\mathsf{S}_1}/2$, and the flow rate through S_1^a equals the density increase due to cell division in S_1^b; therefore, $F^{\mathsf{S}_1^a} = F^{\mathsf{S}_1^b} = \lambda^{\mathsf{S}_1}\rho^{\mathsf{S}_1}$. Analogous equalities hold for the SL and GL subcomponents. There are three independent parameters: the division rates of the two SC populations, λ^{S_1} and λ^{S_2}, and the fraction of BL cells that are S_1, $f^{\mathsf{S}_1} = \rho^{\mathsf{S}_1}/\rho^{\mathsf{BL}}$.

The hybrid model (i.e., $u > 0$) is more complicated since S_1^b has lower density than S_1^a, $\rho^{\mathsf{S}_1^b}(u) < \rho^{\mathsf{S}_1^a}$, because the uncoupled BL→SL transport of cells from S_1^a reduces the flow of cells to S_1^b. (This is a discrete—i.e., two-phase "cell cycle"—echo of the continuous, biological steady-state situation in which the density of cells that have progressed time τ past mitosis is a decreasing function of τ.) We define $\beta(u) = \rho^{\mathsf{S}_1^b}(u)/\rho^{\mathsf{S}_1^b}(0) = 2\rho^{\mathsf{S}_1^b}(u)/\rho^{\mathsf{S}_1}$ and use the constraint $\rho^{\mathsf{S}_1^a} + \rho^{\mathsf{S}_1^b} = \rho^{\mathsf{S}_1}$ and the constancy of $\rho^{\mathsf{S}_1^a}$ and $\rho^{\mathsf{S}_1^b}$ to determine $k^{\mathsf{S}_1\to\mathsf{SL}}(u) = \beta(u) = 2(\sqrt{1+u}-1)/u$. This gives the hybrid 2×SC differential equations

$$
\begin{aligned}
D_t\eta_d^{\mathsf{S}_1^a}(t) &= 2(1+u)\,\lambda^{\mathsf{S}_1}\eta_{d-1}^{\mathsf{S}_1^b}(t) - [2\lambda^{\mathsf{S}_1} + u\,k^{\mathsf{S}_1\to\mathsf{SL}}(u)]\,\eta_d^{\mathsf{S}_1^a}(t) && \eta_d^{\mathsf{S}_1^a}(0) = \delta_{d0}\,\rho^{\mathsf{S}_1^a}(u) \\
D_t\eta_d^{\mathsf{S}_1^b}(t) &= 2\lambda^{\mathsf{S}_1}\eta_d^{\mathsf{S}_1^a}(t) - [2\lambda^{\mathsf{S}_1} + u\,k^{\mathsf{S}_1\to\mathsf{SL}}(u)]\,\eta_d^{\mathsf{S}_1^b}(t) && \eta_d^{\mathsf{S}_1^b}(0) = \delta_{d0}\,\rho^{\mathsf{S}_1^b}(u) \\
D_t\eta_d^{\mathsf{S}_2}(t) &= 2\lambda^{\mathsf{S}_2}\eta_{d-1}^{\mathsf{S}_2}(t) - (\lambda^{\mathsf{S}_2} + k^{\mathsf{S}_2\to\mathsf{SL}})\,\eta_d^{\mathsf{S}_2}(t) && \eta_d^{\mathsf{S}_2}(0) = \delta_{d0}\,\rho^{\mathsf{S}_2} \\
D_t\eta_d^{\mathsf{SL}^a}(t) &= 2(1-u)\,\lambda^{\mathsf{S}_1}\eta_{d-1}^{\mathsf{S}_1^b}(t) + u\,k^{\mathsf{S}_1\to\mathsf{SL}}(u)\,[\eta_d^{\mathsf{S}_1^a}(t) + \eta_d^{\mathsf{S}_1^b}(t)] + \\
&\qquad k^{\mathsf{S}_2\to\mathsf{SL}}\eta_d^{\mathsf{S}_2}(t) - 2k^{\mathsf{SL}\to\mathsf{GL}}\eta_d^{\mathsf{SL}^a}(t) && \eta_d^{\mathsf{SL}^a}(0) = \delta_{d0}\,\rho^{\mathsf{SL}}/2 \\
D_t\eta_d^{\mathsf{SL}^b}(t) &= 2k^{\mathsf{SL}\to\mathsf{GL}}\,[\eta_d^{\mathsf{SL}^a}(t) - \eta_d^{\mathsf{SL}^b}(t)] && \eta_d^{\mathsf{SL}^b}(0) = \delta_{d0}\,\rho^{\mathsf{SL}}/2 \\
D_t\eta_d^{\mathsf{GL}^a}(t) &= 2k^{\mathsf{SL}\to\mathsf{GL}}\,\eta_d^{\mathsf{SL}^b}(t) - 2k^{\mathsf{GL}\to\mathsf{CL}}\eta_d^{\mathsf{GL}^a}(t) && \eta_d^{\mathsf{GL}^a}(0) = \delta_{d0}\,\rho^{\mathsf{GL}}/2 \\
D_t\eta_d^{\mathsf{GL}^b}(t) &= 2k^{\mathsf{GL}\to\mathsf{CL}}\,[\eta_d^{\mathsf{GL}^a}(t) - \eta_d^{\mathsf{GL}^b}(t)] && \eta_d^{\mathsf{GL}^b}(0) = \delta_{d0}\,\rho^{\mathsf{GL}}/2\,.
\end{aligned}
$$

where

$$
\begin{aligned}
\beta(u) &= 2(\sqrt{1+u}-1)/u\,, && [2(\sqrt{2}-1) < \beta(u) \le 1] \\
\rho^{\mathsf{S}_1^a}(u) &= [1 - \beta(u)/2]\,\rho^{\mathsf{S}_1} \\
\rho^{\mathsf{S}_1^b}(u) &= \beta(u)\,\rho^{\mathsf{S}_1}/2 \\
0 &< u \le 1\,.
\end{aligned}
$$

The VGFF is specified by the same $\mathbf{x}$ and C as the semi-coupled model, but with different $\boldsymbol{\rho}$, $\mathbf{F}$, and $T(\xi)$. Noting that the rate of density generation in S_1^b is $\Delta_1 = 2\lambda^{\mathsf{S}_1}\rho^{\mathsf{S}_1^b} = \beta(u)\,\lambda^{\mathsf{S}_1}\rho^{\mathsf{S}_1}$ and that the number of descendants transferred to S_1^a depends linearly on u, we have

$$\begin{aligned}
\boldsymbol{\rho} &= \{[1-\beta(u)/2]\,\rho^{\mathsf{S}_1}, \beta(u)\,\rho^{\mathsf{S}_1}/2, \rho^{\mathsf{S}_2}, \rho^{\mathsf{SL}}/2, \rho^{\mathsf{SL}}/2, \rho^{\mathsf{GL}}/2, \rho^{\mathsf{GL}}/2\} \\
\mathbf{F} &= [(1+u)\beta(u)\lambda^{\mathsf{S}_1}\rho^{\mathsf{S}_1}, \beta(u)\,\lambda^{\mathsf{S}_1}\rho^{\mathsf{S}_1}, \lambda^{\mathsf{S}_2}\rho^{\mathsf{S}_2}, F_\Sigma, F_\Sigma, F_\Sigma, F_\Sigma]
\end{aligned}$$

$$T(\xi) = \begin{bmatrix}
-1 & \xi & 0 & 0 & 0 & 0 & 0 \\
1+u\beta(u)/2 & -1-u\beta(u)/2 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 2(\xi-1) & 0 & 0 & 0 & 0 \\
u[1-\beta(u)/2]\gamma_1(u) & [(1-u)\xi + u\,\beta(u)/2]\gamma_1(u) & \gamma_2(u) & -1 & 0 & 0 & 0 \\
0 & 0 & 0 & 1 & -1 & 0 & 0 \\
0 & 0 & 0 & 0 & 1 & -1 & 0 \\
0 & 0 & 0 & 0 & 0 & 1 & -1
\end{bmatrix},$$

where

$$\begin{aligned}
F_\Sigma(u) &= \beta(u)\,\lambda^{\mathsf{S}_1}\rho^{\mathsf{S}_1} + \lambda^{\mathsf{S}_2}\rho^{\mathsf{S}_2} \\
\gamma_1(u) &= \beta(u)\,\lambda^{\mathsf{S}_1}\rho^{\mathsf{S}_1}/F_\Sigma(u) \\
\gamma_2(u) &= \lambda^{\mathsf{S}_2}\rho^{\mathsf{S}_2}/F_\Sigma(u)\ .
\end{aligned}$$

The $-u\,\beta(u)/2 = -u\,k^{\mathsf{S}_1\to\mathsf{SL}}(u)/2$ term in $T(\xi)_{\mathsf{S}_1^b,\mathsf{S}_1^b}$ corresponds to the stochastic $\mathsf{S}^{\mathsf{S}_1^b} \to \mathsf{SL}^a$ transport. The compensating term in $T(\xi)_{\mathsf{S}^{\mathsf{S}_1^b},\mathsf{S}^{\mathsf{S}_1^a}}$ corresponds to the increased $\mathsf{S}_1^a \to \mathsf{S}_1^b$ flow. The non-linear dependence of the transport rate on u arises from the interplay of the parallel stochastic transport from both S_1 subcomponents with the homeostatic constraints.

Uncombined equations (i.e., analogous to Eqs. (16)) that tracked the SL_1, SL_2, GL_1, and GL_2 cells separately were used for displaying the contributions of the S_1 and S_2 components and their differentiated descendants separately in Fig. 3e and Supplementary Fig. 3b.

Parameter fitting. The best-fit parameters for each model $\mathcal{M}$, $\{\phi^{\mathcal{M}}\}$, were determined by minimizing the statistical discrepancy $\Delta^{\mathcal{M}}$ between the mean experimental proportions $\{\Pi_d^c(t)\}$ and model ($\mathcal{M}$)-predicted proportions $\{\pi_d^{\mathcal{M},c}(t)\}$ using Dirichlet statistics.

$$\Delta^{\mathcal{M}}(\{\phi^{\mathcal{M}}\}) = \sum_{c,t} \log \mathcal{D}\left[\vec{\Pi}^c(t); \vec{\alpha}^c(t)\right] - \sum_{c,t} \log \mathcal{D}\left[\vec{\pi}^{\mathcal{M},c}(t); \vec{\alpha}^c(t)\right]\ ,$$

where the sums are over the experimental measurements at $c \in \{\text{BL, SL,GL}\}$ and $t \in \{3, 7, 21\}$, $\mathcal{D}(\vec{\pi}|\vec{\alpha})$ is the Dirichlet distribution, $\vec{\Pi}^c(t)$ and $\vec{\pi}^{\mathcal{M},c}(t)$ are the $[d_{\max}(t)+1]$-vectors over the experimental and model-predicted proportions, and $\vec{\alpha}^c(t)$ is the $[d_{\max}(t)+1]$-vector with components $\alpha_d^c(t)$ determined from the experimental data as described below. The first term is the log of the Dirichlet probability for a perfect model where the prediction exactly matches the data, while the second term is the log of the probability for the prediction. Therefore, $\Delta^{\mathcal{M}}(\{\phi^{\mathcal{M}}\}) \geq 0$ and smaller values indicate better fitting models.

The $\alpha_d^c(t),\ 0 \leq d \leq d_{\max}(t)$ were determined by the maximum likelihood condition

$$\Pi_d^c(t) = \frac{\alpha_d^c(t) - 1}{\sum_{d'=0}^{d_{\max}(t)}[\alpha_{d'}^c(t) - 1]}$$

{i.e., the mode of $\mathcal{D}[\vec{\Pi}^c(t);\vec{\alpha}^c(t)]$ is fixed to the $\Pi_d^c(t)$}, and the method-of-moments condition that the sum of the Dirichlet component variances equal the sum of the variances of the experimental measurements:

$$\sum_{d=0}^{d_{\max}}(\sigma_d^c)^2 = \sum_{d=0}^{d_{\max}} \alpha_d^c(\alpha_0^c - \alpha_d^c)/[(\alpha_0^c)^2(\alpha_0^c+1)] \ ,$$

where $\alpha_0^c = \sum_d \alpha_d^c$ and the time arguments are implicit.

Relationship to Akaike information criterion. Asymptotically, for $\alpha_d^c(t) \gg 1$, the Dirichlet distribution can be well-approximated by a constrained multinormal distribution and, up to an additive constant, $\Delta^{\mathcal{M}}(\{\phi^{\mathcal{M}}\})$ approximates the negative log-likelihood. In this case, the Akaike information criterion[6] for model $\mathcal{M}$ is

$$\mathrm{AIC} = 2k^{\mathcal{M}} + 2\Delta^{\mathcal{M}}\left(\{\phi^{\mathcal{M}}\}^*\right) \ ,$$

where $k^{\mathcal{M}}$ is the number of adjustable parameters in the model and $\{\phi^{\mathcal{M}}\}^*$ is the best-fit set of parameters. Because some of the $\alpha_d^c(t) \not\gg 1$, this approximation may not be accurate. Nonetheless, it provides a rough guideline for comparing models with different numbers of adjustable parameters: since smaller AICs indicate better models, the model with the minimum $k^{\mathcal{M}} + \Delta^{\mathcal{M}}$ is preferred.

H2B-GFP decay and cell turnover in tail scale and interscale epidermal regions. The decay of H2B-GFP fluorescence density in the interscale (predominantly LRC) epidermal regions was analyzed using the 2×SC model, but with cell density parameters $\rho^{\mathsf{S}_1} = 0$, $\rho^{\mathsf{S}_2} = \eta_{\mathrm{tot}}^{\mathsf{BL}}(0)$, $\rho^{\mathsf{SL}} = \eta_{\mathrm{tot}}^{\mathsf{SL}}(0)$, and $\rho^{\mathsf{GL}} = \eta_{\mathrm{tot}}^{\mathsf{GL}}(0)$, where the $\eta_{\mathrm{tot}}^c(0)$ were the total fluorescence densities measured in the unchased mice. Accounting for the dilution of fluorescence with division, the model-predicted total fluorescence density at later time t is

$$\eta_{\mathrm{tot}}^c(t) = \sum_d \eta_d^c(t)/2^d \ . \tag{18}$$

The best-fit value of λ^{S_2} was determined by least-squares minimization of the predicted values to the data, either for BL alone, or for BL+SL+GL ("total"), as indicated in Supplementary Fig. 3e. The same procedure was used to determine the best-fit λ^{S_1} for the scale (non-LRC) regions, except that the cell density parameters were $\rho^{\mathsf{S}_1} = \eta_{\mathrm{tot}}^{\mathsf{BL}}(0)$, $\rho^{\mathsf{S}_2} = 0$, $\rho^{\mathsf{SL}} = \eta_{\mathrm{tot}}^{\mathsf{SL}}(0)$, and, because there is no GL in the scale regions, $\rho^{\mathsf{GL}} = 0$.

BL→GL transit time. Potten et al.[9] estimated the BL→GL transit-time by measuring the amount of radioactivity that was recovered from the mouse back surface at different times after injection of [^{14}C]thymidine, which is incorporated into the BL. Since tracking radioactive thymidine is mathematically analogous to tracking H2B-GFP, we compared the 2×SC model predictions with these results using Eqs. (17) and the best-fit parameters, but changed the initial boundary conditions to $\eta_d^{\mathsf{S}_1^a}(0) = \delta_{d0}\,\rho^{\mathsf{S}_1}$, $\eta_d^{\mathsf{S}_2}(0) = \delta_{d0}\,\rho^{\mathsf{S}_2}$, and $\eta_d^{\mathsf{S}_1^b}(0) = \eta_d^{\mathsf{SL}^a}(0) = \eta_d^{\mathsf{SL}^b}(0) = \eta_d^{\mathsf{GL}^a}(0) = \eta_d^{\mathsf{GL}^b}(0) = 0$ to reflect the incorporation of [^{14}C]thymidine into the S_1 and S_2 cells during S-phase and the initial absence of radioactivity in SL and GL. The predicted total amount of radioactivity in GL at time t was proportional to $\eta_{\mathrm{tot}}^{\mathsf{GL}}(t)$ as per Eq. (18). The peak of this distribution was compared with the peak of radioactivity recovery measured by Potten et al.

Neutral drift and clone number evolution. The temporal evolution of the number of Dlx1$^{\mathrm{CreER}}$-marked clones observed in the tail interscale lineage tracing experiments was analyzed using a modified hybrid SC model in which the SL and GL compartments were combined into a single SGL compartment having the combined cell density, $\rho^{\mathsf{SGL}} = \rho^{\mathsf{SL}} + \rho^{\mathsf{GL}}$, and division and transport were treated as Poisson processes. (This was adequate because the division and transport rates are fast on the time-scale of interest.) The temporal

evolution of $P_{n_b,n_s}(t)$, the probability that a clone has n_b BL cells and n_s SGL cells at time t, is described by the master equation

$$
\begin{aligned}
&D_t P_{n_b,n_s}(\lambda,u;t) = \\
&\quad (1-u)\,\lambda\, n_b[P_{n_b,n_s-1}(\lambda,u;t) - P_{n_b,n_s}(\lambda,u;t)] + u\,\lambda[(n_b-1)P_{n_b-1,n_s}(\lambda,u;t) - n_b\,P_{n_b,n_s}(\lambda,u;t)] + \\
&\quad u\,k^{\mathsf{S}\to\mathsf{SGL}}[(n_b+1)\,P_{n_b+1,n_s-1}(\lambda,u;t) - n_b\,P_{n_b,n_s}(\lambda,u;t)] + k^{\mathsf{SGL}\to\mathsf{CL}}[(n_s+1)\,P_{n_b,n_s+1}(\lambda,u;t) - \\
&\qquad\qquad n_s\,P_{n_b,n_s}(\lambda,u;t)] \qquad\qquad (19a)\\
&P_{n_s,n_b}(\lambda,u;0) = \delta_{n_s 1}\,\delta_{n_b 0}\ , \qquad\qquad (19b)
\end{aligned}
$$

where u is the probability of uncoupled replication. The positive terms correspond to the inflow of probability into the (n_b, n_s) state from, in order, division coupled to BL→SGL transport, uncoupled division, uncoupled BL→SGL transport, and SGL→CL transport. The negative terms are the corresponding outflows.

We calculate $P_{n_b,n_s}(\lambda,u;t)$ by introducing the generating function

$$
\begin{aligned}
P(\lambda,u;\xi,\chi;t) &= \sum_{\substack{n_s=0\\ n_b=0}}^{\infty} \xi^{n_b}\,\chi^{n_s}\,P_{n_b,n_s}(\lambda,u;t)\\
P_{n_b,n_s}(\lambda,u;t) &= \frac{1}{n_b!\,n_s!}\,\partial_\xi^{n_b}\partial_\chi^{n_s} P(\lambda,u;\xi,\chi;t)\Big|_{\substack{\xi=0\\ \chi=0}}\ ,
\end{aligned}
$$

and summing Eqs. (19) over n_b and n_s. Applying the homeostatic transport rate constraints $k^{\mathsf{S}\to\mathsf{SGL}} = \lambda$ and $k^{\mathsf{SGL}\to\mathsf{CL}} = \rho^{\mathsf{BL}}/\rho^{\mathsf{SGL}}\,\lambda = \beta\,\lambda$, we get

$$
\begin{aligned}
\partial_t P(\lambda,u;\xi,\chi;t) &= \lambda\{[(1-u)\,\xi\,\chi + u\,(\xi^2+\chi) - (1+u)\,\xi]\,\partial_\xi + \beta\,(1-\chi)\,\partial_\chi\}P(\lambda,u;\xi,\chi;t)\\
P(\lambda,u;\xi,\chi;0) &= \xi\ .
\end{aligned}
$$

This homogeneous first-order partial differential equation can be solved by the method of characteristics giving

$$
P(\lambda,u;\xi,\chi;t) = \xi\left[u, (1-\chi)\,e^{-\beta\lambda t}; z(u,1-\chi;\xi)\right]\ ,
$$

where

$$
\begin{aligned}
\xi(u,y;z) &= \frac{\beta\,\mu(u,y)\,f_1(u,y) + y\,\mu(u,-\beta)\,f_2(u,y) + z\,[\beta\,\mu(u,y)\,\psi_1(u,y) - y\,\mu(u,-\beta)\,\psi_2(u,t)]}{\beta\,u\,[f_1(u,y) + z\,\psi_1(u,y)]}\\
z(u,y;\xi) &= \frac{\beta\,[y - u\,(\xi+y-1)]\,f_1(u,y) + y\,\mu(u,-\beta)\,f_2(u,y)}{-\beta\,[y - u\,(\xi+y-1)]\,\psi_1(u,y) + y\,\mu(u,-\beta)\,\psi_2(u,y)}
\end{aligned}
$$

$$
\begin{aligned}
\mu(u,x) &= u + (1-u)\,x\\
f_1(u,y) &= {}_1F_1\left[1 - \frac{u}{\beta(1-u)}; 1; -\frac{(1-u)\,y}{\beta}\right]\\
f_2(u,y) &= {}_1F_1\left[2 - \frac{u}{\beta(1-u)}; 2; -\frac{(1-u)\,y}{\beta}\right]\\
\psi_1(u,y) &= \ \Psi\left[1 - \frac{u}{\beta(1-u)}, 1, -\frac{(1-u)\,y}{\beta}\right]\\
\psi_2(u,y) &= \ \Psi\left[2 - \frac{u}{\beta(1-u)}, 2, -\frac{(1-u)\,y}{\beta}\right]
\end{aligned}
$$

where ${}_1F_1(a;b;z)$ is the confluent hypergeometric function of the first kind and $\Psi(a,b,z)$ is Tricomi's confluent hypergeometric function.

$P_{0,0}(\lambda,u;t) = P(\lambda,u;0,0;t)$ is the probability that a clone has disappeared by time t, so the probability that a clone of any size remains is $1 - P(\lambda,u;0,0;t)$. This was evaluated with the values determined from the tail interscale H2B-GFP experiments, $\beta = 1.6$ (interscale) or 1.5 (scale), and two types of best-fit parameters were determined: either $\lambda = 0.14$/day was fixed at the interscale division rate determined by the H2B-GFP decay experiments, or $u = 1$ was fixed corresponding to completely uncoupled division and BL→SGL transport. In both cases the best-fit value for the other parameter was determined by least-squares minimization.

The probability in the case where the coupling of division and transport is non-stochastically determined by the microenvironment is $1 - u \lim_{v\to 1} P(\lambda,v;0,0;t)$ since the number of clones does not decrease in regions where division and transport are completely coupled.

Code availability

Code for histogram deconvolution has previously been described[3] and is available from B.S.W. All other calculations were performed using Mathematica, Version 10.4, and code is available from D.S.

References

1. Waghmare, S.K. Quantitative proliferation dynamics and random chromosome segregation of hair follicle stem cells. *EMBO J* **27**, 1309-1320 (2008).

2. Tumbar, T. *et al.* Defining the epithelial stem cell niche in skin. *Science* **303**, 359–363 (2004).

3. Lee, J. *et al.* Runx1 and p21 synergistically limit the extent of hair follicle stem cell quiescence in vivo. *Proc Natl Acad Sci U S A* **110**, 4634–4639 (2013).

4. Clayton, E. *et al.* A single type of progenitor cell maintains normal epidermis. *Nature* **446**, 185-189 (2007).

5. Mascre, G. *et al.* Distinct contribution of stem and progenitor cells to epidermal maintenance. *Nature* **489**, 257-262 (2012).

6. Claeskens, G. & Hjort, N. L. *Model Selection and Model Averaging* (Cambridge University Press, 2008).

7. Lechler, T. & Fuchs, E. Asymmetric cell divisions promote stratification and differentiation of mammalian skin. *Nature* **71**, 275–280 (2005).

8. Klein, A.M. & Simons, B.D. Universal patterns of stem cell fate in cycling adult tissues. *Development* **138**, 3103-3111 (2011).

9. Potten, C.S., Saffhill, R. & Maibach, H.I. Measurement of the transit time for cells through the epidermis and stratum corneum of the mouse and guinea-pig. *Cell Tissue Kinet* **20**, 461–472 (1987).