

Supplemental information

Dynamic regulation of tissue fluidity

controls skin repair during wound healing

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Methods S1: Biophysical modelling of dynamics during regeneration, related to Figure 3 and S3

To analyse the clonal fate data under the conditions of DTA mediated cell ablation, we developed a minimal spatial model of basal cell fate in the IFE. Here, we provide further details on the modelling, defining its motivation, implementation and application both to homeostatic and perturbed conditions.

1 Spatial model of the basal layer of skin IFE

1.1 Towards a spatial model of IFE maintenance

Beginning with the pioneering work of Clayton et al.¹⁵, previous studies have shown how statistical modelling-based approaches can provide predictive insights into the functional identity and fate behaviour of the proliferative cells that comprise the basal layer of the mouse skin IFE^{7,8,21}. Despite the success of these approaches, consensus on the nature of the cell fate and dynamics has yet to be reached. Although some argue in favour in a simple one-progenitor model²⁰, and its spatial derivatives¹⁸, such minimal models fail to capture fine but robust features of the clone size data based on the persistent enhancement of even basal cell numbers in clones over odd numbers.

Previously, based on these findings, we have shown that the clonal dynamics of IFE under normal homeostatic conditions and following stretched-mediated expansion of the backskin epidermis is consistent with a two-compartment model of cell fate²¹. In this model, the differentiation and loss of IFE SCs is not direct, but occurs through the terminal division of an intermediate CP cell. Based on the enrichment of even-sized basal clones, we argued that SCs and CP cells are not independent but associate in pairs with correlated timing of cell division. By abstracting a simple lattice-based model, stochastic simulations showed that both fine features of the clone sizes as well as their long-term scaling behaviour under homeostatic and perturbed conditions could be explained quantitatively within this two-progenitor model framework.

While the two-progenitor cell model is essential to capture the fine even-odd features of the clone size distribution, its coarse-grained behaviour over longer times and larger scales - the scaling of the clone size distribution and the linear-like expansion of the average clone size - becomes indistinguishable from a simpler one-progenitor cell model. The committed progenitor cell compartment effectively amplifies the size of the differentiated cell compartment. At the same time, its application to the study of spatial clone dynamics brings a significant overhead, calling for the specification of several parameters that are not constrained by the data. Therefore, to address the spatial dynamics of clonal fate under the injury conditions of the DTA system, in the following, we chose to work within the framework of an effective *one-progenitor* model, where the impact of a the second committed progenitor cell population is absorbed into an extended differentiated basal cell compartment. Within this framework, we can directly compare this model to another competitive theory in which the basal cell layer comprises only equipotent progenitor cells, the model introduced by LeBlond and colleagues in the 1960s.

Previously, to analyse the dynamics of clones under homeostatic conditions, emphasis has been placed zero-dimensional models of cell fate. While such models capture correctly scaling features of clone sizes, they miss small logarithmic corrections to the average clone growth characteristics, a feature of two-dimensional models of cell competition¹⁷. More importantly, such models cannot provide insight into the spatial dynamics of clones, which will be influenced by local cell rearrangements through cell division and active cell migration. While the impact of such effects on cell fate behaviour is expected to be small in the context of a homeostatic tissue, their influence under conditions of DTA mediated cell ablation becomes amplified. Therefore, in the following, we developed a minimal spatial model of IFE fate that could be tailored to the study of homeostatic and regenerative conditions.

Specifically, to model the basal layer of tail skin IFE, we extended the one-progenitor model of cell fate to a spatial vertex (Voronoi) model, where both stem cell fate and proliferate rate are coupled to local cell density (Figure 3A). Vertex models have been widely used to develop predictive insights into the dynamics of epithelial

monolayers *in vitro* and during morphogenesis²⁴. In particular, such models have been successful in connecting the collective behaviour of tissue (e.g., tissue rigidity) to cellular-scale properties (e.g., cell-shape)^{26,27}. Here, we apply the vertex model as a caricature of the basal layer noting that true mechanical properties of the cellular system may not be fully captured by such a minimal theory.

1.2 Vertex model of the basal layer of the skin IFE

With these preliminaries, here we develop a spatial model of the skin IFE integrating a vertex-type model of cell dynamics within the framework of a one-progenitor model of the basal layer. To do so, we modify a well-studied variant of the vertex model known as a self-propelled Voronoi model²⁵ to incorporate cell division and loss. Through the feedback between cell size (as a readout of local cell density), stem cell fate, and cell division rate, the model provides a generic framework to investigate how the basal layer of IFE can recover following injury marked by cell loss, including its application to the DTA system considered in this study.

1.2.1 Tissue mechanics and geometry

In classic implementations of the vertex model, cells of a two-dimensional epithelial monolayer are represented as polygons that tile space. Cells have a preferred perimeter (P_0) and area (A_0). The vertices (corners of the cells) can move until cells find a balance between their cortical tension and cell adhesion. In the absence of cell division or loss, starting from any given configuration, cells will move, adjusting their shape and size until all cells in the tissue have a similar size. Depending on the value of the dimensionless ‘cell shape parameter’, $p_0 = P_0/\sqrt{A_0}$, the tissue can behave as either solid-like (glass-like) or fluid-like. In the solid-like phase, where $p_0 \leq p_0^{(c)} \approx 3.81$ (a critical value applicable when there are no or slow cell fluctuations, such as directed motility), cells remain spatially confined, while in the fluid-like phase ($p_0 > p_0^{(c)}$) cell rearrangements result in cell elongation and diffusion²⁵. In the context of cell proliferation, if the average time scale for cell division is longer than the time scale for mechanical relaxation of cells, then the tissue can show glass-like dynamics on short/intermediate time-scales and will become fluidised (with cells exchanging neighbours and diffusing) on a time-scale determined by the division rate.

Here, to model the mechanics of the basal IFE, we made use of the two-dimensional self-propelled Voronoi (SPV) model²⁵. Given the coordinates of the centres of cells ($\mathbf{r}_i$), a Voronoi tessellation identifies the locations of vertices and hence their polygonal shapes and sizes. The mechanical interactions between cells are described by the energy functional

$$E = \sum_{i=1}^n [k_A(A_i - A_0)^2 + k_P(P_i - P_0)^2] \quad (1)$$

where A_i and P_i are the area and perimeter of cell i , A_0 and P_0 are the target cell area and perimeter, the parameters k_A , k_P represent the area and perimeter moduli, and the sum runs over the n cells that comprise the basal layer. The positions of cells then evolves to minimise the mechanical energy,

$$\frac{d\mathbf{r}_i}{dt} = \mu \mathbf{F}_i = -\mu \nabla_i E \quad (2)$$

where μ is a drag constant consistent with viscous motion of cells at low Reynolds number. Here, we note that the basal layer is not unconstrained, but is confined between the basement membrane and the suprabasal cell layers which, for simplicity, are not modelled explicitly. However, to proceed, we make the simplifying assumption that the impact of the suprabasal layers does not change qualitatively the effective nature of the forces that act between cells, as implied by the energy functional.

1.2.2 Rules of cell fate

In the present study, the IFE undergoes constant turnover with cell loss through differentiation balanced by stem cell division. With a focus on a one-progenitor population, progenitor cells make a fate choice between symmetric duplication, asymmetric division or symmetric differentiation. At homeostasis, the probability of symmetric duplication and symmetric differentiation must be balanced. Moreover, at homeostasis, we propose that stem cells divide stochastically at a fixed rate λ_h with times drawn from an exponential distribution. More generally, to ensure the stability of the basal cell population under regenerative conditions, we propose a feedback between stem cell fate balance and cell density so that the fate probabilities and division rate are

dependent on cell area A_i :

$$S \xrightarrow{\lambda(A_i)} \begin{cases} S + S, & \text{Prob. } rp(A_i) \\ S + D, & \text{Prob. } 1 - r \\ D + D, & \text{Prob. } r(1 - p(A_i)) \end{cases} \quad (3)$$

To enforce an area dependence of the symmetric division probability, we propose a minimal model in which stem cells make a fate decision based on their cell area with a Hill function-like dependence,

$$p(A_i) = h(A_i) = \frac{A_i^{n_{\text{Hill}}}}{A_0^{n_{\text{Hill}}} + A_i^{n_{\text{Hill}}}} \quad (4)$$

To account for the increased proliferation rate (as observed through the rate of EdU incorporation) during regeneration following basal cell ablation, we propose that cells switch between the homeostatic and regenerative division rate depending on the local cell area (again as a read-out for local cell density). Here, we consider a minimal model in which the proliferation rate undergoes a binary switch depending on the value of the local cell area relative to a critical cell area (A_c).

$$\lambda^{(\text{bin})}(A_i) = \begin{cases} \lambda_h & A_i < A_c \\ \lambda_r & A_i > A_c \end{cases} \quad (5)$$

Within the scope of the feedback model, the system is robust to perturbations away from the steady-state density. If the average cell area is low (or high) relative to the homeostatic steady-state density then stem-cells become biased to symmetric duplication over differentiation.

Finally, we considered two variants of the model, one where differentiation is concomitant with delamination (termed the S model) and another where basal differentiated cells have a non-zero residency time (termed the $S+D$ model). Within the framework of the $S+D$ model, the delamination and stratification of differentiated cells from the basal layer is stochastic and modelled as a Poisson process with average rate μ ,

$$D \xrightarrow{\mu} \text{suprabasal} \quad (6)$$

In the S model, there are no differentiated cells in the basal layer, a limit realised by the $S+D$ model when $\mu \rightarrow \infty$. For a given delamination rate μ , the fraction f_s of basal cells that belong to the progenitor compartment at homeostasis can be obtained through the relation

$$\mu = \frac{\lambda_h f_s}{1 - f_s} \quad (7)$$

For either model, once differentiated cells enter the suprabasal cell layer, they undergo progressive differentiation and maturation, before becoming shed from the surface. To capture the clonal dynamics of the suprabasal differentiated cell compartment, we suppose that suprabasal cells (counted experimentally as those that still contain nuclei) are shed at a constant rate Γ . In homeostasis, the ratio of suprabasal cells n_s , compared to the number of basal cells n_b is fixed by the relative division and shed rates according to the relation $n_s/n_b = \lambda/\Gamma$.

1.3 Summary of model parameters

For the numerical simulations of the vertex model, we chose dimensionless units, where lengths are measured in terms of $\sqrt{A_0}$, and time is measured in terms of the characteristic mechanical relaxation times (τ_{mech}). For our modelling of experimental data, we considered the following as possible fit parameters:

- **Mechanical relaxation time:** τ_{mech} , defined in terms of other mechanical parameters $\tau_{\text{mech}} = (\mu k_A A_0)^{-1}$.
- **Fraction of asymmetric stem cell divisions:** $1 - r$
- **Average cell division time at homeostasis:** $\tau = 1/\lambda_h$
- **Average cell division time during regeneration:** $\tau_r = 1/\lambda_r$, for when cell area exceeds A_c .
- **Critical cell area for switching division rate:** A_c
- **Area sensitivity of fate bias:** n_{Hill}

- **Cell shape parameter:** p_0 , which is defined in terms of target cell perimeter and cell area, $p_0 = P_0/\sqrt{A_0}$. At homeostasis, cell rearrangements are minimal, as demonstrated by the low degree of clone fragmentation. Thus, we chose a value of p_0 that lay within the solid-like phase (below the transition point) of $p_0 = 3.5$. We found that fits to the clone fate data were insensitive to p_0 in the solid-like regime.
- **Ratio of area of perimeter moduli:** k_P/k_A , which will not significantly affect the quantities of interest; so we set this parameter to unity.

Some of the mechanical parameters (e.g. p_0 , k_P/k_A , τ_{mech}) do not significantly influence the quantities of interest, so could be held fixed. In the $S+D$ model, the fraction of progenitor cells at homeostasis, f_s , which defines the delamination rate, serves as an additional fit parameter.

1.4 Numerical implementation of self-propelled Voronoi model with turnover

Stochastic simulations of the vertex model were performed with an Euler method with time-steps of size $\Delta t = 0.01$ (with a timescale of 1 corresponding to the non-dimensionalised mechanical relaxation time with periodic boundary conditions on a 15×15 domain. As described above, parameters were chosen such that there were roughly 225 cells at homeostasis. For each simulation, states were initialised with cell centres placed at random initial positions on $[0, L) \times [0, L)$. For the $S+D$ model, the identity of basal cells was chosen at random but consistent with the target value of f_s .

Cell decisions were set to occur on the same discretised time-points. The cell-decision times for stem cell divisions or delaminations of differentiated cells (in the case of the $S+D$ model) were determined by generating times from an exponential distribution with average time τ . We initialised the suprabasal layer with 0 cells, as we are simply interested in the clonal outputs into this layer from labelled basal cells.

Once the simulations were initialised, the simulations were mechanically relaxed in the absence of division for 1,500 simulation time-steps. The tissue was then relaxed with the homeostatic division rules for a further 4,000 simulation time-steps. This relaxed state was taken as the induction time for clonal dynamics, with all cells in the tissue at this time used as part of the clonal ensemble. After this, simulations were run under either homeostatic or cell ablation conditions (using a specified cell death time profile obtained from experiment) for a length of time determined by the corresponding experimental data set. For each time-step of the simulation, we followed the sequence of steps below:

1. Apply periodic boundary conditions using the minimum-image convention.
2. Compute the Voronoi tessellation of cell centres, obtaining the indices of vertices per cell, positions of vertices, lists of neighbouring cells / vertices and areas / perimeters of cells.
3. Compute mechanical forces on each cell (according to the vertex model) as outlined in Bi et al²⁵.
4. Update position based on mechanical forces using the Euler method.
5. Implement cell decisions (divide, delaminate, or die) for times t and $t + \Delta t$.
 - (a) For any stem cells larger than the critical area (A_c), a random number between 0 and 1 was generated. If the number was less than $(\lambda_r - \lambda_h)\Delta t$, then the stem cell is set to divide, with the fate choice made following the rule specified above.
 - (b) If stem cells divide, we initialise the daughter cell centre to be at a small displacement ($\Delta x = 0.01$) from the mother cell at a random angle of division (drawn from a uniform distribution on $[0, \pi]$). When new stem cells were added, their lifetimes from stochastic decisions are generated by drawing from an exponential distribution with average time $\tau = 1/\lambda_h$.
 - (c) If cells die, or delaminate, the cell is removed and the Voronoi tessellation is re-computed.
 - (d) When cells delaminate, the lifetime of the daughter suprabasal differentiated cells are generated from an exponential distribution with lifetime $1/\Gamma$.

1.5 Model assumptions

Before turning to the model fits, we close this section by noting some of the assumptions and limitations of the modelling scheme. In developing the vertex model, we aimed to find the minimal computational scheme that could be constrained by the experimental data. In doing so, we simplified the known proliferative heterogeneity of the biological system. Moreover, the spatial model, based on density-dependent feedback, is still likely to be an over-simplification of a more complex dynamics that are beyond the resolution of the current statistical measures. Here, we highlight the key assumptions and simplifications of the spatial model:

Neglecting proliferative heterogeneity: As stated earlier, tail skin IFE contains a heterogeneous progenitor population. Stem cell differentiation likely occurs via terminal division of a progenitor cell compartment, leading to even-odd correlations in the clone size data²¹. As we focus on short-term dynamics for controls and, as seen below, find a significant degree of clonal fragmentation under ablation conditions, detecting fine features in clone size distributions is beyond the scope of the analysis. Further, the chase times are too short to unveil the proliferative hierarchy present in the interscale region of tail-skin⁸. Thus, it is sensible to simplify the model by incorporating only one progenitor population, given the success that such models have had in capturing the effective dynamics of the renewing compartment^{15,18,20}.

Assuming a 2D character of cells in the basal layer: The focus of the vertex model is only on the cell dynamics within the two-dimensional basal layer and neglects the full three-dimensional nature of the stratified squamous epithelium. In making this simplification, the model is unable to capture the dynamics of cell delamination in which the area of basal attachments is gradually lost while the shape of cells becomes increasingly anisotropic along the basal-apical axis. At the same time, the two-dimensional model neglects potential mechanical influences from the three-dimensional architecture of the tissue, incorporating its effect into the effective parameters of the two-dimensional energy functional. Moreover, for the clonal dynamics of the suprabasal cell layer, we have made use of a zero-dimensional scheme in which the cell dynamics are linked to a constant shed rate.

Mechanical homogeneity: We assume that basal cells are mechanically homogeneous. This is clearly a simplification of the real biological tissue. Mechanical properties of cells vary during the cell-cycle in particular in the growth phase (G0, G1; equivalent to increase of preferred cell area), and the rounding of cells during mitosis. Cells committed to (or in the process of) delamination have lower adhesion to basal cells and to the basement membrane. Further, committed progenitor cells may also have different mechanical parameters. The presence of mechanical heterogeneity affect the solid-fluid transition point^{26,27}.

Feedback on division rate and fate choice is only through local cell density: To explore the effect of density-dependent feedback on tissue dynamics, we have implemented area dependent rules for the stem cell division rate and fate balance. One consequence is that in this model cells can respond (by adjusting their rates) instantaneously to changes in density, whereas a delay between reduction in density and response of increased proliferation is suggested in the data. It is likely that, as well as mechanical influences (e.g., cell size or stress), stem cell fate behaviour and division rate are also likely to be coordinated by biochemical niche factors. Identifying differences between these factors may be difficult within the present data as different models exhibit similar phenomenology on the tissue scale and/or at long times. In this sense, the vertex model should be viewed as an effective theory that captures the clonal dynamics of more complex models.

Division and loss as the main source of noise: Internal biochemical noise leading active stress fluctuations would drive cell shape changes leading to cell shape and size fluctuations over time. If the magnitude of fluctuations were sufficiently high, the tissue would fluidise^{25,28}. Here, we assume that cell intrinsic active stress fluctuations are negligible compared to cell fluctuations from division and loss. The justification is that cell neighbour exchanges (T1 transitions) were found to be rare in the short-term live-imaging of control mice in regions where, by chance, cell division or loss did not occur.

Neglecting temporal correlations between events: Intravital live-imaging indicated that there are strong temporal correlations between sister cells, which either both delaminate or both divide¹⁸. Live-imaging also suggests that, for some fraction of time, cell delamination promotes the division of neighbouring cells¹⁹. The present model at homeostasis assumes that the only temporal correlations between decision timing are from a delaminating cell promoting division, as effected by the increase in cell division rate when cells expand above the critical area. If τ_{mech} is short compared to the average division time, this effect will disappear at homeostasis. Temporal correlations will not influence clonal dynamics except at the shortest time-scales, which are beyond resolution of the data.

Only considering the contribution of basal IFE stem cells: Finally, we know from *Lrig1* tracing in the current study that, under injury conditions, cells from the infundibulum may migrate into the IFE and contribute to repair. However, such influences are difficult to dissect from the clone fate data and, as such, reach beyond the scope of the current implementation of the model.

2 Fitting the control dataset within the one-progenitor Vertex model

With the spatial model for the skin basal layer defined, we turn now to the modelling of the experimental data to see whether a one-progenitor model can recapitulate the experimentally observed dynamics. Before we can understand the applicability of the vertex model to the ablation experiment, we first applied our model to the unperturbed (control) conditions of the tail skin IFE.

2.1 Fitting the models to the control data

2.1.1 Fitting approach

We began modelling the control data from day -5 , half a day after the administration of tamoxifen. To optimise the parameters of each model (*S-only* or *S+D* model), we used a brute force search over parameters r , τ/τ_{mech} , n_{Hill} and A_c . For each parameter set, we re-ran the dynamics including the suprabasal layers for different constant shed rates (Γ) and found the overall rescaling of time (τ_{mech}) that minimises the cost function defined as the product of the average Kolmogorov-Smirnov distance (KSD) for the basal and total clone size over time,

$$\mathcal{C} = \left(\frac{1}{T} \sum_t \sup_n \left| F_{n,B}^{\text{sim}}(t) - F_{n,B}^{\text{exp}}(t) \right| \right) \left(\frac{1}{T} \sum_t \sup_n \left| F_{n,T}^{\text{sim}}(t) - F_{n,T}^{\text{exp}}(t) \right| \right) \quad (8)$$

Here, $F_{n,B/T}^{\text{sim/exp}}(t)$ denotes the cumulative basal or total (basal + suprabasal) clone size distribution from simulations or experiments and $T = 6$ is the number of time-points used in the analysis. We considered only clones with at least one basal cell.

To generate uncertainty estimates on the best fit parameters, we used a bootstrapping procedure. Clones from the best fit simulation dataset were resampled with replacement 1000 times, with each sample using the same number of clones at each time point as the number of clones counted in the scale region. The fitting procedure was repeated for each resampled parameter set, and the standard deviation of the best-fit parameters for each sample was taken as the uncertainty of the fit.

To generate uncertainty estimates for the model's predictions of average clone sizes over time, we estimate the standard error of the mean (S.E.M.) by randomly re-sample with replacement the number of clones from the control experiment using simulations at the best-fit parameters at each time point. The shaded error regions correspond to 95% confidence intervals (i.e., $1.96 \times \text{S.E.M.}$). The same approach is applied for uncertainty estimates in the clone size distributions for the model.

2.1.2 Fitting the S and S+D models

For fitting the control data, we note that the *S* model (all basal cells are from an equipotent stem cell population) is a limiting case of the *S+D* model (basal differentiated cells have a finite residency time), where $f_S = 1$ ($\mu \rightarrow \infty$). Thus, to understand the suitability of both models, we fit the *S+D* model allowing the possibility of $f_S = 1$.

We considered the case where the average division time is slower than the mechanical relaxation time ($\tau/\tau_{\text{mech}} \geq 5$). To begin with, we considered the stem cell division timing to be fully stochastic (a Poisson point process with rate λ_r) and only cell fate is influenced by cell area (a behaviour enforced by setting $A_c = \infty$).

We obtain high quality fits to the control data (Supplementary Figure 3A-C) for the *S* model, or the *S+D* model with a low fraction of differentiated cells ($f_S \gtrsim 0.9$). The best fit parameters obtained were $\tau/r = 1/r\lambda = 20.0_{-1.3}^{+1.8}$, $\Gamma/\lambda = 1.7_{-0.5}^{+0.8}$ (Supplementary Figure 3F), and $f_S = 1.0_{-0.1}^{+0.0}$ (Supplementary Figure 3E). However, the landscape of fits was flat as r (best fit value $0.35_{-0.1}^{+0.05}$, 95% CI: $[0.15, 0.5]$) and τ (best fit value $7.0_{-1.2}^{+2.0}$, 95% CI: $[4.6, 9.6]$) were varied for τ/r and f_S fixed (Supplementary Figure 3D). The quality of fits was also insensitive to changes to n_{Hill} , which influences local correlations between cell decisions. We note that the true fraction of basal differentiated cells may be higher, as we assume here that the K14 promoter does not have a labelling bias between basal cells, whereas likely there is a labelling bias to proliferating cells⁸.

A similarly high quality of fits were achieved if A_c , τ/τ_{mech} or $\lambda_r\tau_{\text{mech}}$ were varied. Altering τ/τ_{mech} affects how quickly cells relax to a steady shape and size after divisions. When τ is comparable to τ_{mech} , we saw a greater variation in cell areas leading to local correlations in cell fate choices. If $\tau \gg \tau_{\text{mech}}$, then cells can all locally relax to areas close to the target cell area between division or delamination events, so there are no local correlations, meaning that each cell effectively ‘tosses a coin’ between division and delamination when symmetric fate outcome is chosen. Hence, the lack of sensitivity to τ/τ_{mech} tells us that these homeostatic static clonal distributions appear insensitive to the presence of local fate correlations, at least for these models.

Altering A_c and $\lambda_r \tau_{\text{mech}}$ affects the extent of divisions triggered by cells being above the critical cell area. In effect, this sets whether we see events where delamination drives cell division, as opposed to randomly timed events. In a wide part of the parameter regime, such as $A_c > 2A_0$ or $\lambda_r \tau_{\text{mech}} \ll 1$, these events are very rare meaning that the cell division rate $\lambda = \lambda_h$. However, when they occur, they lead to correlations in the timing of cell delamination and the subsequent division of a neighbouring stem cell, thus changing the effective stem cell division rate λ . We note that once using the new effective division rate, provided that $1/r\lambda$ takes a similar value to before, very good fits can be obtained to the clone size distribution. Thus, we cannot identify local correlations in the causal timing of events from such a static homeostatic clonal dataset.

2.2 Comparison of fitted values with the literature

We then compared the values of parameters obtained from the fit of the vertex model with those obtained from fits presented in the literature for tail skin IFE based on the study of zero-dimensional models at homeostasis. Proliferation kinetic data in Mascré et al⁷ via BrdU pulse and H2B-GFP label retention determined an average division time of 5.8 days, with data combined from the scale region and interscale region, as we have done here. By contrast, intravital live-imaging of homeostatic tail skin presented in the Supplemented Material by Rompolas et al¹⁸ identified an average division time of 4.0 days (based on a one-progenitor model paradigm) and an average lifetime before cell delamination of 6.5 days. No distinction between scale and interscale was made in this analysis.

For the scale region of the tail skin IFE, Sanchez-Danes et al⁸ previously fit the clonal data to a zero-dimensional one-progenitor model, which can symmetrically duplicate, symmetrically differentiate or asymmetrically divide with an average division time $\tau = 4.0$ days and the fraction of symmetric divisions set at $r = 0.19$.

The insensitivity of the model for varying $\tau = 1/\lambda$ for fixed $r\lambda/f_S$ is a well-known feature of the clone size distribution reflecting the convergence of the dynamics towards a balanced birth-death type process¹⁵. If we consider the long-time ($t \gg \tau$) dynamics, then a model with one type of stem cell, which can give rise to differentiated cells in the basal layer would have a mean clone size increasing as $1 + \frac{rt}{f_S \tau}$, and the clone size distribution would converge to an exponential with a scaling parameter $\frac{rt}{f_S \tau}$. For the model of Sanchez-Danes et al⁸ and the S-only model here, $f_S = 1$ as there are no basal differentiated cells. From the published data $1/r\lambda = 21.1 \pm 1.7$, which is consistent with the values obtained from the S model here $\tau/r = 1/r\lambda \approx 20$.

2.3 Comparing to models with proliferative heterogeneity

We have found that minimal models based on a one-progenitor population work well in explaining the short-term homeostatic data in mouse tail skin IFE. Moreover, the present models do not explicitly contain the large scale proliferative heterogeneity, which is known to be present in the interscale region of the mouse tail skin IFE. The short time-scale data (with no clones exceeding 5 basal cells) would make it difficult to identify any evidence for a proliferative hierarchy, particularly if one population cycled much more slowly than others^{7,8}.

Further, based on previous studies²¹, we could expect a bias towards even clone sizes to emerge if, as in backskin, stem cell differentiation occurs via the terminal division of a committed progenitor. However, comparing basal clone sizes with control measurements from back skin IFE, given the slower average cell division rate, the time-span of the control data is too short for even-odd features to become apparent. Nonetheless, we tested the consistency of this model to the present dataset.

To test whether a two-progenitor cell paradigm could not be ruled out by the current dataset, we adapted the stochastic model from Aragona et al²¹ into a vertex model framework, containing stem cells, committed progenitor (CP) cells and basal differentiated cells in the basal layer. CP cells could divide once to produce differentiated cells. Stem cells could divide symmetrically ($S \rightarrow S + S$), symmetrically differentiate ($S \rightarrow CP + CP$) or asymmetrically ($S \rightarrow CP + S$) divide. As in Aragona et al²¹, CP cells produced by asymmetric division had their cell-cycle synchronised with their sister stem cell. We found if basal differentiated cells instantly delaminated, then the model could not recapitulate both the basal and suprabasal average clone size evolution. However, if basal differentiated cells had a non-zero life-time, then parameter regimes could be found where such a model could re-capitulate the clonal data at a similar level of goodness of fit to the one-progenitor models described above. Thus, the multiple progenitor paradigm noted in previous work is consistent with this dataset, but the present control data cannot distinguish the one from the two-progenitor theories.

It is worth noting that, at the level of basal cell clone dynamics, the $S+D$ model is identical to a model with stem and CP cells in the case where there is no temporal coordination between stem and CP cell divisions. Since, as we had defined, a CP cell can terminally divide just once (concomitant with its delamination), the equivalent shedding rate of the $S+D$ model would be half that of the equivalent $S+CP$ model.

2.4 Insights from the modelling of the control dataset

As in previous studies^{15,7,8}, a one-progenitor model can accurately predict the clone size distributions at early-times in tail-skin IFE, but for this data the presence of basal differentiated cells cannot be distinguished from the model with all basal cells being proliferative. A two-progenitor model (variation of Aragona et al²¹) can also work provided that basal differentiated cells also have a non-zero residency time in the basal layer. Finally, the success of the present models in the context of the control dataset is such that we cannot infer the presence (or absence) of correlations in decision timing (viz. delaminations driving divisions) or local correlations of cell fate, which are proposed to exist^{18,19} (and are possible to achieve in certain parameter regimes of the spatial model).

3 Vertex model for regeneration following the genetic ablation of basal cells

Having adapted the vertex models to control data, we then turned to the experimental data for the basal cell ablation experiment, focusing on measurements of the variation of basal cell density, division rate and clone size distribution over time.

3.1 Modelling cell-death from DTA mediated cell ablation

To model cell death due to Diphtheria toxin (DTA), we introduced a time-varying cell death rate (Supplementary Figure 3G) proportional to the profile specified by the cleaved caspase 3 (CC3) measurements. Empirically, we found that the profile, starting from the initial dose of Doxycyclin (day -2), can be parameterised approximately by the mathematical form

$$D(t) = \begin{cases} 0, & \text{if } t < -2 \\ \frac{D_0 e^{b_1(t+2)}(1-e^{-b_2(t+2)})}{e^{3b_1(1-e^{-3b_2})}}, & \text{if } -2 \leq t < 1 \\ D_0 e^{-b_3(T1)}, & \text{if } t \geq 1 \end{cases} \quad (9)$$

while the overall scale of the death rate (D_0) adds an additional model free parameter. A nonlinear least square fit to the CC3 measurements yields $b_1 = 3.3 \times 10^{-2} \text{ (days)}^{-1}$, $b_2 = 0.88 \text{ (days)}^{-1}$ and $b_3 = 0.52 \text{ (days)}^{-1}$. To model cell death in the vertex model, we simply removed the cell from the tissue and recomputed the Voronoi tessellation.

3.2 Applying the vertex model to the DTA ablation dataset

3.2.1 Fitting approach

To run the simulations under ablation conditions, we first initialised the system by allowing it to achieve a steady-state under homeostatic conditions, which ensures a starting point with the correct homeostatic average cell division rate and basal cell density.

In modelling of the DTA mediated cell ablation experiment, we note that before application of DTA and after the recovery phase, the system is positioned in a homeostatic state. Hence, for parameter fitting we parameter regimes that are consistent with the control clonal dataset in the absence of cell death. However, there is degeneracy in the best fit solution for the control experiment as certain parameters are varied. Thus, we fixed $r\lambda$ (where λ was calculated as the average division time from simulations, which has A_c and λ_r dependence) based on the best fits to the homeostatic solution, and chose $\tau_{\text{mech}} = 12$ hours as the mechanical relaxation time was not significantly constrained by data or the literature. Shorter mechanical relaxation times do not lead to different phenomenology with other parameters adjusted, but take significantly longer to simulate, leading the fitting procedure we used to become computationally infeasible.

To optimise the chosen fitting parameters to the experimental data (basal cell density, division rate, basal and total clone size distribution), we used a simulated annealing procedure. Simulated annealing (SA) enables descent towards an optimal solution, with an ‘effective temperature’ (T) used to avoid becoming stuck in a local minima. The temperature was slowly decreased, linearly with the number of simulated annealing time-steps. Starting from arbitrarily chosen initial parameters, at each iteration, 5 parallel simulations for the same parameter set were run. After each SA iteration, we calculated the cost function

$$\mathcal{C} = \left(\frac{1}{T} \sum_t \sup_n \left| F_{n,B}^{\text{sim}}(t) - F_{n,B}^{\text{exp}}(t) \right| \right) \cdot \left\{ \frac{1}{8} \sum_t \left(\frac{\rho_{\text{sim}}(t)}{\rho_{\text{exp}}(t)} - 1 \right)^2 \right\} \cdot \left\{ \frac{1}{6} \sum_t \left(\frac{\lambda'_{\text{sim}}(t)}{\lambda'_{\text{exp}}(t)} - 1 \right)^2 \right\} \quad (10)$$

which was defined as the product of the sum of squared error of the basal cell density ($\rho_{\text{exp}}(t)$ from experiment and $\rho_{\text{sim}}(t)$ from simulation), the product of the sum of squared error between the relative division rate (defined as the ratio between the homeostatic and basal cell ablation division rates) between experiment ($\lambda'_{\text{exp}}(t)$) and simulation ($\lambda'_{\text{sim}}(t)$), and the KSD between the simulated and experimental basal clone size distributions.

By looking at the ratio of the average basal to suprabasal cell clone sizes in the data, we could infer that the ratio between the basal cell division rate and suprabasal cell shedding rate changes with time during recovery from ablation. Thus, for each SA step to fit the suprabasal layer, we applied a constant shed rate between adjacent time-points and picked the piecewise constant shed rates to optimise the average suprabasal clone size distributions across time.

After each SA step, new parameter sets were then iterated by adjusting a single randomly chosen parameter (of the 4 parameters that were varied), relative to the previous parameter set. If the cost function decreased, then the proposed parameter set was accepted and a step with a change to the same parameter in the same direction (decreased, or increased) was proposed until the cost no longer decreased. Conversely, if the cost increased, the proposed parameter set was accepted with probability $e^{(\mathcal{C}_{\text{new}} - \mathcal{C}_{\text{old}})/T}$. This process was then repeated until a suitable solution was found.

The parameters chosen to fit were those non-constrained by the control, in particular the peak death rate (D_0), the critical area for the switching of the division rate (A_c), the division rate during peak regeneration (λ_r), and the Hill function exponent (n_{Hill}) setting the degree of fate bias depending on area. However, once the optimum parameter set was found for these 4 parameters, we also fitted to r to see if there was a change to the inferred ratio of asymmetric divisions.

As with control, we use bootstrapping to estimate the uncertainty of the model predictions of clone-sizes for the best fit parameters. For basal cell density and division rate, we estimate the S.E.M. of model predictions using the standard deviation of independent simulations at a fixed tissue size.

3.2.2 S model

We first considered a model where all basal cells can proliferate in response to injury (i.e., all basal cells belong to a self-renewing stem cell compartment). However, for the model variant and fitting procedure described we find that the simulated solution never converges to the experimental data and we are left with fits that, from the basal clonal size distribution, density profile and division rate profile, fit one time-course well and two time-courses poorly.

To see whether the poor quality fits emerged as a result of the chosen profile of the stem cell division rate, we tested an additional profile where the proliferation rate increases with increasing cell area above the critical cell area,

$$\lambda^{(\text{relu})}(A_i) = \begin{cases} \lambda_h & A_i < A_c, \\ \lambda_h + (\lambda_r - \lambda_h)(A/A_c - 1) & A > A_c \end{cases} \quad (11)$$

We found that the model gave good fits for the basal cell density over the time-course (Supplementary Figure 3I) and reasonable fits to the division rate at early and late time-points (Supplementary Figure 3J). However, this requires a significantly higher division rate than is observed at the intermediate time-points (peak division rate is different by roughly a factor of 2) and does not accurately capture the basal clone sizes (Supplementary Figure 3H) across the time-course. Thus, this variant is still inadequate to capture the observed dynamics.

A crucial issue in both chosen profiles of division rate vs area, is that an *S-only* model cannot explain why the average basal clone size continues to expand far more rapidly at late time points (from day 11 to day 18) than the homeostatic rate. In any model, where all basal cells are identical in their response during regeneration, the growth in average basal clone size corresponds precisely to the rate of symmetric duplication. So, as the stem cell division rate returns towards its homeostatic value, the basal clone size cannot grow fast enough.

Thus, the *S* model (where all basal cells are proliferative) during ablation fails. We can never reproduce simultaneously the basal and total clone-size distributions, the profile of division rate and basal cell density. One must therefore consider alternative models.

3.2.3 S+D model

To rectify the paradox of the enhanced growth of average basal clone sizes at late times, we note that if basal clones grow as a proliferative unit (i.e., a stem cell can produce differentiated daughter or committed progenitor cells that reside in the basal layer) then the basal clone growth rate is enhanced above the stem cell division rate. More precisely, in the *S+D* model at homeostasis, the basal clone size grows as $r\lambda t/f_s$, whereas it grows as $r\lambda t$ in the *S* model.

Therefore, we tried to fit the $S+D$ model during basal cell ablation using the simulated annealing procedure with the cost function described above. We note that it is likely that the delamination rate would also be density dependent. However, to simplify the model and reduce the number of fit parameters, we assume that differentiated basal cells delaminate at a constant rate, chosen so that the fraction of stem cells at homeostasis $f_s = 0.9$ (consistent with the fits obtained from the control clonal data).

The best fits (best cost of $\mathcal{C} = 0.048$) obtained for each fitting parameter translated to a maximal death rate $D_0 = 1.1 (\text{day})^{-1}$, a peak division rate during regeneration of $\lambda_r = 6$ hrs, a critical cell area $A_0 = 1.25$ and fraction of symmetric duplications ($r = 0.3$, consistent with control fits above). Further, we found the fate balance dependence approached a step-like function (with the best fit value of $n_{\text{Hill}} \approx 10$), indicating that for this model stem cells nearly always choose symmetric duplication over symmetric differentiation. To fit the suprabasal layer, we found a piecewise constant shed rate to re-capitulate the average suprabasal clone-sizes, with $\Gamma = \lambda/0.25$ for $-5 < t < 6$ days, $\Gamma = \lambda/0.3$ for $6 < t < 11$ days and $\Gamma = \lambda/0.5$ for $11 < t < 18$ days. With this fit, the model captured well the qualitative trends in the data with the quantitative predictions capturing satisfactorily the range of dynamic measures during the recovery after basal cell ablation (Figure 3B-D, Supplementary Figure 3L-M).

3.2.4 Fluidisation of tissue after basal cell ablation

Based on the fits of the $S+D$ model, we noted that basal clone sizes were larger at early times in the model compared to experiment (Figure 3D; Supplementary Figure 3L), while the predicted clone persistence was substantially lower as compared to the basal cell ablation experiment (Supplementary Figure 3K). To understand this discrepancy, we considered snapshots of clones at early times (Figure 3F; Supplementary Movie 2). We found that, from the model, we predict a significant degree of cell dispersion and clone fragmentation indicative of increased fluid-like behaviour of the tissue by the high division rate and high death rate, leading to cellular rearrangements and intercalation of clones. We compared this behaviour with that seen in the homeostatic case, which did not show evidence of significant clone fragmentation (Figure 3F; Supplementary Movie 1), as the homeostatic division rate is roughly 10 times lower compared to the peak division rate during recovery from ablation, and there is no substantial rate of cell death. The model suggested that, experimentally, we should expect significant clone fragmentation, which makes it difficult to determine whether nearby fragments are different clones of the same colour, or fragments from the same clone in a lineage tracing assay.

As further evidence for the fluidisation, we quantified the rate of T1 transitions during regeneration vs homeostasis (Figure 3E). In the experiment, topological transitions are segmented into proliferative and non-proliferative events. In the model, division, delamination and death events provide the source of noise so we cannot decouple proliferative events. The model detects T1 events that occur immediately following a cell division that are not observed or counted in experiments, so is qualitatively but not quantitatively comparable to a T1 rate including proliferative and non-proliferative events in experiments. At homeostasis, we observed a low constant rate of T1 transitions, which was proportional to the average basal cell division rate. During regeneration, when there is a dramatic elevation in the cell division rate as well as cell death rate, we observed that the average number of T1 transitions per cell increases significantly. However, this increase follows a profile that mirrors changes in the average basal cell division rate.

We also consider the TPA condition, which is equivalent to homeostasis but with the cell division sped up by a factor of ~ 5 , we fix the delamination rate to the same value as homeostasis. In this case, the T1 transition rate also is sped up by a factor of 5, but we do not observe as substantive fragmentation as in the ablation conditions, increasing the elevated divisions alone is not sufficient for the increased fluid-like behaviour during regeneration.

This effect is generic during homeostasis. If the tissue turnover rate is accelerated by some factor while remaining homeostatic and there is an infinite time-scale separation between mechanical relaxation and division ($\tau_{\text{mech}} \rightarrow \infty$), then the T1 rate will increase by the same factor, without accelerating fragmentation for clones of the same size. However, in the model as time-scale separation is finite, there is a slight increase in the level of clonal fragmentation for an increased division rate with other parameters being held fixed.

The significant observed clonal fragmentation during regeneration occurs because as cells become larger in area during regeneration than in homeostasis, the presence of cell rearrangements (via non-proliferative T1 transitions) take cells physically further apart than at their homeostatic density. As the density recovers, cell divisions from intercalating neighbouring clones can allow clonal fragments to become separated. We note that clonal fragmentation can also be increased by either by increasing p_0 into the fluid-like regime, or by adding substantive levels of cell death or cell motility (leading to active cell rearrangements). Additionally, when there is no significant time scale separation between τ and τ_{mech} , reducing τ/τ_{mech} increases the average rate of T1 transitions per division, increasing levels of clonal fragmentation even at homeostasis. Given this, for plotting of clonal snapshots (Figure 3F) we use $\tau_h/\tau_{\text{mech}} = 25$. Whereas $\tau_h/\tau_{\text{mech}} = 5$ was used above to

speed up simulation and hence fitting time, but results in a greater extent of clonal fragmentation than is seen experimentally.

3.2.5 Delay between drop of basal cell density and increased proliferation rate

Based on the fit of the model, we noted that the temporal profile of the cell division rate could be improved to more closely align with the observed experimental profile by adding a time delay of 2.0 days (Figure 3C). This occurs because in the ablation experiment there is a delay between drop in basal cell density and increase in the proliferation rate, so in reality the simple switch based on cell area is a simplification. We could incorporate this effect directly in our models by using different critical cell areas for switching elevated division rate on (when $A > A_1$) or off (when $A > A_2$, for $A_2 < A_1$). However, this would add further free parameters to the modelling framework, so to keep the framework minimal was not included here.

3.3 Implication for models with proliferative heterogeneity

As noted above, in the basal layer, the clonal dynamics of the $S+CP$ model can mirror that of the $S+D$ model, for suitable adjustment of the delamination rate. In this sense, the $S+CP$ model is also compatible with the ablation data. For the model variants of the $S+D$ model and the $S+CP$ model we considered, the present ablation experiment could not provide a discernible advantage of one model over the other. It is possible to introduce other approximations concerning how the D or CP cell adjust their fate decision rates in response to cell ablation. Although this could lead to slight improvements in the quality of fits to clone-size distributions, it would do so at the expense of bring additional parameters that would be difficult to constrain by the clonal data, i.e., there would be parameter combinations in which the quality of the fits remained equally strong.

3.4 Summary of findings from modelling ablation experiment

From the superiority of the $S+D$ model (or the equivalent model with a committed progenitor cell compartment) over the S model, we can conclude that a heterogeneous response of basal cells is required to reproduce the observed recovery from basal cell ablation. The differentiated cell (or CP cell compartment) acts as a buffer to the basal cell population in response to significant cell death and during recovery. While the data used to fit the model is consistent with proliferative heterogeneity in the basal layer, it cannot be used to reliably infer changes in the proliferation kinetics of the putative CP cell compartment during ablation. Additionally, we can conclude that a local density dependent feedback is sufficient to capture the observed recovery to homeostasis.

All the models explored predict significant clonal fragmentation and intercalation during recovery from ablation, which was confirmed in live-imaging experiments.