
Supplementary information

Sexual dimorphism in epidermal stem cell dynamics

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Supplementary Note

In the following, we provide further details on the statistical modelling-based approach used to investigate the long-term clone dynamics of the mouse ear skin IFE. Aspects of the approach have been discussed in prior studies by our group and others [1–6], including a variant of the spatial model that forms the foundation of the current analysis [7]. Here, we provide essential details of the model and the fits to the experimental data, referring to the prior literature for details of the many competitive models used to address stem cell dynamics in squamous epithelia.

Background

To set the current analysis in context, it is helpful to summarise briefly the nature of previous approaches to address stem cell identity and fate in the squamous epithelia of the mouse skin IFE and oesophagus. Here, to model clonal dynamics, the majority of studies have placed emphasis on non-spatial – “zero-dimensional” – models of cell fate (for a review see, e.g., Refs. [8, 9]). Here, the proliferative kinetics and fate behaviour of stem cell populations and their progenies are represented by stochastic models based on fixed rate constants and cell fate probabilities. In the context of lineage tracing studies, such models can provide a good quantitative description of the long-term clonal dynamics. This success is due in part to the nature of clonal evolution in a homeostatic context: under conditions of balanced stochastic stem cell loss and replacement, the distribution of clone sizes is found to converge rapidly towards hallmark statistical scaling behaviours that depend only on the dimensionality of tissue (tubular, planar or volumnar), but are otherwise insensitive to details of the underlying cellular hierarchy [8]. In particular, in the context of planar tissues such as the skin IFE, the distributions take an exponential-like form in the long-term.

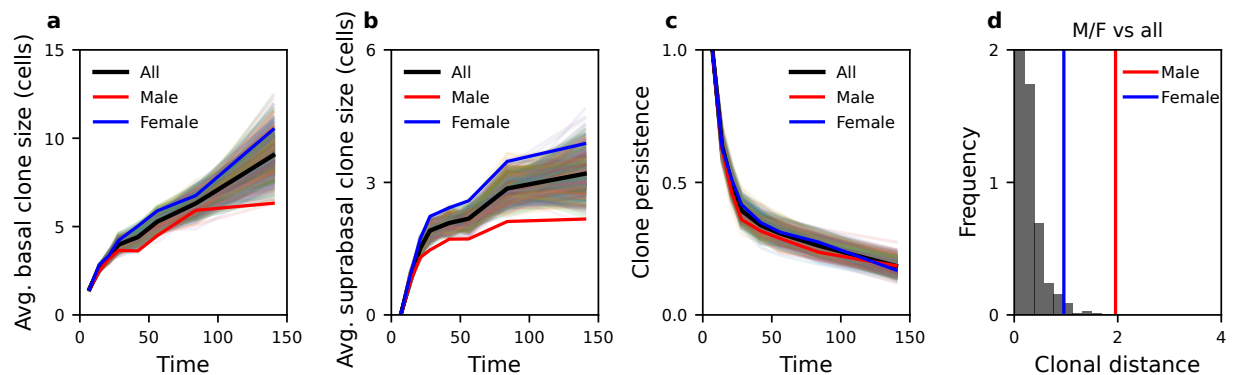
Here, taking advantage of the time-lapse nature of the clonal data, our aim is to go beyond these heuristic models of stem cell fate and determine details of the underlying proliferative heterogeneity and fate behaviour of the cells that comprise the mouse ear skin IFE. To do so, it is necessary to focus on features that lie beyond the statistical scaling regime, emphasizing the transient dynamics of clonal evolution and the robust even clone size correlations that are found to persist over long timescales in female mice (see main text). To capture these features, it is necessary to develop models that can accommodate potential spatial dependencies in the regulation of cell fate, and a more detailed description of temporal correlations of events.

To model the dynamics of the basal layer of the IFE, we therefore made use of a variant of the spatial vertex model, known as a Voronoi model. Such models have been used extensively to study the phase behaviour of confluent epithelial monolayers [10–13]. In this framework, cells are represented as polygons that tile two-dimensional space. In vertex models, the vertices of cells constitute the independent degrees of freedom, while in Voronoi models the locations of cell vertices and hence their polygonal shapes and sizes are determined as a Voronoi tessellation based on the coordinates of cell centres [14]. Here, to model the basal cell layer, we develop a Voronoi model similar to that introduced in Sarate et al. to study the regeneration of the tail skin IFE following genetic cell ablation [7]. In the following, we outline the basis of the spatial model and its adaptation to the current system. Later, we

will discuss the strengths and limitations of the spatial model, and when it can be safely substituted for a simpler zero-dimensional model. However, before turning to the modelling-based approach, we first apply statistical significance tests to the data, finding that male and female animals show distinct patterns of growth.

Statistical analysis of mouse-to-mouse variability

A crucial advantage of the current time-lapse imaging approach over static clonal tracing methods is that, here, we retain temporal information on individual cell lineages. However, by focussing on the dynamics of the same set of clones, the clonal data may exhibit a higher level of mouse-to-mouse variability for a given sample size than static tracing data, where each time point represents a distinct statistical ensemble. Before turning to the quantitative modelling of the long-term clonal data, it is important to understand whether variability by mouse or sex may indicate true biological variability, or arises merely as an artifact from the limited sampling of a stochastic process. We therefore assessed the extent of variability from sampling clonal trajectories by introducing a statistical test, with our results indicating that male and female mice have statistically distinct dynamics.



Supplementary Fig. 1: **Bootstrap test indicates a statistically significant difference in clonal trajectories between male and female mice.** Trajectories from pooled male and female mice (1,000 trajectories shown - faint lines) were randomly sampled and the average basal and suprabasal clone size compared to data by sex. a-c) Average basal (a), suprabasal (b) clone size and persistence (c) for male (M), female (F) and all (All) clones with individual clone trajectories from random sampling shown in grey. Specifically, we assessed the “clonal distance” (d) between pooled male and female mice with mice for each individual sex based on basal and suprabasal clone sizes (see Supplementary text).

Testing whether clonal trajectories differ by sex

Formally, we questioned whether differences in the time-dependence of the average basal and suprabasal clone size between the sexes could be accounted for by small number fluctuations. We therefore used the data to randomly sample clonal trajectories using experimental sample sizes (718 clonal trajectories for females and 418 clonal trajectories for males) from the pool of all mice (Supplementary Fig. 1), weighting samples from male and female animals to

avoid potential bias from the majority female cohort. To assess the dispersion between clonal samples from the pooled data, we defined a distance metric based on the average basal and suprabasal clone size between a sampled ensemble of trajectories (indexed i) and the combined dataset (all mice combined, with equal weighting of males and females),

$$D_{\text{clonal}}^{(i)} = \sum_t \frac{(\langle n_{\text{bas}}^{(i)}(t) \rangle - \langle n_{\text{bas}}^{(\text{all})}(t) \rangle)^2}{\sigma_{\text{bas}}^{(\text{all})}(t)} + \frac{(\langle n_{\text{sup}}^{(i)}(t) \rangle - \langle n_{\text{sup}}^{(\text{all})}(t) \rangle)^2}{\sigma_{\text{sup}}^{(\text{all})}(t)}.$$

Here, $\langle n_{\text{bas}}(t) \rangle$ and $\langle n_{\text{sup}}(t) \rangle$ denote, respectively, the average basal and suprabasal clone size at timepoint t , and $\sigma_{\text{bas}}^{(\text{all})}(t)$ and $\sigma_{\text{sup}}^{(\text{all})}(t)$ are the standard deviations of basal and suprabasal clone sizes. To assess statistical significance, we computed this metric for $N = 100,000$ randomly sampled clone trajectories ($D_{\text{clonal}}^{(i)}$), as well as the experimentally obtained samples for each sex $D_{\text{clonal}}^{(M/F)}$. This allows us to obtain a p-value,

$$p_{M/F} = \frac{1}{N} \sum_i (D_{\text{clonal}}^{(i)} \geq D_{\text{clonal}}^{(M/F)}).$$

Based on this analysis, we found that the clonal dynamics of both male ($p = 0.0014$) and female mice ($p = 0.0012$) cannot be accounted for by the dynamics of the pooled population, rejecting the null hypothesis (to a statistical significance level of 0.01) that the average clone dynamics of male and female animals are consistent with the combined data set.

Male and female mice differ in fine-features of the clonal distributions

As noted in the main text, differences between male and female animals were not simply restricted to average basal and suprabasal clone sizes (Fig. 1e). Indeed, clones showed striking differences in the detailed size distributions, with more even-sized clones than odd observed in both male and female animals (Fig. 1k, Extended Data Fig. 2e). To quantify the scale and statistical significance of this enhancement, we took advantage of the long-term scaling nature of the clone dynamics, which showed convergence of the size distribution towards an exponential-like dependence [1, 8]. We therefore defined the degree of even bias as the relative proportion of even clone sizes compared to that predicted from an exponential distribution with the same average clone size,

$$\Delta_{\text{even}} = \frac{F_e(t) - F_g(t)}{1 - F_g(t)}.$$

Here, $F_e(t)$ denotes the fraction of even sized clones at timepoint t and $F_g(t) = (\langle n(t) \rangle - 1) / (2\langle n(t) \rangle - 1)$ is the fraction of even clones expected for a geometric (i.e., a discrete exponential) distribution with the same mean $\langle n(t) \rangle$ as given by the data. When there is no statistical bias towards even sizes, $\Delta_{\text{even}} = 0$, whereas a bias towards even sizes relative to the exponential distribution is reflected by a positive value of Δ_{even} . The normalisation constant is chosen such that if all clones had even sizes, $\Delta_{\text{even}} = 1$. To formally assess whether the measured enhancements of even sizes are statistically significant, we used a one-sided one-sample proportion test, with results shown in Supplementary Note Table 1.

	Male basal	Female basal	Male supra.	Female supra.
D7	2.1×10^{-20}	9.4×10^{-29}	1	1
D14	1.4×10^{-20}	2.1×10^{-70}	1	1
D21	1.2×10^{-7}	9.0×10^{-59}	1	1
D28	6.7×10^{-12}	2.9×10^{-47}	1	5.4×10^{-28}
D42	8.8×10^{-3}	9.3×10^{-39}	1	3.8×10^{-21}
D56	9.1×10^{-6}	2.6×10^{-37}	1	9.1×10^{-24}
D84	5.1×10^{-3}	2.8×10^{-35}	0.58	1.1×10^{-25}
D140	5.7×10^{-2}	4.8×10^{-10}	0.15	9.4×10^{-4}

Supplementary Note Table 1: **p-values for testing enhancement of even clone sizes for male and female mice for basal and suprabasal clone sizes.** Hypothesis testing uses a one-sided one-sample proportion test to determine whether the fraction of even clone sizes is greater than that expected for an exponential distribution of the same mean as the data. For details, see Supplementary text.

Together, these findings show that female mice maintain a strong degree of bias towards even clone sizes across time in both the basal ($p < 10^{-9}$ across time) and suprabasal compartment ($p < 10^{-3}$ from D28) (Fig. 1h). Males also show a statistical bias, but the overall scale is weaker with significance reached only in the basal and not the suprabasal layer.

Potential mouse-to-mouse variability within the male and female population

For completeness, we also considered the potential for variability between individual mice (Supplementary Fig. 5a-f). We found that, at most time-points, individual mice fell within the 95% confidence intervals of mice pooled by sex, with a few exceptions of transient excursions. Individual mice of the same sex followed a common statistical behaviour in terms of the scaling of clone size distributions (Supplementary Fig. 5g-j). Transient differences in average clone sizes could be associated with transient wounding events or other effects associated with local environmental changes. At the same time, differences in clone persistence at early times (Supplementary Fig. 5c,f) may result from the differential labelling of basal cell populations under the K14 promoter. To avoid potential biasing of the data, we chose to accommodate this random variability by combining data across all mice of each given sex.

Based on this analysis, we conclude that male and female mice have statistically distinct clonal dynamics. Female mice have a strong enhancement of even clone sizes, whereas male mice have only a weak – but statistically significant – enhancement. The observation of these robust fine-grained features provides the opportunity to challenge existing modelling paradigms, and test for sex-specific differences in the dynamics of cell populations, which we address through a spatial model below.

Spatial Voronoi model of the basal layer of the skin IFE

We now turn to a spatial Voronoi model in order to capture the clonal dynamics. Within the Voronoi model, the arrangement of cells i are defined by the spatial coordinates $\mathbf{r}_i$ of their cell centres. To accommodate the effects of cell incompressibility, cortical tension, cell-cell

adhesion and cell contractility, the mechanical interactions between cells are described by the energy functional [14],

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

where A_i and P_i denote, respectively, the area and perimeter of cell i , A_0 and P_0 represent the target cell area and perimeter, the parameters k_A and k_P represent the area and perimeter elastic moduli, and the sum runs over all cells in the basal layer. The first term corresponds to volume incompressibility (though cell height can fluctuate). If we expand the second perimeter term, the quadratic perimeter term ($k_P P_i^2$) corresponds to the active contractility of the cell cortex, and the linear perimeter term ($-2k_P P_i P_0$) captures the balance between cortical tension (reducing perimeter) and cell-cell adhesion (cells sticking together try to increase the length of interface). Here, for simplicity, we set the moduli equal $k_A = k_P$ noting that small differences between these parameters do not impact significantly on the model dynamics. The position of cells then evolves to minimise the mechanical energy,

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

where μ is a drag constant consistent with the viscous motion of cells at low Reynolds number. In contrast to a cell monolayer, the basal layer is not isolated, but is confined between the basement membrane and the suprabasal cell layers. Here, for simplicity, we do not model the suprabasal cell layers explicitly but will assume that, in the regime of interest, their presence will not change qualitatively the effective nature of the forces that act between cells, as implied by the energy functional. Importantly, our focus here is not on quantifying the mechanical properties of the basal layer, but in developing a phenomenology that can capture the clonal dynamics over timescales comparable to the cell division times and longer.

Previously, in the context of non-dividing cell populations, it has been shown that, when subject to small fluctuations, the vertex [15] and Voronoi [14] models have a rigidity transition at a critical value of the shape index, $p_0 \equiv P_0/\sqrt{A_0}$. When $p_0 < p_0^c \approx 3.81$, the tissue is positioned in a solid-like phase, where neighbour exchange through T1-type transitions of cells becomes strongly inhibited. For $p_0 > p_0^c$ the tissue transitions into a fluid-like phase, where fluctuations drive T1 transitions leading to cell diffusion.

To adapt the Voronoi model to study cell turnover, several refinements are necessary to accommodate different cell types as well as the process of cell delamination and division. To model delamination, a cell is simply removed from the basal layer, leading to an instantaneous expansion of neighbouring cells (as defined by the Voronoi tessellation) followed by their progressive remodelling and relaxation to accommodate the loss. Although the process of cell delamination in tissue is not abrupt, as long as the timescale for a cell to exit the basal layer once it has started its delamination process (typically less than a day) is much faster than the typical cell division time (around a week), this approximation would not impact significantly on the clonal dynamics.

Similarly, to model the cell division process, a new cell is added to the population, positioned at a short distance (formally $0.01A_0$) from the dividing mother cell with a random orientation within the layer. Such events will again lead to an instantaneous repositioning

of cell boundaries followed by a progressive remodelling of the surrounding tissue to accommodate the additional cell. Although such a model represents a simplification of the mechanical rearrangements that occur during cell division, if the mechanical relaxation time $\tau_{\text{mech}} = (\mu k_A A_0)^{-1}$ is faster than the average cell division time, such an approximation is also not expected to impact significantly on the clonal dynamics. Note that, in the context of cell turnover for a solid-like tissue, cell rearrangements are driven predominantly by cell division and delamination, obviating the need to impose additional dynamical fluctuations on cell positions.

One-proliferative compartment *SD*-model of epidermal maintenance

To adapt the Voronoi model to the renewing IFE, we first considered its implementation in the framework of a one-proliferative compartment model of epidermal maintenance. In early seminal work, Marques-Pereira and LeBlond proposed a simple model of epithelial maintenance in which the basal cell layer of the – in their case, rat oesophageal – epithelium comprised a single proliferative cell population [16]. In their model, stochastic cell loss through delamination and differentiation is compensated by cell duplication in the basal layer, leading to clonal dynamics of a Voter model type. Later, Clayton et al. proposed a refinement of this model in which differentiated basal cells have a non-zero residency time in the basal layer before delamination, opening the possibility for basal cell divisions that lead to symmetric or asymmetric fate outcomes [1]. In the following, we will adapt the model of Clayton et al. to the spatial Voronoi construction, noting that the model of Marques-Pereira and LeBlond represents a limiting case in which the residency time of differentiated basal cells vanishes.

To this end, we therefore considered a model in which the basal layer is comprised of two states, a progenitor state denoted *SC* to acknowledge its stem cell-like self-renewal potential and a terminally differentiated state denoted *D* (Extended Data Fig. 3a). Here, for simplicity, we assume that both cell states have the same mechanical properties, noting that small differences would not impact significantly on the resulting clone dynamics. To ensure homeostasis, differentiated cells lost from the basal layer through delamination at rate Γ are replenished by cell division at rate λ . Following Clayton et al., stem cell division leads to stochastic – probabilistic – fate outcome, with daughter cells adopting one of three possible fates: cell duplication, asymmetric division and terminal division. Within this framework, cells then follow the stochastic process

$$SC \xrightarrow{\lambda} \begin{cases} SC + SC & \text{Pr. } r p(A_i) \\ SC + D & \text{Pr. } 1 - r \\ D + D & \text{Pr. } r (1 - p(A_i)) \end{cases}, \quad D \xrightarrow{\Gamma} D^*,$$

where D^* denotes a suprabasal cell. To ensure stem cell density homeostasis, the frequency of stem cell duplication must precisely balance the frequency of differentiation. Here, we propose a model in which fate balance is achieved through density-dependent feedback, placing the frequencies of symmetric divisions under a local area-based rule. Setting the probability

$$p(A_i) = h(A_i/A_0)$$

with $h(x) \equiv x^n/(1+x^n)$ denoting a Hill-like function, when the local density is high, the area of the cell is small $A_i < A_0$, and stem cell fate is tilted towards terminal division. Conversely, when the density is low, $A_i > A_0$, and fate is biased towards cell duplication. Later, we will comment on the viability of alternative models of fate balance based not on density but on chemical feedback through local contact or competition for limiting niche factors. Finally, suprabasal cells become enucleated and therefore lost at rate γ ,

$$D^* \xrightarrow{\gamma} \emptyset.$$

As defined, the one-proliferative compartment model would not give rise to enhanced and persistent even clone sizes. Such features imply temporal coordination both in the timing of cell division and delamination, and/or in the coordination of these events between sister cells. Previously, attempts to address cell fate from clonal data have largely focused on a minimal scheme in which the timing between consecutive cell division and delamination events is statistically uncorrelated (Markovian) and drawn at random from an exponential distribution. Although this choice overlooks the impact of refractory periods during cell division, or the potential maturation time of differentiated basal cells, such effects have minimal impact on the dynamics of clones over multiple rounds of division and delamination. However, at shorter timescales, such effects could contribute to the observed enhancement of even-odd correlations in the clone size distribution. Therefore, to accommodate the impact of such effects, cell division and delamination times were modelled using a Gamma distribution, similar to the framework considered by Piedrafita et al. [9]. Here, we parametrise the distribution of cell lifetimes of these processes by an average lifetime τ and coefficient of variation ($cv = 1/\sqrt{k}$),

$$f_{\Gamma}(t; k, \tau) = \frac{1}{\Gamma(k)} \left(\frac{k}{\tau}\right)^k t^{k-1} e^{-\frac{kt}{\tau}},$$

where shape parameter $k = 1$ ($cv = 1$) corresponds to an exponential distribution and $\Gamma(k) = \int_0^\infty t^{k-1} e^{-t} dt$ denotes the Gamma function. Note that, later, to initialise numerical simulations of the model dynamics, we will assume that the cell labelling time is chosen in an unbiased manner across phases of the cell cycle. Thus, the time of the first division event is proportional to the probability that longer cell cycle periods than t exist, which is determined by drawing numbers from the following probability distribution function,

$$P_0(t) = C(1 - \text{GamCDF}(t; k, \tau)),$$

where $\text{GamCDF}(t; k, \tau) = \int_0^t f_{\Gamma}(t'; k, \tau) dt'$ and C denotes a normalisation constant.

To accommodate the potential for statistical correlations in the timing of sister cell fates, we introduced independent parameters for symmetric stem cell divisions (c_{SS}), asymmetric divisions (c_{SD}), and symmetric differentiation (c_{DD}) events. These correlation parameters are defined such that sister cells A and B will have uncorrelated lifetimes if $c_{AB} = 0$ and maximally correlated lifetimes if $c_{AB} = 1$. Formally, $c_{AB} = \rho_{AB}/\rho_{AB}^{\max}$, where ρ_{AB} is the Pearson correlation coefficient and $\rho_{AB}^{\max}$ is the maximum possible Pearson correlation coefficient for sisters A and B . For symmetric divisions, sisters have the same shape parameter and average lifetime. In this case, correlation parameters will be equal to the Pearson correlation coefficients. Thus, for symmetric divisions, a correlation of $c_{AB} = 1$ corresponds to the full

synchronization of sister cell events. However, for asymmetric divisions, sisters could have different shape parameters ($k_A \neq k_B$, which is equivalent to $cv_A \neq cv_B$). In such cases, the Pearson correlation coefficient is bounded by $0 \leq \rho_{AB} \leq \min(\sqrt{k_A/k_B}, \sqrt{k_B/k_A})$, while the normalized correlation coefficient c_{AB} ranges from 0 to 1.

To implement correlations numerically, we exploited properties of Gamma distribution. If $X_1 \sim \text{Gamma}(k_1, \theta)$ and $X_2 \sim \text{Gamma}(k_2, \theta)$ are independent Gamma random variables with the same scale parameter θ , then their sum follows a Gamma distribution $X_1 + X_2 \sim \text{Gamma}(k_1 + k_2, \theta)$. For sister cells A and B with average lifetimes τ_A and τ_B , shape parameters k_A and k_B , and desired Pearson correlation coefficient ρ_{AB} , we may generate three independent Gamma distributed random variables with scale parameter of unity: $X_0 \sim \text{Gamma}(\rho_{AB}\sqrt{k_A k_B}, 1)$, $X_1 \sim \text{Gamma}(k_A - \rho_{AB}\sqrt{k_A k_B}, 1)$, and $X_2 \sim \text{Gamma}(k_B - \rho_{AB}\sqrt{k_A k_B}, 1)$. Cell lifetimes can then be constructed as: $T_A = (X_0 + X_1)\tau_A/k_A$ and $T_B = (X_0 + X_2)\tau_B/k_B$. This construction ensures that $T_A \sim \text{Gamma}(k_A, \tau_A/k_A)$ with mean τ_A and coefficient of variation $1/\sqrt{k_A}$, and $T_B \sim \text{Gamma}(k_B, \tau_B/k_B)$ with mean τ_B and coefficient of variation $1/\sqrt{k_B}$. The resulting Pearson correlation coefficient between T_A and T_B is ρ_{AB} . To implement the normalized correlation coefficient, we therefore set $\rho_{AB} = c_{AB} \times \min(\sqrt{k_A/k_B}, \sqrt{k_B/k_A})$, which ensures that $c_{AB} = 1$ corresponds to the maximum possible correlation.

This completes the definition of the modelling framework for the one-proliferative compartment *SD*-model. In the limit $n \rightarrow 0$, the spatial model recapitulates the clonal dynamics of the zero-dimensional model introduced by Clayton et al. [1]: Here, cells mechanically relax following cell division or delamination to reduce spatial inhomogeneities, but cell fate decisions are not informed by the local environment. For $n > 0$, basal cell fate becomes locally coordinated, with the chance increase in local cell density due to cell division or decrease due to cell delaminations biasing stem cell fate so that local density homeostasis is maintained. To mimic the observed dynamics of the basal layer of the ear skin IFE, in the following we set the shape index $p_0 = 3.0$, which positions the system in a solid-like phase. Here, cell rearrangements are driven predominantly through loss and replacement events and not through T1 transitions, leading to clones that remain cohesive, consistent with experimental observations. Finally, noting the approximate uniformity of the basal cell density, we will assume that the mechanical relaxation rate $1/\tau_{\text{mech}}$ is much faster than the cell division rate, setting the ratio $\lambda\tau_{\text{mech}} = 1/10$. The Hill function exponent is then effectively left as an adjustable parameter. In the limit $n \rightarrow \infty$, the model converges towards an off-lattice Voter-type dynamics, where cell loss is compensated by the division of neighbouring cells.

Parameter constraints based on mean-field parameters

As defined, the *SD*-model contains a range of adjustable parameters. However, we can use average – “mean-field” – properties of the clonal data to constrain several key parameters. To do so, it is helpful to first segregate clonal lineages according to whether they contain a single cell at most over the time course. Such clonal lineages – termed “D-like” – represent clones induced predominantly in the terminally differentiated cell population. (Note that, based on the time-lapse nature of the data, such lineages may contain a small number of stem cell-derived clones, where divisions, delamination and shedding events are missed within the first week when the first timepoint is measured.) Similarly, lineages that expand

beyond a single cell over the timecourse – termed “SC-like” – must belong to clones induced in the stem cell compartment. For SC-like clones, measurements of the labelled basal cell density – defined as the average number of basal cells per clone, including clones of zero size (i.e., extinct) – show a rapid convergence over time towards a plateau (Fig. 1g,j), consistent with homeostatic turnover. From the magnitude of this plateau, $b_{\infty}^{(\text{SC})}$, we thus obtained an estimate for the **fraction of cells in the basal layer that belong to the stem cell compartment**,

$$f_{\text{SC}} = \frac{\Gamma}{\lambda + \Gamma} = \frac{1}{b_{\infty}^{(\text{SC})}} \equiv 1 - f_{\text{D}}.$$

Similarly, from measurements of the corresponding labelled suprabasal cell density (Extended Data Fig. 3d,h), we obtained an estimate of the **ratio of cells in the suprabasal layer n_D^* to the number of cells in the basal cell layer n_{bas}** in terms of the plateaus of basal ($b_{\infty}^{(\text{SC})}$) and suprabasal ($s_{\infty}^{(\text{SC})}$) labelled cell density for SC-like clones,

$$\frac{n_D^*}{n_{\text{bas}}} = \frac{s_{\infty}^{(\text{SC})}}{b_{\infty}^{(\text{SC})}}$$

Applied to the experimental data, for male mice we obtained $f_{\text{SC}} = 0.58 \pm 0.03$, and $n_D^*/n_{\text{bas}} = 0.40 \pm 0.02$, and for female mice $f_{\text{SC}} = 0.40 \pm 0.01$, and $n_D^*/n_{\text{bas}} = 0.49 \pm 0.01$. Note that, here, we have used a weighted linear least-squares fit with zero-gradient (fitting to the y-intercept only) to the late time-points (D28 and after), weighting by the inverse variance at a given time-point.

Similarly, we could use the early-time clonal growth characteristics for SC-like clones to estimate the turnover rate. At homeostasis, the average total clone size will grow linearly at early times. This linear growth characteristic will turn over towards a plateau as cells become lost through first enucleation and then shedding. Using a straight line interpolation of the average total clone size between D2 (noting that, based on the D7 and D14 clone growth data, induction under the K14 promoter takes around 2 days to label cells) and D7, we obtained an estimate of the **cell division rate of $\lambda = 0.13 \pm 0.02 \text{ d}^{-1}$ for both male and female mice**. This is equivalent to an average stem cell division time of $\tau = 1/\lambda = 7.7 \pm 2.4 \text{ d}$. Given the large uncertainty, for fitting simulations we chose to use the division rate as an adjustable fit parameter.

Finally, we could use the values obtained above to estimate the **average rate of cell loss** – defined in the current experiment as the rate at which suprabasal cells become enucleated. Noting that at steady-state the net basal cell differentiation rate must match the loss rate from the suprabasal layer, we have

$$\gamma = \lambda \frac{n_{\text{SC}}}{n_{\text{D}^*}} = \lambda f_{\text{SC}} \frac{n_{\text{b}}}{n_{\text{sb}}}.$$

From this result, it follows that the shedding time in the *SD*-model is given by $1/\gamma = 5.6 \pm 1.9$ days for male mice and 9.4 ± 3.2 days for female mice, noting that the high uncertainty arises from the uncertainty in the cell division rate. The lower value of the shedding rate in female animals (when the division rates are similar between sexes) reflects significant differences in the size of the suprabasal cell compartment between sexes.

Numerical implementation of Voronoi model with turnover

Simulations were implemented in python, with the code to reproduce the modelling and analysis provided on Zenodo (<https://doi.org/10.5281/zenodo.21997810>). As in our prior work [7], Voronoi model simulations used periodic boundary conditions, and were performed using an Euler method (time-steps of size $\Delta t = 0.01$ (with a timescale of 1 corresponding to the non-dimensionalised mechanical relaxation time). Cell fate decisions occur on the same discretised time-points.

Spatial simulations used a 32×32 domain (approximately 1024 cells) unless otherwise stated. To initialise simulations simulation, cell centres placed at random initial positions on $[0, L) \times [0, L)$, and cell life-times were generated according to the distributions described below. The proportion of cells of each type is initialised to match the homeostatic composition at the mean-field level (outlined above). The suprabasal layer was initialised with zero cells, since clones originating in the suprabasal layer are not tracked.

To equilibrate the simulation, simulations were ran for 2500 time-steps in the absence of division/differentiation/loss to mechanically relax cells, followed by 5000 timesteps with full reaction rules to ensure the correct tissue composition and lifetime correlations between sisters.

To simulate the tissue, we follow the scheme defined in our prior work [7] (adapted here for completeness),

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 Voronoi model) as outlined in Bi et al [14].
4. Update position based on mechanical forces using the Euler method.
5. Implement cell decisions (divide, differentiate, or delaminate) for times t and $t + \Delta t$.
 - (a) 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 cells were added, their lifetimes from stochastic decisions are generated by drawing from a Gamma distribution with the shape parameter and sister correlations outlined above.
 - (b) If cells delaminate, the cell is removed and the Voronoi tessalation is re-computed.
 - (c) When cells delaminate, the lifetime of the daughter suprabasal differentiated cells are generated from a Gamma distribution with lifetime $1/\gamma$ and coefficient of variation parameter (cv_γ).

For parameter sets in the zero-dimensional limit (no mechanical feedback on cell fate decisions: $n = 0$), simulations used a discrete-event scheme instead of fixed time-stepping: the time of each cell's future fate decision was drawn at birth from the specified lifetime

distribution and stored in a priority queue. Simulation time was advanced directly to the earliest scheduled time in the queue. All cells scheduled to decide at that time were resolved as a batch, before that time was removed from the queue and the simulation advanced to the next scheduled event.

Fits of the *SD*-model to the time-lapse clonal data

We now turn to the fitting of the long-term clonal dataset with the *SD*-model. Since the average long-term clonal dynamics converge to statistical scaling behaviour (Supplementary Fig. 5g-j), the long-term average clonal growth characteristics will be governed by a single scaling parameter $r\lambda/f_{\text{SC}}$ [1]. Thus, the cell division rate λ can be obtained by temporally re-scaling the simulated clonal data (using the average basal clone size) to best match the experimental clonal data. Further, using the mean-field arguments above, one may fix the proportion of stem cells f_{SC} and the ratio of basal to suprabasal cells (which provides an estimate of the shed rate γ , given λ). The presence of spatial coordination ($n > 0$) or not ($n = 0$) will determine whether the average clone growth characteristic follows a critical birth-death process (with the average surviving basal clone size $\langle n_b \rangle(t)$ growing linearly with time t) or follows a Voter model-type dynamics (with the average basal clone size growing as $\langle n_b \rangle(t) \sim t/\ln t$). Since, empirically, the dynamics were found to be rather insensitive to the specific values of n , we choose to consider specific values for the fitting of n , considering only the cases $n = 0, 2, 8$ (translating to no, weak and strong spatial coordination of stem cell fate). Finally, simulations of the suprabasal layer dynamics could be achieved straightforwardly and efficiently, since cell shedding doesn't influence the basal dynamics in the model. Thus, to optimise the fits to the suprabasal distributions, we simply varied cv_Γ as a post-processing step without re-running simulations of basal cell dynamics.

Sex	r	$1/\lambda$ (d)	cv_λ	cv_Γ	cv_γ	c_{SS}	c_{SD}	c_{DD}	n
M	$0.35^{+0.05}_{-0.1}$	$5.8^{+0.1}_{-1.3}$	$0.45^{+0.3}_{-0.05}$	$0.55^{+0.25}_{-0.4}$	$0.45^{+0.15}_{-0.1}$	0	1	0	8
F	$0.3^{+0.15}_{-0.1}$	$5.6^{+1.1}_{-0.1}$	$0.45^{+0.15}_{-0.1}$	$0.55^{+0.25}_{-0.4}$	$0.1^{+0.45}_{-0.1}$	$\frac{3}{4}$	1	$\frac{3}{4}$	8

Supplementary Note Table 2: **Fits of the one-proliferative compartment *SD*-model to the clonal data.** Best fit parameters obtained from fitting the full clonal data (minimising joint clone size distribution for SC-like and D-like clones). For each parameter, best fits were obtained using simulated annealing with uncertainties (95% confidence intervals) estimated using bootstrapping of the experimental data based on a grid search (1,000 bootstrap samples). To make the uncertainty estimation procedure computationally feasible, we used a reduced parameter space, where temporal coordination parameters were fixed to a coarse grid with $c_{SS} = c_{DD}$, and c_{SS}, c_{SD} varying over $(0, \frac{3}{4}, 1)$ (moving between no and strong temporal correlation of sister cell fate). Although we don't have accurate uncertainty estimates for these parameters, variations showed that the best fit parameters were relatively insensitive to these values so long as the correlations were positioned in this regime.

To fit the model to the data, we thus optimised over the remaining 6-dimensional space of parameters $\theta_{\text{fit}} = (r, \text{cv}_\lambda, \text{cv}_\Gamma, c_{SS}, c_{SD}, c_{DD})$ by using simulated annealing to minimise the sum of the log-likelihoods of the joint clone size distribution $\mathcal{L}_{SD} = \mathcal{L}_{\text{SC}} + \mathcal{L}_{\text{D}}$ for SC-like

($\mathcal{L}_{\text{SC}}$) and D-like ($\mathcal{L}_{\text{D}}$) clones, where

$$\begin{aligned}\mathcal{L}_{\text{SC/D}} &= \log P(D_{\text{SC/D}} | \theta_{\text{fit}}, \theta_{\text{fix}}, \theta_{\text{post}}) \\ &= C + \sum_{t,b,s} N^{\text{SC/D}} P_{b,s}^{\text{SC/D}}(t) \log(p(b_t, s_t | \theta_{\text{fit}}, \theta_{\text{fix}}, \theta_{\text{post}})).\end{aligned}$$

Here, $P_{b,s}^{\text{SC/D}}(t)$ represents the experimental probability density for basal and suprabasal clone sizes for a given clone type (S,D) at time t post-induction. The constants C and $N^{\text{SC/D}}$ (denoting the experimental number of SC-like/D-like clones, respectively) are included in the definitions for completeness, but their values do not influence the numerical optimisation procedure. $p(b_t, s_t | \theta_{\text{fit}}, \theta_{\text{fix}}, \theta_{\text{post}})$ represents the joint clone size distribution obtained from model simulations at time t for variable model parameters θ_{fit} , fixed modelling parameters $\theta_{\text{fix}} = (f_{\text{SC}}, n, \gamma)$ and post-run optimisation parameters $\theta_{\text{post}} = (\lambda, cv_\gamma)$. Estimates of optimal parameters and uncertainty estimates obtained using bootstrapping over a grid are provided in Supplementary Note Table 2.

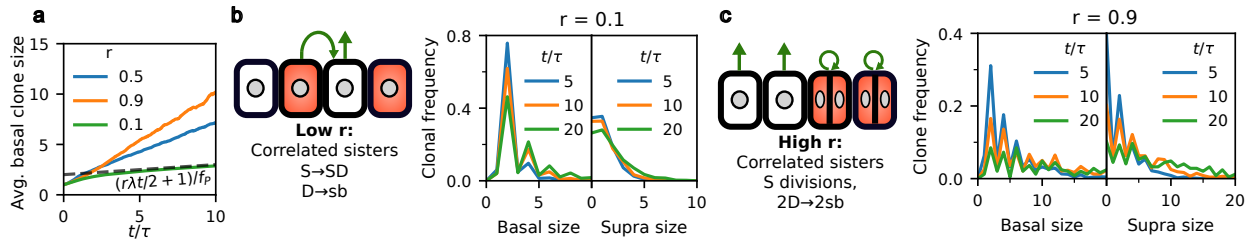
While the *SD*-model could recapitulate the time-dependence of the average clone sizes, labelled cell densities and clone persistence for male and female mice (Extended Data Fig. 3b-i), it was completely unable to capture the robust even-odd features in the static and temporal clonal dynamics (Extended Data Fig. 3j-l). In contrast to female mice, the *SD*-model provided a satisfactory fit to the clone size distributions for male animals across the entire timecourse. We therefore turned to consider temporal features of the data as a more refined test of the viability of the model. In this case, we found that for both male and female mice, we could not account simultaneously for the dynamics of SC-like and D-like clones in terms of clone size distributions, clone persistence and labelled cell densities (Extended Data Fig. 3b-i). In particular, for the male mice, to accurately capture the dynamics of SC-like clones, the clonal persistence decayed too rapidly compared to that observed in experiment.

We further challenged the model using temporal features of the data by plotting the clone transition probabilities combined across time-points (Extended Data Fig. 3l). The transition data for female mice, displays a checkerboard pattern reflecting clone sizes growing and shrinking in multiples of ± 2 basal and suprabasal cells across time-points, and biased to moving to even clone sizes from odd sizes. However, the *SD*-model was unable to capture the checkerboard pattern for female mice. Additionally, for male mice, the model prediction failed to match the most dominant observed transitions.

Based on these fits, we then questioned the origin of the discrepancies between model predictions and the data. In accordance with previous clone fate studies [1, 2, 9], the average basal clone size had a characteristic profile, showing a rapid linear-like growth reflecting the average cell division rate, followed by a slower linear-like growth in the long-term reflecting the average stem cell loss/replacement rate. Together, these dependences imply a strong bias towards asymmetric fate outcome, which is reflected in the fitting values of $r \approx 0.3$ for both male and female mice. Indeed, with an even stronger bias towards asymmetric fate ($r \approx 0 - 0.2$), we showed theoretically that one can in principle maintain a bias to even *basal* clone sizes (Supplementary Fig. 2b) if sister stem cell divisions and sister cell delamination of differentiated cells are tightly synchronised ($\lambda \approx \Gamma$ and $c_{\text{SD}} = 1$). Yet, even with a best fit value of $c_{\text{SD}} = 1$, in the observed parameter regime of f_{SC} and r we could not obtain a strong bias to even *basal* clone sizes, as observed in the data. Further, with

mostly asymmetric divisions, we were unable to produce the robust enhancements of even suprabasal sizes visible in the long-term data in female mice (Extended Data Fig. 3k).

Based on a parameter sweep of model regimes, we found that to obtain an enhancement in even clone sizes in both the basal and suprabasal layer, there needed to be a preponderance of symmetric divisions (Supplementary Fig. 2c), with a tight coordination between sister cell divisions ($c_{SS} \sim 1$) and a high coordination between sister cell delaminations ($c_{DD} \sim 1$). In this regime, the model could not capture the observed profile of average clone size over time (Supplementary Fig. 2a). With this result in mind, we note that early time clonal data retains statistical correlations from recent divisions so can be used to provide distinct estimates of fate coordination parameters. When repeating fits to only the D7 and D14 clonal data, a strong bias towards symmetric fate outcome $r = 0.8^{+0.05}_{-0.05}$ was found for female mice (vs. $r = 0.45^{+0.05}_{-0.05}$ for males), which recapitulated enhancements in even sizes in the basal and suprabasal clone sizes, but was inconsistent with the value obtained from fitting the full time-course.



Supplementary Fig. 2: Generation of even-odd clone size correlations in the framework of an *SD*-model a, Typical time-dependence of the average basal clone sizes for an *SD*-model for varying degrees of bias towards symmetric stem cell fate outcome. When the bias towards asymmetric fate is high ($r < 0.5$), the growth characteristic shows a fast initial rise followed by slower growth. b, A strong bias towards asymmetric fate outcome combined with strong temporal correlations between the timing of stem cell division and cell delamination of sister SC-differentiated cell pairs creates even clone size enhancements in the basal, but not suprabasal, layer. c, A strong bias towards symmetric fate outcome combined with strong coordination between sister stem cell divisions and sister cell delaminations can also create robust even-odd features in the basal and suprabasal clone size data.

Taken together, these results show that while the one-proliferative compartment *SD*-model is successful at capturing various features of clone dynamics, it struggles to explain others. Specifically, while the model can capture the time-evolution of average clone sizes for both male and female mice, the model fails to capture the fine-features of the clone size distributions, especially for female mice. Moreover, the model struggles to capture simultaneously the short-term dynamics of SC-like and D-like clones. Finally, the predicted preponderance of symmetric cell divisions from fits to the early-time clone size data is incompatible with the long-term clonal dynamics, which predicts a strong degree of bias towards asymmetric stem cell fate. Given these limitations, we turned to consider a refined two-proliferative compartment model of IFE maintenance.

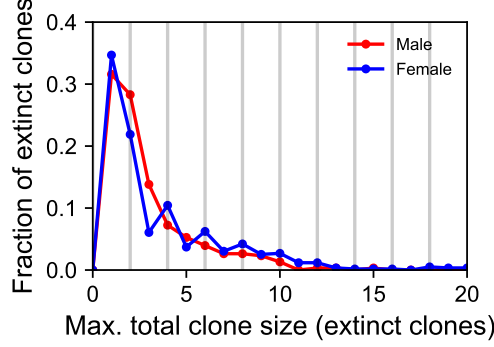
Two-proliferative compartment model of epidermal maintenance

To refine the spatial model, we placed emphasis on key features of the clonal data: First, to capture the observed long-term scaling behaviour of clone size observed here (Supplementary Fig. 5g-j) and in prior studies, the renewing stem cell population was required to undergo balanced stochastic loss and replacement. Moreover, to capture the time-dependence of the average basal clone size, showing a rapid increase followed by a slower linear-like growth characteristic, it follows that the renewing stem cell population must experience a strong bias towards asymmetric fate outcome. Based on the observed enrichment of even clone sizes in female animals, it therefore follows that for each asymmetric stem cell division, two differentiated cells must be produced on average - to maintain enhancements of even clone sizes. We therefore proposed that (a) stem cell differentiation is not direct, but occurs through an intermediate transit-amplifying (TA)-like state TA , and (b) that the timing between the division and delamination of sister cells must be temporally coordinated to maintain even clone size correlations. Moreover, to account for the enrichment of even clone sizes in the suprabasal layer of female mice (Extended Data Fig. 2e), we proposed that the majority of TA cell divisions are terminal with the delamination of sister cells temporally correlated. Based on these considerations, we therefore proposed the following minimal refinement of the SD -model,

$$\begin{aligned} SC &\xrightarrow{\lambda_S} \begin{cases} SC + SC & \text{Pr. } r p(A_i) \\ SC + TA & \text{Pr. } 1 - r \\ TA + TA & \text{Pr. } r(1 - p(A_i)) \end{cases} \\ TA &\xrightarrow{\lambda_T} \begin{cases} TA + D & \text{Pr. } 1 - q \\ D + D & \text{Pr. } q \end{cases} \end{aligned}$$

Here, consistent with the observed abundance of clones that achieve a maximum size of 3 cells (Supplementary Fig. 3), we considered the potential for both symmetric (terminal) and asymmetric divisions of the TA cell compartment.

While this STD -model engages a broad range of parameters, the time-lapse data provides multiple constraints that limit strongly the available phase space. To implement these constraints, it was useful once again to define the ensemble of D-like clones, as clones that contain a maximum of one cell over the timecourse; TA-like clones as ones that contain a maximum of 3 cells over the timecourse; and SC-like clones as ones that contain 4 or more cells over the timecourse. Here, for simplicity, TA cells were allowed to undergo only a single round of asymmetric division, which implies that all SC-like clones are stem cell-derived. To engineer the strong and persistent enhancement of even clone sizes observed in female mice, as well as enforcing the correlation $c_{ST} \simeq 1$, we set the cell division rates to be equal, $\lambda_T \simeq \lambda_S \equiv \lambda$. Consistent with this assumption, SC-like and TA-like clones showed a similar average size dependence at short times (Fig. 1j). Alongside correlations between sister stem and TA cells, we also accommodated further correlations in the timing of symmetric stem and TA cell divisions ($c_{SS} = c_{TT} \equiv c_{\text{sym div}}$, which we assume for simplicity to be equal), as well as in the delamination time of sister differentiated cells (c_{DD}).



Supplementary Fig. 3: **Distribution of maximum total clone size for clones that become lost (extinct) over the 140 day time course** for male (red) and female (blue) mice. Evidence of even-odd size correlations are present in female mice, suggestive of a bias towards stem cell divisions that change the number of differentiated cells by units of two. Such behaviour mirrors that expected for a model in which stem cell differentiation occurs through the terminal division of a TA-like cell, as implied by the *STD*-model (in the situation when the frequency of asymmetric TA cell divisions is small or altogether absent).

Fits of the *STD*-model to the time-lapse clonal data

As with the analysis of the *SD*-model, to fit the data, we first constrained the fraction of stem cells f_{SC} (which now only includes cells growing to 4 or more cells) and the ratio of basal to suprabasal cells (which provided the shed rate γ). We then obtained an estimate for the cell division rate λ by temporal rescaling of the simulated average basal clone sizes to best match experiment. By re-running the simulations for the optimal parameter set, we modelled the dynamics of the suprabasal layer to obtain the shed variability cv_γ . Once again, parameter estimates were obtained by optimising over discrete parameter regimes for the Hill parameter $n = 0, 2, 8$. We then fit the data over the 6-dimensional parameter space for the adjustable parameters $\theta_{fit} = (r, q, cv_\lambda, cv_\gamma, c_{SS}, c_{DD})$, the fixed parameters $\theta_{fix} = (f_{SC}, n, c_{ST}, \gamma)$ and post-run parameters $\theta_{post} = (\lambda, cv_\gamma)$, using a simulated annealing approach to minimise the sum of the log-likelihood $\mathcal{L}_{STD} = \mathcal{L}_{SC} + \mathcal{L}_{TA} + \mathcal{L}_D$ of the joint clone size distribution for SC-like, TA-like and D-like clones, where

$$\begin{aligned} \mathcal{L}_{SC/TA/D} &= \log P(D_{SC/TA/D} | \theta_{fit}, \theta_{fix}, \theta_{post}) \\ &= C + \sum_{t,b,s} N^{SC/TA/D} P_{b,s}^{SC/TA/D}(t) \log (p^{SC/TA/D}(b_t, s_t | \theta_{fit}, \theta_{fix}, \theta_{post})). \end{aligned}$$

Here, $P_{b,s}^{SC/TA/D}(t)$ denotes the experimental probability distribution of basal and suprabasal clone sizes for a given clone type (SC/TA/D) at time t post-induction. As before, the constants C and $N^{SC/TA/D}$ (defined as the experimental number of SC-like/TA-like/D-like clones) are included for completeness, but their values do not influence the numerical optimisation. $p^{SC/TA/D}(b_t, s_t | \theta_{fit}, \theta_{fix}, \theta_{post})$ represents the joint clone size distribution obtained from model simulations at time t for fixed modelling parameters $\theta_{fix} = (f_{SC}, n, c_{ST}, \gamma)$. Estimates of optimal parameters and uncertainty estimates obtained using bootstrapping over a grid for male, female and castrated mice are provided in Supplementary Note Table 3.

Based on these definitions and constraints, we found that the *STD*-model could accurately recapitulate the clone dynamics, including the average time-dependence of SC-like clones (Fig. 1i-j), average sizes of TA-like and D-like clones (Fig. 1j), the clone persistence (Extended Data Fig. 4a,c), and the fine-features of the full basal and suprabasal clone size distributions across the entire timecourse (Fig. 1k, Extended Data Fig. 4b,d).

From these results, it followed that the differences in the clonal dynamics of male and female mice can be accounted for by:

- differences in the proportion of TA cell divisions that result in asymmetric fate with $1 - q = 0.3$ for males and $1 - q = 0$ for females. In the context of male animals, the non-zero rate of asymmetric TA cell division serves to erode even-odd features in the size distribution. It also results in a larger TA cell compartment and smaller differentiated cell compartment for males compared to females ($f_{SC}/f_{TA}/f_D = 0.3/0.4/0.3$ for males and $0.275/0.275/0.45$ for females).
- a higher degree of coordination in the delamination of sister cells for female mice ($c_{DD} = 0.9$ for females, and 0.6 for males), which also erodes even-odd features in males.
- differential shedding dynamics between male and female mice, with male mice having a faster cell loss rate ($1/\gamma = 4.4$ days for males and 5.7 days for females), with a low degree of variability for females ($cv_\gamma = 0.1$), but not males ($cv_\gamma = 0.7 - 1.0$). This reflects the fact that male mice have fewer nucleated suprabasal cells than females, suggesting that differentiated cells mature more rapidly in the former.

Finally, to further challenge the *STD*-model, we used the model fits to predict the temporal correlations in the clonal data. Notably, the *STD*-model predicted qualitatively the observed enhancements in the transitions of clone sizes between consecutive time points, integrated across all timepoints. Specifically, the model predicted both the existence and scale of the changes in clone size at even integers, leading to a hallmark checkerboard pattern in the joint clone size distribution (Fig. 1l, Extended Data Fig. 4h). This contrasts with the predictions of the one-compartment *SD*-model, where these temporal features were not enhanced (Extended Data Fig. 3l). However, the model overestimated the extent of checkerboard pattern for male mice for the best fit parameters. We note that these features are fragile and easily lost from the experimental data, indicating that male mice may have a lower degree of temporal coordination (c_{ST}, c_{SS}, c_{DD}) than our best fit model suggests. Notably, for our fitting approach, this difference could not be distinguished for male mice, as there is a range of model coordination parameters with very similar $\mathcal{L}_{STD}$ that would have different extents of temporal enhancements of even clone sizes.

In summary, the *STD*-model accounts for robust fine-features of the clonal dynamics that lie beyond the *SD*-model, suggesting that the mouse ear skin IFE is characterised by a stem-TA cell-like proliferative hierarchy.

Sex	r	q	f_{SC}	$1/\lambda$ (d)	$1/\gamma$ (d)	c_{SS}	c_{DD}
K14 M	$0.1^{+0.1}_{-0.05}$	$0.7^{+0.15}_{-0.2}$	$0.31^{+0.04}_{-0.04}$	$6.7^{+0.5}_{-1.0}$	$4.4^{+0.3}_{-0.9}$	$0.3^{+0.7}_{-0.2}$	$0.6^{+0.4}_{-0.4}$
K14 F	$0.15^{+0.025}_{-0.025}$	$1.0^{+0.0}_{-0.2}$	$0.27^{+0.02}_{-0.02}$	$6.3^{+0.45}_{-0.3}$	$5.7^{+0.05}_{-0.6}$	$0.2^{+0.8}_{-0.0}$	$0.9^{+0.05}_{-0.1}$
K14 M castr.	$0.1^{+0.025}_{-0.05}$	$0.8^{+0.2}_{-0.15}$	$0.27^{+0.03}_{-0.03}$	$5.3^{+0.6}_{-0.6}$	$4.0^{+0.6}_{-0.4}$	$0.2^{+0.6}_{-0.1}$	$0.7^{+0.3}_{-0.2}$
K14 F ovx.	$0.225^{+0.025}_{-0.125}$	$0.725^{+0.275}_{-0.175}$	$0.32^{+0.04}_{-0.04}$	$5.5^{+0.1}_{-1.6}$	$3.3^{+0.3}_{-0.7}$	$0.4^{+0.1}_{-0.4}$	$0.5^{+0.5}_{-0.4}$
Lgr6 M	$0.15^{+0.10}_{-0.05}$	$0.65^{+0.35}_{-0.13}$	$0.26^{+0.04}_{-0.04}$	$3.2^{+0.4}_{-0.6}$	$2.6^{+0.6}_{-0.6}$	$0.5^{+0.5}_{-0.5}$	$0.9^{+0.1}_{-0.7}$
Lgr6 F	$0.125^{+0.075}_{-0.075}$	$1.0^{+0.0}_{-0.38}$	$0.26^{+0.06}_{-0.06}$	$3.0^{+0.6}_{-0.7}$	$2.6^{+1.0}_{-0.7}$	$0.5^{+0.5}_{-0.5}$	$0.6^{+0.4}_{-0.1}$
Notch1 M	$0.15^{+0.25}_{-0.05}$	$1.0^{+0.0}_{-0.25}$	$0.34^{+0.08}_{-0.08}$	$4.5^{+1.75}_{-1.0}$	$3.4^{+0.7}_{-0.9}$	$0.5^{+0.5}_{-0.5}$	$0.6^{+0.4}_{-0.4}$
Notch1 F	$0.15^{+0.125}_{-0.05}$	$0.8^{+0.12}_{-0.23}$	$0.26^{+0.05}_{-0.05}$	$4.2^{+0.25}_{-0.5}$	$3.6^{+0.1}_{-0.8}$	$0.25^{+0.75}_{-0.25}$	$0.6^{+0.2}_{-0.2}$

Supplementary Note Table 3: **Fits and uncertainty analysis for the two-proliferative compartment *STD*-model to the clonal data.** Best fit values are obtained using simulated annealing with uncertainty estimates (presented as 95% confidence intervals) found by bootstrapping experimental data (1,000 bootstraps) and refitting simulation data using a grid search. During simulated annealing, we found that best fits were largely insensitive to the Hill coefficient n (see Supplementary text). (We therefore set $n = 0$ to make the uncertainty analysis more computationally efficient). Due to computational intensity, we performed uncertainty analysis with a reduced parameter space with cv_λ , cv_Γ and cv_γ fixed, using the values based on the best fits from simulated annealing of $cv_\lambda = 0.5$ and $cv_\Gamma = 1.0$. From simulated annealing, we found $cv_\Gamma = 0.7 - 1.0$ depending on sister coordination parameters, $cv_\gamma \approx 1.0$ for males, castrated males and ovariectomized females and $cv_\gamma \approx 0.1$ for females.

Parameter summary and sensitivity analysis

While the *STD*-model has multiple parameters, only certain parameter combinations are critical for capturing the key experimental observations, leaving a degree of degeneracy. Here, we outline the meaning of each parameter in the final model, describe its impact on the dynamics and present a sensitivity analysis.

We first note that for the *STD*-model, the spatial feedback mechanism has a minimal impact on the long-term clonal dynamics. Since we are in a regime where stem cells are relatively dilute within the basal cell layer ($f_{SC} \approx 0.3$) and their fate is biased towards asymmetry ($r \approx 0.1$), clonal dynamics are not particularly sensitive to changes to the Hill parameter n . However, the best fits to the *SD*-model (which was incompatible with the fine-grained features of the data) were with high n , highlighting that spatial competition matters in models where stem cells are not dilute. Thus, for the regime of the best fit parameters to the *STD*-model, the predictions of the clonal dynamics for the spatial Voronoi model depart relatively little from a zero-dimensional model over the timescales considered here. As such, the Voronoi model parameters p_0 and $\lambda\tau_{mech}$ would not affect the average clonal dynamics. However, we can deduce that p_0 is in the solid phase due to clonal contiguity [7].

All of the remaining parameters influence the quality of the fits, but certain parameters and parameter combinations have greater influence. Here, for clarity, we specify the influence and sensitivity of each model parameter before summarising the most critical ones. For both the *STD* and *SD* models, the dynamics of the SC compartment are well constrained by the data:

- The relative proportion of stem cells in the basal layer (f_S) is fully constrained by the data and hence not fit, as the plateau in the labelled cell density of SC-like clones provides a read-out of $1/f_S$.
- The proportion of SC divisions that result in asymmetric fate (r) is critical to capturing the profile of average clonal growth: the observed fast initial growth followed by a slower growth rate requires a significant bias towards asymmetric fate outcome. The optimal value of r is influenced by the correlation parameters below, which strongly affect the initial rate of clonal growth.
- Given f_S and r , the division rate λ is constrained by the data, since linear-like long-term clonal growth occurs at rate $r\lambda/f_S$, as reported in prior studies [1]. In the fitting procedure, with r and f_S fixed, the optimal λ is obtained from the temporal rescaling between simulation time and experimental data that makes the average clone sizes match.
- The coefficient of variation of the cell-lifetime distribution (cv_λ) is constrained by the data, with exponential lifetimes overestimating the number of clones in the tail of the clone-size distribution. However, the particular values are not tightly constrained, as there is a broad uncertainty interval owing to the collective influence of other parameters.
- The correlation parameters for daughters of stem cell divisions (c_{ST} , $c_{SS} = c_{TT}$) are not precisely constrained by the data. Since most SC divisions are asymmetric, c_{ST} must be high (~ 1), otherwise even-odd features would be rapidly erased; for simplicity we fixed this parameter to unity to reduce the dimensionality of the parameter space. Conversely, the correlation for symmetric stem cell divisions c_{SS} is poorly constrained, since the proportion of symmetric SC events is small (95% CI [0.1, 1.0] for males and [0.0, 1.0] for females).

For the TA and D cell populations, the parameters are less precisely constrained, with degeneracies along certain parameter combinations:

- Since the short-term growth characteristic of TA-cell clones is similar to that of SC-derived clones, the division rate of TA cells is $\sim \lambda$, the rate of SC division. For simplicity we assume the coefficient of variation of division time for all dividing cells is the same (cv_λ).
- The proportion of asymmetric TA cell divisions ($1 - q$) is treated as a free parameter. The model is sensitive to it, as without it one cannot explain the 3- and 5-cell clones observed in female mice. In the log-likelihood fit there is some degeneracy, as q covaries with certain parameters (c_{DD} , cv_Γ).
- The D-cell delamination rate (Γ) is uniquely defined once λ , f_S and q are fixed. The quality of fits is sensitive to the delamination rate if c_{DD} is small, since a slower delamination rate would erase even-odd features. The fits are relatively insensitive to cv_Γ , given other parameter combinations of c_{DD} and Γ/q .

- For the correlation parameters, we fixed $c_{TD} = 0$ and treated c_{DD} as a fitting parameter. For male mice the fits were relatively insensitive to c_{DD} and c_{TD} , as there is no even-odd fine structure in the clonal data. In contrast, female mice fits are strongly sensitive to c_{DD} , requiring a high degree of sister-cell coordination to maintain the observed even-odd features. Coordination between delamination and TA cell divisions following asymmetric division (c_{TD}) alters the best-fit values of q/Γ and c_{DD} , but does not increase the quality of fits to the present data.

For the suprabasal layer, parameters are well constrained by the data:

- The suprabasal shedding rate (γ) is fixed by the data, with the quality of suprabasal-layer fits highly sensitive to its value.
- The suprabasal shedding variability (cv_γ) influences the degree of even-odd features in the suprabasal layer, so is strongly constrained in the female data (low variability is needed for even-odd suprabasal features to persist), but is not well constrained by the male data.

The parameter sensitivity of the free fitting parameters can be assessed by bootstrapping on the grid. The free fitting parameters r and q were sampled over a uniform 20-point grid, and the parameters c_{SS} and c_{DD} over a 10-point grid. Over 1000 resamples of the experimental data, we found that 0.5% of the unique parameter combinations were best fits for male mice and 0.1% for female mice. Principal Component Analysis of the male bootstrap best-fit parameters revealed that c_{SS} is a “nuisance” parameter, with 40% of the total parameter variance explained by varying it alone. In contrast, 58% of the parameter variance is explained by a combination of q and c_{DD} , reflecting a sensitivity of fits to the sister delamination correlation when the delamination rate is slow: higher c_{DD} is required if the delamination rate is slower (q is smaller, since (f_S, λ, q) uniquely determine Γ at the mean-field/0D level). Variation in r explains only 0.5% of the total parameter variance, reflecting that the proportion of asymmetric divisions is strongly constrained by the profile of the average basal clone size. For female mice the picture is strikingly different: 95% of the parameter variance is explained by a single principal component, which depends mostly on c_{SS} with weak dependence on q . This means equally high-quality fits can be found along a manifold where c_{SS} increases and q decreases. These four fitting parameters therefore correspond to two soft parameter directions in male mice and one soft parameter direction in female mice.

To summarise, the average model dynamics for SC-like clones are determined by the division rate (λ), the proportion of stem cells (f_S), the proportion of asymmetric divisions (r) and the shed rate (γ). To capture the fine-grained features of the clone-size distributions, fits are strongly sensitive to c_{ST} and c_{DD} (reducing either destroys even-odd correlations) and cv_λ (which sets the maximum clone sizes), with a contribution from cv_Γ and q (where decreasing q destroys even-odd correlations when c_{TD} is not high). To simultaneously capture the TA-like and D-like dynamics together with the SC-like dynamics, the fits are sensitive to q , cv_Γ , c_{TD} and c_{DD} along particular parameter combinations. Overall, the model robustly explains the male dynamics over a wider parameter regime, whereas female mice require fine-tuning to maintain the fragile even-odd features through a high degree of coordination with a low degree of TA cell division asymmetry.

Fits to the clonal dynamics of castrated mice

We repeated the fitting procedure for the *STD*-model in the context of the castrated male mice (Fig. 3i, Extended Data Fig. 6a-e). Castration restored even clone size enhancements in the basal, but not the suprabasal layer. This was reflected in our model (Supplementary Note Table 3) by a slight increase in the proportion of asymmetric divisions ($q \approx 0.8$ for the best fit to castrated mice versus 0.7 for male mice). Further, we observed an increased cell division rate for castrated mice ($1/\lambda = 5.3$ days for castrated animals versus ~ 6.5 days for male and female animals). The other parameters are equivalent within 1 SEM uncertainty estimates of parameters.

Fits to the clonal dynamics of ovariectomized mice

For ovariectomized female mice we repeated the fitting procedure for the *STD*-model (Fig. 3m, Extended Data Fig. 6f-j). Ovariectomy destroyed the strong even-odd features present in female mice in both the basal and suprabasal layer. The *STD*-model predicts that the destruction of even-odd features originates from either the increase of TA cell asymmetric divisions, or desynchronization of sister divisions and delaminations (Supplementary Note Table 3). However, the fits have a significant degeneracy for varying q , c_{SS} and c_{DD} indicating an uncertainty in the precise contribution of TA asymmetric divisions and sister desynchronization in the loss of even-odd features. Further, stem cell fate is affected by ovariectomy with an enhanced proportion of SC symmetric divisions (r) and slight acceleration of division rate. Additionally, delamination rate is significantly increased under the perturbation relative to regular female mice (2 d vs 5.1 d).

Fits to lineage tracing under the *Lgr6* promoter

The *Lgr6* promoter was slightly more biased towards labelling the stem cell population than the *K14* promoter: for male mice 50% of labelled *Lgr6* clones were SC-like relative to 41% for *K14* clones, and for female mice 52% of *Lgr6* clones are SC-like, compared with 50% of *K14* clones. Across SC-like, TA-like and D-like clones the dynamics were captured well with an *STD*-model (Extended Data Fig. 10a-b,e-f,i-j,m,o). The inferred division rate was substantially elevated relative to the *K14* population (Supplementary Note Table 3). A potential explanation is spatial heterogeneities in the proliferation rate. For *Lgr6* tracing, the basal clonal size distributions (Extended Data Fig. 10m) exhibited stronger even-size enhancement in female than male mice, but these features were significantly more dilute relative to *K14*. The dilution may be partially accounted for by smaller sample sizes ($N = 149$ for *Lgr6* males and $N = 110$ for *Lgr6* females, compared with $N = 384$ for *K14* males and $N = 745$ for *K14* females), but there is a potential change in the degree of sister coordination and proportion of TA asymmetric divisions in this dataset (uncertainty intervals overlap with *K14* data, so no precise statements on the differences of these parameters can be made). Further, we note that there are some discrepancies with the clonal persistence of TA-like clones (Extended Data Fig. 10i-j). The origin of the discrepancy may be accounted for by a correlation between sister division and delamination following TA cell asymmetric divisions.

Fits to lineage tracing under the Notch1 promoter

For lineage tracing under the Notch1 promoter, most of the labelled clones are differentiated cells (73% for males and 75% for females). Of the remaining clones there is a roughly equal proportion of SC-like (10% for males and 12% for females) and TA-like clones (18% for males and 13% for females). This indicates that Notch1 is predominantly a differentiated cell marker, as expected, with some capacity to label TA and stem cells. However, we cannot rule out that labelled stem cells are induced from the de-differentiation of cells on a differentiation path. SC-like clones under the Notch1 promoter showed similar dynamics to K14 clones captured well by the SC population from the *STD*-model (Extended Data Fig. 10c-d,g-h,k-l,n,p), with diluted even-odd features relative to lineage tracing with the K14 promoter, but showing a similar relative trend of females vs. males (even sizes enhanced in females). D-like clones were captured well by the model, but some small discrepancies existed for TA-like clones, with a similar trend to the Lgr6 data.

Summary of conclusions and limitations of the modelling-based analysis

Based on our analysis, we conclude that under normal homeostatic conditions the clonal dynamics of ear skin IFE in male and female mice are statistically distinct with notable correlations in the coordination of cell division, delamination and fate, leading to an enrichment of even-sized clones in female animals. Notably, we found that models based on a single proliferative basal cell compartment (*S*-model and *SD*-model) struggle to capture the fine features of the clonal data. Notably, the strong basal and suprabasal even size enhancement is fragile and requires a high degree of coordination between sister cell divisions and delaminations, with a strong bias toward a symmetric fate outcome within a one progenitor paradigm. However, at the same time, the profile of average basal clone size implies a bias of stem cell fate toward asymmetric fate outcomes.

Based on these findings, our results suggest that epidermal stem cell differentiation is not direct, but proceeds through an intermediate transit-amplifying cell with strictly limited proliferative potential. Crucially, to capture the observed even-size correlations, we found that stem and TA cells are not independent but involve a tight temporal coordination between sister cells. Whether this coordination is encoded in cell-intrinsic factors or involves cell-cell interaction is unclear. Within the modelling framework, the differences between male and female mice could be accounted for by the potency of TA cells (with male mice showing evidence for amplification through an additional asymmetric TA cell division) and differences in differentiated cell delamination (with male mice having a shorter residency time with less coordination than in females).

While fine-features of the clonal data point to a proliferative hierarchy, we note that many features of the dynamics are consistent with prior one-proliferative compartment models [1, 9]. Thus, in the following, we summarise which features can be captured by an *SD*-model, and which require the implementation of the *STD*-model:

- The emergence of **statistical scaling behaviour of clone size in the long-term** represents a conserved feature of any homeostatic model based on stochastic loss-replacement, and therefore follows from both the *SD* and *STD* models.

- The time-dependence of both the **average basal and suprabasal clone size** can be captured well by both *SD* and *STD* models, calling for a strong bias of the stem cell compartment towards asymmetric fate outcome.
- While the *SD*-model can show a **persistent enhancement in the frequency of even clone sizes** for strongly synchronised sister cell dynamics, such behaviour cannot be recovered from a regime in which the stem cell bias is towards asymmetric fate outcome, as observed. Nor does the model predict the observed enhancement in even clone sizes in the suprabasal cell layer. Thus, to capture persistent even clone size correlations, we require the stem-TA cell hierarchy of the *STD*-model, with tightly controlled cell division and delamination events, and a low proportion of asymmetric TA cell fate.
- In the *SD*-model, where all proliferative cells are equipotent, the **temporal dynamics of SC-like and D-like clones** cannot be obtained simultaneously.
- Finally, the strong **enhancement of temporal “transition” probabilities in the joint clone size data at even cell numbers** cannot be recovered within the framework of an *SD*-model when tilted towards asymmetric stem cell fate. By contrast, such features emerge naturally within the framework of the *STD*-model in the regime of high spatiotemporal coordination and low TA asymmetric division probability.

Based on the modelling, the following features of stem cell dynamics are demanded by the experimental data:

- *Bias to asymmetric divisions for the SC population:* To capture long-time clone sizes, a bias to asymmetric fate for the stem cell population is needed.
- *Bias to symmetric divisions for the TA cell population for female mice:* To maintain even-odd features in both the basal and suprabasal layer, the final division before differentiation must be mostly symmetric.
- *Non-exponential cell cycle times:* To capture the tail of the basal clone-size distribution, a peaked distribution of division and delamination times (or a refractory period) is needed.
- *Tightly coordinated timings of sister cell fate decisions for female mice:* To capture the bias to even clone sizes in female mice, the following temporal coordinations are demanded: strong correlation between SC and TA cell divisions among sisters, and strong correlation in D sister cell delamination. Also, the shedding of sister differentiated cells from the suprabasal layer must be coordinated (this can be captured through a Gamma distributed lifetime or correlation parameter).
- *Stem and progenitor cells proliferate at a similar rate:* TA-like clones (which grow to 2-3 cells) have the same initial clonal growth as SC-like clones (4+ cells), implying SCs and TA cells divide at similar rates. This is incompatible with a paradigm of slow-cycling stem cells [4], which would cause the larger clones to have an initially slower clonal growth.

The *STD*-model is related to the hierarchical models put forward in the tail skin [4, 17], with the added assumption that TA cells have no symmetric self-renewing division channel ($TA \rightarrow TA + TA$), and that the SC and TA cell division rates are equal. The model is a refinement of those models with two additional features: (i) Gamma distributed lifetimes for division, delamination and shedding, and (ii) temporal correlations between the events of sisters (SS, ST, TT, DD) following division.

Other proposed two-compartment models with similar complexity will not explain the data. A prior model based on two independent equipotent stem cell populations [18] can also be ruled out as follows. If these populations have no intrinsic bias towards asymmetric fate outcome, the model cannot capture the profile of long-term average basal clone sizes. Even allowing a bias of each population to asymmetry, this model is unable to capture the long-term even-odd features in both the basal and suprabasal layers. Since both stem cell populations are long-lived and the clone-size distribution is the linear mixture of each population’s distributions, both populations would have to have a bias towards symmetric fate outcome, or the even-odd features would rapidly be washed away. However, this parameter regime is incompatible with the profile of clonal growth, which demands asymmetry, as we saw earlier. Thus, the two-SC models from the literature can be excluded as possible candidates to explain the dynamics. We cannot rule out multiple independent populations with similar division rates, where one has an SC-TA hierarchy and the other has mostly symmetric division, but there is no evidence that demands this level of complexity. Thus, the *STD*-model is the most minimal one that can explain the data.

As with any phenomenological theory, the current *STD*-model also has important limitations and assumptions:

Evidence for spatial coordination between basal and suprabasal cell layers

There are a few temporal features of the clonal data where the model shows minor but statistically significant deviation. For male mice, the model struggles to capture the dynamics of clones which grow to a maximum size of 3 cells. This is reflected in the clone persistence leading to an underestimate in the labelled cell density in TA cell-like clones (Fig. 1j and Extended Data Fig. 4a). Further, the predicted temporal data of male mice shows a significantly higher degree of even clone size transitions (viz. checkerboard pattern) than is observed experimentally (Fig. 1m, Extended Data Fig. 4h). Moreover, for female mice, the predicted D14 suprabasal clone size distribution doesn’t accurately match experiment (Fig. 1k, Extended Data Fig. 4d), and at later times (D42 and beyond) the extent of even clone size enhancement is weaker in the model than seen in experiment. Together, these discrepancies in fine-scale features suggest that the turnover of suprabasal cells (shedding) may be tightly coupled with cell delamination, and that upwards migration of sister differentiated cells through suprabasal layers is temporally coordinated. This would allow suprabasal clone sizes to maintain even cell numbers, as seen in female mice, while the behaviour in male mice reflects the relative abundance of asymmetric TA cell divisions.

Mechanics of cell delamination

More realistic models of delamination and transit within the suprabasal layers may enable better capture of spatiotemporal correlations across layers. Indeed, in this context, variants of the Vertex-type models into 3D have been considered in recent studies [19–21]. Moreover, various mechanisms for delamination have been proposed: either driven by crowding, leading to loss of basement membrane contact [19], or by changes in the mechanical state of a basal cell, involving altered interfacial tension with neighbouring cells and the basement membrane [20]. Our paradigm requires cell-type dependent mechanical changes along the lines of the latter model, since stem and TA cells cannot directly delaminate, and any model in which any basal cell could delaminate independently of cell type would quickly erase the observed strong even-odd clone size correlations. Density-dependent, crowding-induced feedback on the delamination of basal differentiated cells may also contribute to temporal correlations between sister-cell events (sister delaminations, or one sister dividing and the other sister delaminating following an asymmetric TA cell division), as well as correlations between basal delamination and suprabasal shedding. However, we opted against incorporating such additional complexities in the modelling of delamination here since they require adding further free parameters to the model that are not well constrained by the data. Nonetheless, 3D mechanical models incorporating proliferative heterogeneity are a compelling direction for future work.

Determination of spatiotemporal correlation parameters

The long-term time-lapse data admits regions of degeneracy in parameter space for the *STD*-model, including some of the correlation parameters for symmetric divisions $c_{SS} = c_{TT}$ and c_{DD} , which leads to the high uncertainty estimates (Supplementary Note Table 3). Since stem cell divisions are mostly asymmetric, these events are rare and the data is therefore not very sensitive to a presence or absence of coordination. Further, while we found highly coordinated stem and sister TA cell divisions ($c_{ST} \approx 1$) are necessary to capture the clonal dynamics of female mice, we cannot rule out a lower degree of coordination for male animals, even though the best fits correspond to high values (with $c_{ST} \approx 1$).

We cannot rule out higher level correlations, such as the presence of correlations between asymmetric TA cell division and sister differentiated cell delamination (c_{TD}). Indeed, we found similar quality of fits (as measured by $\mathcal{L}$) when $c_{TD} > 0$, and that high values ($c_{TD} \approx 1$) allow the even clone sizes in the basal layer to be maintained even following asymmetric TA cell division. This allows the inferred q values to be lowered for males to $q \approx 0.5$ and for females to $q \approx 0.8$.

Information on correlation parameters can be more accurately accessed using short-term live-imaging, when the imaging window is long enough to resolve lineage behaviour (that is, whether the division is for an SC-like or TA-like cell). The work of Rompolas et al. [22] presents estimates for correlations following division, where both sisters divide (0.64 in ear skin IFE and 0.61 in paw skin IFE; viz. $c_{SS} = c_{TT}$ in the current model), both sisters delaminate (0.92 in ear and 0.98 in paw; viz. c_{DD} here), or asymmetric fate behaviour (0.52 in ear and 0.24 in paw; which we assume to be zero here), figures that are compatible with our fitted values. Here, our experimental analysis based on short-term time-lapse imaging

(see Methods and main text) found a high degree of coordination between sisters for all types of events, providing a direct measurement of coordination parameters under the assumption that the cell fate rules follow the model. For male mice, the sister cell lifetime correlation coefficient was 0.89 ± 0.03 (78 pairs of events) between delaminating sisters (c_{DD}), 0.7 ± 0.15 (86 pairs of events) between dividing sisters (this is a combination of division modes, so is equivalent to $rc_{SS} + (1 - r)c_{ST}$) and 0.75 ± 0.19 (24 pairs of events) between one sister dividing and one sister delaminating (c_{TD}). For female mice, the sister cell lifetime correlation coefficient was 0.82 ± 0.14 (62 pairs of events) between delaminating sisters (c_{DD}), 0.8 ± 0.12 (74 pairs of events) between dividing sisters and 0.3 ± 0.3 (15 pairs of events) between one sister dividing and one sister delaminating (c_{TD}). However, we could not decouple c_{ST} from c_{SS} and c_{TT} due to the small sample sizes. Further, we frequently observed two delamination events within the 12 hour time window within a clone. For male mice 84% (42/50) of these events were sisters, whereas for female mice 98% (45/46) of these double delamination events were sisters. These results provide direct experimental support for the model’s prediction of a high degree of coordination of fate events between sisters.

We note that parameters from short term live-imaging may not perfectly correspond to parameters inferred from fitting long-term data due to invasiveness of imaging: the bi-daily imaging (cell division every 2 days on average for dividing cells) has a faster division rate compared to long-term imaging (cell division every 6 days on average for dividing cells), implying that there may be inflammation caused by daily imaging which may quantitatively affect the inferred coordination and fate parameter values. Nonetheless, based on our inference, we are confident that a high degree of coordination in sister fate decisions in both the short-term and long-term imaging in female mice is present, since in the model a reduction in the degree of temporal coordination between sister cells or an increase in the proportion of asymmetric TA divisions will diminish and eventually erase the even-odd signature.

Spatial correlations are difficult to disentangle from long-term time-lapse data, and may call for short-term live-imaging to resolve. The work of Mesa et al. [23] suggests that delamination triggers cell division in paw skin, while for ear skin (which has a lower basal cell density than paw) Yamamoto et al. [24] found evidence that both delamination can trigger division, and that division can trigger delamination. In the present modelling framework, we do not expect significantly different results in the case where the timing of division and delamination are locally correlated, as opposed to correlations within sibling pairs.

TA cell fate

In the present analysis, we considered the situation in which TA cells were, for simplicity, limited to a single round of asymmetric division before terminal division. With this condition, the *STD*-model was capable of capturing the clonal dynamics for male and female mice (where in the latter case the constraint was academic since the best fit had only terminal TA cell divisions). However, we cannot rule out more complex behaviour, where TA cells are equipotent, capable of undergoing multiple rounds of asymmetric division. This would admit one additional free parameter, since we can no longer constrain f_{SC} based on clones that grow to a maximum size of only 4 cells. For completeness, we repeated fitting with simulated annealing in this situation (which we term the *SPD*-model in the code) finding comparable fits based on values of log-likelihood $\mathcal{L}$. Here, we chose to present the simpler

model. Further, while we can't rule out the potential for symmetric TA cell division, we found that their inclusion led to fits with a frequency of events predicted to be at $< 5\%$.

Once again, information on TA dynamics could in principle be obtained from short-term live-imaging. Indeed, prior live-imaging studies by Cockburn et al. [25] indicated that, while cells labelled under the K10 promoter mostly delaminate, they can also undergo a round of terminal division or a single round of asymmetric division followed by terminal division, consistent with the *STD*-model.

Mechanism of stem cell density regulation

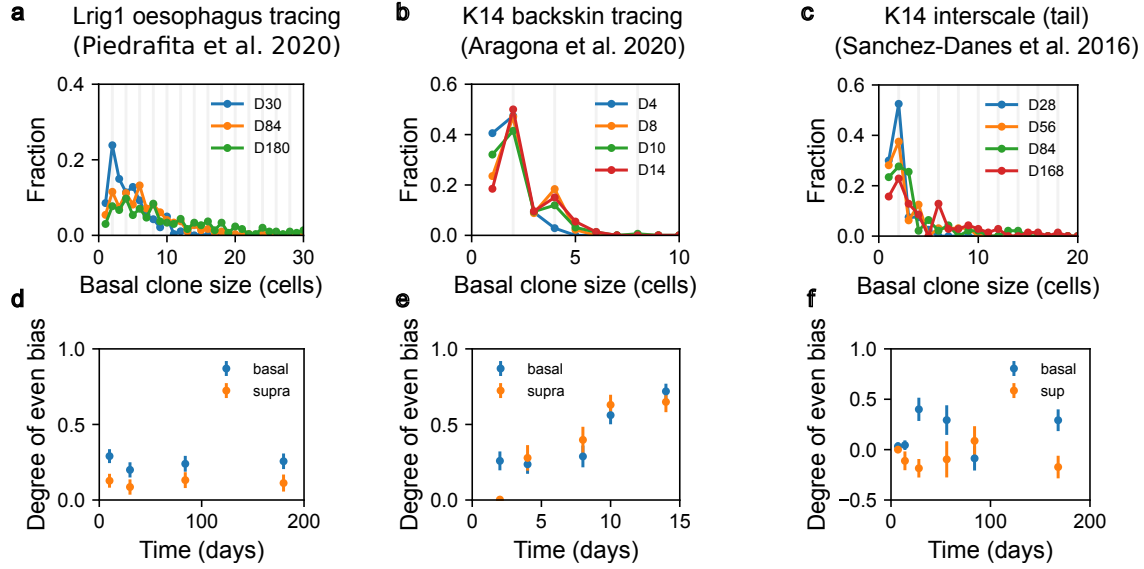
To enforce stem cell density regulation, we made use of a mechanical feedback mechanism based on the sensing of local density. However, a model of stem cell fate based on the reception and consumption of diffusible niche factors would translate to a qualitatively similar dynamics [26]. However, from the present experimental design, it is challenging to disentangle local spatial correlations during the course of stem cell division and differentiation. As a result, it is difficult to resolve the potential role of mechanical and/or biochemical niche factors in the regulation of stem cell density homeostasis.

Since we are in a regime where stem cells are relatively dilute within the basal cell layer ($f_{SC} \approx 0.3$) and their fate is biased towards asymmetric fate ($r \approx 0.1$), clonal dynamics are not particularly sensitive to changes to the Hill parameter n . Thus, for the regime of the best fit parameters, the predictions of the clonal dynamics for the spatial Voronoi model depart relatively little from a zero-dimensional model, at least over the timescales considered here.

Long-term clonal dynamics of other squamous tissues

Our findings raise the question of the relevance of the hierarchical *STD*-model to other squamous tissues including other skin IFE compartments and oesophagus. Beyond the mouse ear skin IFE studied here, in prior studies even-odd clone size correlations have been observed in static short-term lineage tracing of the mouse backskin at homeostasis and under TPA treatment [6]. Notably, such correlations are also visible in long-term (6-month) lineage tracing data from the mouse oesophagus [9], as well as the interscale tail skin IFE [5] (Supplementary Fig. 4). We confirmed that back skin and tail skin are sexually dimorphic (Extended Data Fig. 2i-l), with female mice showing strong even-odd features, with male mice only showing weak features. Such observations point to a hierarchical model of tissue maintenance in other epithelial contexts, consistent with the dynamics of the *STD*-model.

However, we note that in contexts where even-odd features are weak or absent (such as the male ear skin IFE considered here), aspects of the clonal dynamics might mirror the behaviour of the one-proliferative compartment *SD*-model even when the underlying dynamics is hierarchical. This is because even-odd features are extremely fragile, and will be greatly diminished if cell division or delamination events become desynchronised, or if the proportion of TA cell asymmetric division is high.



Supplementary Fig. 4: **Evidence for the enhancement of even clone sizes during homeostasis in other squamous tissues.** Basal clone size distribution (a) and degree of even bias (d) for long-term Lrig1 lineage tracing reproduced from Piedrafita et al. [9]. Basal clone size distributions (b) and degree of even bias (e) for short-term K14 back skin lineage tracing from Aragona et al. [6]. Basal clone size distributions (c) and degree of even bias (f) for K14 tail skin (interscale) lineage tracing reproduced from Sanchez-Danes et al. [5].

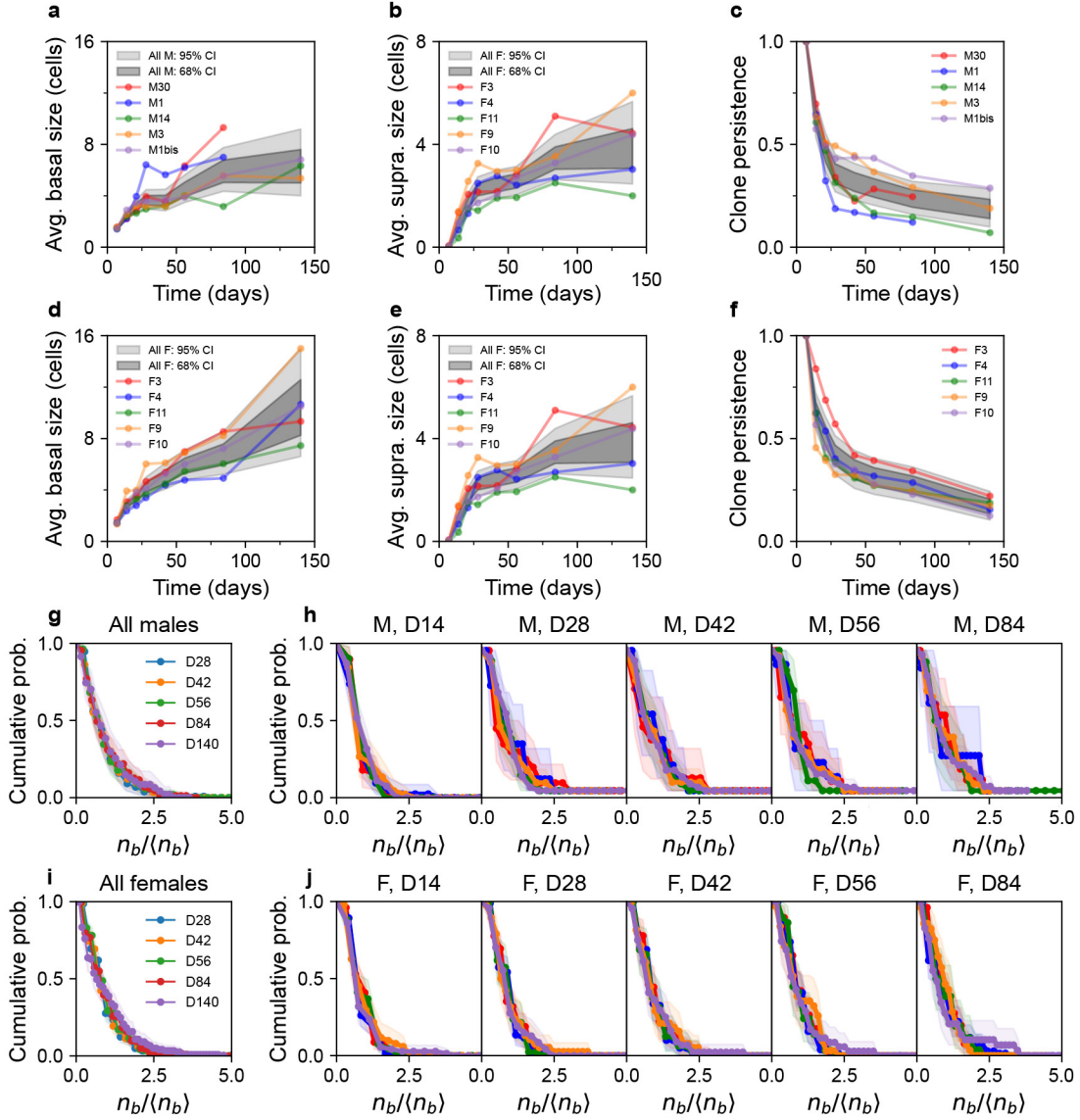
Regional stem cell heterogeneity

While the timelapse imaging data in the current study can be accounted for by a model in which stem and TA cells are homogeneous, we cannot rule out the potential for further heterogeneity of the cell compartments. In particular, previous lineage tracing studies based on distinct promoters show evidence for stem cell populations marked by differences in long-term renewal potential. In the mouse tail skin interscale epidermis, clones induced under a K14 promoter show on average a higher long-term survival potential than clones induced under the involucrin promoter [4, 5]. The functional differences between the targeted populations are reinforced by studies of mutant clone dynamics following activation of Hedgehog signalling [5]: only clones induced under a K14 promoter transition to invasive basal cell carcinoma while those induced under the involucrin promoter fail to transform. If stem cell fate were regulated by the reception and consumption of niche factors, such behaviour could be explained within the same *STD*-model paradigm through a local enrichment of niche factor concentration in regions not targeted by the involucrin promoter. While interesting to note, such effects lie beyond the scope of the present study.

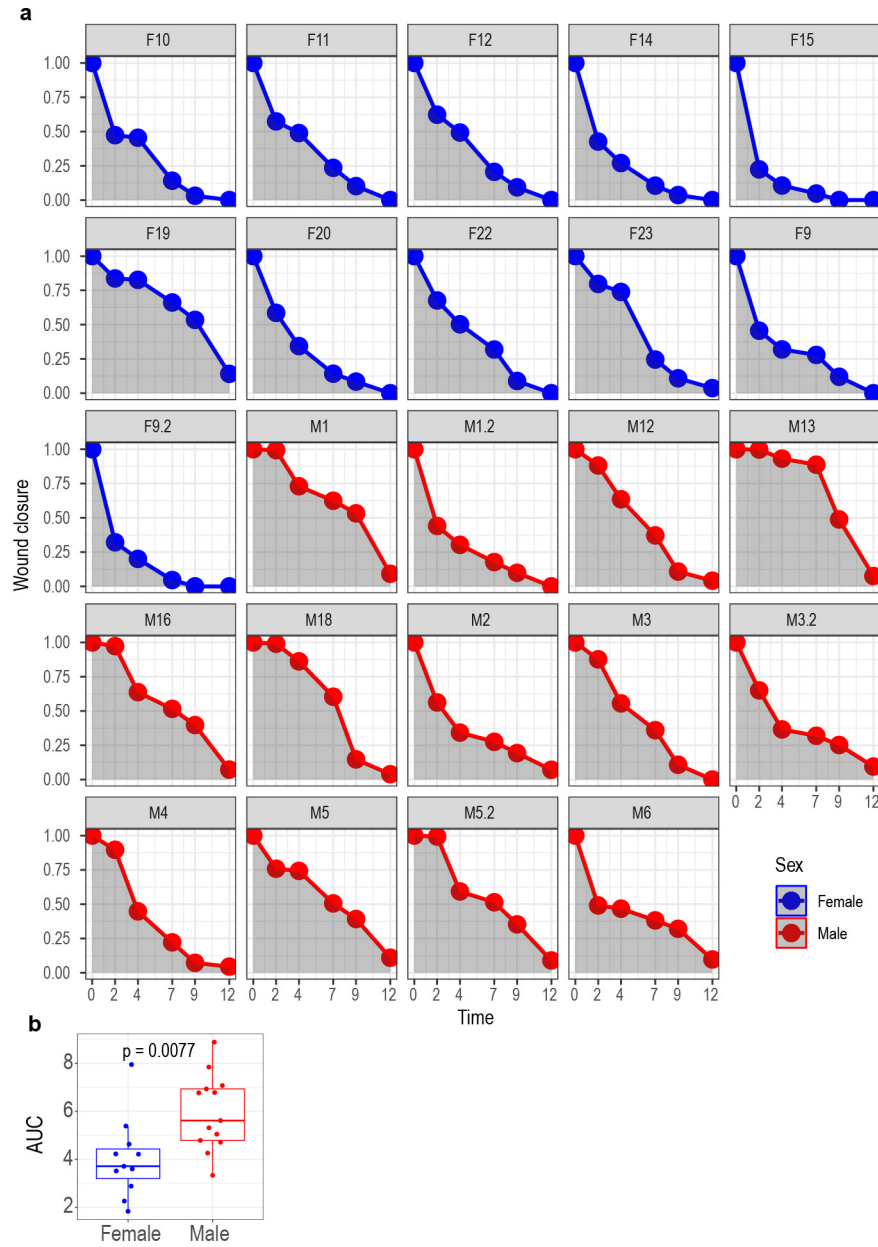
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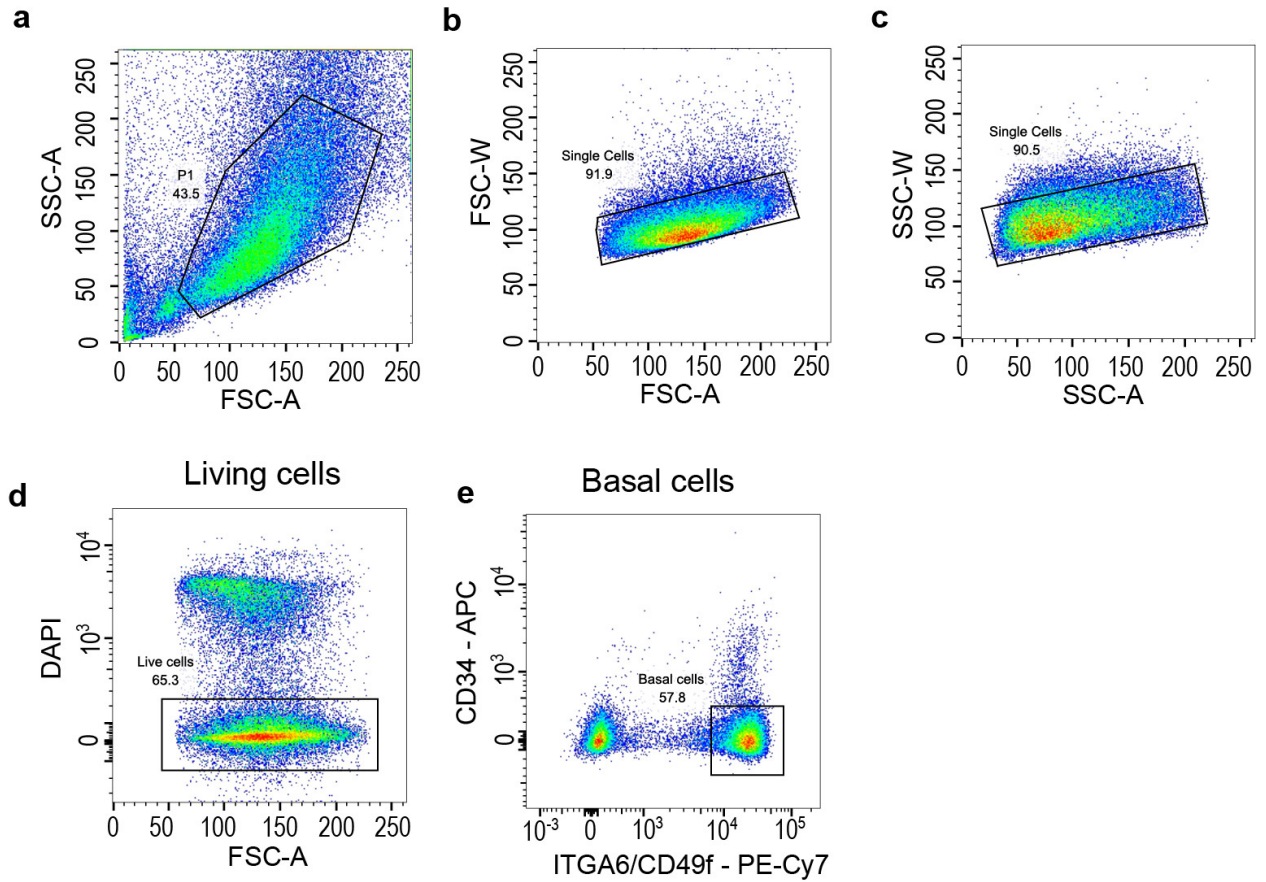
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Supplementary Fig. 5: **Statistical analysis of mouse-to mouse-variability** **a-f**, Average basal clone size (a male, d female), average supra-basal clone size (b male, e female) and clone persistence (c male, f female) for individual mice, with shaded uncertainty regions indicating 95 % and 68% confidence intervals from 1000 ensembles of randomly sampled trajectories with the number of samples set by the average number of clones per mouse for the sex (for details see Supplementary Notes). Colours correspond to different mice. Lines within uncertainty region indicate mouse dynamics are consistent with pooled mice for that sex. **g-j**, Cumulative basal clone size distribution, with the size rescaled by the mean. Data from the combination of all males (g) and all females (i) show evidence of statistical scaling behaviour, marked by collapse of the cumulative size distribution at different timepoints onto the same curve at long times. This scaling property is consistent with a model of balanced stochastic stem cell loss and replacement.¹ Further, individual male (h) and female (j) mice also show evidence of statistical scaling across time.



Supplementary Fig. 6: **Individual data for sex-specific differences in wound healing.** **a**, Fraction of wound area closure over time in individual female and male mice highlighting AUC used as an estimate of wound closure speed. **b**, AUC for wound closure of female and male mice. N=11 female and N=12 male mice. Box plots show the median (line), 25th–75th percentiles (box), and min/max (whiskers), two-sided Wilcoxon rank sum test.



Supplementary Fig. 7: **IFE basal cells isolation strategy.** **a-e**, Representative fluorescence activated cell sorting (FACS) strategy used to isolate basal cells, excluding doublets (a-c), selecting living cells as DAPI negative cells (d) and Itga6+ (Pe-Cy7) marking basal cells and CD34- (APC) to exclude hair follicle cells (e).