Supplemental Theory, Singh et al.

In the following supplementary theory section, we provide more details on the analysis of the fate mapping data in the extramural glands and surface epithelium using a genetic lineage tracing strategy based on the *Mki67* promoter (Figure 5A). To begin the analysis, we will not consider the overall induction frequency and whether there is clonal information in the data. Instead, our focus is first on what we can learn from measurements of the overall fraction of lineage labelled cells in each of the sub-compartments of the extramural glands (base, body, and pit) and the surface epithelium (Figure 5B-I). The fate mapping assay comprises data from 4 chase times: 14 days, 30 days, 90 days, and 180 days.

Evidence for compartmentalization of the extramural peribiliary glands

Before questioning the cell fate behaviour of the individual compartments, a first key question is their inter-relation. Here, the 14 day time point is revealing: Comparing the fraction of lineage labelled cells in the body and base region, there is only a weak degree of correlation within individual glands (Figure S5E) with many glands showing a higher level of base labelling than the body. By contrast, at 90 days, the fraction of labelled cells in the base and body is now more correlated within individual glands with the base and body clones scaling in equal proportion (Figure S5F). Together, such behaviour fits well with the results of the *Rosa26:Cre^{ERT2}; Confetti* tracing (Figure 2) and is consistent with a model in which renewing cells in the base region replenish cells in the body region.

By contrast, when we look for correlations in the fraction of labelled cells in the base or body region with cells in the pit region, the correlation is weak even at later time points (Figure S5G). Consistent with the results of the *Rosa26:Cre^{ERT2}*; Confetti labelling, this suggests that the base/body region forms a closed anatomical unit that is lineage-decoupled from the pit region.

Self-renewal of the glandular epithelium in the base/body region

Based on these findings, let us then consider whether the lineage tracing data can provide insight into the renewal dynamics of the gland base region. When averaged across all glands that were studied, including those that contain no lineage labelled cells, the total fraction of tdTomato$^+$ cells rises from around 9% to 19% within the base, showing an approximate doubling over the 6 month chase period. This is consistent with *Mki67* induction resulting in an enrichment of labelling in the self-renewing and proliferative population that gives rise to an approximately equal number of cells that are non-cycling.

To gain insight into the underlying fate behaviour of individual cells, it is necessary to assess the degree of clonality of the lineage tracing assay. If we assume that the labelling efficiency of the *Mki67* promoter is equivalent for all glands (i.e., representative), then we can use the frequency of fully unlabelled gland compartments to assess the overall induction frequency within each compartment. Setting p_{base} as the average number of cells induced per gland base, the probability P_n that a gland has n base clones at induction is given approximately by a binomial distribution,

$$P_n = \frac{p_{base}^n}{n!} e^{-p} \qquad (1)$$

Therefore, with some 24% of glands found to have no tdTomato+ cells in the base region at 14 days post-labelling, this translates to an induction probability of $p_{base} = 1.4$. In this case, we would expect some 34% of glands to have just 1 base clone at the time of induction and 41% of glands to have more than one (Figure S5D). Fortunately, although this implies that the lineage tracing data is not fully clonal, if clones are lost and replaced through competition among the renewing base cells, we can expect that, over time, the dynamics becomes effectively clonal since glands that had two clones at the time of induction will typically have lost at least one from the base through rounds of stochastic cell loss and replacement.

With this in mind, we can look at the statistical distribution of the labelled cell fractions to assess whether there is evidence for stem cell loss and replacement in the base compartment. Here, we can take inspiration from previous studies of clonal dynamics in the mouse intestinal crypt. Here, clone dynamics fits well with a minimal model in which renewing cells at the crypt base compete neutrally for niche access. Cells that are lost from the base region through displacement and differentiation are replaced by the symmetric duplication of neighbours leading to neutral clone dynamics around the crypt circumference until the clone is altogether lost or the crypt becomes monoclonally fixed. Prior to fixation, when clones occupy only a fraction of the crypt, the clone dynamics enters a scaling regime, where the chance of finding a clone with a size n larger than some multiple of the average $\langle n(t) \rangle$ becomes constant and given by[1]

$$C_n(t) = \exp\left[-\frac{\pi}{4}\left(\frac{n}{\langle n(t) \rangle}\right)^2\right] \qquad (2)$$

It follows that, when plotted as a function of $f/\langle f(t) \rangle$, where $f = n/N$ denotes the fraction of labelled cells in the base, the cumulative distribution of clone sizes should fall onto the parameter free curve $\exp[-\pi x^2/4]$. The dependence on the effective cell loss and replacement rate, λ, enters only through the average surviving clone size, which is predicted to increase as $\langle n(t) \rangle = \sim\sqrt{\lambda t}$. (It is worth noting that an estimate of the "effective" cell number N is usually not straightforward.)

To apply the neutral drift model to the current data, we have to exercise some caution. Since 41% of glands have more than one base clone at the time of induction, we can expect the distribution of labelled base cell fractions to differ at short chase times from the predicted statistical scaling form (2), a problem that could be further exacerbated by chance clone merger events. However, at longer chase times, we can expect ongoing clone loss at a rate $\sim 1/\langle n(t) \rangle$ to lead to an enrichment in the contribution of single cell-derived clones. Indeed, applied to the data, we find that the size distributions match well with the predicted scaling dependence (Figure 5J and 5K), suggesting that the base region is maintained a process of stochastic cell loss and replacement at the base of the gland similar to that seen in the intestinal crypt.

Self-renewal of the glandular epithelium in the pit region

Based on similar reasoning, we can then question the clonal dynamics of the renewing population in the pit region. Here, at 32%, the frequency of unlabelled glands at 14 days post-induction leads to an average pit clone number per gland of around $p_{pit} = 1.1$. This implies that some 31% of glands will have more than one clone in the pit at the time of induction (Figure S5D). Over the 180 day time course, the average fraction of labelled cells rises from around 9% to 29% suggesting that, in this case, self-renewing pit cells support a total average of around 2 or more terminally differentiated cells or proliferating cells with no renewal potential. Moreover, in common with the inferred dynamics in the base region, the cumulative clone size distribution also shows convergence to the statistical scaling form (2) pointing to a pattern of neutral cell competition in the pit region of the gland (Figure 5K and S5L). Together, these results suggest that the pit region is renewed by cells that line the circumference of the gland and undergo a process of stochastic cell loss and replacement, giving rise to cells that occupy the higher regions of the pit before escaping onto the surface epithelium. Indeed, such clonal dynamics mirrors the behaviour of stem cells localized in the isthmus region of the stomach corpus.[2]

Dynamics of epithelial cell fate on the surface epithelium

Finally, we turn to consider the results of fate mapping on the surface epithelium. Following induction under the *Mki67* promoter, very few cells are found to be tdTomato$^+$ on the surface epithelium. At the 14 day time point, only 3% of cells are labelled, and many of these cells may have derived from cells labelled in the pit region at the time of induction that have subsequently migrated onto the surface. However, by 180 days, some 30% of cells on the surface epithelium are lineage labelled, a figure roughly equal to the fraction of pit cells labelled at the same timepoint. These findings are consistent with a model in which the migration of cells from the peribiliary pit region supports the slow and steady turnover of the surface epithelium.

To challenge this hypothesis, it is useful to look at the quantifications of the labelled cell fractions on the surface epithelium. For any given patch of surface epithelium, there will be several extramural peribiliary glands that contribute to the turnover of tissue. Each of these glands supports a pit region that provides a steady production of new epithelial cells that move onto the surface epithelium. Let us then consider what we would expect if, having migrated on the surface epithelium, epithelial cells compete neutrally for survival – cells lost through exhaustion or damage are replenished by the duplication of neighbouring epithelial cells on the surface epithelium.

Based on this hypothesis, we can develop a minimal model of the labelled cell dynamics in the following manner: Since several glands contribute to a given region and the level of pit labelling is relatively high (at around 1 or more clones per gland on average), then the total fraction of labelled pit cells in a given neighbourhood of the surface epithelium would be approximately self-averaging, comprised of cells from multiple clones, and fixed at the average labelling density – a figure that can be read off empirically from the pit cell data at around 29% at the 180 day time point. Within the framework of the model, labelled cells that migrate from the pit region onto the surface then undergo a process of stochastic loss and replacement. Such behaviour translates to a stochastic birth-death process with

"immigration" – viz. the arrival of new pit cells, and can be represented as the dynamical process

$$P \longrightarrow P + E \quad \text{rate } \omega$$

$$E \longrightarrow \begin{cases} E + E & \text{Pr. } (1-\delta)/2 \\ \text{Loss} & \text{Pr. } (1+\delta)/2 \end{cases} \quad \text{rate } \kappa$$

where P denotes self-renewing pit cells and E represent cells of the surface epithelium. Here δ represents a small imbalance in fate to compensate for the arrival of new pit cells.

For a near-balanced stochastic birth-death process with immigration, the distribution of labelled "clone" sizes – the amalgamation of all labelled cells on the surface epithelium in a given region – takes the form of a negative binomial with the chance of finding a clone of size n given by[3]

$$Q_n(t) \approx \frac{A(t)}{(n/\kappa t)^{1-\frac{\omega}{\kappa}}} \exp\left[-\frac{n}{\kappa t}\right]$$

where $A(t)$ denotes the normalization. If the immigration rate ω is small compared to loss/replacement rate κ, the clonal distribution would be biased towards small clone sizes: Most clones on the surface epithelium would be derived recently from pits with the majority of those cells that migrated further back in time having been lost through cell competition. However, when the arrival rate of new cells from the pit is faster than the loss/replacement rate, the distribution becomes Gaussian-like, with

$$Q_n(t) \approx B(t) \exp\left[-\frac{1}{2}\frac{1}{\omega\kappa t^2}(n-\omega t)^2\right]$$

with $B(t)$ the normalisation. In this limit, the average labelled cell fraction is predicted to rise linearly with time (at least until the domain becomes clonally saturated), scaling with the net immigration rate of cells from the pit region while the variance of the distribution expands in proportion to t^2. To apply this result to the lineage tracing data, we have to consider the fraction of labelled cells, which is linked to the cell number through an unknown constant of proportionality, $f = n/M$ that is linked to the unknown ratio of self-renewing pit to surface epithelial cells in steady state. Once again, applied to the lineage tracing data, the predictions of the model fit remarkably well with the measured average fractional size dependence of labelled cells on the surface epithelium, its variance, and the cumulative fractional size distributions (Figure 5M). From a fit of the average labelled cell fraction to the predicted linear time dependence, we obtain $\omega/M = 0.16$ per day. From a fit of the standard deviation of labelled cell fraction to the predicted linear time dependence, we obtain $\sqrt{\omega\kappa}/M = 0.062$ per day. From this result, it follows that $\omega/\kappa \approx 7$, providing a consistency check on the model limit.

Supplemental References

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